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5-Acyloxy-5-hydroxymethyltetrahydro-2-furancarboxylate as a novel template for protein kinase C (PKC) binding

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Abstract

A series of alkyl tetrahydrofuran-2-carboxylates (**1**–**4**) bearing a new set of three pharmacophoric groups were tested as protein kinase C (PKC) ligands. The compounds were synthesized from commercially available glycidyl 4-methoxyphenyl ether. The correlation between their binding affinities for PKC-α and a conformational fit to phorbol ester indicates they mimic a pharmacophore model comprising the C_{20}–OH, C_3–C=O and C_9–OH rather than that including the C_{13}–C=O moiety. © 2001 Elsevier Science S.A. All rights reserved.

Keywords: Protein kinase C; Phorbol ester; Pharmacophore model; Diacylglycerol–lactones; PKC ligands

1. Introduction

Protein kinase C (PKC) is a family of phospholipid-dependent, serine/threonine-specific kinases consisting of 12 isozymes which play pivotal roles in cellular signal transduction pathways and disease states [1–3]. Most isozymes are activated endogenously by diacylglycerol (DAG) and exogenously by several xenobiotic ligands such as phorbol ester, bryostatin, teleocidin and ingenol esters [4]. These ligands bind competitively to a tandem repeat of two cysteine-rich zinc-finger motifs, so-called C1a and C1b domains, present in the regulatory domain of the enzyme [5]. Because of their exceptionally high binding affinity, phorbol esters have been used as pharmacological tools to activate PKC. Their specific interaction with the C1 domain of PKC has been the subject of extensive structure–activity relationship (SAR) analyses [6–10], molecular modeling [11] and X-ray crystallography studies [12] in search of the essential pharmacophore groups responsible for high affinity binding. SAR analysis, however, is complicated because modeling and X-ray crystallography have been restricted to the binary ligand–C1 domain complex, whereas the physiological situation is represented by a the ternary complex including the phospholipid bilayers. From the various approaches, several pharmacophore models of phorbol esters have been suggested based on different combinations of pharmacophoric elements comprising C_3–C=O, C_4–OH, C_9–OH, C_{20}–OH [6–12]. In particular, the pharmacophore containing the subset of C_3–C=O, C_9–OH and C_{20}–OH [10] has been useful in the design of the potent DAG–lactone series based on template I [13,14] (Fig. 1).

Recently, a new pharmacophore model proposed by Hecker et al. incorporates C_{13}–C=O ester based on the irritant and tumor-promoting capacity of several phorbol esters [15] in mouse skin, and in accordance with the diminished activity of phorbol analogues lacking the C_{13}–C=O ester reported by the group of Shibasaki and coworkers [16]. Guided by this new model, we recently investigated simple substituted tetrahydrofuran templates (II and III) with a conformationally rigid glycerol backbone [17]. These compounds retained two of the three pharmacophoric groups present in the very

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potent DAG–lactones (template I) that are considered isopharmacophoric to the C_{20}–OH and the C_3–C=O groups in phorbol. In addition, templates II and III contained an ester group that was designed to function as a surrogate pharmacophoric group equivalent to the C_{13}–C=O of the phorbol esters. Although the lowest energy conformers of both the *E*- and *Z*-isomers of template II fit nicely to all three pharmacophoric groups of phorbol-12,13-diacetate (i.e. C_{20}–OH, C_3–C=O and C_{13}–C=O) with RMS values of 0.002 and 0.009, respectively, their binding affinities for PKC were only in the submicromolar range (ca. 300 nM) [17]. Compounds based on the *cis*- and *trans*-template III showed poorer fit to phorbol which resulted in ligands with even weaker binding affinities [17]. The weak binding affinities observed for templates II and III stand in sharp contrast to the potent, low nanomolar binding affinities that have been achieved with compounds built on template I.

In an effort to continue exploring the importance of phorbol ester's pharmacophores through conformationally constrained DAG template, we investigated some 5-acyloxy-5-hydroxymethyltetrahydro-2-furancarboxylate analogues (**1**–**4**) as PKC ligands. The *cis*-isomers **1** and **3** (*cis*-template IV) and the *trans*-isomers **2** and **4** (*trans*-template IV) are defined relative to the stereochemistry of the two ester groups at C2 and C5 on the tetrahydrofuran ring. We herein describe synthesis, PKC binding affinity and pharmacophoric analysis of template IV analogues with phorbol ester to investigate whether the pharmacophores match with a model of C_3, C_{20}, C_9 as template I or that of C_3, C_{20}, C_{13} as template II (or III) [12,15].

