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# 2-Benzyl and 2-phenyl-3-hydroxypropyl pivalates as protein kinase C ligands

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**Abstract**—A series of 2-benzyl and 2-phenyl-3-hydroxypropyl pivalates designed to incorporate the principal pharmacophores of phorbol esters have been synthesized and tested as PKC-α ligands. Among the analogues, **13c** exhibited the most potent binding affinity with a $K_i = 0.7$ μM. The synthesized analogues were subjected to molecular modeling analysis based on two alternative models of the phorbol pharmacophore and a docking study of **13c** was carried out.


## 1. Introduction

Protein kinase C (PKC) comprises a family of serine/threonine kinases playing a pivotal role in cellular signal transduction.[1,2] These enzymes are activated by diacylglycerol (DAG) generated either by phospholipase C (PLC) mediated hydrolysis of phosphatidylinositol-4,5-bisphosphate ($PIP_2$) or indirectly via phospholipase D and phosphatidic acid hydrolase.[3] DAG binds to the C1 domains of both the calcium-dependent classical PKC isoforms α, β, and γ and the novel or calcium-independent PKC isoforms δ, ε, η, and θ. The binding activates these enzymes and promotes their association with the membrane phospholipids, inducing the translocation of cytosolic PKC to the inner leaflet of the cellular membrane.[4] Besides the PKCs, other C1 domain-containing receptors including members of the chimaerin, RasGRP, MUNC13, PKD, and DAG kinase families also function in DAG signaling and therefore represent potential sites of action for DAG analogs.[5]

Phorbol esters bind competitively to the same DAG-binding site on the C1 domains but with affinities several orders of magnitude greater than those of DAGs and have provided powerful pharmacological tools for studying PKC function.[6,7] Phorbol esters function as potent and metabolically stable DAG surrogates because their conformationally rigid scaffold, unlike the flexible glycerol backbone of DAG, is able to specifically direct the hydrophilic pharmacophores of the ligand.

Over the last few years, we have synthesized a series of conformationally constrained DAG analogues embedded in a variety of lactone templates designed to reduce part of the entropic penalty associated with the binding of the flexible glycerol backbone of DAG,[8,9] and we have obtained a series of 'ultrapotent' DAG–lactone analogues built on a 5-[(acyloxy)methyl]-5-(hydroxymethyl)tetrahydro-2-furanone template. Depending on the type of hydrophobic substitution, some of the compounds built with this template displayed low-nanomolar binding affinities, thus displaying substantially improved affinities for PKC-α compared to, for example, dioleoylglycerol.[10]

A comprehensive molecular modeling study of the 5-[(acyloxy)methyl]-5-(hydroxymethyl)tetrahydro-2-furanone template in comparison with the phorbol esters indicated that the two C=O groups (acyloxy and lactone) and the primary OH overlapped almost perfectly with the $C_3$–C=O, $C_9$–OH, and $C_{20}$–OH of the diterpene.[11] This result supported a previous pharmacophore model in which the combined interaction of

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these three groups was considered essential for the strong binding of phorbol esters.[12–14]

As an alternative approach, we recently reported a series of substituted tetrahydrofurans with an embedded glycerol backbone carrying additional tetrahydrofuranylidene acetate or tetrahydrofuranyl acetate motifs which were designed to mimic three essential pharmacophores ($C_3$–C=O, $C_{20}$–OH, and $C_{13}$–C=O) of the phorbol esters according to a new, revised model.[15] Molecular modeling analysis of the templates showed that the binding affinities of the series correlated with the rms values when fitted to phorbol ester. This finding confirmed that the $C_{13}$–C=O pharmacophore of phorbol is very likely to be involved in interacting with PKC, in the presence of phospholipid, at the DAG-binding site. However, since the $C_9$–OH and $C_{13}$–C=O in the phorbol esters appear to form an intramolecular hydrogen bond that functions as a combined pharmacophore and since the tetrahydrofuran template still yielded less potent ligands compared to the equivalent DAG–lactone template, it seemed possible that the combined $C_9$–OH/$C_{13}$–C=O motif of the phorbol ester might be needed for efficient interaction with the enzyme at the membrane interface.

In our continuing effort to design DAG analogues as PKC ligands with potencies similar to those of the phorbol esters, we set out to investigate 2-benzyl and 2-phenyl-3-hydroxypropyl pivalate templates with an embedded glycerol backbone carrying a surrogate pharmacophore for the $C_9$–OH or $C_{13}$–C=O of the phorbol esters. The two prototypes (templates I and II) were designed to retain the putative recognition domain of the phorbol ester, namely, the $C_3$–C=O and $C_{20}$–OH (Fig. 1). Since, from our previous work, the C=O of the pivaloyloxymethyl group and the primary OH group of the potent lactone template overlaid nicely with the $C_3$–C=O and $C_{20}$–OH groups, respectively, these groups were retained as a 3-hydroxypropyl pivalate scaffold.

In the designed DAG analogues, benzyl (template I) or phenyl (template II) carrying hydrogen bonding groups, such as hydroxyl (**6** and **14**), benzyloxy (**5** and **13**), and hexanoyl (**7** and **15**) groups, at different positions were incorporated into the 2-position of 3-hydroxypropyl pivalate. The substituents were investigated as surrogate pharmacophores of the $C_9$–OH or $C_{13}$–C=O of the phorbol esters.

The syntheses and binding affinities of the newly designed templates and their SAR analysis by molecular modeling are reported here.

## 2. Chemistry

The syntheses of 2-benzyl-3-hydroxypropyl pivalate analogues (template I, **5**–**7**) are represented in Scheme 1. Starting from 2 (3 or 4)-hydroxybenzyl alcohols, the phenolic hydroxyl group was selectively protected by a benzyl group and then the benzylic alcohol was transformed

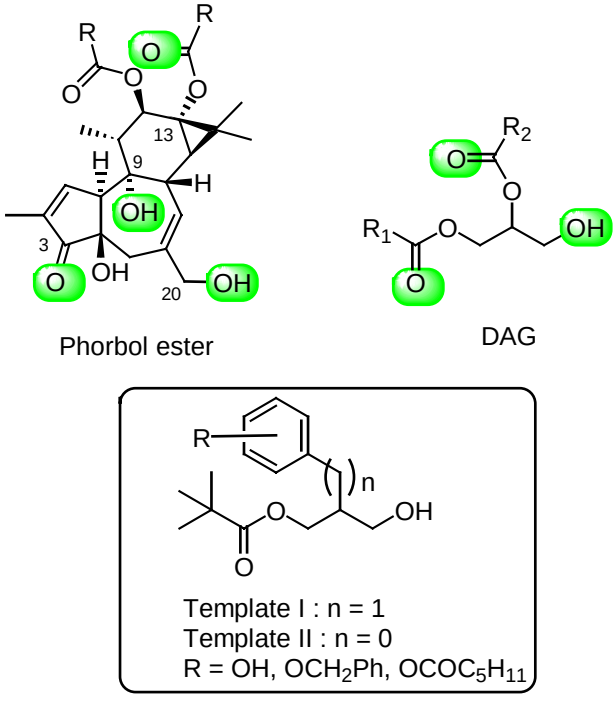



**Figure 1.**

to the corresponding chloride **2** by thionyl chloride. The benzyl chloride was alkylated with diethyl malonate to afford **3**, whose diesters were reduced to the corresponding alcohols **4**. A hydroxyl group of **4** was selectively acylated by pivaloyl chloride to afford the final target **5**. The debenzylation and selective hexanoylation of **5** provided the target compounds **6** and **7**.

