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Design and Synthesis of Bioisosteres of Ultrapotent Protein Kinase C (PKC) Ligand, 5-Acetoxymethyl-5-hydroxymethyl-3-alkylidene tetrahydro-2-furanone

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Three compounds, 5-(acetoxymethyl)-5-(hydroxymethyl)-3-tetradecyl-2,5-dihydro-2-furanone (**3**), 5-(acetoxymethyl)-5-(hydroxymethyl)-3,3-dihexyltetrahydro-2-furanone (**4**) and 5-(acetoxymethyl)-5-(hydroxymethyl)-3,3-dioctyltetrahydro-2-furanone (**5**), were designed and synthesized as surrogates of the ultrapotent DAG analogue, 5-(acetoxymethyl)-5-(hydroxymethyl) 3-[(Z)-tetradecylidene]tetrahydro-2-furanone (**1**), a compound that showed high affinity for PKC-α (K_i=35 nM) in a competition binding assay with [^{3}H-20]phorbol-12,13-dibutyrate (PDBU). In an attempt to overcome the problem of generating geometrical E- and Z- isomers, as encountered with **1**, the double bond was moved to an endocyclic location as in **3**, or an additional alkyl chain was appended to C3 to give the corresponding 3,3-dialkyl saturated lactones (**4** and **5**). The lactone was constructed from glycidyl-4-methoxyphenyl ether in 5 steps. The target compounds showed reduced binding affinities for PKC-α with K_i values of 192 nM (**3**), 4,829 nM (**4**), and 2,812 nM (**5**), respectively. These results indicate that constrained DAG analogues having a tetrahydro-2-furanone template are effectively discriminated by PKC-α in terms of the direction of the long alkyl chain connected to the 3-position.

Key words : Protein kinase C ligand, Diacylglycerol, Phorbol ester, 5,5-Disubstituted tetrahydro-2-furanone template, Bioisosteres

INTRODUCTION

Protein kinase C (PKC) represents a family of phospholipid-dependent, serine/threonine-specific protein kinase comprising 12 isozymes. These isozymes are believed to play important roles in cellular signal transduction pathways involved in cellular processes such as cell differentiation, proliferation, modulation of gene expression and multiple-drug resistance (MDR) (Hug *et al.*, 1993, Lester *et al.*, 1992, Clemens *et al.*, 1992 and Rahmsdorf *et al.*, 1990). Most PKC isozymes are activated endogenously by diacylglycerol (DAG) released from the receptor-activated hydrolysis of phosphatidylinositol 4,5-diphosphate (PIP_2), or from phosphatidyl choline (PC) hydrolysis (Stabel *et al.*, 1991 and Nishizuka *et al.*, 1995), and exogenously by tumor-promoting diterpene esters such as the phorbol esters (Leach *et al.*, 1983). The binding site of these ligands has been identified as a region of a tandem repeat of two cysteine-rich zinc-finger motifs, so-called Cys-1 and Cys-2, present in the regulatory C1 domain of the enzyme (Ono *et al.*, 1989). In general, the phorbol esters display several orders of magnitude greater binding affinities for PKC than DAG. However, the phorbol esters can act supraphysiologically and activate responses that are not normally elicited by DAG (Wilkinson *et al.*, 1994). In order to solve this problem, a series of "ultrapotent" DAG analogues that displayed ca. 100-fold greater affinity for PKC-α than DAG were synthesized (Lee *et al.*, 1996). These compounds were designed as conformationally constrained DAG analogues embedded in a lactone template. Thus, by restricting the flexible glycerol backbone of the DAGs, part of the entropic penalty associated with their binding was reduced. Among them, 5-(acetoxymethyl)-5-(hydroxymethyl)-3-[(Z) or (E)-tetradecylidene]tetrahydro-2-furanone (**1,2**) represent lead compounds in this series. Interestingly, the (Z)-isomer consistently had higher binding affinity than (E)-isomer. Molecular modeling studies on both isomers compared with the phorbol esters indicated that the alkyl chain of the (Z)-isomer showed a better overlap with the alkyl chains of the C-12,13 diesters of phorbol. A synthetic problem, however, is that formation of the more active (Z)-isomer is always accom-

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panied by the less potent, and often more abundant (E)-isomer. This problem prompted the search for a (Z)-isomer surrogate with a similar biological profile. In this paper, the synthesis and biological activity of three possible 2-furanone surrogates, one with an endocyclic doble bond (**3**), and two bearing identical 3, 3-dialkyl chains (**4,5**), were designed to search for an equivalent, and possibly optimal alkyl chain orientation.

