BEYOND THE AFFINITY FOR PROTEIN KINASE C: EXPLORING 2-PHENYL-3-HYDROXYPROPYL PIVALATE ANALOGUES AS C1 DOMAIN-TARGETING LIGANDS.

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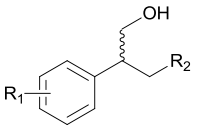
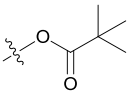
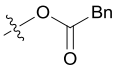
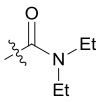
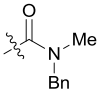
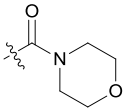
1. Molecular Simulations

The docking studies were performed with the crystal structure of PKCδ C1b domain complexed with phorbol-13-*O*-acetate (Protein Data Bank code: 1PTR).[1] The crystal structure was modified as previously described.[2] Briefly, phorbol-13-*O*-acetate was removed from the crystal structure, and the hydrogens and the side chains of Lys 234, Arg 273, and Glu 274 were added to the protein. The structure was then energy minimized and used for ligand docking.

$SOMA_2$, the Open Source Molecular Modelling Workflow Environment of the Finnish Center for Scientific Computing,[3] was used in the ligand geometry optimization and ligand docking studies. Firstly, the structures of the ligands were cleaned and the hydrogens added to the compounds. CORINA v3.2 [4] was used in the 2D to 3D optimization of the ligands and the basic settings were utilized, except that CORINA was forced to generate stereoisomeric compounds from the input files. Next, the docking simulations were performed with GOLD v5.1 [5] where the binding cavity of the protein was set to the carbonyl oxygen atom of Leu 251 (atom number 168) with an active site radius of 10 Å. With the predefined settings in GOLD, eight docking runs of each ligand were executed. The docking scores can be found in Table 1.

Table 1. Docking scores of PKCδ C1b domain for the enantiomers of the designed compounds.



Compound	R_1	R_2	Docking score
1	**4-BnO**		**57.02 (*S*)** **62.45 (*R*)**
2	**4-BnO**		**66.54 (*S*)** **68.34 (*R*)**
3	**4-BnO**		**67.24 (*S*)** **69.19 (*R*)**
4	**3-BnO**		**69.09 (*S*)** **66.29 (*R*)**
5	**biphen-4-yl**		**57.86 (*S*)** **60.69 (*R*)**
6	**4-BnO**		**56.09 (*S*)** **64.42 (*R*)**
7	**3-BnO**		**59.95 (*S*)** **61.16 (*R*)**
8	**biphen-4-yl**		**48.29 (*S*)** **56.31 (*R*)**
9	**4-BnO**		**64.76 (*S*)** **65.16 (*R*)**
10	**3-BnO**		**68.54 (*S*)** **64.88 (*R*)**
11	**biphen-4-yl**		**54.92 (*S*)** **59.26 (*R*)**
12	**4-BnO**		**56.15 (*S*)** **64.69 (*R*)**
13	**3-BnO**		**61.90 (*S*)** **62.09 (*R*)**
14	**biphen-4-yl**		**47.89 (*S*)** **55.93 (*R*)**

2. Chemistry

2.1 General

Reagents and solvents for synthesis were obtained from Aldrich (Italy). Unless otherwise specified, the commercially available reagents were used as received from the supplier. Solvents were purified according to the guidelines in Purification of Laboratory Chemicals.[6] Microwave dielectric heating

was performed in a Discover® LabMate instrument (CEM Corporation) specifically designed for organic synthesis and following an appropriate microwave program. Melting points were measured on SMP3 Stuart Scientific apparatus and are uncorrected. Analytical thin-layer-chromatography (TLC) was carried out on silica gel precoated glass-backed plates (Fluka Kieselgel 60 F254, Merck) and visualized by ultra-violet (UV) radiation, acidic ammonium molybdate (IV), or potassium permanganate. Flash chromatography (FC) was performed with Silica Gel 60 (particle size 230–400 mesh, purchased from Nova Chimica). IR spectra were recorded on a Jasco FT/IR-4100 spectrophotometer with ATR module; only noteworthy absorptions are given. Proton nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 400 spectrometer operating at 400.13 MHz or JEOL JNM-LA 300 at 300 MHz. Proton chemical shifts (δ) are reported in ppm with the solvent reference relative to tetramethylsilane (TMS) employed as the internal standard ($CDCl_3$, δ = 7.26 ppm; CD_2Cl_2, δ = 5.32 ppm; [D_6]acetone, δ = 2.05 ppm). The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal, dd = doublet-doublet, td = triplet-doublet. The coupling constant values are reported in Hz. ^{13}C NMR spectra were recorded on a 400 MHz spectrometer operating at 100.56 MHz, with complete proton decoupling. Carbon chemical shifts (δ) are reported in ppm relative to TMS with the respective solvent resonance as the internal standard ($CDCl_3$, δ = 77.23 ppm; CD_2Cl_2, δ = 54.00 ppm; [D_6]acetone, δ = 29.84 ppm).