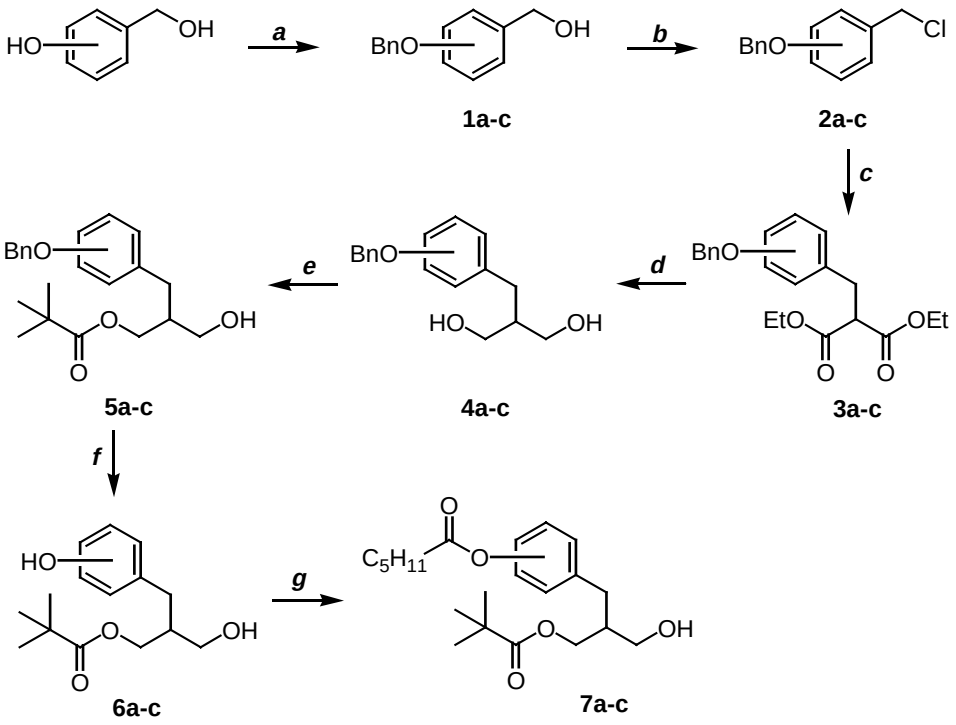
The syntheses of 2-phenyl-3-hydroxypropyl pivalate analogues (template II, **13**–**15**) are outlined in Scheme 2. The chloride **2** was converted to the corresponding nitrile **8**, which was hydrolyzed and then esterified to afford ester **10**. Acylation of **10** with dimethylcarbonate produced phenylmalonate **11**, which was readily converted to target compounds **13**–**15** by following the same protocol described in Scheme 1.

## 3. Results and discussion

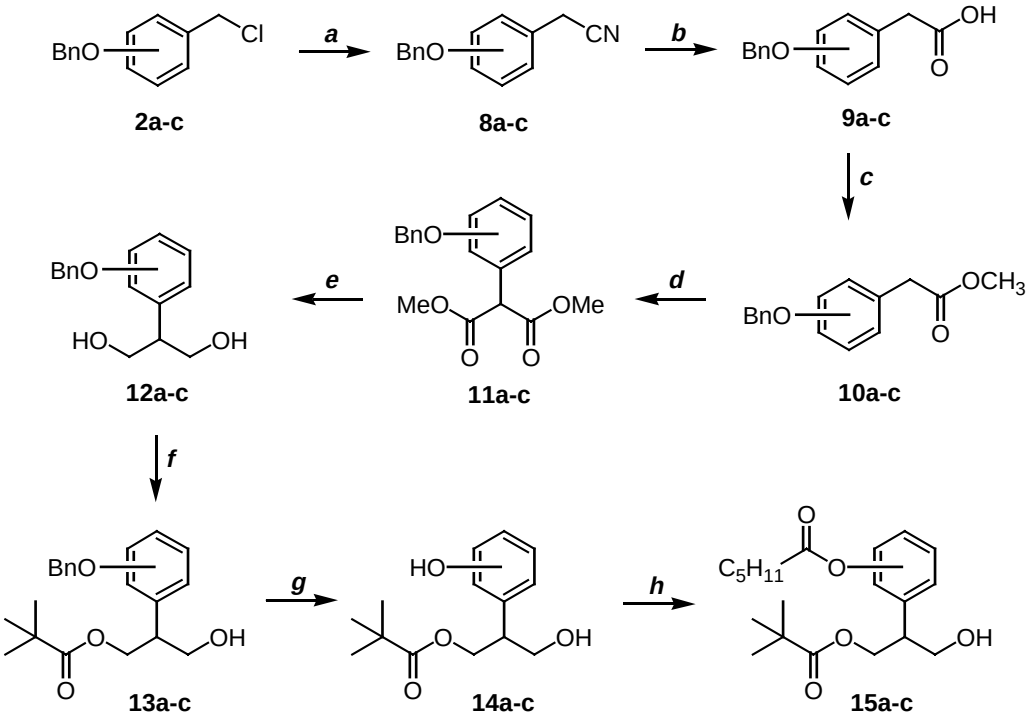
### 3.1. Biological activity

The interaction of the target DAG–lactones with PKC was assessed in terms of the ability of the ligand to displace bound [20-$^3$H] phorbol 12,13-dibutyrate (PDBu) from the recombinant single isozyme, PKC-α, in the presence of phosphatidylserine as previously described.[10] The $IC_{50}$ values were determined by fitting the data points to the theoretical competition curve. The $K_i$ values for inhibition of binding were calculated from the corresponding $IC_{50}$ values (Table 1).

The hydroxyl analogues (R = H, **6a**–**c** and **14a**–**c**) showed moderate to weak binding affinities (17.5–136 μM) for both templates. Their low binding affinities



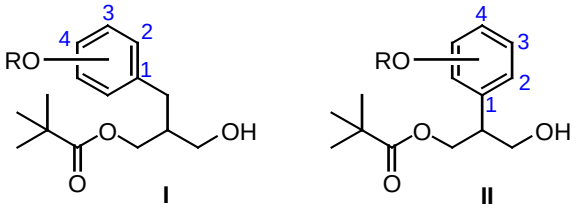

**Scheme 1.** Synthesis of DAG template I. Reagents and conditions: (a) BnBr, $K_2CO_3$, acetone, reflux; (b) $SOCl_2$, $CH_2Cl_2$, 0 °C; (c) $CH_2(CO_2Et)_2$, NaH, DMF, rt; (d) $LiAlH_4$, THF, 0 °C to rt; (e) $(CH_3)_3CCOCl$, pyridine, $CH_2Cl_2$, rt; (f) $H_2$, Pd–C, MeOH, rt; (g) $(CH_3(CH_2)_4CO)_2O$, DMAP, $CH_2Cl_2$, 0 °C.




**Scheme 2.** Synthesis of DAG template II. Reagents and conditions: (a) NaCN, DMF, 100 °C; (b) NaOH, $H_2O$, reflux; (c) $H_2SO_4$, MeOH, reflux; (d) $(CH_3O)_2CO$, NaH; (e) $LiAlH_4$, THF, 0 °C to rt; (f) $(CH_3)_3CCOCl$, pyridine, $CH_2Cl_2$, rt; (g) $H_2$, Pd–C, MeOH, rt; (h) $(CH_3(CH_2)_4CO)_2O$, DMAP, $CH_2Cl_2$, 0 °C.

are probably attributed to their low lipophilicities (c$\log P$ = 2.90 and 2.41). For that reason, lipophilic groups (benzyl and hexanoyl groups) were incorporated to provide enhanced binding. The activities of the resulting derivatives were a function both of the template and its isomers.