MATERIALS AND METHODS

General Experimental

All chemical reagents were commercially available. Column chromatography was performed on silica gel 60, 230-400 mesh (E. Merck). ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz and JEOL JNM-GCX 400 at 400 MHz. Chemical shift are reported in ppm units with Me_4Si as reference standard. Infrared spectra were recorded on a Perkin-Elmer 1710 FT spectrometer. Mass spectra were recorded with VG TRIO-2 GC-MS.

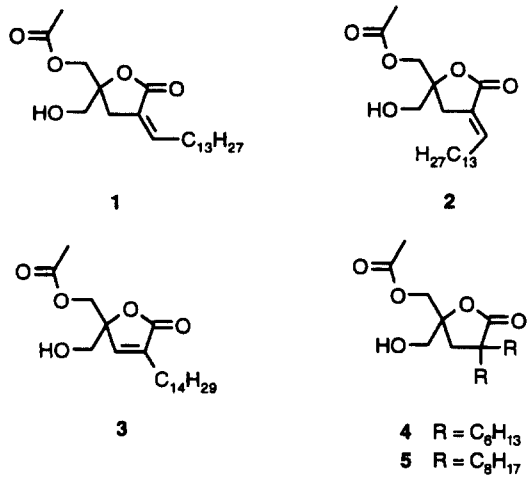
Analysis of inhibition of [^{3}H]PDBU binding by non-radioactive ligands 3-5

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.

1-(Benzyloxy)-3-(4-methoxyphenoxy)acetone (6)

A solution of benzyl alcohol (8.6 ml, 83.2 mmol) in DMF (40 ml) was treated portionwise with NaH (60%, 3.33 g, 83.2 mmol) while maintained at O°C. After stirring for 20 min at room temperature, the mixture was treated with glycidyl-4-methoxyphenyl ether (10 g, 55.5 mmol). The reaction mixture was heated at 80°C for 4 h and cooled to room temperature. The mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:2) as eluant to give the intermediate alcohol as an oil (14.46 g, 90%).

^{1}H NMR ($CDCl_3$) δ 7.27~7.37 (m, 5 H, phenyl), 6.80~6.86 (m, 4 H, Ar), 4.58 (s, 2 H, $PhCH_2O$), 4.17 (m, 1 H, $CH_2CH(OH)CH_2$), 4.00 (dd of AB, 1 H, CH_2OAr, J= 4.8 and 10 Hz), 3.96 (dd of AB, 1 H, CH_2OAr, J=6.0 and 10 Hz), 3.76 (s, 3 H, OCH_3), 3.67 (dd of AB, 1 H, $BnOCH_2$, J=4.4 and 10 Hz), 3.61 (dd of AB, 1 H, $BnOCH_2$, J=6.0 and 10 Hz), 2.60 (bs, 1 H, OH); IR (neat) 3436 (OH) cm^{-1}.



Scheme 1.

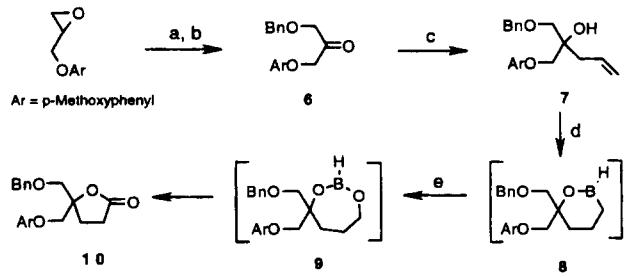
The above alcohol (14.46 g, 50 mmol) was dissolved in CH_2Cl_2 (50 ml) and slowly added via syringe to a mixture of 4 Å molecular sieves (32.3 g) and pyridinium chlorochromate (32.3 g, 150 mmol) in CH_2Cl_2 (150 ml). After stirring for 24 h at room temperature, the reaction mixture was quenched with ether and celite and stirred for 30 min. The mixture was filter through a short pad of silica gel and the filtrate was concentrated. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:2) as eluant to give **6** as an oil (13.26 g, 92%).