HPLC-UV-ESI/MS analyses were carried out on a Thermo Scientific LCQ FLEET system (LCQ FLEET ion trap mass spectrometer, Surveyor MS Pump/Autosampler/PDA Detector) using an ESI source operating in positive ion mode, controlled by Xcalibur software 1.4 (Thermo Finnigan). Analyses were run on a Synergi Fusion-RP 80A (0.2 cm diameter × 5 cm length, 4 μm) column, at room temperature, with gradient elution (solvent A: acetonitrile containing 0.1% of formic acid; solvent B: water containing 0.1% of formic acid; gradient: 10% A in B to 100% A in 4 min, followed by isocratic elution 100% A for 3 min) at a flow rate of 0.3 mL min^{-1}. All of the final compounds had 95% or greater purity.

Chiral HPLC runs were conducted on a Jasco HPLC system equipped with a Jasco AS-2055 plus autosampler, a PU-2089 plus quaternary gradient pump, and an MD-2010 plus multi-wavelength detector. Experimental data were acquired and processed by Jasco Borwin PDA and Borwin Chromatograph Software. Solvents used were HPLC grade and supplied by Carlo Erba.

Optical rotation values were measured on a Jasco photoelectric polarimeter DIP 1000 using a 0.5 dm cell and a mercury lamp (λ=405 nm); sample concentration values (c) are given in 10^{-2}g mL^{-1}

2.2 Synthesis of 1 and 2

*Synthesis of 4-(benzyloxy)benzyl alcohol (**15**)*

A solution of 4-benzyloxy benzaldehyde (1.5 g, 7.1 mmol) in diethyl ether was treated with lithium aluminum hydride (0.794 g, 21.2 mmol) at 0 ºC and then refluxed for 30 min. The reaction mixture was allowed to reach room temperature, cooled at 0 ºC, carefully quenched with ice-water followed by 15% aqueous sodium hydroxide and finally extracted with diethyl ether. The organic phase was washed with water, dried over magnesium sulfate, and concentrated *in vacuo*. The residue was purified by FC on silica gel eluting with ethyl acetate/*n*-hexane (30/70, v/v), yielding **15** as a white solid, 1.36 g (90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.62 (d, 2H, *J* = 5.88 Hz, C*H*$_2$OH), 5.07 (s, 2H, OC*H*$_2$Ph), 6.95 (d, 2H, *J* = 8.58 Hz, Ar*H*), 7.24-7.45 (m, 7H).

*Synthesis of 4-(benzyloxy)benzyl chloride (**16**)*

A solution of **15** (1.20 g, 5.6 mmol) in dichloromethane (DCM) was treated with thionyl chloride (0.911 mL, 11.2 mmol, 2 equiv.) at 0 ºC and stirred for 1 h at the same temperature. The reaction mixture was then concentrated *in vacuo*. The residue was diluted in DCM and the resulting solution was washed with water, dried over magnesium sulfate and concentrated *in vacuo*, yielding compound **16**, as a white solid, 1.18 g (91%), that was used for the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.60 (s, 2H, C*H*$_2$Cl), 5.07 (s, 2H, OC*H*$_2$Ph), 6.95 (d, 2H, *J* = 8.58 Hz, Ar*H*), 7.24-7.45 (m, 7H).

*Synthesis of 2-[4-(benzyloxy)phenyl]acetonitrile (**17**)*

A mixture of **16** (1.0 g, 4.3 mmol) and sodium cyanide (0.526 g, 10.8 mmol, 2.5 equiv.) in *N*,*N*-dimethylformamide (DMF) was heated at 100 °C for 2 h. The reaction mixture was allowed to reach room temperature, diluted with water and then extracted with ethyl acetate. The combined organic layers were washed with water, dried over magnesium sulfate and concentrated *in vacuo*. The residue was purified by FC on silica gel eluting with ethyl acetate/*n*-hexane (20/80, v/v), furnishing **17** as a white solid, 0.940 g (98%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.66 (s, 2H, C*H*$_2$CN), 5.05 (s, 2H, OC*H*$_2$Ph), 6.95 (d, 2H, *J* = 8.60 Hz, Ar*H*), 7.21 (d, 2H, *J* = 8.60 Hz, Ar*H*), 7.31-7.41 (m, 5H).

