Arch. Pharm. Res. Vol.21, No.2, pp. 164-167, 1998

Conformationally Constrained Analogues of Diacylglycerol Having a Perhydrofuro[3,4-c]furan-1,4-dione Bis-γ-butyrolactone Skeleton

Jeewoo Lee[1], Nancy E. Lewin[2], Peter M. Blumberg[2] and Victor E. Marquez[3]

[1]College of Pharmacy, Seoul National University, Shinlim-Dong, Kwanak-Ku, Seoul, Korea 151-742, [2]Laboratories of Cellular Carcinogenesis and Tumor Promotion, and [3]Medicinal Chemistry, Division of Basic Sciences, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

(Received November 7, 1997)

Bis-γ-lactones (**1, 2**) having a perhydrofuro[3,4-c]furan-1,4-dione skeleton were designed as conformationally constrained diacylglycerol analogues. They were synthesized from D-apiose in 11 steps, and evaluated as PKC-α ligands by measuring their ability to displace bound [^{3}H]-PDBU from the enzyme. The compounds showed moderate binding affinities with K_i values of 13.89 (±5.67) μM and 11.47 (±0.89) μM, respectively. Their similar binding affinities indicate that these two bicyclic compounds were not effectively discriminated by PKC-α in terms of the direction of the side chain as other ligands built on similar bis-γ-lactones.

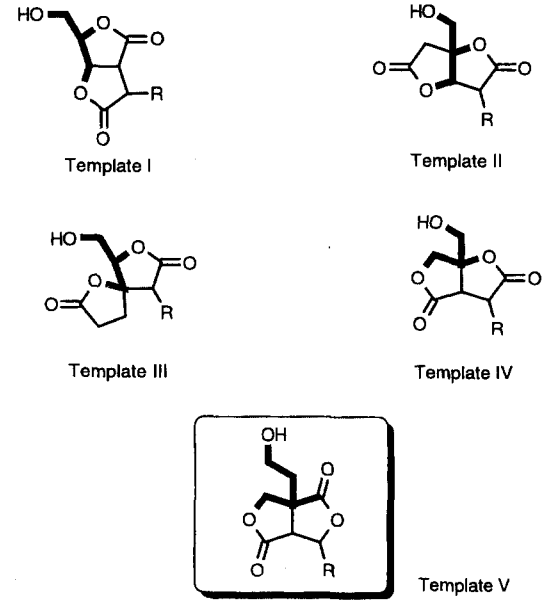
Key words : Protein kinase C, Ligand, Diacylglycerol, Phorbol ester, Bis-γ-butyrolactone

INTRODUCTION

Protein kinase C (PKC) is a family of phospholipid-dependent, serine/threonine-specific kinases which is believed to play a pivotal role in cell signal transduction pathways (Lester et al., 1992). Activation of PKC by diacylglycerol (DAG), which is released by phospholipase C-mediated hydrolysis of membrane-resident phosphatidylinositol 4,5-diphosphate, is a critical step in the signal transduction pathways controlling processes such as cellular proliferation and differentiation (Stabel *et al.*, 1991). The fact that this enzyme has been implicated in the progression of a wide variety of diseases accentuates the importance of PKC ligands as attractive therapeutic targets, especially as anticancer agents (Blobe *et al.*, 1994 and Gescher *et al.*, 1989).