2. Chemistry

The synthesis of the four target compounds was accomplished from 5,5-disubstituted tetrahydrofurancarboxylic acid (**11**) as a key intermediate (Scheme 1). Commercially available glycidyl 4-methoxyphenyl ether (**5**) was converted to lactol **8** in four steps. One carbon elongation from **8** was achieved in two steps via 2-chlorotetrahydrofuran intermediate to give 2-cyanotetrahydrofuran (**10**). Hydrolysis and esterification of **10** afforded methyl ester **12** and tetradecyl ester **13**, respectively. Removal of the 4-methoxyphenyl group from **12** and **13** produced separable isomers **14** and **16**, **15** and **17** whose stereochemistry was derived from those of final compounds, respectively. Acylation of alcohols (**14**–**17**) with the appropriate acid chloride gave the corresponding final components (**1**–**4**) after hydrogenolytic debenzylation. These compounds were fully characterized, and the relative stereochemistry at C-2 in the final compounds was assigned based on the strong NOE observed between H_2 and $H_{6''}$ in *cis*-isomers (e.g. **1**) or between H_2 and $H_{6'}$ in trans-isomers (e.g. **4**) [18].

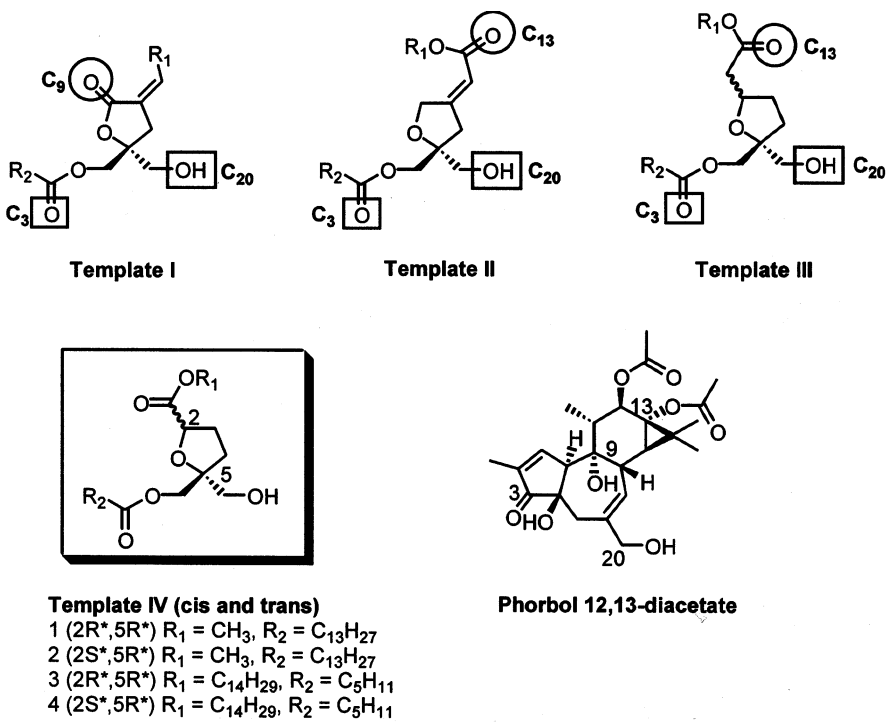
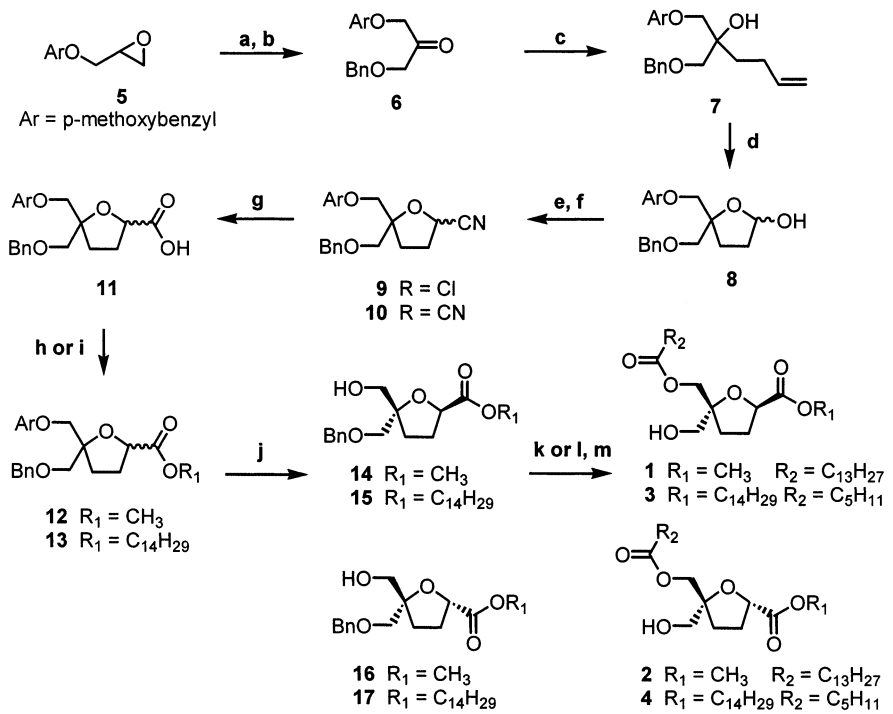


Fig. 1.