*Synthesis of 2-[4-(benzyloxy)phenyl]acetic acid (**18**)*

A solution of **17** (0.92 g, 4.1 mmol) in 30% aq. sodium hydroxide (19 mL) was refluxed overnight. The reaction mixture was allowed to reach room temperature, neutralized with 1N aqueous hydrochloric acid, added with 1N aqueous sodium hydroxide (pH~10) and then extracted with ethyl

acetate. The aqueous layer was made acidic by adding 1N aqueous hydrochloric acid (pH~1) and the resulting mixture was then extracted with ethyl acetate. The organic layer was washed with water, dried over magnesium sulfate and concentrated in *vacuo*, yielding **18** as pale yellow solid, 0.898 g (90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.60 (s, 2H, C*H*$_2$CN), 5.05 (s, 2H, OC*H*$_2$Ph), 6.94 (d, 2H, *J* = 8.79 Hz, Ar*H*), 7.21 (d, 2H, *J* = 8.79 Hz, Ar*H*), 7.31-7.41 (m, 5H).

*Synthesis of methyl 2-[4-(benzyloxy)phenyl]acetate (**19**)*

The carboxylic acid **18** (0.88 g, 3.6 mmol) was dissolved in methanol and the resulting solution was treated with sulfuric acid (a couple of drops) at 0 ºC. The mixture was refluxed for 2 h and then allowed to reach room temperature. The reaction mixture was concentrated in *vacuo*, diluted with water and extracted with ethyl acetate. The organic layer was washed with water, dried over magnesium sulfate and evaporated. The residue was purified by FC on silica gel eluting with ethyl acetate/*n*-hexane (30/70, v/v) to furnish **19** as yellow oil, 0.763 g (82%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.56 (s, 2H, C*H*$_2$CO$_2$CH$_3$), 3.69 (s, 3H, CO$_2$C*H*$_3$), 5.05 (s, 2H, OC*H*$_2$Ph), 6.94 (d, 2H, *J* = 8.61 Hz, Ar*H*), 7.20 (d, 2H, *J* = 8.61 Hz, Ar*H*), 7.31-7.44 (m, 5H).

*Synthesis of dimethyl 2-[4-(benzyloxy)pheny]malonate (**20**)*

A solution of **19** (0.750 g, 2.9 mmol) in 1,4-dioxane was treated with sodium hydride (0.210 g, 8.7 mmol, 3equiv.) at 0 ºC and stirred at room temperature for 30 min. Dimethyl carbonate (29.4 ml) was then added at 0 ºC and the resulting mixture was refluxed for 2 days. The reaction mixture was then diluted with water at 0 ºC and extracted with ethyl acetate. The organic layer was washed with water, dried over magnesium sulfate and concentrated in *vacuo*. The residue was purified by FC on silica gel eluting with ethyl acetate/*n*-hexane (20/80, v/v), yielding **20** as pale yellow solid, 0.559 g (61%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.75 (s, 6H, 2x CO$_2$C*H*$_3$), 4.59 (s, 1H, ArC*H*), 5.05 (s, 2H, OC*H*$_2$Ph), 6.96 (d, 2H, *J* = 8.61 Hz, Ar*H*), 7.29-7.44 (m, 7H).

*Synthesis of 2-(4-(benzyloxy)phenyl)propane-1,3-diol (**21**)*

A solution of lithium aluminium hydride (0.170 g, 5.1 mmol, 3 equiv.) in tetrahydrofuran (THF) was cooled at 0 ºC. A solution of **20** (0.540 g, 1.71 mmol) in THF was then added dropwise to the reaction mixture. The resulting mixture was stirred at room temperature for 2 h, cooled at 0 ºC and then carefully diluted with water. A 15% aqueous solution of sodium hydroxide was then added dropwise and the resulting mixture was stirred for 1 h, filtrated and concentrated in *vacuo*. The residue was purified by FC eluting with ethyl acetate/*n*-hexane (50/50, v/v), furnishing **21** as white solid, 0.310 g (70%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.07 (m, 1H, ArC*H*), 3.80 (m, 4H, 2x C*H*$_2$OH),

5.04 (s, 2H, OC*H*$_2$Ph), 6.88 (d, 2H, *J* = 8.60 Hz, Ar*H*), 7.05 (d, 2H, *J* = 8.60 Hz, Ar*H*), 7.29-7.44 (m, 5H).