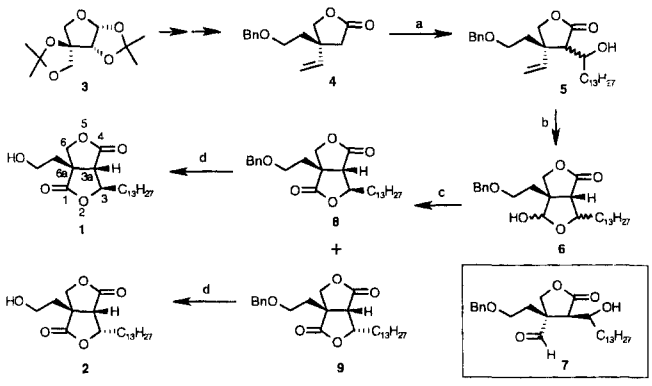
Recent studies in our laboratories have focused on the construction of conformationally constrained diacylglycerol analogues in which glycerol backbone of DAG is embedded within a lactone template (Lee *et al.*, 1996a). Until now, we have reported four distinct bis γ-lactone templates (Template I-IV) as restricted DAG surrogates in which the glycerol backbone is extended into the two fused γ-lactone rings (scheme 1). Compounds constructed with these templates have shown moderate PKC binding affinity that is sensitive to the stereochemistry of the alkyl chain, R (Lee *et al.*, 1992, 1993a, 1993b, 1994, 1996b).

To expand our investigation into additional bis γ-lactones as constrained templates for the construction of potent PKC ligands, we explored a perhydrofuro[3,4-c]furan-1,4-dione ring system (Template V) where



Scheme 1.

Correspondence to: Jeewoo Lee, College of Pharmacy, Seoul National University, Shinlim-Dong, Kwanak-Ku, Seoul, Korea 151-742



Reagent and Conditions: (a) LiHMDS, THF, - 78 °C; $C_{13}H_{27}CHO$ (85%) (b) OsO_4, 4-NMO, $NaIO_4$, H_2O-THF (50% for 6, 48% for 7) (c) PDC, AcOH, CH_2Cl_2 (38% for 8, 58% for 9) (d) H_2, Pd-C, MeOH (95% for 1, 2)

Scheme 2.

the two ester carbonyl pharmacophores are in an antiparallel orientation as in Templates II and III. In order to maintain a proper fit to DAG, the typical hydroxymethyl pharmacophore present in Templates I-IV was increased to the next higher hydroxyethyl homologue. In this paper, we report the synthesis of compound **1** and **2**, as well as their binding affinity towards PKC-α, the most of abundant isozyme of PKC.

MATERIALS AND METHODS

General experimental

All chemical were commercially available. Melting points were determined on a Mel-Temp II apparatus, Laboratory Devices, USA, and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230~400 mesh (E. Merck). ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-250 instrument at 250 and 62.9 MHz, respectively. Spectra were referenced to the solvent in which they were run (7.24 ppm for $CDCl_3$). Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

Analysis of inhibition of [^{3}H]PDBU binding by nonradioactive ligands

Enzyme-ligand interactions were analyzed by competition with [^{3}H]PDBU binding essentially as described in our previous work, except that the PKC preparation used here was the single isozyme PKC-α (Teng *et al.*, 1992). This recombinant PKC-α was expressed in the baculovirus system and was isolated as described (Kazanietz *et al.*, 1993). The ID_{50} values were determined from the competition curves, and the corresponding K_i values for the ligands were calculated from the ID_{50} values as described before. Values represent the mean ± standard error (three determinations).

(3*R*,3a*S*,6a*S*)-3-Tridecanyl-6a-[2-(benzyloxy)ethyl]perhydrofuro[3,4-c]furan-1,4-dione (8) and (3*S*,3a*S*,6a*S*)-3-Tridecanyl-6a-[2-(benzyloxy)ethyl]-perhydrofuro[3,4-c]furan-1,4-dione (9)

A stirred solution of **4** (1.65 g, 6.69 mmol) in THF (35 ml) was cooled to -78°C and treated slowly with sodium bis(trimethylsilyl)amide (1 M, 8.02 ml, 8.02 mmol). After 30 min, tetradecanyl aldehyde (80%, 2.66 g, 10.03 mmol) dissolved in THF (10 ml) was added and stirring was continued for 1 h at -40°C. The reaction mixture was quenched with saturated NH_4Cl solution, and diluted with diethyl ether and water. The organic phase was separated, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel with EtOAc:hexane (1:5 to 1:2) as eluant to give compound **5** (2.0 g, 65%), as a colorless oil, plus some recovered starting material (**4**, 0.33 g, 20%).