**Table 1.** Binding affinities of synthesized DAG analogues




| Compound | R | Template | Isomers | $K_i$ (μM) | c Log *P* |
|---|---|---|---|---|---|
| OAG[a] | | | | 0.050[c] | 7.0 |
| DiC8[b] | | | | 0.033[c] | 5.3 |
| **6a** | H | I | 1,2 | 17.5 (±1.3) | 2.90 |
| **6b** | H | I | 1,3 | 136 (±11) | 2.90 |
| **6c** | H | I | 1,4 | 106 (±16) | 2.90 |
| **14a** | H | II | 1,2 | 97 (±15) | 2.41 |
| **14b** | H | II | 1,3 | 37.7 (±0.8) | 2.41 |
| **14c** | H | II | 1,4 | 123 (±9) | 2.41 |
| **5a** | $CH_2Ph$ | I | 1,2 | 34.4 (±4.3) | 5.17 |
| **5b** | $CH_2Ph$ | I | 1,3 | 3.82 (±0.29) | 5.17 |
| **5c** | $CH_2Ph$ | I | 1,4 | 4.4 (±0.6) | 5.17 |
| **13a** | $CH_2Ph$ | II | 1,2 | 12.6 (±0.9) | 4.68 |
| **13b** | $CH_2Ph$ | II | 1,3 | 0.86 (±0.05) | 4.68 |
| **13c** | $CH_2Ph$ | II | 1,4 | 0.70 (±0.05) | 4.68 |
| **7a** | $COC_5H_{11}$ | I | 1,2 | 40.0 (±2.0) | 4.94 |
| **7b** | $COC_5H_{11}$ | I | 1,3 | 17.0 (±0.2) | 4.94 |
| **7c** | $COC_5H_{11}$ | I | 1,4 | 6.0 (±0.2) | 4.94 |
| **15a** | $COC_5H_{11}$ | II | 1,2 | 122 (±2) | 4.45 |
| **15b** | $COC_5H_{11}$ | II | 1,3 | 2.6 (±0.2) | 4.45 |
| **15c** | $COC_5H_{11}$ | II | 1,4 | 1.75 (±0.08) | 4.45 |

[a] 1-Oleoyl-2-acetate-*sn*-glycerol.
[b] 1,2-Dioctanoyl-*sn*-glycerol.
[c] Ref. 21.

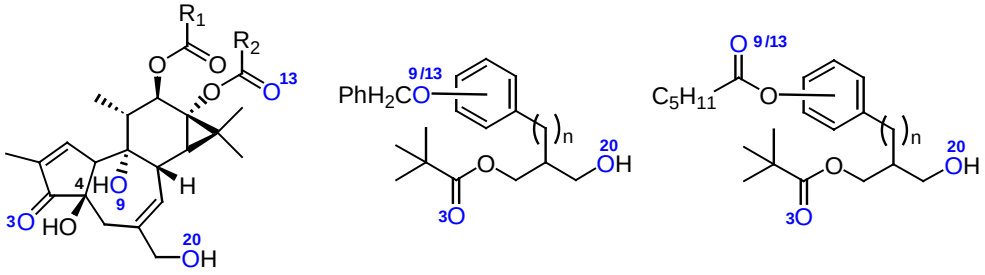
First, the binding affinities of template II (2-phenyl-3-hydroxypropyl pivalate) derivatives were consistently better than those of the corresponding template I (2-benzyl-3-hydroxypropyl pivalate) (**13a–c** > **5a–c** and **15b**,**c** > **7b**,**c**), except in the case of **15a** (1,2-isomer, R = $COC_5H_{11}$). The exceptional low potency of **15a** is probably derived from steric repulsion between the hexanoyl group and the pivalate (or hydroxyl) resulting in disposition of pharmacophores in an unfavorable position. Second, the binding affinities depended on the substituent position. The 1,4-isomers were the most potent and the 1,3-isomers were next in potency. The 1,2-isomers exhibited a dramatic reduction in binding affinity compared to the 1,4- and 1,3-isomers, probably due to steric repulsion as discussed above. Among the analogues, **13c** exhibited the most potent binding affinity with a $K_i$ = 0.7 μM, and, for proper comparison with the diacylglycerols, 1-oleoyl-2-acetate-*sn*-glycerol (OAG) and *sn*-1,2-dioctanoylglycerol (DiC8), its intrinsic potency would be further enhanced if only the optically active isomer and the relatively lower lipophilicity of **13c** were taken into consideration.

In order to investigate the structure–activity relationships of these analogues in detail relative to the phorbol pharmacophores, we carried out a molecular modeling study of the analogues. These studies support the model of three pharmacophores, including the hydroxyl, carbonyl oxygen of pivalate, and the ether oxygen of phenyl in benzyl-substituted analogues (or carbonyl oxygen in hexanoyl-substituted analogues).

### 3.2. Molecular modeling

The series of synthesized DAG analogues were designed to mimic three essential pharmacophores of the phorbol esters ($C_3$–C═O, $C_{20}$–OH, and $C_9$–OH or $C_{13}$–C═O). In order to search the principal pharmacophores of the DAG analogues, the energy-minimized conformations of all analogues were determined and the positions of their three pharmacophores were matched with those of phorbol ester. The pharmacophore model has two hydrogen bond acceptors and a hydroxyl group. The center points of the three pharmacophoric groups of the DAG derivatives, including the carbonyl of the 3-pivaloyloxy group, the hydroxyl group, and the ether oxygen atom (R = benzyl) or the ester carbonyl (R = hexanoyl), were superimposed with the $C_3$-keto carbonyl, the $C_{20}$-hydroxyl group, and the $C_9$-hydroxyl or the $C_{13}$-ester carbonyl of phorbol esters, respectively. The respective rms values after fitting were calculated for two types of chemical modification (R = benzyl or hexanoyl) as shown in Table 2. For the comparison with the binding affinities and the rms values, the ranking of the PKC ligands based on quality of fit to phorbol ester was determined (see the number in parentheses, Table 2,

**Table 2.** rms values after fitting into the pharmacophores of phorbol ester




| Compound | $K_i$ (μM) | rms value (3–9–20) | rms value (3–13–20) |
|---|---|---|---|
| *Benzyl* | | | |
| **5a** | 34.4 (6) | 0.797 (3) | 1.372 (5) |
| **5b** | 3.82 (3) | 0.832 (4) | 0.610 (3) |
| **5c** | 4.4 (4) | 0.855 (5) | 0.614 (4) |
| **13a** | 12.6 (5) | 0.374 (1) | 1.431 (6) |
| **13b** | 0.86 (2) | 0.778 (2) | 0.480 (2) |
| **13c** | 0.7 (1) | 1.056 (6) | 0.370 (1) |
| *Hexanoyl* | | | |
| **7a** | 40 (5) | 1.354 (4) | 1.040 (4) |
| **7b** | 17 (4) | 0.890 (1) | 1.320 (5) |
| **7c** | 6 (3) | 1.388 (6) | 0.711 (3) |
| **15a** | 122 (6) | 1.022 (2) | 1.374 (6) |
| **15b** | 2.6 (2) | 1.357 (5) | 0.253 (2) |
| **15c** | 1.75 (1) | 1.305 (3) | 0.137 (1) |

The number in parentheses indicates the order of rank.

the lowest number indicates the best fit). The rank order of binding affinities of the PKC ligands correlated very well with those of the rms values in the 3–13–20 model. However, for the three point fitting of the 3–9–20 model, no clear correlation was found. The result indicates that the 3–13–20 triplet of hydrophilic groups appear to be principal pharmacophores of phorbol ester for binding with the complex of enzyme and phospholipid.