Scheme 1. Reagents and conditions: (a) BnOH, NaH, DMF, reflux, 99%; (b) PCC, 4 Å mol sieves, CH_2Cl_2, 90%; (c) CH_2=$CHCH_2CH_2Br$, Mg, THF, 80%; (d) OsO_4, 4-NMO, $NaIO_4$, acetone–H_2O, 85%; (e) HCl, AcOH; (f) Et_2AlCN, toluene–THF, 50%; (g) 15% NaOH, EtOH, 72%; (h) $TMSCHN_2$, THF–MeOH, 84%; (i) $C_{14}H_{29}OH$, DCC, DMAP, CH_2Cl_2, 76%; (j) CAN, CH_3CN–H_2O, 44–58%; (k) $CH_3(CH_2)_{12}COCl$, NEt_3, DMAP, CH_2Cl_2, 73%; (l) $C_5H_{11}COCl$, NEt_3, DMAP, 59%; (m) H_2, Pd–C, EtOAc, 82–92%.

3. Results and discussion

The binding affinities of the target compounds (**1**–**4**) were assessed in terms of their ability to displace bound [^{3}H-20]phorbol-12,13-dibutyrate (PDBU) from recombinant PKC-α. The resulting values expressed as K_i values (Table 1) indicated differences between *cis*-isomers **1** and **3**, and *trans*-isomers **2** and **4**. In agreement with what was previously observed with template III, the *cis*-isomers showed better binding affinities than the *trans*-isomers. However, compounds **1** and **3** were only slightly more potent than their homologous *cis*-isomers built on template III bearing identical R_1 and R_2 alkyl groups (K_i values 2.72 and 2.38 μM, respectively) [17]. This means that bringing the ester pharmacophore closer to the tetrahydrofuran ring had only a marginal benefit. Relative to compounds constructed on template II, the new template IV did not offer any significant advantage since the K_i values are in the same submicromolar range [17].

The conformation of two truncated ($R_1 = R_2 = CH_3$) template IV compounds, IV-*cis* (2*R**,5*R**) and IV-*trans* (2*S**,5*R**) were energy-minimized using the SYBYL 6.4 program and compared to two sets of phorbol ester pharmacophores involving different functional groups as shown in Figs. 2 and 3. The result was summarized in Table 1. The more potent *cis*-isomers **1** and **3** better matched the original C_{20}–OH, C_3–C=O and C_9–OH

Table 1
Apparent K_i values for ligands as inhibitors of PDBU binding to PKC-α and rms values after fitting to three-point pharmacophores of phorbol 12,13-diacetate

Compounds	K_i ± S.E.M. (μM)	RMS (C_3, C_{20}, C_9)	RMS (C_3, C_{20}, C_{13})	Log *P*
1	1.68 (±0.22)	0.577	1.431	6.283
2	2.39 (±0.51)	0.966	0.895	6.283
3	0.534 (±0.032)	0.577	1.431	8.928
4	11.71 (±0.28)	0.966	0.895	8.928

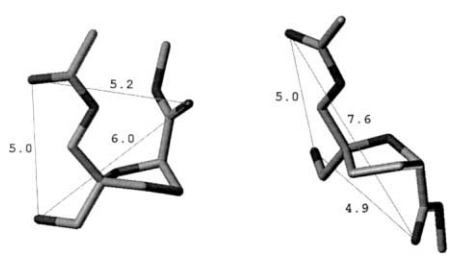


Fig. 2. Lowest energy conformers of IV-*cis* (left) and IV-*trans* (right) (distances between pharmacophores in Å).