*General procedure for synthesis of **1** and **2***

A solution of **21** (0.150 g, 0.6 mmol) in DCM was cooled at 0 °C and pyridine (0.045 mL, 0.6 mmol, 1 equiv.) was added to this solution. The resulting solution was stirred for 10 min. Pivaloyl chloride (0.067 mL, 0.6 mmol, 1 equiv., for **1**) or 2-phenylacetyl chloride (0.75 mL, 0.6 mmol, 1 equiv., for **2**) was then added to the solution at 0 °C and the resulting mixture was stirred for 2 h at room temperature. The reaction mixture was washed with water and brine, dried over magnesium sulfate and concentrated in *vacuo*. The residue was purified by FC eluting with ethyl acetate/*n*-hexane (20/80, v/v) for compound **1** or ethyl acetate/hexane (50/50, v/v) for compound **2.**

*2-[4-(Benzyloxy)phenyl]-3-hydroxypropyl pivalate (**1**)*. White solid, 0.127 g (64%). Mp 49.7-53.0 ºC; IR (ν_{max}/cm^{-1}): 1725 (m s C=O$_{ester}$), 3435 (m b OH $_{alcohol}$). ^{1}H-NMR data comply with those reported in the literature. [7] ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.6, 39.3 (s), 47.1, 64.3 (t), 65.4 (t), 70.4 (t), 115.5, 127.9, 128.4, 129.0, 129.6, 131.6 (s), 137.4 (s), 158.4 (s), 179.3 (s). t_R 3.50 min, λ: 254 nm. MS (ESI) m/z 343.01 $[M+H]^+$, m/z 704.26 $[4M+NH_4^+ + Na^+]^{m/2}$.

*2-[4-(Benzyloxy)phenyl]-3-hydroxypropyl 2-phenylacetate (**2**)*. White solid, 0.111 g (51%)**.** Mp 42.6-44.5 ºC. IR (ν_{max}/cm^{-1}): 1732 (m s C=O$_{ester}$), 3469 (m b OH $_{alcohol}$).^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.72–1.91 (br s, 1H, *OH*, *exchanges with D_2O*), 3.10 (quin, *J* = 6.3 Hz, 1H, –*CH*CH_2OCO), 3.65 (s, 2H, Ph*C*H_2CO), 3.72–3.81 (m, 2H, –CH*C*H_2OH), 4.34 (dd, *J* = 7.0, 11.1 Hz, 1H, –CH*C*H_2OCO), 4.44 (dd, *J* = 5.9, 11.1 Hz, 1H, –CH*C*H_2OCO), 5.07 (s, 2H, –O*C*H_2Ph), 6.93 (d, *J* = 8.7 Hz, 2H, ArH), 7.10 (d, *J* = 8.6 Hz, 2H, ArH), 7.22–7.50 (m, 10H, ArH) ^{13}C NMR ($CDCl_3$, 100 MHz) δ 41.9 (t), 46.9, 64.3 (t), 66.0 (t), 70.4 (t), 115.5, 127.6, 127.9, 128.5, 129.1 (2C), 129.6, 129.7, 131.4 (s), 134.2 (s), 137.4 (s), 158.4 (s), 172.2 (s). t_R : 3.47min, λ: 274 nm. MS (ESI) m/z 377.01 $[M+H]^+$, m/z 772.51 $[4M+NH_4^+ + Na^+]^{m/2}$

2.3 Synthesis of compounds 3-14

*General procedure for synthesis of **28-30**.*

To a solution of the appropriate aryl bromide (**22**, **23** or **24**, 2.5 g, 1 equiv.) in DMF (40 mL), tetra-*n*-butylammonium chloride (TBAC, 2 equiv.), anhydrous sodium acetate (2 equiv.), palladium (II) acetate (0.05 equiv.) and methyl but-2-enoate (1.5 equiv.) were sequentially added. The reaction

mixture was refluxed overnight under stirring, filtered through Celite eluting with DCM, concentrated in *vacuo* and finally extracted with water. The organic layer was dried over sodium sulfate and evaporated in *vacuo*. The residue was dissolved in 1,2-dichloroethane (120 mL, DCE) and to this solution was added selenium dioxide (0.8 equiv.) and *tert*-butyl hydroperoxide (4 equiv.). The resulting mixture was refluxed overnight under stirring, filtered, concentrated under *vacuo* (~ 40 mL) and extracted with water. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified with FC eluting with *n*-hexane/ethylacetate (70/30, v/v).