Compound **5** (2.0 g, 4.36 mmol) was dissolved in THF:H_2O (1:1, 20 ml) and treated with 4-methylmorpholine N-oxide (1.02 g, 8.72 mmol), sodium periodate (1.86 g, 8.72 mmol), and osmium tetroxide (2.5 wt% in 2-methyl-2-propanol, 2 ml). After stirring for 16 h at room temperature, the reaction mixture was diluted with EtOAc. The organic layer was washed with sodium thiosulfate solution and H_2O, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel with EtOAc:hexane (1:5 to 1:3) as eluant to give **6** (1.0 g, 50%, R_f=0.4 and 0.38, EtOAc:hexane=1:2) and **7** (0.96 g, 48%, R_f=0.5 and 0.32, EtOAc:hexane=1:2) as a colorless oils.

Compound **6** (1.0 g, 2.17 mmol) was dissolved in CH_2Cl_2 (40 ml) and treated with pyridinium dichromate (2.45 g, 6.51 mmol), 4 Å molecular sieve (3.0 g), and acetic acid (0.25 ml, 4.34 mmol). After stirring for 1 h at room temperature, the reaction mixture was quenched with ether and celite, and stirred for 30 min. The suspension was filtered through a short pad of silica gel with EtOAc and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel with EtOAc:hexane (1:3) as eluant to give **8** (0.378 g, 38%, R_f=0.5, EtOAc:hexane=1:2) and **9** (0.577 g, 58%, R_f=0.4, EtOAc:hexane=1:2) as a white solids.

Compound **8**: ^1H NMR ($CDCl_3$) δ 7.20~7.40 (m, 5 H, phenyl), 4.60 (m, 1 H, H_3), 4.53 (d, 1 H, *J*=10 Hz, $H_{6'}$), 4.46 (dd, 2 H, $PhCH_2O$), 4.33 (d, 1 H, *J*=10 Hz, $H_{6''}$), 3.64 (m, 2 H, $C\underline{H}_2OBn$), 3.24 (d, 1 H, *J*=3.1 Hz, H_{3a}), 2.02~2.24 (m, 2 H, $C\underline{H}_2CH_2OBn$), 1.20~1.80 (m, 24 H, $(C\underline{H}_2)_{12}CH_3$), 0.86 (distorted t, 3 H, $(CH_2)_{12}C\underline{H}_3$).

Compound **9**: ^1H NMR ($CDCl_3$) δ 7.20~7.40 (m, 5 H, phenyl), 4.60 (m, 1 H, H_3), 4.57 (d, 1 H, *J*=9.75 Hz, $H_{6'}$), 4.46 (s, 2 H, $PhCH_2O$), 4.12 (d, 1 H, *J*=9.75 Hz, $H_{6''}$), 3.60 (m, 2 H, $C\underline{H}_2OBn$), 3.36 (d, 1 H, *J*=7.4 Hz,

H_{3a}), 1.80~2.24 (m, 2 H, $C\underline{H}_2CH_2OBn$), 1.15~1.70 (m, 24 H, $(C\underline{H}_2)_{12}CH_3$), 0.86 (distorted t, 3 H, $(CH_2)_{12}C\underline{H}_3$).

(3*R*,3a*S*,6a*S*)-3-Tridecanyl-6a-(2-hydroxyethyl) perhydrofuro[3,4-*c*]furan-1,4-dione (1)