^{1}H NMR ($CDCl_3$) δ 7.30~7.40 (m, 5 H, phenyl), 6.83 (bs, 4 H, Ar), 4.71 (s, 2 H, CH_2OAr), 4.62 (s, 2 H, $PhCH_2O$), 4.36 (s, 2 H, CH_2OBn), 3.77 (s, 3 H, OCH_3); IR (neat) 1753 (C=O) cm^{-1}.

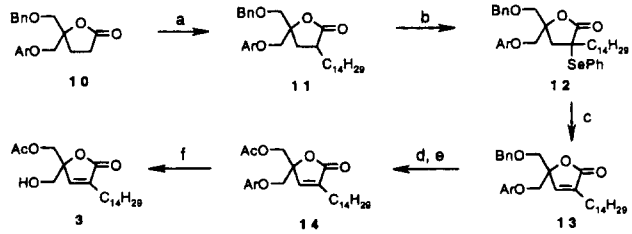
1-(Benzyloxy)-2-[(4-methoxyphenoxy)methyl]-4-penten-2-ol (7)

Allyl magnesium chloride (2 M in THF, 60 ml, 120 mmol) was slowly added to a cooled solution of **6** (11.53 g, 40 mmol) in THF (40 ml) at O°C. The reaction mixture was warmed to room temperature and stirred for 2 h. The mixture was quenched with 1 N HCl and concentrated to a small volume, which was diluted with H_2O and extracted with ether several times. The combined organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:4) as eluant to give **7** as an oil (11.52 g, 88%).

^{1}H NMR ($CDCl_3$) δ 7.25~7.35 (m, 5 H, phenyl), 6.75~6.86 (m, 4 H, Ar), 5.89 (m, 1 H, $CH=CH_2$), 5.10~5.20



Scheme 2. (a) BnOH, NaH, DMF, 80°C, 90% (b) PCC, 4 Å molecular sieve, CH_2Cl_2, 92% (c) CH_2=$CHCH_2MgBr$, THF, 0°C-rt, 88% (d) BH_3-SMe_2, THF, 2 h, (e) PCC, CH_2Cl_2, 48 h, 64%.



Scheme 3. (a) i) LiHMDS, THF, -78°C ii) $C_{14}H_{29}Br$, HMPA, 40% (b) i) LiHMDS, THF, -78°C ii) PhSeCl, 60%, (c) H_2O_2, pyridine, CH_2Cl_2, 90% (d) BCl_3, CH_2Cl_2, -78°C, 90% (e) Ac_2O, NEt_3, CH_2Cl_2, 95% (f) CAN, CH_3CN-H_2O, 90%.

(m, 2 H, CH=C$\underline{H}_2$), 4.55 (s, 2 H, $PhCH_2O$), 3.87 (s, 2 H, CH_2OAr), 3.76 (s, 3 H, OCH_3), 3.57 (d, 1 H, $BnOCH_2$, J=9.2 Hz), 3.48 (d, 1 H, $BnOCH_2$, J=9.2 Hz), 2.60 (bs, 1 H, OH), 2.44 (d, 2 H, $CH_2C\underline{H}$=CH_2, J =7.3 Hz); IR (neat) 3468 (OH) and 1641 (C=C) cm^{-1}.

5-[(Benzyloxy)methyl]-5-[(4-methoxyphenoxy)methyl] tetrahydro-2-furanone (10)

BH_3-SMe_2 complex (22.5 ml, 2M in THF, 45 mmol) was slowly added to a cooled solution of **7** (9.82 g, 30 mmol) in THF (100 ml) at -78°C. The reaction mixture was then allowed to reach room temperature, stirred for 24 h, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (300 ml) and treated with pyridinium chlorochromate (64.7 g, 300 mmol). After stirring for 3 days, the mixture was quenched with ether and celite and stirred for 30 min. The suspension was passed through a short pad of silica gel and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:1) as eluant to give **10** as a white solid (6.53 g, 64%).