*4-(4-Benzyloxyphenyl)-5H-furan-2-one (**28**).*

Compound **28** was synthesized according to the general procedure using **22** (2.5 g, 9.5 mmol) to yield 1.02 g (41%), white powder, mp 146.6-149.1 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.15 (s, 2H, -OC*H*$_2$Ph), 5.21 (d, *J* = 1.6 Hz, 2H, O-CH*H*), 6.27 (t, *J* = 1.6 Hz, 1H, -C(O)-C*H*=C), 7.06 (d, *J* = 8.8 Hz, 2H, ArH), 7.35-7.51 (m, 7H, ArH).

*4-(3-Benzyloxyphenyl)-5H-furan-2-one (**29**).*

Compound **29** was synthesized according to the general procedure using **23** (2.5 g, 9.5 mmol) to yield 0.834 g (33%), yellow powder, mp 108.7-110.2 °C.^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.14 (s, 2H, -OC*H*$_2$Ph), 5.21 (d, *J* = 1.6 Hz, 2H, O-CH*H*), 6.37 (t, *J* = 1.6 Hz, 1H, -C(O)-C*H*=C), 7.09-7.16 (m, 3H, ArH), 7.35-7.53 (m, 6H, ArH).

*4-Biphenyl-4-yl-5H-furan-2-one (**30**).*

Compound **30** was synthesized according to the general procedure using **24** (2.5 g, 10.7 mmol) to yield 1.36 g (54%), yellow powder, mp 164.6-165.8 °C (lit.,[8] 176 °C).^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.30 (d, *J* =1.5 Hz, 2H, O-CH*H*), 6.44 (t, *J* =1,5 Hz, 1H, -C(O)-C*H*=C), 7.39-7.47 (m, 1H, ArH), 7.51 (t, *J* = 7.7 Hz, 2H, ArH), 7.58–7.68 (m, 4H, ArH), 7.73 (d, *J* = 8.3 Hz, 2H, ArH).

*General procedure for synthesis of **31-33**.*

To a solution of the appropriate α,β-unsaturated γ-butyrolactone (**28**, **29** or **30**, 0.4 g, 1 equiv.) in absolute ethanol (40 mL), ammonium formate (5 equiv.) and 10% Pd/C (0.07 equiv.) were added. The reaction mixture was irradiated with a microwave power of 100 W, at 100 °C for 180 s, then filtered through Celite eluting with DCM and concentrated in *vacuo*. The residue was dissolved in ethyl acetate and washed with water. The organic phase was then dried over sodium sulfate and evaporated to dryness.

*4-(4-Hydroxyphenyl)dihydrofuran-2-one (**31**).*

Compound **31** was synthesized according to the general procedure using **28** (0.4 g, 1.5 mmol) to yield 0.253 g (95%), yellow powder, mp 119.4-121 °C (lit.,[9] 120-121 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.65 (dd, *J* = 9.2, 17.5 Hz, 1H, -C(O)-C*H*H); 2.92 (dd, *J* = 8.6, 17.5 Hz, 1H, -C(O)-CH*H*), 3.70-3.80 (m, 1H, ArCH), 4.21-4.28 (m, 1H, O-CH*H*), 4.63-4.70 (m, 1H, , O-CH*H*), 4.80-5.96 (brs, 1H, ArH), 6.85 (d, *J* = 8.5 Hz, 2H, ArH), 7.12 (d, *J* = 8.6 Hz , 2H, ArH).

*4-(3-Hydroxyphenyl)dihydrofuran-2-one (**32**).*

Compound **32** was synthesized according to the general procedure using **29** (0.4 g, 1.5 mmol) to yield 0.253 g (95%), yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.69 (dd, *J* = 8.7, 17.6 Hz, 1H, -C(O)-C*H*H), 2.94 (dd, *J* = 8.7, 17.6, 1H, -C(O)-CH*H*), 3.69–3.82 (m, 1H, ArCH), 4.25–4.34 (m, 1H, O-CH*H*), 4.63–4.73 (m, 1H, O-CH*H*), 5.80–6.46 (brs, 1H, ArH), 6.73–6.76 (m, 1H, ArH), 6.76–6.83 (m, 2H, ArH), 7.24 (t, *J* = 7.9 Hz, 1H, ArH).

