

## A New Class of Simplified Phorbol Ester Analogs: Synthesis and Binding to PKC and $\eta$ PKC-C1B ( $\eta$ PKC-CRD2)

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### Supplementary Information:

#### Experimental Procedures

**General Techniques.** The following general procedures were used in all reactions unless otherwise noted. Oxygen- and moisture-sensitive reactions were carried out in oven-dried ( $T > 110^\circ \text{C}$ ) glassware sealed under a positive pressure of dry nitrogen from a manifold or balloon. Sensitive liquids and solutions were transferred by syringe through rubber septa. Reactions were run at room temperature and stirred with a Teflon-covered magnetic stirring bar unless otherwise stated.

All commercial available reagents were used without further purification with the following exceptions. Phorbol was purchased from LC-laboratories and stored at  $-4^\circ \text{C}$  (the purchased sample contained 1 molequivalent ethanol). Ether, THF, and DME were distilled from sodium-benzophenone ketyl under nitrogen.  $\text{CH}_2\text{Cl}_2$  and Pyridine were distilled from  $\text{CaH}_2$  under nitrogen prior to use. Chromatography grade hexanes were distilled through a 50 cm packed column.

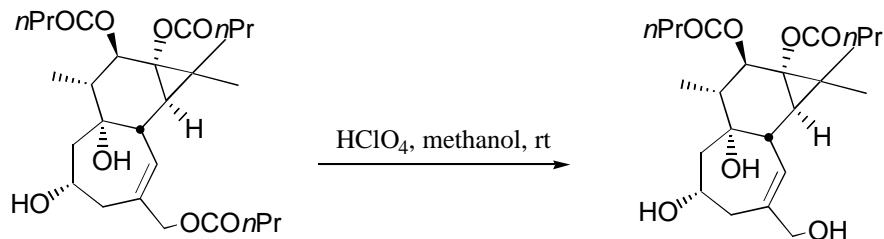
Analytical TLC was performed with 0.25 mm silica gel 60 plates with a 254 nm fluorescent indicator from Merck. Plates were developed in a covered chamber and visualized by UV light and by treatment with acidic anisaldehyd stain followed by heating. Flash chromatography refers to column chromatography following the procedure of Still and co-workers using Merck Silica Gel 60, 230-240 mesh (40-60 mm) as stationary phase. Solvent was eluted at a flow rate of approximately two inches/min with 6-9 pounds of nitrogen pressure.

Infrared spectra were measured on a Perkin-Elmer model 1605 Fourier transform spectrometer (FT-IR) and are reported in wavenumbers ( $\text{cm}^{-1}$ ). IR spectra were collected as solutions in  $\text{CDCl}_3$  against a background of  $\text{CDCl}_3$ . The instrument was calibrated using a 0.05 mm polystyrene film. The measured values of the IUPAC reference bands were  $1601.2 \text{ cm}^{-1}$  and  $1583.0 \text{ cm}^{-1}$ . The IUPAC values for these bands are  $1601.4 \text{ cm}^{-1}$  and  $1583.1 \text{ cm}^{-1}$ .

NMR spectra were measured on a Varian Unity Inova ( $^1\text{H}$  at 500 MHz,  $^{13}\text{C}$  at 125 MHz), Varian XL-400 ( $^1\text{H}$  at 400 MHz,  $^{13}\text{C}$  at 100 MHz), or a Varian Gemini-300 ( $^1\text{H}$  at 300 MHz,  $^{13}\text{C}$  at 75 MHz) magnetic resonance spectrometer. Proton NMR spectra are reported as chemical shifts in parts-per-million (ppm) downfield from tetramethylsilane (0 ppm). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad, dd = doublet of doublets, dt = doublet of triplets, dq = doublet of quartets,

and ddd = doublet of doublet of doublets; other combinations derive from those listed. Coupling constants (J) are reported in Hertz (Hz). Carbon-13 NMR spectra are reported as chemical shifts in ppm based on the middle peak of chloroform-d (77.0 ppm) and are recorded with complete heterodecoupling.