^{1}H NMR ($CDCl_3$) δ 7.30~7.37 (m, 5 H, phenyl), 6.82 (m, 4 H, Ar), 4.59 (s, 2 H, $PhCH_2O$), 4.04 (dd, 2 H, CH_2OAr), 3.76 (s, 3 H, OCH_3), 3.68 (s, 2 H, $BnOCH_2$), 2.65~2.71 (m, 2 H, H-3), 2.24~2.31 (m, 2 H, H-4); IR (KBr) 1779 cm^{-1} (C=O).

5-[(Benzyloxy)methyl]-5-[(4-methoxyphenoxy)methyl]-3-tetradecyl-2,5-dihydro-2-furanone (13)

A solution of **10** (0.34 g, 1 mmol) in THF (2 ml) was added via syringe to a cooled solution of lithium bis(trimethylsilyl)amide (1M in THF, 1.2 ml, 1.2 mmol) and THF (1.2 ml) at -78°C. After 30 min stirring at - 78 °C, the reaction mixture was treated with a solution of 1-bromotetradecane (0.42 g, 1.5 mmol) and hexamethylphosphoramide (0.27 g, 1.5 mmol). After additional stirring at -78°C at 1 h and at -40°C for 1 h, the mixture was quenched with saturated NH_4Cl solution and diluted with ether. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:5) as eluant to give **11** as a white solid. (0.215 g, 40%).

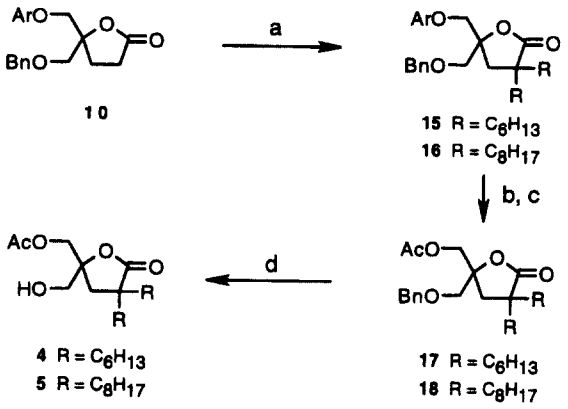
A solution of intermediate **11** (0.215 g, 0.4 mmol) in THF (2 ml) was added via syringe to a cooled solution of lithium bis(trimethylsilyl)amide (1M in THF, 0.48 ml, 0.48 mmol) and THF (0.5 ml) at -78°C. After 30 min stirring at -78°C, the reaction mixture was treated with a solution of phenylselenenyl chloride (0.114 g, 0.6 mmol) in THF (1.2 ml). After stirring at -78°C for 1 h and at -40°C for 1 h, the mixture was quenched with saturated NH_4Cl solution and diluted with ether. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:7) as eluant to give **12** as an oil. (0.166 g, 60%).

A solution of pyridine (0.024 ml) and 30% H_2O_2 (0.288 ml) was added to a cooled solution of **12** (0.166 g, 0.24 mmol) in CH_2Cl_2 (5 ml) at 0°C. After stirring for 1 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:5) as eluant to give **13** as a white solid (0.116 g, 90%).

^{1}H NMR ($CDCl_3$) δ 7.21~7.33 (m, 5 H, phenyl), 7.08 (s, 1 H, CH=C<), 6.75~6.80 (m, 4 H, Ar), 4.55 (dd, 2 H, J=12, 6 Hz, $PhCH_2O$), 4.20 (d of AB, 1 H, J=9 Hz, CH_2OAr), 4.01 (d of AB, 1 H, J=9 Hz, CH_2OAr), 3.77 (d of AB, J=9 Hz, CH_2OBn), 3.74 (s, 3 H, OCH_3), 3.68 (d of AB, J=9 Hz, CH_2OBn), 2.28 (t, 2 H, J=6 Hz, $C\underline{H}_2$-$(CH_2)_{12}Me$), 1.23 (m, 24 H, $(CH_2)_{12}$), 0.86 (distorted t, 3 H, J=6 Hz, $(CH_2)_{12}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 172.72, 154.38, 152.42, 146.84, 137.46, 136.53, 128.44, 127.86, 127.62, 115.83, 114.64, 86.62, 73.82, 70.69, 69.37, 55.69, 31.92, 29.65, 29.51, 29.34, 29.31, 29.07, 27.33, 25.41, 22.69, 14.11; IR (KBr) 1768 (C=O), 1662 (C=C) cm^{-1}; MS EI *m/e* 536 (M^++1)