*4-Biphenyl-4-yldihydrofuran-2-one (**33**).*

Compound **33** was synthesized according to the general procedure using **30** (0.4 g, 1.7 mmol) to yield 0.384 g (95%), white powder, mp 164.6-165.3 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.70 (dd, *J* = 9.1, 17.5 Hz, 1H, -C(O)-C*H*H); 2.94 (dd, *J* = 8.7, 17.5 Hz, 1H, -C(O)-CH*H*), 3.76-3.88 (m, 1H, ArCH), 4.25-4.33 (m, 1H, O-CH*H*), 4.65-4.72 (m, 1H, O-CH*H*), 7.27-7.37 (m, 3H, ArH), 7.43 (t, *J* = 7.3 Hz, 2H, ArH), 7.53-7.61 (m, 4H, ArH).

*General procedure for the synthesis of **34-35***

To a solution of the appropriate precursor (**31** or **32**, 0.240 g, 1 equiv.) in acetonitrile (25 mL), caesium carbonate (1 equiv.) and benzyl bromide (1.2 equiv.) were subsequently added. The reaction mixture was irradiated with a microwave power of 60 W, at 100 °C for 40 min, then filtered and evaporated to dryness. The residue was purified by FC eluting with *n*-hexane to *n*-hexane/ethylacetate (70/30, v/v).

*4-(4-Benzyloxyphenyl)dihydrofuran-2-one (**34**).*

Compound **34** was synthesized according to the general procedure using **31** (0.240 g, 1.34 mmol) to yield 0.344 g (95%), white powder, mp 131.0-132.3 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.65 (dd, *J* = 9.3, 17.5 Hz, 1H, -C(O)-C*H*H), 2.92 (dd, *J* = 8.6, 17.5 Hz, 1H, -C(O)-CH*H*), 3.70-3.81 (m, 1H, O-CH*H*), 4.21-4.28 (m, 1H, O-CH*H*), 4.63-4.69 (m, 1H, ArC*H*), 5.09 (s, 2H, -OCH_2Ph), 6.99 (d, *J* = 8.7 Hz, 2H, ArH), 7.17 (d, *J* = 8.6 Hz, 2H, ArH), 7.34-7.38 (m, 1H, ArH), 7.38-7.47 (m, 4H, ArH).

*4-(3-Benzyloxy-phenyl)-dihydro-furan-2-one (**35**).*

Compound **35** was synthesized according to the general procedure using **32** (0.240 g, 1.34 mmol) to yield 0.344 g (95%), brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.68 (dd, *J* = 9.1, 17.4 Hz, 1H, -C(O)-C*H*H); 2.93 (dd, *J* = 8.7, 17.4 Hz, 1H, -C(O)-C*H*H), 3.73-3.83 (m, 1H, O-CH*H*), 4.24-4.31 (m, 1H, O-CH*H*), 4.64-4.70 (m, 1H, ArC*H*), 5.09 (s, 2H, -OCH_2Ph), 6.83-6.88 (m, 2H, ArH), 6.91-6.95 (m, 1H, ArH), 7.30-7.49 (m, 6H, ArH).

*General procedure for synthesis of retro-ester derivatives **3-5***

To a solution of the appropriate γ-butyrolactone (**33**, **34** or **35,** 0.1 g, 1 equiv.) in methanol (18 mL), a 10% aqueous solution of sodium hydroxide (0.420 mL) was added. The mixture was refluxed for 3 h and then evaporated *in vacuo.* The residue was dissolved in water/DMF (1/10, v/v, 10 mL) and potassium carbonate (1 equiv.) and benzyl bromide (5 equiv.) were then sequentially added to the solution. The resulting mixtures was stirred at room temperature overnight, filtered and evaporated to dryness in *vacuo* at room temperature. The residue was dissolved in diethyl ether and washed with water. The organic layer was dried over sodium sulfate and evaporated to dryness in *vacuo* at room temperature. The residue was purified by FC eluting with DCM/methanol/diethylamine (99/1/0.1, v/v/v). The final products were stored at -20 °C under nitrogen.