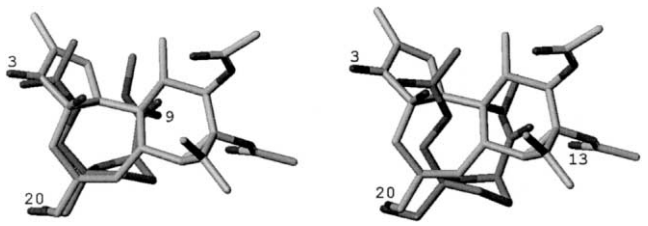


Fig. 3. Superimposition of IV-*cis* on three pharmacophores of phorbol 12,13-diacetate (matched with C_3, C_{20}, C_9 (left) or C_3, C_{20}, C_{13} (right)).

pharmacophore model than the one incorporating the C_{20}–OH, C_3–C=O and C_{13}–C=O. This situation was reversed for the less potent *trans*-isomers **2** and **4**; however, the quality of the fit in this case was poorer. The calculated log *P* values [19] show that for the better IV-*cis* template (**1** and **3**) the more lipophilic compound is the more potent of the two. This situation is reversed for the IV-*trans* template compounds (**2** and **4**) (Table 1). This controversy can be explained by our previous finding in which binding affinity of PKC ligands as DAG surrogates parabolically correlated with liphophilicity of their acyl chains and they therefore required optimal liphophilicity for maximal binding affinity [20].

We conclude that finding a single isopharmacophoric group equivalent to phorbol's C_{13}–C=O ester on simple conformationally constrained glycerol templates remains a challenge. Since in all the templates studied (I–IV) the C_{13}–C=O···HO–C_9 hydrogen bonding motif characteristic of the phorbol esters has been neglected, and only individual isopharmacophoric groups to either the C_9–OH (template I) or the C_{13}–C=O (templates II and III) have been considered independently, perhaps a new generation of conformationally constrained glycerol must include a bifunctional pharmacophore structurally capable of mimicking this important intramolecular hydrogen bond.

4. Experimental

4.1. Synthesis

All chemical reagents were commercially available. Melting points were determined on a melting point Büchi B-540 apparatus, and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. Proton and carbon NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz and JEOL JNM-GCX 400 at 100 MHz, respectively. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Infrared spectra were recorded on a Perkin–Elmer 1710 Series FTIR. Mass spectra were recorded on a VG Trio-2 GC-MS. Elemental analyses were performed with an EA 1110 Automatic Elemental Analyzer, CE Instruments. Analyses indicated by the symbols of the elements or functions were within $\pm 0.4\%$ of theoretical values.

4.1.1. 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 0°C. After stirring for 20 min at room temperature (r.t.), the mixture was treated with glycidyl-4-methoxyphenyl ether (**5**) (10 g, 55.5 mmol). The reaction mixture was heated at 80°C for 4 h and cooled to r.t. The mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were 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, 5H, phenyl), 6.80–6.86 (m, 4H, Ar), 4.58 (s, 2H, PhCH2O), 4.17 (m, 1H, $CH_2CH(OH)CH_2$), 4.00 (dd of AB, 1H, CH_2OAr, $J =$ 4.8 and 10 Hz), 3.96 (dd of AB, 1H, CH_2OAr, $J = 6.0$ and 10 Hz), 3.76 (s, 3H, OCH_3), 3.67 (dd of AB, 1H, $BnOCH_2$, $J = 4.4$ and 10 Hz), 3.61 (dd of AB, 1H, $BnOCH_2$, $J = 6.0$ and 10 Hz), 2.60 (bs, 1H, OH); IR (neat) 3436 (OH) cm^{-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 r.t., the reaction mixture was quenched with ether and Celite and stirred for 30 min. The mixture was filtered 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, 5H, phenyl), 6.83 (bs, 4H, Ar), 4.71 (s, 2H, CH_2OAr), 4.62 (s, 2H, $PhCH_2O$), 4.36 (s, 2H, CH_2OBn), 3.77 (s, 3H, OCH_3); IR (neat) 1753 (C=O) cm^{-1}.

4.1.2. 1-(Benzyloxy)-2-[(4-methoxyphenoxy)methyl]-4-hexen-2-ol (7)

A solution of 4-bromo-1-butene (2.5 ml, 23.9 mmol) in THF (20 ml) at -10°C was treated portionwise with dried magnesium turnings (0.782 g, 32.2 mmol) followed by a solution of **6** (2.3 g, 8.0 mmol) in THF (25 ml). The reaction mixture was warmed to r.t. and added a catalytic amount of dibromoethane three times at 30-min intervals. After stirring for 14 h at r.t., the reaction mixture was quenched with 1 N aqueous NH_4Cl, acidified with 1 N HCl and extracted with diethyl ether several times. The combined organic layers

were washed with water, brine and dried over $MgSO_4$. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (3:1) as eluant to afford **7** (2.20 g, 80%) as an oil. ^{1}H NMR ($CDCl_3$): δ 7.36–7.26 (m, 5H, phenyl), 6.83 (bs, 4H, Ar), 5.85 (m, 1H, C*H*=CH_2), 5.00 (m, 2H, CH=CH_2), 4.55 (s, 2H, $PhCH_2O$), 3.88 (s, 2H, $ArOCH_2$), 3.77 (s, 3H, OCH_3), 3.57 (d, 1H, $J = 9.0$ Hz, $BnOCH_2$), 3.47 (d, 1H, $J = 9.0$ Hz, $BnOCH_2$), 2.60 (s, 1H, OH), 2.19 (m, 2H, CH_2CH=), 1.75 (m, 2H, C–CH_2); IR (neat) 3468 (OH), 1641 (C=C) cm^{-1}.