5-(Acetoxymethyl)-5-[(4-methoxyphenoxy)methyl]-3-tetradecyl-2,5-dihydro-2-furanone (14)

A solution of BCl_3 (1M in CH_2Cl_2, 0.6 ml, 0.6 mmol)



Scheme 4. (a) i) LiHMDS, THF, -78°C ii) $C_6H_{13}I$ or $C_8H_{17}I$, HMPA, 92% for **15**, 60% for **16** (b) CAN, CH_3CN-H_2O, 80% from **15**, 95% for **16** (c) Ac_2O, NEt_3, CH_2Cl_2, 85% for **17**, 90% for **18** (d) H_2, Pd-C, MeOH, 90% for **4**, 88% for **5**.

was added slowly to a cooled solution of **13** (0.107 g, 0.2 mmol) in CH_2Cl_2 (4 ml) at -78°C. After stirring for 2 h at -78°C, the reaction mixture was quenched with saturated $NaHCO_3$ and diluted with CH_2Cl_2. The organic layer was washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:2) as eluant to give the intermediate alcohol as a white solid (0.08 g, 90%).

A solution of the intermediate alcohol (0.08 g, 0.18 mmol) in CH_2Cl_2 (10 ml) was treated with Et_3N (0.073 g, 0.72 mmol), Ac_2O (0.037 g, 0.36 mmol) and DMAP (2 mg, 0.018 mmol). After stirring for 2 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:4) as eluant to give **14** as a white solid (0.083 g, 95%).

^{1}H NMR ($CDCl_3$) δ 7.05 (s, 1 H, CH=C<), 6.80 (m, 4H, Ar), 4.46 (d of AB, 1 H, J=12 Hz, CH_2OAc), 4.34 (d of AB, 1 H, J=12 Hz, CH_2OAc), 4.18 (d of AB, J=9 Hz, CH_2OAr), 3.93 (d of AB, J=9 Hz, CH_2OAr), 3.74 (s, 3 H, OCH_3), 2.27 (t, 2 H, J=6 Hz, $C\underline{H}_2(CH_2)_{12}Me$), 2.02 (s, 3 H, CH_3C=O), 1.23 (m, 24 H, $(CH_2)_{12}$), 0.86 (distorted t, 3 H, J=6 Hz, $(CH_2)_{12}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 172.19, 170.23, 154.58, 152.07, 145.69, 145.58, 137.11, 115.72, 114.71, 85.12, 69.54, 63.65, 55.66, 31.88, 29.62, 29.49, 29.32, 29.27, 29.05, 27.37, 25.36, 22.67, 20.64, 20.55, 14.09.

5-(Acetoxymethyl)-5-(hydroxymethyl)-3-tetradecyl-2,5-dihydro-2-furanone (3)

Ammonium cerium (IV) nitrate (0.164 g, 0.3 mmol) was added to a cooled solution of **14** (0.073 g, 0.15 mmol) in CH_3CN-H_2O (4:1, 5 ml) at 0°C. After stirring for 30 min at 0°C, the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:2) as eluant to give **3** as a white solid (0.083 g, 90%).

^{1}H NMR ($CDCl_3$) δ 6.93 (s, 1 H, CH=C<), 4.32 (dd of AB, 2 H, J=12, 3 Hz, CH_2OAc), 3.74 (s, 2 H, CH_2OH), 2.27 (t, 2 H, J=6 Hz, $C\underline{H}_2(CH_2)_{12}Me$), 2.05 (s, 3 H, CH_3C=O), 1.78 (bs, 1 H, OH), 1.23 (m, 24 H, $(CH_2)_{12}$), 0.86 (distorted t, 3 H, J=6 Hz, $(CH_2)_{12}C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 172.51, 170.69, 145.60, 137.22, 87.04, 63.78, 63.29, 31.89, 29.64, 29.62, 29.53, 29.48, 29.32, 29.26, 29.07, 27.37, 25.36, 22.66, 20.61, 14.09; IR (KBr) 3379 (OH), 1733 (C=O), 1653 (C=C) cm^{-1}; MS EI *m/e* 383 (M^+).