*3-(4-Benzyloxyphenyl)-4-hydroxybutyric acid benzyl ester (**3**)*

Compound **3** was synthesized according to the general procedure using **34** (0.1 g, 0.37 mmol) to yield 0.048 g (34%), yellow oil. IR (ν_{max}/cm^{-1}): 1156 (m s C-C(=O)-O), 1729 (m s C=O_{ester}), 2871 and 2920 (w C-H_{arom}), 3421(m b OH). ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ* 1.63–1.93 (br s, *OH*, partially overlapped with H_2O signal, *exchanges with D_2O*), 2.70 (dd, *J* = 8.0, 15.5 Hz, 1H, –CH*C*H_2CO), 2.87 (dd, *J* = 7.0, 15.5 Hz, 1H, –CH*C*H_2CO), 3.35 (quin, *J* = 6.9 Hz, 1H, –*C*HCH$_2$CO), 3.73 (dd, *J* = 6.9, 10.9 Hz, 1H, –CH*C*H_2OH), 3.78 (dd, *J* = 6.6, 10.9 Hz, 1H, –CH*C*H_2OH), 5.06 (s, 2H, –*C*H_2O), 5.08 (s, 2H, –*C*H_2OCO), 6.95 (d, *J* = 8.7 Hz, 2H, ArH), 7.16 (d, *J* = 8.7 Hz, 2H, ArH), 7.23–7.28 (m, 2H, ArH), 7.32–7.48 (m, 8H, ArH). t_R: 3.51 min, λ: 273 nm. MS (ESI): *m/z* 376.93 [M+H]$^+$, *m/z* 772.40 [4M+NH_4^++Na^+]$^{m/2}$.

*3-(3-Benzyloxyphenyl)-4-hydroxybutyric acid benzyl ester (**4**)*

Compound **4** was synthesized according to the general procedure using **35** (0.1 g, 0.37 mmol) to yield 0.041 g (29%), white oil. IR (ν_{max}/cm^{-1}): 694, 736, 1024, 1151 (m s C-C(=O)-O), 1729 (m s C=O_{ester}), 2870 and 2924 (w C-H_{arom}), 3426 (m b OH). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.73 (dd, *J* = 7.6, 15.7 Hz, 1H, –CH*C*H_2CO), 2.87 (dd, *J* = 7.6, 15.7 Hz, 1H, –CH*C*H_2CO), 3.37 (quin, *J* = 6.9

Hz, 1H, –*CH*CH_2CO), 3.76 (dd, *J* = 6.5, 10.9 Hz, 1H, –$CHCH_2OH$), 3.81 (dd, *J* = 6.5, 10.9 Hz, 1H, –$CHCH_2OH$), 5.04 (s, 2H, –CH_2O), 5.09 (s, 2H, –CH_2OCO), 6.83–6.92 (m, 3H, ArH), 7.23–7.30 (m, 3H, ArH), 7.31–7.48 (m, 8H, ArH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.6 (t), 44.9, 66.9 (t), 67.3 (t), 70.4 (t), 113.7, 115.0, 120.7, 128.0, 128.4, 128.6 (2C), 128.9, 129.0, 130.2, 136.2 (s), 137.3 (s), 142.9 (s), 159.5 (s), 172.7 (s). t_R: 3.47 min, λ: 273 nm. MS (ESI): *m/z* 377.02 $[M+H]^+$, *m/z* 772.78 $[4M+NH_4^+ + Na^+]^{m/2}$.

*3-Biphenyl-4-yl-4-hydroxy-butyric acid benzyl ester (**5**)*

Compound **5** was synthesized according to the general procedure using **33** (0.1 g, 0.41 mmol) to yield 0.123 g (84%), yellow oil. IR (ν_{max}/cm^{-1}): 1149 (m s C-C(=O)-O), 1727 (m s $C=O_{ester}$), 2942 (w $C-H_{arom}$), 3402 (m b OH). ^1H NMR (DMSO-d_6, 400 MHz) δ 2.68 (dd, *J* = 9.8, 15.6 Hz, 1H, –$CHCH_2CO$), 2.95 (dd, *J* = 5.3, 15.6 Hz, 1H, –$CHCH_2CO$), 3.16–3.26 (m, 1H, –*CH*CH_2CO), 3.47–3.55 (m, 1H, –$CHCH_2OH$, dd after exchange with D_2O, *J* = 7.6, 10.5 Hz), 3.55–3.62 (m, 1H, –$CHCH_2OH$, dd after exchange with D_2O, *J* = 6.0, 10.5 Hz), 4.85 (t, *J* = 5.3 Hz, 1H, *OH*, exchanges with D_2O), 5.00 (s, 2H, –CH_2OCO), 7.14–7.21 (m, 2H, ArH), 7.25–7.39 (m, 6H, ArH), 7.47 (t, *J* = 7.5 Hz, 2H, ArH), 7.57 (d, *J* = 8.3 Hz, 2H, ArH), 7.62–7.68 (m, 2H, ArH). t_R: 3.46 min, λ: 254 nm. MS (ESI): m/z 346.98 $[M+H]^+$, m/z 712.75 $[4M+NH_4^+ + Na^+]^{m/2}$.