High resolution mass spectrometry (HRMS) was performed by the Mass Spectrometry Facility, University of California-San Francisco supported by the NIH Division of Research Resources. Numbers are given as mass ion and relative abundance (%) with the base peak assigned as 100%. Elemental Analysis were obtained from Desert Analytics, Tucson, Arizona, USA.

**Alcohol 7a**

Alcohol **6a** (2.6 mg, 5.1  $\mu\text{mol}$ ) was dissolved in methanol (0.4 mL) under a N<sub>2</sub>-atmosphere. A 0.028 M HClO<sub>4</sub> solution in methanol (0.4 mL) was added and stirring at room temperature was continued with exclusion of light until all the starting material was consumed. Solid Na<sub>2</sub>CO<sub>3</sub> (10.0 mg, 94  $\mu\text{mol}$ ) was added and stirring was continued for 15 min. The crude reaction mixture was filtered (silica gel, EtOAc) and concentrated under reduced pressure. Purification of the resultant residue by flash chromatography (silica gel, 60% EtOAc in hexanes) afforded triol **7a** (1.5 mg, 67%) as a colorless material.

<sup>1</sup>H-NMR (300MHz, CDCl<sub>3</sub>):  $\delta$  = 5.58 (d, J = 2.96 Hz, 1H), 5.36 (d, J = 10.99 Hz, 1H), 4.67 (s, 1H), 4.53 (s, 1H), 4.11 (m, 1H), 4.04 (m, 2H), 2.78-2.20 (m, 8H), 1.70-1.55 (m, 4H), 1.42 (m, 2H), 1.24 (s, 3H), 1.20 (s, 3H), 1.04-0.88 (m, 10H).

<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 173.2, 172.8, 139.7, 132.5, 74.9, 74.4, 68.5, 66.0, 64.4, 43.9, 43.7, 40.1, 39.6, 36.4, 36.2, 35.4, 24.4, 24.3, 18.6, 17.9, 16.1, 13.6, 13.4, 11.5.

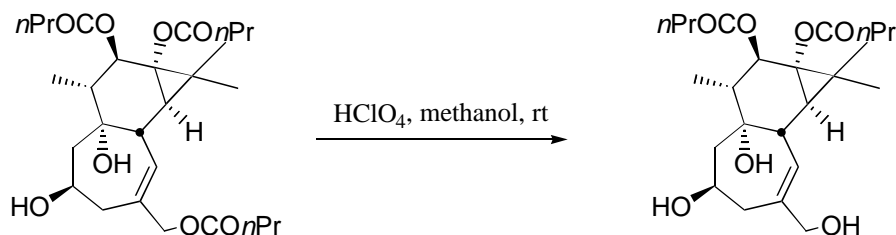
FTIR (CDCl<sub>3</sub>): 3674, 3410, 2926, 1725, 1601, 1458, 1374, 1257, 1187 cm<sup>-1</sup>.

MS: 420 (1), 351 (2), 350 (4), 349 (4), 285 (4), 262 (19), 244 (22), 83 (21), 71 (100), 69 (20).

HRMS: Calculated for C<sub>24</sub>H<sub>36</sub>O<sub>6</sub> [M<sup>+</sup>-H<sub>2</sub>O] 420.2511

Found: 420.2527

### Alcohol 7b



Alcohol **6b** (2.6 mg, 5.1  $\mu$ mol) was dissolved in methanol (0.4 mL) under a N<sub>2</sub>-atmosphere. A 0.028 M HClO<sub>4</sub> solution in methanol (0.4 mL) was added and stirring at room temperature was continued with exclusion of light till all the starting material was consumed. Solid Na<sub>2</sub>CO<sub>3</sub> (20.0 mg, 188  $\mu$ mol) was added and stirring was continued for 15 min. The crude reaction mixture was filtered (silica gel, EtOAc) and concentrated under reduced pressure. Purification of the resultant residue by flash chromatography (silica gel, 60% - 100% EtOAc in hexanes) afforded triol **7b** (1.7 mg, 75%) as a colorless material.

<sup>1</sup>H-NMR (300MHz, CDCl<sub>3</sub>):  $\delta$  = 5.45 (d, J = 2.54 Hz, 1H), 5.39 (d, J = 10.87 Hz, 1H), 4.50 (s, 1H), 4.06 (m, 2H), 3.85 (m, 1H), 2.45-2.20 (m, 8H), 1.78-1.56 (m, 4H), 1.48 (m, 1H), 1.35 (m, 1H), 1.24 (s, 3H), 1.18 (s, 3H), 1.10-0.85 (m, 10H).