4.1.3. 5-[(Benzyloxy)methyl]-5-[(4-methoxyphenoxy)-methyl]tetrahydro-2-furanol (**8**)

A solution of **7** (2.20 g, 6.4 mmol) in acetone and water (1:1, 40 ml) was treated with 4-methylmorpholine *N*-oxide (1.51 g, 12.5 mmol), sodium periodate (1.38 g, 6.5 mmol) and a catalytic amount of osmium tetroxide and stirred for 30 h at r.t. The reaction mixture was filtered through Celite with ethyl acetate and the filtrate was concentrated in vacuo. The residue was partitioned with CH_2Cl_2 and aqueous $Na_2S_2O_3$ solution and the aqueous phase was extracted with additional CH_2Cl_2. The combined organic phase was washed with water, brine and dried over $MgSO_4$. The filtrated was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (1:1) as the eluent to afford the anomeric mixture **8** (1.88 g, 85%) as an oil. ^{1}H NMR ($CDCl_3$): δ 7.36–7.26 (m, 5H, phenyl), 6.78–6.84 (m, 1H, H-2), 4.5–4.6 (m, 2H, $PhCH_2O$), 3.78–4.05 (m, 2H, ArO), 3.77 (m, 3H, OCH_3), 3.45–3.6 (m, 2H, $BnOCH_2$), 2.20–1.97 (m, 4H, H-3 and H-4); IR (neat) 3460 (OH) cm^{-1}.

4.1.4. 5-[(Benzyloxy)methyl]-5-[(4-methoxyphenoxy)-methyl]tetrahydro-2-furancarbonitrile (**9**)

A solution of **8** (1.88 g, 5.5 mmol) in 0.09 M HCl (diluted with 1 M acetic acid, 14.6 ml) was stirred for 5 min at r.t. The reaction mixture was concentrated in vacuo, diluted with CH_2Cl_2 and concentrated in vacuo several times until most of acetic acid was removed. The residue was purified by flash column chromatography over silica gel with hexane/EtOAc (3:1) as eluant to afford the corresponding chloride **9** (1.64 g, 83%) as an oil. ^{1}H NMR ($CDCl_3$): δ 7.4–7.2 (m, 5H, phenyl), 6.9–6.8 (m, 4H, Ar), 6.37 (d, 1H, H-2), 4.55–4.58 (m, 2H, $PhCH_2O$), 3.8–4.1 (m, 2H, $ArCH_2O$), 3.77 (d, 3H, OCH_3), 3.4–3.7 (m, 2H, $BnOCH_2$), 2.3–1.9 (m, 4H, H-3 and H-4).

A solution of **9** (1.64 g, 4.5 mmol) in toluene and THF (5:4, 9 ml) was treated dropwise with diethyl aluminum cyanide (1.0 M in toluene, 12.4 ml) at 0°C and warmed to r.t. After stirring for 7 h, the reaction mixture was quenched with aqueous NaCl solution in ice bath and extracted with ethyl acetate for three times. The combined organic phase was washed with water, brine and dried over Na_2SO_4. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (3:1) as eluant to afford **10** as an oil (0.80 g, 50%). ^{1}H NMR ($CDCl_3$): δ 7.4–7.2 (m, 5H, phenyl), 6.9–6.8 (m, 4H, Ar), 4.81 (m, 1H, H-2), 4.55–4.75 (m, 2H, $PhCH_2O$), 3.66–3.95 (m, 2H, $ArCH_2O$), 3.52–3.92 (m, 2H, $BnOCH_2$), 3.75 (s, 3H, OCH_3), 2.14–2.43 (m, 4H, H-3 and H-4); IR (neat) 2250 (CN) cm^{-1}.

4.1.5. Methyl 5-[(benzyloxy)methyl]-5-[(4-methoxyphenoxy)methyl]tetrahydro-2-furancarboxylate (**12**)

A solution of **10** (0.80 g, 2.3 mmol) in ethyl alcohol (5 ml) was treated with 15% NaOH solution (13 ml) and refluxed for 8 h. The reaction mixture was cooled to r.t. and concentrated in vacuo with additional toluene for three times. The residue was acidified with 1 N HCl in ice bath and extracted with CH_2Cl_2 several times. The combined organic layers were washed with water, brine and dried over $MgSO_4$. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (1:1) as eluant to afford acid **11** (0.606 g, 72%) as an oil.