5-[(Benzyloxy)methyl]-3,3-dihexyl-5-[(4-methoxyphenoxy)methyl]-tetrahydro-2-furanone (15) and 5-[(Benzyloxy)methyl]-3,3-dioctyl-5-[(4-methoxyphenoxy)methyl]tetrahydro-2-furanone (16)

A solution of **10** (0.17 g, 0.5 mmol) in THF (1 ml) was added via syringe to a cooled solution of lithium bis(trimethylsilyl)amide (1M in THF, 1.2 ml, 1.2 mmol) and THF (1.2 ml) at -78°C. After 30 min stirring at -78 °C, the reaction mixture was treated with a solution of 1-iodohexane (0.265 g, 1.25 mmol) and hexamethylphosphoramide (0.225 g, 1.25 mmol) for 1 h at -78°C and was then allowed to warm to room temperature. After stirring overnight, the mixture was quenched with saturated NH_4Cl solution and diluted with ether. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:5) as eluant to give **15** as an oil. (0.224 g, 92%).

^{1}H NMR ($CDCl_3$) δ 7.26~7.34 (m, 5 H, phenyl), 6.80 (bs, 4 H, Ar), 4.57 (dd of AB, 2 H, J=12, 9 Hz, $PhCH_2$-O), 3.99 (dd of AB, 2 H, J=9, 3 Hz, CH_2OAr), 3.75 (s, 3 H, OCH_3), 3.61 (dd of AB, 2 H, J=9, 6 Hz, $BnOCH_2$), 2.14 (dd of AB, 2 H, J=15, 15 Hz, H-4), 1.56~1.66 (m, 4 H, $2\times C\underline{H}_2(CH_2)_4CH_3$), 1.19~1.24 (m, 16 H, $2\times CH_2(C\underline{H}_2)_4CH_3$), 0.85 (m, 6 H, $2\times CH_2(CH_2)_4C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 180.86, 154.23, 152.55, 137.52, 128.42, 127.85, 127.80, 115.53, 114.66, 82.26, 73.67, 72.91, 70.93, 55.71, 47.57, 37.57, 37.50, 35.58, 31.64, 31.61, 29.66, 29.57, 24.34, 22.58, 14.04; IR (neat) 1770 (C=O) cm^{-1}; MS EI *m/e* 487 (M^+).

Compound **16** (60% yield) was prepared from **10** by following the same procedure used for the synthesis of **15**.

^{1}H NMR ($CDCl_3$) δ 7.24~7.30 (m, 5 H, phenyl), 6.76 (bs, 4 H, Ar), 4.56 (dd of AB, 2 H, J=16, 8 Hz, $PhCH_2$-O), 3.99 (dd of AB, 2 H, J=8, 4 Hz, CH_2OAr), 3.75 (s, 3 H, OCH_3), 3.61 (dd of AB, 2 H, J=12, 8 Hz, $BnOCH_2$), 2.14 (dd of AB, 2 H, J=20, 16 Hz, H-4), 1.54~1.57 (m, 4 H, $2\times C\underline{H}_2(CH_2)_6CH_3$), 1.22~1.24 (m, 24 H, $2\times CH_2(C\underline{H}_2)_6CH_3$), 0.85 (m, 6 H, $2\times CH_2(CH_2)_6C\underline{H}_3$); ^{13}C

NMR ($CDCl_3$) δ 180.86, 154.22, 152.54, 137.52, 128.41, 127.84, 127.79, 115.52, 114.64, 82.25, 73.66, 72.51, 70.92, 55.69, 47.56, 37.58, 37.48, 35.59, 31.82, 30.00, 29.92, 29.41, 29.38, 29.34, 29.26, 29.23, 24.39, 22.64, 22.61, 14.10, 14.08; IR (neat) 1769 (C=O) cm^{-1}; MS EI *m/e* 567 (M^+).

5-(Acetoxymethyl)-5-[(benzyloxy)methyl]-3,3-dihexyltetrahydro-2-furanone (17) and 5-(Acetoxymethyl)-5-[(benzyloxy)methyl-3,3-dioctyltetrahydro-2-furanone (18)

Ammonium cerium(IV) nitrate (0.329 g, 0.6 mmol) was added to a cooled solution of **15** (0.14 g, 0.3 mmol) in CH_3CN-H_2O (4:1, 10 ml) at 0°C. After stirring for 30 min at 0°C, the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:3) as eluant to give the intermediate alcohol as a colorless oil (0.11 g, 80%).