<sup>13</sup>C-NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  = 175.8, 173.3, 138.2, 131.1, 75.2, 74.8, 67.8, 65.4, 64.5, 49.7, 44.4, 39.7, 39.4, 38.7, 36.4, 36.2, 24.5, 24.3, 18.6, 17.9, 16.1, 13.6, 13.4, 11.6.

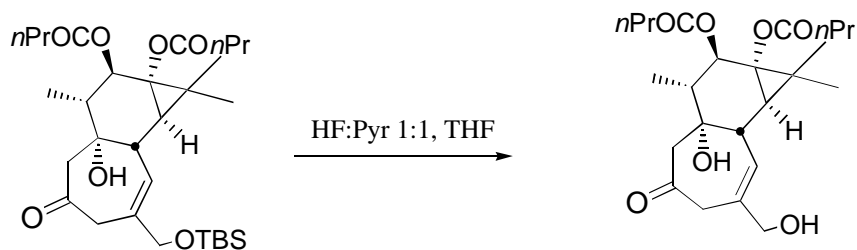
FTIR (CDCl<sub>3</sub>): 3606, 3411, 2965, 2929, 1725, 1601, 1460, 1377, 1256, 1186 cm<sup>-1</sup>.

MS: 351 (4), 350 (2), 349 (2), 325 (2), 262 (18), 244 (23), 125 (14), 105 (11), 83 (21), 71 (100), 69 (19).

HRMS: Calculated for C<sub>20</sub>H<sub>31</sub>O<sub>5</sub> [M<sup>+</sup>-OButyrate] 351.2171

Found: 351.2170

## Alcohol 11



Ketone **10** (1.8 mg, 3.2  $\mu\text{mol}$ ) was dissolved in THF (1.5 mL) under a gentle  $\text{N}_2$ -stream. Pyridine (0.25 mL) was added followed by a solution of HF in pyridine (70%, 100  $\mu\text{L}$ ). The reaction mixture was stirred at room temperature for 5 hrs. After this period the mixture was diluted with EtOAc (2.0 mL) and washed with sat. aqueous  $\text{CuSO}_4$  solution (2 x 1.0 mL) and brine (1.0 mL) and dried over  $\text{NaSO}_4$ . The crude reaction mixture was filtered and concentrated under reduced pressure. Purification of the resultant residue by flash chromatography (silica gel, 60% EtOAc in hexanes) afforded **11** (1.4 mg, 100%) as a colorless material.

$^1\text{H-NMR}$  (300MHz,  $\text{CDCl}_3$ ):  $\delta$  = 5.76 (d,  $J$  = 5.49 Hz, 1H), 5.39 (d,  $J$  = 10.38 Hz, 1H), 5.27 (s, 1H), 4.13 (m, 2H), 3.35 (d,  $J$  = 18.40 Hz, 1H), 3.07 (d,  $J$  = 18.07 Hz, 1H), 2.92, (d,  $J$  = 12.98 Hz, 1H), 2.58 (d,  $J$  = 12.70 Hz, 1H), 2.35-2.20 (m, 5H), 2.14 (m, 1H), 1.71-1.58 (m, 4H), 1.19 (s, 3H), 1.17 (s, 3H), 1.00-0.90 (m, 10H).

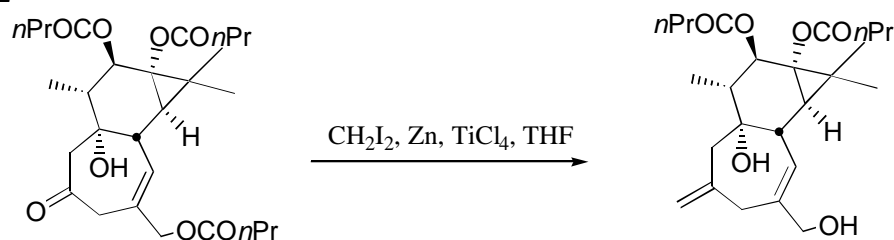
$^{13}\text{C-NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 207.1, 183.8, 176.1, 134.9, 129.5, 78.3, 75.3, 71.4, 67.0, 49.7, 45.4, 42.9, 41.0, 36.4, 36.2, 35.8, 25.3, 23.8, 18.6, 18.0, 16.4, 13.6, 13.5, 12.3.