The pharmacophoric importance of the $C_{13}$–C═O of phorbol esters was proposed by Hecker and co-workers who provided evidence in support of the carbonyl for irritant and tumor-promoting potency in mouse skin.[16] Similarly, Shibasaki and co-workers demonstrated indirectly the importance of the $C_{13}$–C═O with phorbol ester analogues lacking this functionality.[17] In a more direct fashion, photocross-linking experiments of a phorbol ester with a diazoacetyl group at $C_{13}$ suggested that this group was indeed located in close proximity to the protein in the ligand–enzyme–phospholipid complex.[18] Blumberg and co-workers also reported that the removal of the acyl group at $C_{13}$ caused a significant drop in binding affinity when comparing phorbol 12- and 13-monoesters.[19]

To obtain detailed information concerning PKC ligand binding, we examined the binding mode of the most potent compound (**13c**). The docked model of PKC-α compound **13c** is shown in Figure 2. Compound **13c** interacts with Thr12 and Gly23 in the C1b domain of PKC-α. In the complex, the hydroxyl group acts as a hydrogen bond acceptor for the backbone nitrogen of

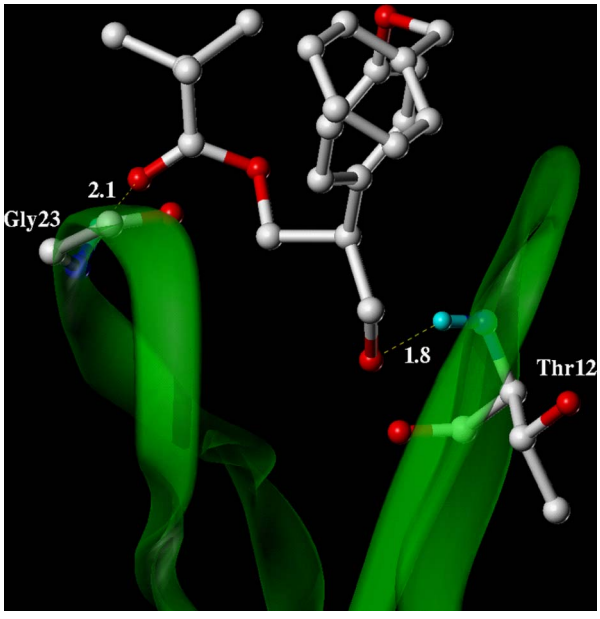



**Figure 2.** The docking of compound **13c** to the C1b domain of PKC-α.

Thr12 (1.8 Å) and the carbonyl of 3-acyloxy forms a hydrogen bond with Gly23 (2.1 Å). However, as found in the crystal structure of phorbol ester with the C1b domain of PKC-δ, the ether oxygen of benzyloxy may interact with the interface of membrane/phospholipid rather than with the protein.

## 4. Conclusion

A series of 2-benzyl (template I) and 2-phenyl (template II)-3-hydroxypropyl pivalates carrying the principal pharmacophores of phorbol esters, including $C_3$–C═O, $C_{20}$–OH and $C_9$–OH/$C_{13}$–C═O, were designed and investigated as PKC-α ligands. The binding affinities of the two templates were optimized by incorporating three hydrogen bonding groups, such as hydroxy, benzyloxy, and hexanoyl groups, and by changing their positions on the phenyl ring. Compound **13c** exhibited the most potent binding affinity with $K_i = 0.7$ μM. The comparative molecular modeling analysis of benzyl and hexanoyl analogues with the two sets of phorbol models based on their binding affinities indicated that the rank order of binding affinities of this series of PKC ligands correlated very well with the rank order of rms values in the 3–13–20 model. The docked model of PKC-α compound **13c** demonstrated that the hydroxyl and the carbonyl of the pivaloyloxy group form hydrogen bonds with the backbone nitrogen of Thr12 (1.8 Å) and Gly23 (2.1 Å), respectively.

## 5. Experimental

### 5.1. Chemistry

All chemical reagents were commercially available. Melting points were determined with 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 NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz. Chemical shifts are reported in parts per million 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.

**5.1.1. General procedure for benzylation.** A mixture of hydroxybenzyl alcohol (500 mg, 4.03 mmol), potassium carbonate (835 mg, 6.04 mmol) in acetone (40 mL) was treated with benzyl bromide (0.48 mL, 4.03 mmol) and refluxed for 4 h. The reaction mixture was diluted with water and then extracted with EtOAc several times. The combined organic layers were concentrated in vacuo and the residue was purified by flash chromatography using EtOAc–hexanes = 2:1 as eluant to afford **1**.

**5.1.1.1. 2-(Benzyloxy)benzyl alcohol (1a).** 94% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.15–7.4 (m, 7H), 6.9 (m, 2H), 5.06 (s, 2H, $OCH_2Ph$), 4.68 (s, 2H, $CH_2OH$), 2.24 (br s, 1H, OH).

**5.1.1.2. 3-(Benzyloxy)benzyl alcohol (1b).** 95% yield, white solid, mp = 49 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.2–7.45 (m, 6H), 6.85–7.0 (m, 3H), 5.04 (s, 2H, $OCH_2Ph$), 4.63 (s, 2H, $CH_2OH$), 1.90 (br s, 1H, OH).

**5.1.1.3. 4-(Benzyloxy)benzyl alcohol (1c).** 96% yield, white solid, mp = 86 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.2–7.5 (m, 7H), 6.86 (d, 2H), 5.04 (s, 2H, $OCH_2Ph$), 4.57 (s, 2H, $CH_2OH$).

**5.1.2. General procedure for chlorination.** A cooled solution of **1** (800 mg, 3.73 mmol) in $CH_2Cl_2$ (20 mL) at 0 °C was treated with thionyl chloride (0.41 mL, 5.6 mmol) and stirred at the same temperature for 3 h. The reaction mixture was concentrated in vacuo and the residue was purified by flash chromatography using EtOAc–hexanes = 4:1 as eluant to afford **2**.

**5.1.2.1. 2-(Benzyloxy)benzyl chloride (2a).** 92% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.25–7.5 (m, 7H), 6.9 (m, 2H), 5.10 (s, 2H, $OCH_2Ph$), 4.69 (s, 2H, $CH_2Cl$).

**5.1.2.2. 3-(Benzyloxy)benzyl chloride (2b).** 94% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.1–7.45 (m, 6H), 6.85–7.0 (m, 3H), 4.93 (s, 2H, $OCH_2Ph$), 4.45 (s, 2H, $CH_2Cl$).

**5.1.2.3. 4-(Benzyloxy)benzyl chloride (2c).** 93% yield, white solid, mp = 79–80 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.25–7.5 (m, 7H), 6.95 (d, 2H), 5.06 (s, 2H, $OCH_2Ph$), 4.56 (s, 2H, $CH_2Cl$).

**5.1.3. General procedure for alkylation.** A mixture of diethyl malonate (826 mg, 5.16 mmol) and sodium hydride (60%, 275 mg, 6.88 mmol) in DMF (5 mL) was stirred at room temperature for 30 min and then treated with **2** (800 mg, 3.44 mmol). After being stirred for 3 h, the reaction mixture was diluted with $H_2O$ and then extracted with EtOAc several times. The combined organic layers were washed with $H_2O$ and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography using EtOAc–hexanes = 4:1 as eluant to afford **3**.