A solution of **11** (0.528 g, 1.42 mmol) in THF and MeOH (2:1, 30 ml) was treated with trimethylsilyl–diazomethane (2.0 M in hexane, 1.2 ml, 2.4 mmol) at −10°C dropwise and warmed to room temperatue. After stirring for 2 h, the reaction mixture was quenched with acetic acid and diluted with diethyl ether. The mixture was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (2:1) as eluant to afford 12 as an oil (0.458 g, 84%). ^{1}H NMR ($CDCl_3$): δ 7.25–7.35 (m, 5H, phenyl), 6.8–6.9 (m, 4H, Ar), 4.55–4.65 (m, 3H, H-2 and $PhCH_2O$), 4.0–4.1 (m, 2H, $ArCH_2O$), 3.78 (s, 3H, $ArOCH_3$), 3.70 (s, 3H, $CO2CH_3$), 3.60 (m, 2H, $BnOCH_2$), 1.95–2.35 (m, 4H, H-3 and H-4); IR (neat) 1735 (C=O) cm^{-1}.

4.1.6. Tetradecyl 5-[(benzyloxy)methyl]-5-[(4-methoxyphenoxy)methyl]tetrahydro-2-furancarboxylate (**13**)

A stirred solution of **11** (0.255 g, 0.7 mmol), a catalytic amount of dimethylamino pyridine and tetradecanol (0.221 g, 1 mmol) in CH_2Cl_2 (10 ml) was treated with 1,3-dicyclohexylcarbodiimide (1.0 M in CH_2Cl_2, 1.38 ml, 1.38 mmol) and stirred for 1 h at r.t. The reaction mixture was quenched with acetic acid and concentrated in vacuo. The residue was diluted with ether:hexane (1:1), filtered and the filtrate was concentrated in vacuo. The residue was purified by

flash column chromatography over silica gel with hexane/EtOAc (3:1) as eluant to afford 13 (0.286 g, 76%) as an oil. ^{1}H NMR ($CDCl_3$): δ 7.25–7.30 (m, 5H, phenyl), 6.8–6.86 (m, 4H, Ar), 4.5–4.65 (m, 3H, H-2 and $PhCH_2O$), 4.1 (m, 2H, $CO2CH_2$), 3.9–4.02 (m, 2H, $ArCH_2O$), 3.76 (s, 3H, OCH_3), 3.55–3.65 (m, 2H, $BnOCH_2$), 1.95–2.4 (m, 4H, H-3 and H-4), 1.26 (bs, 24H), 0.89 (distorted t, 3H, CH_3); IR (neat) 1734 (C=O) cm^{-1}.

*4.1.7. (rel-2R,5S and rel-2S,5S) Methyl 5-[(benzyloxy)methyl]-5-(hydroxymethyl) tetrahydro-2-furancarboxylate (**14** and **16**)*

A solution of **12** (0.142 g, 0.37 mmol) in acetonitrile (9.2 ml) and water (2.3 ml) was cooled to 0°C, treated with ammonium cerium nitrate (0.403 g, 0.74 mmol) and stirred for 30 min. The reaction mixture was quenched with aqueous $NaHCO_3$ solution and extracted with diethyl ether several times. The combined organic layers were washed with water, brine and dried over $MgSO_4$. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (3:1) as eluant to afford **14** (0.057 g, 56%) and **16** (0.035 g, 34%) as oils, respectively.

14: R_f: 0.4 (Hex:EtOAc = 1:1); ^{1}H NMR ($CDCl_3$): δ 7.25–7.35 (m, 5H, phenyl), 4.61 (dd, 1H, J = 3.5 and 8.8 Hz, H–2), 4.53 (dd, 2H, $PhCH_2O$), 3.78–3.80 (m, 4H, CO_2CH_3 and 1H of CH_2OH), 3.56 (d, 1H, 1H of CH_2OH), 3.42 (s, 2H, $BnOCH_2$), 1.94–2.44 (m, 4H, H-3 and H-4); IR (neat) 3470 (OH), 1735 (C=O) cm^{-1}.

16: R_f: 0.2 (Hex:EtOAc = 1:1); 1H NMR ($CDCl_3$): δ 7.25–7.35 (m, 5H, phenyl), 4.55–4.6 (m, 3H, H-2 and $PhCH_2O$), 3.76 (s, 3H, $CO2CH_3$), 3.62 (m, 2H, CH_2OH), 3.42 (s, 2H, $BnOCH_2$), 1.92–2.42 (m, 4H, H-3 and H-4); IR (neat) 3470 (OH), 1735 (C=O) cm^{-1}.