FTIR ( $\text{CDCl}_3$ ): 3606, 3409, 2928, 1720, 1601, 1459, 1378, 1260, 1189  $\text{cm}^{-1}$ .

MS: 436 (0.1), 418 (0.6), 349 (5), 261 (5), 260 (14), 243 (24), 242 (20), 199 (13), 125 (7), 121 (7), 119 (7), 95 (9), 83 (21), 72 (9), 71 (100), 69 (13).

HRMS: Calculated for  $\text{C}_{24}\text{H}_{36}\text{O}_7$  436.2461  
Found: 436.2466

Compound **12**



$\text{CH}_2\text{I}_2$  (100  $\mu\text{L}$ , 1241  $\mu\text{mol}$ ) was added to a slurry of zinc dust (148.6 mg, 2262  $\mu\text{mol}$ ) in THF (2.5 mL) under an argon atmosphere and stirring at room temperature was continued for 30 min. The reaction mixture was cooled to 0  $^\circ\text{C}$ . A solution of  $\text{TiCl}_4$  (1 M in  $\text{CH}_2\text{Cl}_2$ , 250  $\mu\text{L}$ , 250  $\mu\text{mol}$ ) was added. The cooling bath was removed and stirring was continued for 45 min. A solution of the starting ketone **8** (3.3 mg, 6.5  $\mu\text{mol}$ ) and cycloheptanone (20.9 mg, 188  $\mu\text{mol}$ ) in THF (0.5 mL) was added dropwise via syringe. Stirring was continued for 120 min. The reaction mixture was diluted with diethylether (2.0 mL), washed with 2 N HCl (2 x 2.0 mL), water (2 x 2.0 mL), brine (2 x 2.0 mL) and was dried over  $\text{MgSO}_4$ . The crude reaction mixture was filtered and concentrated under reduced pressure. Purification of the resultant residue by flash chromatography (silica gel, 30% EtOAc in hexanes) afforded **12** (0.9 mg, 31%) as a colorless material.

$^1\text{H-NMR}$  (300MHz,  $\text{CDCl}_3$ ):  $\delta$  = 5.54 (d,  $J$  = 5.74 Hz, 1H), 5.42 (d,  $J$  = 10.22 Hz, 1H), 4.95 (s, 1H), 4.83 (s, 1H), 4.66 (s, 1H), 4.06 (m, 2H), 3.10 (d,  $J$  = 16.48 Hz, 1H), 2.95 (d,  $J$  = 16.30 Hz, 1H), 2.57, (d,  $J$  = 13.19 Hz, 1H), 2.49 (m, 1H), 2.36-2.25 (m, 5H), 1.75-1.58 (m, 5H), 1.22 (s, 1H), 1.16 (s, 3H), 1.16 (s, 3H), 1.00-0.89 (m, 10H).

$^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 185.5, 173.2, 144.0, 139.0, 128.5, 111.2, 78.1, 76.3, 76.0, 67.5, 64.8, 42.8, 41.7, 38.3, 36.4, 36.4, 36.2, 25.1, 23.9, 18.6, 18.0, 16.3, 13.5, 13.4, 12.3.

FTIR ( $\text{CDCl}_3$ ): 3620, 3459, 2975, 2931, 2895, 1723, 1602, 1447, 1390, 1252, 1186  $\text{cm}^{-1}$ .)

MS: 416 (0.6), 345 (8), 259 (10), 258 (20), 257 (16), 243 (9), 241 (10), 240 (23), 227 (11), 225 (11), 163 (11), 149 (14), 125 (13), 105 (23), 83 (22), 71 (100), 69 (20).

HRMS: Calculated for  $\text{C}_{25}\text{H}_{36}\text{O}_5$  [ $\text{M}^+ - \text{H}_2\text{O}$ ] 416.2562  
 Found: 416.2551