**5.1.3.1. Diethyl [2-(benzyloxy)benzyl]malonate (3a).** 97% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.15–7.5 (m, 7H), 6.8 (m, 2H), 5.12 (s, 2H, $OCH_2Ph$), 4.10 (q, 4H, 2× $OCH_2CH_3$), 3.90 (t, 1H, $J = 7.8$ Hz, $ArCH_2CH$), 3.10 (d, 1H, $J = 7.8$ Hz, $ArCH_2CH$), 1.17 (t, 6H, 2× $OCH_2CH_3$).

**5.1.3.2. Diethyl [3-(benzyloxy)benzyl]malonate (3b).** 98% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.1–7.4 (m, 6H), 6.65–6.8 (m, 3H), 4.94 (s, 2H, $OCH_2Ph$), 4.08 (q, 4H, 2× $OCH_2CH_3$), 3.55 (t, 1H, $J = 7.8$ Hz, $ArCH_2CH$), 3.27 (d, 1H, $J = 7.8$ Hz, $ArCH_2CH$), 1.15 (t, 6H, 2× $OCH_2CH_3$).

**5.1.3.3. Diethyl [4-(benzyloxy)benzyl]malonate (3c).** 98% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.25–7.45 (m, 5H), 7.10 (d, 2H), 6.86 (m, 2H), 5.01 (s, 2H, $OCH_2Ph$), 4.10 (q, 4H, 2× $OCH_2CH_3$), 3.57 (t, 1H, $J = 7.8$ Hz, $ArCH_2CH$), 3.13 (d, 1H, $J = 7.8$ Hz, $ArCH_2CH$), 1.17 (t, 6H, 2× $OCH_2CH_3$).

**5.1.4. General procedure for reduction.** A cooled suspension of lithium aluminum hydride (255 mg, 6.74 mmol) in THF (20 mL) at 0 °C was treated dropwise with **3**

(800 mg, 2.245 mmol) in THF (20 mL). After being stirred for 2 h at room temperature, the reaction mixture was quenched with $H_2O$ (0.25 mL), 15% NaOH (0.5 mL), and $H_2O$ (0.75 mL) successively and then stirred for 1 h. The suspension was filtered and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography using EtOAc–hexanes = 1:2 as eluant to afford **4**.

**5.1.4.1. 2-[2-(Benzyloxy)benzyl]-1,3-propanediol (4a).** 58% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.25–7.5 (m, 5H), 7.15 (m, 2H), 6.9 (m, 2H), 5.06 (s, 2H, $OCH_2Ph$), 3.65 (ddd of AB, 4H, 2× $CH_2OH$), 2.72 (d, 1H, $J$ = 7.3 Hz, Ar$CH_2$CH), 2.30 (br s, 2H, OH), 1.90 (m, 1H, Ar$CH_2CH$).

**5.1.4.2. 2-[3-(Benzyloxy)benzyl]-1,3-propanediol (4b).** 60% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.15–7.5 (m, 6H), 6.8 (m, 3H), 5.04 (s, 2H, $OCH_2Ph$), 3.70 (ddd of AB, 4H, 2× $CH_2OH$), 2.58 (d, 1H, $J$ = 7.5 Hz, Ar$CH_2$CH), 2.34 (br s, 2H, OH), 2.04 (m, 1H, Ar$CH_2CH$).

**5.1.4.3. 2-[4-(Benzyloxy)benzyl]-1,3-propanediol (4c).** 60% yield, white solid, mp = 84 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.25–7.5 (m, 5H), 7.08 (d, 2H), 6.90 (m, 2H), 5.01 (s, 2H, $OCH_2Ph$), 3.70 (ddd of AB, 4H, 2× $CH_2OH$), 2.65 (br s, 2H, OH), 2.53 (d, 1H, $J$ = 7.5 Hz, Ar$CH_2$CH), 1.98 (m, 1H, Ar$CH_2CH$).

**5.1.4.4. 2-[2-(Benzyloxy)phenyl]-1,3-propanediol (12a).** 74% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.3–7.45 (m, 5H), 7.15–7.25 (m, 2H), 6.9–7.0 (m, 2H), 5.08 (s, 2H, $OCH_2Ph$), 3.97 (m, 4H, 2× $CH_2OH$), 3.59 (m, 1H, ArCH).

**5.1.4.5. 2-[3-(Benzyloxy)phenyl]-1,3-propanediol (12b).** 78% yield, white solid, mp = 73–74 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.25–7.45 (m, 6H), 6.8–6.9 (m, 3H), 5.06 (s, 2H, $OCH_2Ph$), 3.96 (m, 4H, 2× $CH_2OH$), 3.10 (m, 1H, ArCH).

**5.1.4.6. 2-[4-(Benzyloxy)phenyl]-1,3-propanediol (12c).** 76% yield, white solid, mp = 124–125 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.3–7.45 (m, 5H, Ph), 7.16 (dt, 2H, Ar), 6.95 (dt, 2H, Ar), 5.05 (s, 2H, $OCH_2Ph$), 3.93 (m, 4H, 2× $CH_2OH$), 3.07 (m, 1H, ArCH).

**5.1.5. General procedure for pivaloylation.** A cooled solution of **4** (350 mg, 1.285 mmol) and pyridine (0.2 mL, 2.57 mmol) in $CH_2Cl_2$ (25 mL) 0 °C was treated dropwise with pivaloyl chloride (0.15 mL, 1.285 mmol) and was stirred at room temperature for 16 h. The reaction mixture was diluted with $H_2O$ and then extracted with EtOAc several times. The combined organic layers were washed with $H_2O$ and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography using EtOAc–hexanes = 1:2 as eluant to afford **5**.

**5.1.5.1. 2-[2-(Benzyloxy)benzyl]-3-hydroxypropyl pivalate (5a).** 64% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.1–7.5 (m, 7H), 6.9 (m, 2H), 5.08 (s, 2H, $OCH_2Ph$), 4.12 (ddd of AB, 2H, 2× $CH_2OCO$), 3.48 (ddd of AB, 2H, 2× $CH_2OH$), 2.72 (ddd of AB, 2H, Ar$CH_2$CH), 2.30 (br s, 1H, OH), 2.15 (m, 1H, Ar$CH_2CH$), 1.20 (s, 9H, $C(CH_3)_3$); IR (neat) 3445 (OH), 1725 (C=O); MS (FAB) $m/z$ 357 ($MH^+$); Anal. Calcd for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.36; H, 7.95.

**5.1.5.2. 2-[3-(Benzyloxy)benzyl]-3-hydroxypropyl pivalate (5b).** 67% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.15–7.5 (m, 6H), 6.8 (m, 3H), 5.05 (s, 2H, $OCH_2Ph$), 4.14 (ddd of AB, 2H, 2× $CH_2OCO$), 3.50 (ddd of AB, 2H, 2× $CH_2OH$), 2.63 (ddd of AB, 2H, Ar$CH_2$CH), 2.10 (m, 1H, Ar$CH_2CH$), 1.23 (s, 9H, $C(CH_3)_3$); IR (neat) 3445 (OH), 1725 (C=O); MS (FAB) $m/z$ 357 ($MH^+$); Anal. Calcd for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.35; H, 7.94.

**5.1.5.3. 2-[4-(Benzyloxy)benzyl]-3-hydroxypropyl pivalate (5c).** 65% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.25–7.4 (m, 5H), 7.02 (d, 2H), 6.84 (d, 2H), 4.97 (s, 2H, $OCH_2Ph$), 4.05 (ddd of AB, 2H, 2× $CH_2OCO$), 3.45 (ddd of AB, 2H, 2× $CH_2OH$), 2.52 (ddd of AB, 2H, Ar$CH_2$CH), 2.0 (m, 1H, Ar$CH_2CH$), 1.75 (br s, 1H, OH), 1.16 (s, 9H, $C(CH_3)_3$); IR (neat) 3442 (OH), 1726 (C=O); MS (FAB) $m/z$ 357 ($MH^+$); Anal. Calcd for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 74.33; H, 7.95.