A solution of the alcohol (0.11 g, 0.24 mmol) in CH_2Cl_2 (12 ml) was treated with NEt_3 (0.1 g, 0.96 mmol), Ac_2O (0.05 g, 0.48 mmol) and DMAP (3 mg, 0.024 mmol). After stirring for 2 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:5) as eluant to give **17** as a colorless oil (0.102 g, 95%).

^{1}H NMR ($CDCl_3$) δ 7.27~7.35 (m, 5 H, phenyl), 4.54 (dd of AB, 2 H, *J*=12, 4 Hz, $PhCH_2O$), 4.16 (dd of AB, 2 H, *J*=16, 12 Hz, CH_2OAc), 3.51 (dd of AB, 2 H, *J*=20, 8 Hz, $BnOCH_2$), 2.13 (d of AB, 1 H, *J*=14 Hz, H-4a), 2.04 (s, 3 H, CH_3COO), 1.92 (d of AB, 1 H, *J*=14 Hz, H-4b), 1.50~1.60 (m, 4 H, 2×$C\underline{H}_2(CH_2)_4CH_3$), 1.14~1.29 (m, 16 H, 2×$CH_2(C\underline{H}_2)_4CH_3$), 0.85 (m, 6 H, 2×$CH_2(CH_2)_4C\underline{H}_3$).

Compound **18** (77% yield) was prepared from **16** by following the same procedure used for the synthesis of **17**.

^{1}H NMR ($CDCl_3$) δ 7.27~7.33 (m, 5 H, phenyl), 4.54 (dd of AB, 2 H, *J*=12, 3 Hz, $PhCH_2O$), 4.16 (dd of AB, 2 H, *J*=12, 9 Hz, CH_2OAc), 3.51 (dd of AB, 2 H, *J*=12, 9 Hz, $BnOCH_2$), 2.13 (d of AB, 1 H, *J*=12 Hz, H-4a), 2.04 (s, 3 H, CH_3COO), 1.92 (d of AB, 1 H, *J*=12 Hz, H-4b), 1.50~1.60 (m, 4 H, 2×$C\underline{H}_2(CH_2)_6CH_3$), 1.20~1.25 (m, 24 H, 2×$CH_2(C\underline{H}_2)_6CH_3$), 0.85 (m, 6 H, 2×$CH_2(CH_2)_6C\underline{H}_3$).

5-(Acetoxymethyl)-5-(hydroxymethyl)-3,3-dihexyltetrahydro-2-furanone (4) and 5-(Acetoxymethyl)-5-(hydroxymethyl)-3,3-dioctyltetrahydro-2-furanone (5)

A solution of **17** (0.09 g, 2 mmol) in MeOH (5 ml) was treated with 10% Pd-C (0.045 g) and hydrogenated under a ballon of hydrogen for 2 h. The mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:3) as eluant to give **3** as a colorless oil (0.064 g, 90%).

^{1}H NMR ($CDCl_3$) δ 4.25 (d of AB, 1 H, *J*=12 Hz, CH_2OAc), 4.10 (d of AB, 1 H, *J*=12 Hz, CH_2OAc), 3.62 (s, 2 H, CH_2OH), 2.54 (bs, 1 H, OH), 2.08 (d of AB, 1 H, *J*=12 Hz, H-4a), 2.07 (s, 3 H, CH_3COO), 1.93 (d of AB, 1 H, *J*=12 Hz, H-4b), 1.50~1.60 (m, 4 H, 2×$C\underline{H}_2(CH_2)_4CH_3$), 1.22~1.24 (m, 16 H, 2×$CH_2(C\underline{H}_2)_4CH_3$), 0.84 (m, 6 H, 2×$CH_2(CH_2)_4C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 180.70, 170.74, 82.24, 65.71, 65.27, 47.64, 37.52, 37.45, 35.34, 31.57, 29.59, 29.53, 24.27, 24.25, 22.53, 20.71, 13.96; IR (neat) 3469 (OH), 1749 (C=O) cm^{-1}; MS EI *m/e* 357 (M^+)

Compound **4** (88% yield) was prepared from **18** by following the procedure used for the synthesis of **17**.