*4.1.8. (rel-2R,5S and rel-2S,5S) Tetradecyl 5-[(benzyloxy)methyl]-5-(hydroxymethyl) tetrahydro-2-furancarboxylate (**15** and **17**)*

These compounds were prepared from 13 following the same procedure for the synthesis of **14** and **16**.

15: 42% yield, R_f: 0.7 (Hex:EtOAc = 3:1); ^{1}H NMR ($CDCl_3$): δ 7.25–7.40 (m, 5H, phenyl), 4.5–4.6 (m, 3H, H-2 and $PhCH_2O$), 4.16 (t, 2H, $CO2CH_2$), 3.66 (m, 2H, CH_2OH), 3.39 (s, 2H, $BnOCH_2$), 1.9–2.45 (m, 4H, H-3 and H-4), 1.60 (m, 2H, $CO_2CH_2CH_2$), 1.26 (bs, 22H), 0.88 (distorted t, 3H, CH_3); IR (neat) 3470 (OH), 1736 (C=O) cm^{-1}.

17: 47% yield, R_f: 0.45 (Hex:EtOAc = 3: 1); ^{1}H NMR ($CDCl_3$): δ 7.25–7.40 (m, 5H, phenyl), 4.5–4.63 (m, 3H, H-2 and $PhCH_2O$), 4.15 (m, 2H, $CO2CH_2$), 3.48–3.70 (m, 4H, CH_2OH and $BnOCH_2$), 1.9–2.35 (m, 4H, H-3 and H-4), 1.60 (m, 2H, $CO_2CH_2CH_2$), 1.26 (bs, 22H), 0.88 (distorted t, 3H, CH_3); IR (neat) 3470 (OH), 1736 (C=O) cm^{-1}.

*4.1.9. General procedure for the synthesis of **1**–**4***

A solution of alcohol (**14**–**17**) (0.15 mmol), triethylamine (0.06 ml, 0.60 mmol) and a catalytic amount of dimethylamino pyridine in CH_2Cl_2 (10 ml) at 0°C was treated with acyl chloride (0.30 mmol) dropwise. After stirring for 2 h at r.t., the reaction mixture was quenched with aqueous $NaHCO_3$ solution and extracted with diethyl ether several times. The combined organic layers were washed with water, brine and dried over $MgSO_4$. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (3:1) as eluant to afford the corresponding esters.

A mixture of above ester (0.1 mmol) and 10% Pd/C (20 mg) in ethyl acetate (10 ml) was hydrogenated under a balloon of hydrogen at r.t. for 1 h. The reaction mixture was filtered through Celite with ethyl acetate. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (3:1) as eluant to afford **1**–**4**.

*4.1.10. (rel-2R,5R) Methyl 5-(hydroxymethyl)-5-[(tetradecanoyloxy)methyl]tetrahydro-2-furancarboxylate (**1**)*

67% yield; a coloreless oil; ^{1}H NMR ($CDCl_3$): δ 4.59 (dd, 1H, J = 5.7, 7.8 Hz, H-2), 4.34 (d, 1H, J = 11.5 Hz, CO_2CH_2), 4.17 (d, 1H, J = 11.5 Hz, CO_2CH_2), 3.75 (s, 3H, CO_2CH_3), 3.54 (d, 1H, J = 11.8 Hz, CH_2OH), 3.48 (d, 1H, J = 11.8 Hz, CH_2OH), 2.34 (m, 3H, CH_2CO_2 and H-3a), 2.16 (m, 1H, H-3b), 2.01 (m, 1H, H-4a), 1.85 (m, 1H, H-4b), 1.60 (m, 2H, $CH_3(CH_2)_{10}CH_2CH_2CO_2$), 1.2–1.35 (m, 20H, $CH_3(CH_2)_{10}CH_2CH_2CO_2$), 0.88 (t, 3H, $CH_3(CH_2)10CH_2CH_2CO_2$); IR (neat) 3456 (OH), 1739 (C=O) cm^{-1}; MS (CI) m/z 401 ($M^+ + 1$); *Anal.* ($C_{22}H_{40}O_6$) C, H.