**5.1.5.4. 2-[2-(Benzyloxy)phenyl]-3-hydroxypropyl pivalate (13a).** 76% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.25–7.45 (m, 5H), 7.15–7.25 (m, 2H), 6.9–7.0 (m, 2H), 5.08 (s, 2H, $OCH_2Ph$), 4.41 (ddd of AB, 2H, $CH_2OCO$), 3.87 (d, 2H, $CH_2OH$), 3.68 (m, 1H, ArCH), 1.15 (s, 9H, $C(CH_3)_3$); IR (neat) 3444 (OH), 1725 (C=O) $cm^{-1}$; MS (FAB) 365 ($MNa^+$); Anal. Calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.88; H, 7.68.

**5.1.5.5. 2-[3-(Benzyloxy)phenyl]-3-hydroxypropyl pivalate (13b).** 79% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.2–7.45 (m, 6H), 6.82–6.9 (m, 3H), 5.05 (s, 2H, $OCH_2Ph$), 4.36 (ddd of AB, 2H, $CH_2OCO$), 3.83 (br t, 2H, $CH_2OH$), 3.14 (m, 1H, ArCH), 1.86 (br s, 1H, OH), 1.17 (s, 9H, $C(CH_3)_3$); IR (neat) 3445 (OH), 1725 (C=O) $cm^{-1}$; MS (FAB) 365 ($MNa^+$); Anal. Calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.90; H, 7.67.

**5.1.5.6. 2-[4-(Benzyloxy)phenyl]-3-hydroxypropyl pivalate (13c).** 78% yield, white solid, mp = 57 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.3–7.45 (m, 5H, Ph), 7.18 (dt, 2H, Ar), 6.95 (dt, 2H, Ar), 5.05 (s, 2H, $OCH_2Ph$), 4.34 (ddd of AB, 2H, $CH_2OCO$), 3.81 (d of AB, 2H, $CH_2OH$), 3.12 (m, 1H, ArCH), 1.92 (br s, 1H, OH), 1.16 (s, 9H, $C(CH_3)_3$); IR (neat) 3435 (OH), 1725 (C=O) $cm^{-1}$; MS (EI) 342 ($M^+$); Anal. Calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.89; H, 7.67.

**5.1.6. General procedure for debenzylation.** A suspension of **5** (110 mg, 0.31 mmol) in MeOH (6 mL) was treated with 10% palladium on carbon (11 mg) and hydrogenated under a balloon pressure of hydrogen for 16 h at room temperature. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue

was purified by flash chromatography using EtOAc–hexanes = 1:2 to afford **6**.

**5.1.6.1. 3-Hydroxy-2-(2-hydroxybenzyl)propyl pivalate (6a).** 85% yield, white solid, mp = 61–62 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.04–7.15 (m, 2H), 6.83 (m, 2H), 4.12 (ddd of AB, 2H, 2× $CH_2OCO$), 3.50 (ddd of AB, 2H, 2× $CH_2OH$), 2.70 (ddd of AB, 2H, Ar$CH_2$CH), 2.10 (m, 1H, Ar$CH_2CH$), 1.21 (s, 9H, $C(CH_3)_3$); IR (neat) 3395 (OH), 1704 (C=O); MS (FAB) *m*/*z* 267 ($MH^+$); Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.86; H, 8.36.

**5.1.6.2. 3-Hydroxy-2-(3-hydroxybenzyl)propyl pivalate (6b).** 88% yield, white solid, mp = 95–96 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.16 (t, 1H), 6.65–6.77 (m, 2H), 5.18 (br s, 1H, OH), 4.10 (ddd of AB, 2H, 2× $CH_2OCO$), 3.53 (ddd of AB, 2H, 2× $CH_2OH$), 2.60 (ddd of AB, 2H, Ar$CH_2$CH), 2.11 (m, 1H, Ar$CH_2CH$), 1.23 (s, 9H, $C(CH_3)_3$); IR (neat) 3396 (OH), 1704 (C=O); MS (FAB) *m*/*z* 267 ($MH^+$); Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.88; H, 8.35.

**5.1.6.3. 3-Hydroxy-2-(2-hydroxybenzyl)propyl pivalate (6c).** 90% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.02 (d, 2H), 6.74 (d, 2H), 5.2 (br s, 1H, OH), 4.12 (ddd of AB, 2H, 2× $CH_2OCO$), 3.50 (ddd of AB, 2H, 2× $CH_2OH$), 2.56 (ddd of AB, 2H, Ar$CH_2$CH), 2.06 (m, 1H, Ar$CH_2CH$), 1.21 (s, 9H, $C(CH_3)_3$); IR (neat) 3398 (OH), 1704 (C=O); MS (FAB) *m*/*z* 267 ($MH^+$); Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 67.89; H, 8.37.

**5.1.6.4. 2-(2-Hydroxyphenyl)-3-hydroxypropyl pivalate (14a).** 92% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 8.24 (br s, 1H, OH), 7.05–7.2 (m, 2H), 6.8–6.9 (m, 2H), 4.65 (dd of AB, 1H, $CH_2OCO$), 4.25 (dd of AB, 1H, $CH_2OCO$), 3.95 (ddd of AB, 2H, $CH_2OH$), 3.26 (m, 1H, ArCH), 1.19 (s, 9H, $C(CH_3)_3$); IR (neat) 3395 (OH), 1704 (C=O) $cm^{-1}$; MS (FAB) *m*/*z* 275 ($MNa^+$); Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.65; H, 7.99. Found: C, 66.90; H, 8.01.

**5.1.6.5. 2-(3-Hydroxyphenyl)-3-hydroxypropyl pivalate (14b).** 93% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.17 (m, 1H), 6.7–6.8 (m, 3H), 6.47 (br s, 1H, OH), 4.34 (ddd of AB, 2H, $CH_2OCO$), 3.82 (d of AB, 2H, $CH_2OH$), 3.11 (m, 1H, ArCH), 2.51 (br s, 1H, OH), 1.15 (s, 9H, $C(CH_3)_3$); IR (neat) 3395 (OH), 1706 (C=O) $cm^{-1}$; MS (EI) *m*/*z* 252 ($M^+$); Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.65; H, 7.99. Found: C, 66.88; H, 8.02.

**5.1.6.6. 2-(4-Hydroxyphenyl)-3-hydroxypropyl pivalate (14c).** 94% yield, white solid, mp = 123–124 °C: $^1$H NMR ($CDCl_3$) $\delta$ 7.09 (dt, 2H, Ar), 6.77 (dt, 2H, Ar), 5.81 (br s, 1H, OH), 4.32 (ddd of AB, 2H, $CH_2OCO$), 3.82 (ddd of AB, 2H, $CH_2OH$), 3.11 (m, 1H, ArCH), 2.05 (br s, 1H, OH), 1.17 (s, 9H, $C(CH_3)_3$); IR (neat) 3169 (OH), 1723 (C=O) $cm^{-1}$; MS (FAB) *m*/*z* 253 ($MH^+$); Anal. Calcd for $C_{14}H_{20}O_4$: C, 66.65; H, 7.99. Found: C, 66.92; H, 8.00.