A solution of **8** (0.3 g, 0.66 mmol) in MeOH (20 ml) was treated with 10% Pd-C (0.15 g) and hydrogenated under a hydrogen-filled balloon for 4 h. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography over silica gel with EtOAc:hexane (1:1) as eluant to give **1** (0.236 g, 98%) as a white solid: mp=72°C; $[\alpha]_D$=-31.5 (c 1.08, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.66 (m, 1 H, H_3), 4.61 (d, 1 H, *J*=10 Hz, $H_{6'}$, 4.36 (d, 1 H, *J*=10 Hz, $H_{6''}$, 3.88 (m, 2 H, $C\underline{H}_2OH$), 3.22 (d, 1 H, *J*=3.0 Hz, H_{3a}), 2.00~2.24 (m, 2 H, $C\underline{H}_2CH_2OH$), 1.20~1.90 (m, 24 H, $(C\underline{H}_2)_{12}CH_3$), 0.86 (distorted t, 3 H, $(CH_2)_{12}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 177.89 (s), 175.94 (s), 81.45 (d), 74.09 (t), 58.27 (t), 52.10 (s), 50.49 (d), 36.40 (t), 36.03 (t), 31.91, 29.67 (t), 29.64 (t), 29.59 (t), 29.49 (t), 29.39 (t), 29.35 (t), 29.03 (t), 25.23 (t), 22.68 (t), 14.11 (q); IR (KBr) 3568, 1785, 1654 cm^{-1}. Anal. Calcd for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 68.21; H, 9.89. FAB MS m/e 369 (MH^+).

(3*S*,3a*S*,6a*S*)-3-Tridecanyl-6a-(2-hydroxyethyl)perhydrofuro[3,4-*c*]furan-1,4-dione (2)

Following the same procedure described for compound **1**, compound **2** was prepared from **9** and obtained as a white solid in 98% yield; mp=59°C; $[\alpha]_D$=-102 (c 2.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.78 (m, 1 H, H_3), 4.61 (d, 1 H, *J*=9.75 Hz, $H_{6'}$, 4.17 (d, 1 H, *J*=9.75 Hz, $H_{6''}$, 3.84 (m, 2 H, $C\underline{H}_2OH$), 3.40 (d, 1 H, *J*=7.63 Hz, H_{3a}), 1.90~2.24 (m, 2 H, $C\underline{H}_2CH_2OH$), 1.20~1.80 (m, 24 H, $(C\underline{H}_2)_{12}CH_3$), 0.86 (distorted t, 3 H, $(CH_2)_{12}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 178.19 (s), 173.21 (s), 79.98 (d), 73.81 (t), 58.75 (t), 52.10 (s), 48.28 (d), 35.31 (t), 31.91 (t), 31.44 (t), 29.66 (t), 29.64 (t), 29.53 (t), 29.43 (t), 29.35 (t), 29.27 (t), 26.33 (t), 22.68 (t), 14.11 (q); IR (KBr) 3752, 1785, 1654 cm^{-1}. Anal. Calcd for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 68.27; H, 9.83. FAB MS m/e 369 (MH^+).

RESULTS AND DISCUSSION

The synthesis of target compounds **1** and **2** is shown in scheme 2. Compound **4** was prepared from 1,2:3,5-di-*O*-isopropylidene-α-D-threo-apiofuranose **3** in 7 steps as previously reported (Lee *et al.*, 1996c). Reaction of the lithium enolate of **4** with myristyl aldehyde afforded a diastereomeric mixture of alcohol **5**, which on subsequent treatment with OsO_4, 4-NMO and $NaIO_4$ gave a mixture of **6** and **7** in a 1:1 ratio. The correct assignment of **6** or **7** was based on the presence of a resonance peak that corresponded to the aldehyde proton in the ^1H NMR spectrum of the opened product **7**. Oxidation of **6** by pyridinium dichromate afforded the corresponding lactones, **8** and **9**, whose stereochemistry was discerned on the basis of the value of the coupling constant between the H_{3a} and H_3 protons (**8**: trans, *J*=3.1 Hz, **9**: cis, *J*=7.4 Hz). Finally, each compound (**8** and **9**) was debenzylated to give the respective final products **1** and **2**.

The binding affinities of **1** and **2** to PKC were measured in terms of their ability to displace bound [^3H]-phorbol-12,13-dibutyrate (PDBU) from the recombinant single isozyme of PKC, PKC-α. Both compound showed similar and moderate affinities with K_i values of 13.89 (±5.67) μM (1) and 11.47 (±0.89) μM (**2**). The binding of DAG and other bis-γ-lactones to the regulatory domain of the PKC is very sensitive to the direction of alkyl side chain. The present results, however, indicate that this distinction does not occur in the case of compounds constructed with this new template.