*4.1.11. (rel-2S,5R) Methyl 5-(hydroxymethyl)-5-[(tetradecanoyloxy)methyl]tetrahydro-2-furancarboxylate (**2**)*

66% yield; a colorless oil; ^{1}H NMR ($CDCl_3$): δ 4.59 (dd, 1H, J = 5.7, 7.8 Hz, H-2), 4.34 (d, 1H, J = 11.5 Hz, CO_2CH_2), 4.17 (d, 1H, J = 11.5 Hz, CO_2CH_2), 3.75 (s, 3H, CO_2CH_3), 3.54 (d, 1H, J = 11.8 Hz, CH_2OH), 3.48 (d, 1H, J = 11.8 Hz, CH_2OH), 2.34 (m, 3H, CH_2CO_2 and H-3a), 2.16 (m, 1H, H-3b), 2.01 (m, 1H, H-4a), 1.85 (m, 1H, H-4b), 1.60 (m, 2H, $CH_3(CH_2)_{10}CH_2CH_2CO_2$), 1.2–1.35 (m, 20H, $CH_3(CH_2)_{10}CH_2CH_2CO_2$), 0.88 (t, 3H, $CH_3(CH_2)_{10}CH_2CH_2CO_2$); IR (neat) 3472 (OH), 1740 (C=O) cm^{-1}; MS (CI) m/z 401 ($M^+ + 1$); *Anal.* ($C_{22}H_{40}O_6$) C, H.

*4.1.12. (rel-2R,5R) Tetradecyl 5-[(hexanoyloxy)-methyl]-5-(hydroxymethyl)tetrahydro-2-furancarboxylate (**3**)*

56% yield; a colorless oil; ^{1}H NMR ($CDCl_3$): δ 4.57 (dd, 1H, J = 5.4, 7.8 Hz, H-2), 4.36 (d, 1H, J = 11.5 Hz,

$C_5H_{11}CO_2CH_2$), 4.18 (d, 1H, $J = 11.5$ Hz, C_5H_{11}-CO_2CH_2), 4.13 (m, 2H, $CO_2CH_2C_{13}H_{27}$), 3.50 (ddd of AB, 2H, CH_2OH), 2.35 (t, 2H, CH_2CO_2), 2.28 (m, 1H, H-3a), 2.13 (m, 1H, H-3b), 2.00 (m, 1H, H-4a), 1.84 (m, 1H, H-4b), 1.6–1.7 (m, 4H), 1.2-1.4 (m, 26H), 0.85–0.9 (m, 6H, $2 \times CH_3$); IR (neat) 3470 (OH), 1740 (C=O) cm^{-1}; MS (CI) m/z 471 ($M^+ + 1$); *Anal.* ($C_{27}H_{50}O_6$) C, H.

4.1.13. (rel-2S,5R) Tetradecyl 5-[(hexanoyloxy)-methyl]-5-(hydroxymethyl)tetrahydro-2-furancarboxylate (**4**)

57% yield; a colorless oil; ^1H NMR ($CDCl_3$): δ 4.58 (dd, 1H, $J = 3.4$, 9.0 Hz, H-2), 4.15 (bt, 2H, $CO_2CH_2C_{13}H_{27}$), 4.05 (d, 1H, $J = 11.4$ Hz, $C_5H_{11}CO_2$-CH_2), 3.99 (d, 1H, $J = 11.4$ Hz, $C_5H_{11}CO_2CH_2$), 3.78 (d, 1H, $J = 11.5$ Hz, CH_2OH), 3.50 (d, 1H, $J = 11.5$ Hz, CH_2OH), 2.45 (m, 1H, H-3a), 2.33 (t, 2H, CH_2CO_2), 2.1–2.2 (m, 2H, H-3b and H-4a), 1.80 (m, 1H, H-4b), 1.55–1.7 (m, 4H), 1.2-1.36 (m, 26H), 0.85–0.9 (m, 6H, $2 \times CH_3$); IR (neat) 3470 (OH), 1740 (C=O) cm^{-1}; MS (CI) m/z 471 ($M^+ + 1$); *Anal.* ($C_{27}H_{50}O_6$) C, H.

4.2. Analysis of Inhibition of [^{3}H-20]PDBU binding by nonradioactive ligand

Enzyme–ligand interactions were analyzed by competition with [^{3}H-20]phorbol dibutyrate binding essentially as described previously with the single isozyme PKC-α [21]. This recombinant isozyme was expressed in the baculovirus system and was isolated as described in Ref. [4]. 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 [22]. Values represent the mean ± standard error (three determinations).

4.3. Molecular modeling

The structures of template IV-*cis* and IV-*trans* were built and optimized using SYBYL 6.4, Tripos. All calculations including the conformational search were performed on a Silicon Graphics O_2 R10000 workstation.

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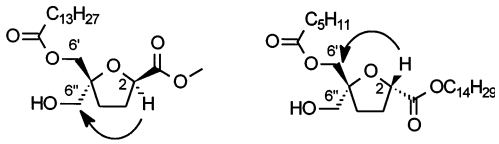
[18] 

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