**5.1.7. General procedure for hexanoylation.** A cooled solution of **6** (48 mg, 0.18 mmol) and a catalytic amount of 4-dimethylaminopyridine in $CH_2Cl_2$ (2 mL) at 0 °C was treated with *n*-hexanoic anhydride (0.05 mL, 0.22 mmol) and stirred for 30 min. The reaction mixture was diluted with $H_2O$ and then extracted with EtOAc several times. The combined organic layers were concentrated in vacuo and the residue was purified by flash chromatography using EtOAc–hexanes = 1:4 to afford **7**.

**5.1.7.1. 2-[2-(Hexanoyloxy)benzyl]-3-hydroxypropyl pivalate (7a).** 91% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.15–7.3 (m, 3H, Ar), 7.03 (m, 1H, Ar), 4.20 (dd, 1H, $CH_2OCO$), 4.07 (dd, 1H, $CH_2OCO$), 3.54 (dd, 1H, $CH_2OH$), 3.44 (dd, 1H, $CH_2OH$), 2.5–2.65 (m, 4H, Ar$CH_2$CH and $COCH_2$), 2.15 (m, 1H, Ar$CH_2CH$), 1.7–1.8 (m, 2H, $COCH_2CH_2$), 1.35–1.45 (m, 4H, $(CH_2)_2CH_3$), 1.22 (s, 9H, $C(CH_3)_3$), 0.93 (distorted t, 3H, $CH_3$); IR (neat) 3443 (OH), 1758, 1727 (C=O); MS (FAB) *m*/*z* 365 ($MH^+$); Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.40; H, 8.87.

**5.1.7.2. 2-[3-(Hexanoyloxy)benzyl]-3-hydroxypropyl pivalate (7b).** 93% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.30 (dt, 1H, Ar), 7.05 (br d, 1H, Ar), 6.9–6.96 (m, 2H, Ar), 4.21 (dd, 1H, $CH_2OCO$), 4.06 (dd, 1H, $CH_2OCO$), 3.58 (dd, 1H, $CH_2OH$), 3.46 (dd, 1H, $CH_2OH$), 2.66 (ddd of AB, 2H, Ar$CH_2$CH), 2.54 (t, 2H, $COCH_2$), 2.11 (m, 1H, Ar$CH_2CH$), 1.7–1.8 (m, 2H, $COCH_2CH_2$), 1.3–1.45 (m, 4H, $(CH_2)_2CH_3$), 1.23 (s, 9H, $C(CH_3)_3$), 0.94 (distorted t, 3H, $CH_3$); IR (neat) 3440 (OH), 1760, 1728 (C=O); MS (FAB) *m*/*z* 365 ($MH^+$); Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.42; H, 8.88.

**5.1.7.3. 2-[4-(Hexanoyloxy)benzyl]-3-hydroxypropyl pivalate (7c).** 92% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.19 (d, 2H, Ar), 7.00 (d, 2H, Ar), 4.21 (dd, 1H, $CH_2OCO$), 4.05 (dd, 1H, $CH_2OCO$), 3.58 (dd, 1H, $CH_2OH$), 3.46 (dd, 1H, $CH_2OH$), 2.64 (ddd of AB, 2H, Ar$CH_2$CH), 2.54 (t, 2H, $COCH_2$), 2.10 (m, 1H, Ar$CH_2CH$), 1.7–1.8 (m, 2H, $COCH_2CH_2$), 1.35–1.45 (m, 4H, $(CH_2)_2CH_3$), 1.23 (s, 9H, $C(CH_3)_3$), 0.94 (distorted t, 3H, $CH_3$); IR (neat) 3447 (OH), 1760, 1728 (C=O); MS (FAB) *m*/*z* 365 ($MH^+$); Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.44; H, 8.88.

**5.1.7.4. 2-[2-(Hexanoyloxy)phenyl]-3-hydroxypropyl pivalate (15a).** 90% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.2–7.4 (m, 3H), 7.04 (m, 1H), 4.34 (ddd of AB, 2H, $CH_2OCO$), 3.81 (ddd of AB, 2H, $CH_2OH$), 3.34 (m, 1H, ArCH), 2.60 (t, 2H, $COCH_2$), 2.32 (dt, 1H, OH), 1.7–1.8 (m, 2H, $COCH_2CH_2$), 1.3–1.45 (m, 4H, $(CH_2)_2CH_3$), 1.16 (s, 9H, $C(CH_3)_3$), 0.93 (distorted t, 3H, $CH_3$); IR (neat) 3524, 1760, 1730 $cm^{-1}$; MS (FAB) *m*/*z* 373 ($MNa^+$); Anal. Calcd for $C_{20}H_{30}O_5$: C, 68.54; H, 8.63. Found: C, 68.78; H, 8.66.

**5.1.7.5. 2-[3-(Hexanoyloxy)phenyl]-3-hydroxypropyl pivalate (15b).** 89% yield, colorless oil: $^1$H NMR ($CDCl_3$) $\delta$ 7.34 (m, 1H), 7.13 (m, 1H), 6.96–7.02 (m, 2H), 4.38 (ddd of AB, 2H, $CH_2OCO$), 3.84 (d, 2H, $CH_2OH$), 3.17 (m, 1H, ArCH), 2.55 (t, 2H, $COCH_2$), 2.34 (t, 1H, OH), 1.7–1.8 (m, 2H, $COCH_2CH_2$), 1.3–1.45 (m, 4H, $(CH_2)_2CH_3$), 1.16 (s, 9H, $C(CH_3)_3$), 0.93 (distorted t, 3H, $CH_3$); IR (neat) 3524, 1760,

1730 $cm^{-1}$; MS (FAB) *m/z* 351 ($MH^+$); Anal. Calcd for $C_{20}H_{30}O_5$: C, 68.54; H, 8.63. Found: C, 68.75; H, 8.65.

**5.1.7.6. 2-[4-(Hexanoyloxy)phenyl]-3-hydroxypropyl pivalate (15c).** 86% yield, colorless oil: $^1H$ NMR ($CDCl_3$) $\delta$ 7.26 (dt, 2H), 7.05 (dt, 2H), 4.37 (ddd of AB, 2H, $CH_2OCO$), 3.83 (dd, 2H, $CH_2OH$), 3.17 (m, 1H, ArCH), 2.55 (t, 2H, $COCH_2$), 1.92 (t, 1H, OH), 1.7–1.8 (m, 2H, $COCH_2CH_2$), 1.35–1.45 (m, 4H, $(CH_2)_2CH_3$), 1.17 (s, 9H, $C(CH_3)_3$), 0.93 (distorted t, 3H, $CH_3$); IR (neat) 3502, 1758, 1728 $cm^{-1}$; MS (FAB) *m/z* 351 ($MH^+$); Anal. Calcd for $C_{20}H_{30}O_5$: C, 68.54; H, 8.63. Found: C, 68.80; H, 8.65.

**5.1.8. General procedure for cyanation.** A mixture of **2** (800 mg, 3.44 mmol) and sodium cyanide (337 mg, 6.88 mmol) in DMF (5 mL) was heated at 100 °C for 2 h. The reaction mixture was diluted with $H_2O$ and then extracted with EtOAc several times. The combined organic layers were washed with $H_2O$ and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography using EtOAc–hexanes = 1:4 as eluant to afford **8**.

**5.1.8.1. 2-(Benzyloxy)benzyl cyanide (8a).** 96% yield, white solid, mp = 77 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 7.25–7.45 (m, 7H), 6.9–7.0 (m, 2H), 5.12 (s, 2H, $OCH_2Ph$), 3.73 (s, 2H, $CH_2CN$).

**5.1.8.2. 3-(Benzyloxy)benzyl cyanide (8b).** 98% yield, colorless oil: $^1H$ NMR ($CDCl_3$) $\delta$ 7.25–7.45 (m, 6H), 6.85–6.95 (m, 3H), 5.06 (s, 2H, $OCH_2Ph$), 3.70 (s, 2H, $CH_2CN$).