ACKNOWLEDGMENT

This work was partially supported by Seoul National University Posco Research Fund, 1996 and Research Institute of Pharmaceutical Science, College of Pharmacy, Seoul National University.

REFERENCES CITED

Blobe, G. C., Obeid, L. M. and Hannum, Y. A., Regulation of protein kinase C and role in cancer biology. *Cancer and Metastasis Reviews* 13, 411-431 (1994).

Gescher, A. and Dale, I. L., Protein kinase C-A novel target for rational anti-cancer drug design ? *Anti-Cancer Drug Design*, 4, 93-105 (1989).

Kazanietz, M. G., Areres, L. B., Bahador, A., Mischak, H., Goodnight, J., Mushinski, J. F. and Blumberg, P. M., Characterization of ligand and substrate specificity for the calcium-dependent and calcium-independent protein kinase C isozymes. *Mol. Pharmacol.* 44, 298-307 (1993).

Lee, J., Teng, K. and Marquez, V. E., Synthesis of two rigid diacylglycerol analogues having a bis-butyrolactone skeleton. *Tet. Lett.* 33, 1539-1542 (1992).

Lee, J., Marquez, V. E., Lewin, N. E. and Blumberg, P. M., Synthesis of two rigid diacylglycerol analogues having a perhydrofuro[3,4-b]furan bis-γ-butyrolactone skeleton 2. *Tet. Lett.*, 34, 4313-4316 (1993a).

Lee, J., Marquez, V. E., Bahador, A., Kazanietz, M. G. and Blumberg, P. M., Synthesis of two rigid diacylglycerol analogues having a perhydrofuro[3,2-b]furan bis-γ- butyrolactone skeleton 3. *Tet. Lett.*, 34, 4317-4320 (1993a).

Lee, J., Marquez, V. E., Lewin, N. and Blumberg, P. M., Synthesis of two rigid diacylglycerol analogues having a 1,7-dioxaspiro[4,4]nonane bis-γ-butyrolactone skeleton. 4. *Synlett*, 4, 206-208 (1994).

Lee, J., Sharma, R., Wang, S., Milne, G. W. A., Lewin, N. E., Szallazi, Z., Blumberg, P. M., George, C. and Marquez, V. E., Conformationally constrained analogues of diacylglycerol. 12. Ultrapotent protein kinase C ligands based on a chiral 5-disubstituted heptono-1,4-lactone template. *J. Med. Chem.*, 39, 36-45 (1996a) and references cited therein.

Lee, J., Lewin, N. E., Blumberg, P. M. and Marquez, V. E., Synthesis of bis-γ-butyrolactones containing conformationally constrained (*S*)- and (*R*)-diacylglycerol structures. *Bioorg. Med. Chem.*, 4, 1299-1305 (1996b).

Lee, J., Lewin, N. E., Acs, P., Blumberg, P. M. and Marquez, V. E., Conformationally constrained analogues of diacylglycerol. 13. Protein kinase C ligands based on templates derived from 2,3-dideoxy-L-erythro(threo)-hexono-1,4-lactone and 2-deoxyapiolactone. *J. Med. Chem.* 39, 4912-4919 (1996c).

Lester, D. S. and Epand, R. M. Eds., *Protein kinase C. current and future perspectives.* Ellis Horwood: New York. (1992)

Stabel, S. and Parker, P. J., Protein kinase C. *Pharmacol Ther.*, 51, 71-95 (1991)

Teng, K., Marquez, V. E., Milne, G. W. A., Barchi, Jr. J. J., Kazanietz, M. G., Lewin, N. E., Blumberg, P. M. and Abushanab, E., Conformationally constrained analogues of diacylglycerol. Interaction of γ-lactones with the phorbol ester receptor of protein kinase C. *J. Am. Chem. Soc.* 114, 1059-1070 (1992).