^{1}H NMR ($CDCl_3$) δ 4.27 (d of AB, 1 H, *J*=12 Hz, CH_2OAc), 4.11 (d of AB, 1 H, *J*=12 Hz, CH_2OAc), 3.63 (dd of AB, 2 H, *J*=12, 8 Hz, CH_2OH), 2.10 (d of AB, 1 H, *J*=12 Hz, H-4a), 2.08 (s, 3 H, CH_3COO), 1.94 (d of AB, 1 H, *J*=12 Hz, H-4b), 1.52~1.66 (m, 4 H, 2×$C\underline{H}_2(CH_2)_6CH_3$), 1.22~1.26 (m, 24 H, 2×$CH_2(C\underline{H}_2)_6CH_3$), 0.85 (m, 6 H, 2×$CH_2(CH_2)_6C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 180.59, 170.77, 82.14, 65.68, 65.34, 47.63, 37.58, 37.52, 35.43, 31.78, 29.97, 29.91, 29.40, 29.38, 29.22, 24.34, 22.60, 20.75, 14.06; IR (neat) 3467 (OH), 1749 (C=O) cm^{-1}; MS EI *m/e* 414 (M^++1).

RESULTS AND DISCUSSION

For the synthesis of target compounds **3-5**, a key intermediate, 5,5-disubstituted tetrahydro-2-furanone **10** was prepared as shown in scheme II. Commercially available glycidyl-4-methoxyphenyl ether was reacted with the sodium salt of benzyl alcohol in DMF to give the corresponding alcohol. The intermediate alcohol was immediately oxidized to ketone **6** with pyridinium chlorochromate (PCC). Ketone **6** was converted into the tertiary homoallylic alcohol **7** by reaction with allyl magnesium bromide in THF. Following the methodology of Mandal and Mahajan, a one-pot synthesis of lactone **10** was achieved via hydroboration of the double bond and PCC oxidation of the intermediate boron complexes **8** and **9** (Mandal *et al.*, 1991).

The target compound **3** was synthesized from lactone **10** by consecutive alkylation and selenenylation reactions followed by elimination of PhSeO as shown in scheme III. Alkylation of lactone **10** with tetradecanyl bromide afforded a diastereomeric mixture of **11**, which on subsequent reaction with phenyl selenenyl chloride gave the 3,3-disubstituted tetrahydro-2-furanone **12**. Elimination of **12** with hydrogen peroxide and pyridine proceeded smoothly to give 3-alkyl-2,5-dihydro-2-furanone **13**. The characteristic proton NMR spec-

trum indicated the presence of a distinctive singlet at 7.08 ppm indicative of a H-4 vinyl proton. Debenzylation of **13** with BCl_3 and subsequent acetylation afforded **14**, whose p-methoxyphenyl group was finally deprotected to give **3**.

Syntheses of target 3,3-dialkyl analogues **4** and **5** are shown in scheme IV. Reaction of the lithium enolate of **10** with iodohexane, or iodooctane, afforded 3, 3-dialkyltetrahydro-2-furanones **15** and **16**, respectively. Removal of the p-methoxyphenyl group of **15** and **16**, followed by acetylation and debenzylation gave final compounds **4** and **5**.

PKC binding affinities for **3-5** were assessed in terms of their ability to displace bound [^{3}H-20]-phorbol-12, 13-dibutylate (PDBU) from a recombinant single isozyme PKC-α. The inhibition curves obtained for these ligands were of the type expected for competitive inhibition, and ID_{50} values were determined by fit of data points to the theoretical noncooperative competitive curve. The K_i's for inhibition of binding were calculated from the ID_{50} values. The results indicated that the new compounds showed lower affinities for PKC-α with K_i values of 192 nM (**3**), 4,830 nM (**4**), and 2,813 nM (**5**). A comparison with lead compounds **1** and **2** (K_i=35 nM for **1**, 78 nM for **2**) revealed that the direction of 3-alkyl chain on tetrahydro-2-furanones is very important to retain high binding affinity for PKC.

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