**5.1.8.3. 4-(Benzyloxy)benzyl cyanide (8c).** 98% yield, white solid, mp = 68–69 °C; $^1H$ NMR ($CDCl_3$) $\delta$ 7.3–7.45 (m, 5H, Ph), 7.22 (dt, 2H, Ar), 6.96 (dt, 2H, Ar), 5.05 (s, 2H, $OCH_2Ph$), 3.66 (s, 2H, $CH_2CN$).

**5.1.9. General procedure for esterification.** A solution of **8** (700 mg, 3.14 mmol) and sodium hydroxide solution (30%, 10 mL) was refluxed overnight. The reaction mixture was neutralized with 1 N HCl and extracted with EtOAc several times. The combined organic layers were concentrated in vacuo to afford **9** which was used for the next step without further purification. The acid was dissolved in MeOH (10 mL) and treated with a couple of drops of $H_2SO_4$. After being refluxed for 2 h, the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography using EtOAc–hexanes = 1:4 as eluant to afford **10**.

**5.1.9.1. 2-[2-(Benzyloxy)phenyl]acetic acid (9a).** 97% yield, colorless oil: $^1H$ NMR ($CDCl_3$) $\delta$ 7.2–7.4 (m, 7H), 6.9–7.0 (m, 2H), 5.09 (s, 2H, $OCH_2Ph$), 3.72 (s, 2H, $CH_2CO_2H$).

**5.1.9.2. 2-[3-(Benzyloxy)phenyl]acetic acid (9b).** 98% yield, white solid, mp = 119 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 7.25–7.45 (m, 6H), 6.85–6.95 (m, 3H), 5.06 (s, 2H, $OCH_2Ph$), 3.64 (s, 2H, $CH_2CO_2H$).

**5.1.9.3. 2-[4-(Benzyloxy)phenyl]acetic acid (9c).** 98% yield, white solid, mp = 122–123 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 7.3–7.45 (m, 5H, Ph), 7.20 (dt, 2H, Ar), 6.94 (dt, 2H, Ar), 5.05 (s, 2H, $OCH_2Ph$), 3.59 (s, 2H, $CH_2CO_2H$).

**5.1.9.4. Methyl 2-[2-(benzyloxy)phenyl]acetate (10a).** 90% yield, colorless oil: $^1H$ NMR ($CDCl_3$) $\delta$ 7.15–7.4 (m, 7H), 6.9–6.95 (m, 3H), 5.03 (s, 2H, $OCH_2Ph$), 3.68 (s, 3H, $CO_2CH_3$), 3.62 (s, 2H, $CH_2CO_2Me$).

**5.1.9.5. Methyl 2-[3-(benzyloxy)phenyl]acetate (10b).** 94% yield, colorless oil: $^1H$ NMR ($CDCl_3$) $\delta$ 7.3–7.45 (m, 5H, Ph), 7.24 (m, 1H, Ar), 6.85–6.95 (m, 3H), 5.05 (s, 2H, $OCH_2Ph$), 3.68 (s, 3H, $CO_2CH_3$), 3.60 (s, 2H, $CH_2CO_2Me$).

**5.1.9.6. Methyl 2-[4-(benzyloxy)phenyl]acetate (10c).** 93% yield, white solid, mp = 94–95 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 7.3–7.45 (m, 5H, Ph), 7.20 (dt, 2H, Ar), 6.94 (dt, 2H, Ar), 5.05 (s, 2H, $OCH_2Ph$), 3.69 (s, 3H, $CO_2CH_3$), 3.57 (s, 2H, $CH_2CO_2Me$).

**5.1.10. General procedure for methoxycarbonylation.** A cooled solution of **10** (700 mg, 2.73 mmol) in dimethylcarbonate (5 mL) at 0 °C was treated portionwise with sodium hydride (60%, 328 mg, 8.19 mmol) and stirred overnight at room temperature. The reaction mixture was diluted with $H_2O$ and then extracted with EtOAc several times. The combined organic layers were concentrated in vacuo and the residue was purified by flash chromatography using EtOAc–hexanes = 1:4 to afford **11**.

**5.1.10.1. Dimethyl [2-(benzyloxy)phenyl]malonate (11a).** 96% yield, colorless oil: $^1H$ NMR ($CDCl_3$) $\delta$ 7.25–7.4 (m, 7H), 6.92–7.0 (m, 2H), 5.20 (s, 1H, ArCH), 5.08 (s, 2H, $OCH_2Ph$), 3.72 (s, 6H, 2× $CO_2CH_3$).

**5.1.10.2. Dimethyl [3-(benzyloxy)phenyl]malonate (11b).** 95% yield, white solid, mp = 69 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 7.25–7.45 (m, 6H), 6.9–7.05 (m, 3H), 5.06 (s, 2H, $OCH_2Ph$), 4.62 (s, 1H, ArCH), 3.75 (s, 6H, 2× $CO_2CH_3$).

**5.1.10.3. Dimethyl [4-(benzyloxy)phenyl]malonate (11c).** 94% yield, white solid, mp = 87–88 °C: $^1H$ NMR ($CDCl_3$) $\delta$ 7.25–7.45 (m, 7H), 6.97 (dt, 2H, Ar), 5.05 (s, 2H, $OCH_2Ph$), 4.59 (s, 1H, ArCH), 3.75 (s, 6H, 2× $CO_2CH_3$).

### 5.2. Molecular modeling

The structures of all chemicals, R = benzyl (**5a**–**c** and **13a**–**c**) and hexanoyl (**7a**–**c** and **15a**–**c**) analogues, were built using the Sybyl 6.9 molecular modeling program (Tripos, Inc.), and then the geometries were fully optimized using the Tripos force fields with the following non-default options (method: conjugate gradient, termination: gradient 0.01 kcal/mol Å, and max iterations: 10,000). The partial atomic charges were calculated by the Gasteiger–Hückel method in the Sybyl program. The 3D structure of phorbol ester was extracted from

the crystal structure of the cys2 activator-binding domain of PKC-δ in complex with phorbol ester.[20] Three hydrogen bond-forming pharmacophores ($C_3$–C═O, $C_{20}$–OH, and $C_9$–OH or $C_{13}$–C═O) in phorbol ester were superimposed with the corresponding type of features (the carbonyl of ester, the hydroxyl group, and the ether oxygen atom or the ester carbonyl) in DAG analogues, respectively.

Docking of the most potent compound (**13c**, $K_i = 0.7\ \mu M$) was carried out using the FlexiDock function of the Sybyl program. The active site of PKC-α was defined using the key amino acids which play an important role in the specific binding of phorbol ester. All amino acid residues within a 4 Å radius of three amino acids at positions 12, 21, and 23 were considered. Compound **13c** was pre-positioned using least-squares fitting on the three pharmacophore features (3–13–20). To estimate the interaction energy between the ligand and the receptor-binding pocket, the Tripos force fields with default FlexiDock parameters were used along with Gasteiger–Hückel and Kollman partial atomic charges for the ligand and enzyme, respectively. Compound **13c** was docked into the binding pocket of PKC-α, which was kept rigid, with 3000 genetic algorithm (GA) runs throughout the simulation. Based on the fitness score (energy), only the energetically favorable structures were analyzed and the lowest energy structure of compound **13c** in the C1b domain of PKC-α was selected for further refinement. Then, the obtained complex was fully optimized by energy minimization using Tripos force fields with the above minimization criteria. All calculations were performed on a Silicon Graphics O2 R10000 workstation.

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