*General procedure for synthesis of retro-amide derivatives **6-14***

To a suspension of aluminium chloride (1.3 equiv.) in DCE (1 mL) a solution of the appropriate amine (5 equiv.) in DCE (1 mL) and a solution of the appropriate γ-butyrolactone (**33**, **34** or **35,** 0.05 g, 1 equiv.) in DCE (1 mL) were sequentially added. The reaction mixture was irradiated with a microwave power of 60 W, at 100 °C for 25 min, and evaporated to dryness. The residue was dissolved in ethyl acetate and washed with 0.1 M hydrochloric acid. The organic layer was dried over sodium sulfate and evaporated *in vacuo*. The residue was purified by FC eluting with ethyl acetate/methanol (95/5, v/v).

*3-(4-Benzyloxy-phenyl)-4-hydroxy-but-2-enoic acid diethylamide (**6**).*

Compound **6** was synthesized according to the general procedure using **34** (0.07 g, 0.29 mmol) to yield 0.056 g (61%), white powder, mp: 83.6-85.5 °C. IR (ν_{max}/cm^{-1}): 1240 (w C-N-H), 1608 (n $C=O_{amide}$), 2929 (m NH), 3443 (m b OH). ^1H NMR (DMSO-d_6, 400 MHz), a mixture of rotamers, δ 0.91 (t, *J* = 7.0 Hz, 3H, –NCH_2CH_3), 1.02 (t, *J* = 7.0 Hz, 3H, –NCH_2CH_3), 2.48 (dd, *J* = 8.4, 15.4 Hz, 1H, –$CHCH_2CO$), 2.68 (dd, *J* = 5.7, 15.4 Hz, 1H, –$CHCH_2CO$), 3.10–3.27 (m, 5H, –$N(CH_2CH_3)_2$ + –*CH*CH_2CO), 3.48 (t, *J* = 6.1 Hz, 2H, –$CHCH_2OH$), 4.65 (t, *J* = 5.3 Hz, 1H, OH,

exchanges with D_2O), 5.06 (s, 2H, –O*CH*$_2$Ph), 6.89 (d, *J* = 8.6 Hz, 2H, ArH), 7.13 (d, *J* = 8.6 Hz, 2H, ArH), 7.29–7.47 (m, 5H, ArH). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz), a mixture of rotamers, δ 13.9, 15.2, 36.1 (t), 39.8 (t), 42.1 (t), 44.6, 66.1 (t), 69.9 (t), 115.1, 128.4, 128.6, 129.3, 129.7, 136.3 (s), 138.2 (s), 157.5 (s), 170.9 (s). t_R: 3.08 min, λ: 273 nm. MS (ESI): *m/z* 342.28 $[M+H]^+$, *m/z* 683.08 $[2M+H]^+$.

*3-(3-Benzyloxyphenyl)-4-hydroxybut-2-enoic acid diethylamide (**7**).*

Compound **7** was synthesized according to the general procedure using **35** (0.05 g, 0.19 mmol) to yield 0.039 g (60%), yellow oil. IR (ν_{max}/cm^{-1}): 1258 (w C-N-H), , 1607 (n C=O$_{amide}$), 2870, 2931 and 2972 (m N-H) , 3366 (m b O-H) ; ^{1}H NMR (DMSO-*d*$_6$, 400 MHz). a mixture of rotamers, δ 0.92 (t, *J* = 7.0 Hz, 3H, –NCH$_2$*CH*$_3$), 1.02 (t, *J* = 7.0 Hz, 3H, –NCH$_2$*CH*$_3$), 2.52 (dd, *J* = 8.2, 15.6 Hz, 1H, –CH*CH*$_2$CO), 2.70 (dd, *J* = 5.8, 15.6 Hz, 1H, –CH*CH*$_2$CO), 3.13–3.29 (m, 5H, –N(*CH*$_2$CH$_3$)$_2$ + –*CH*CH$_2$CO), 3.52 (t, *J* = 6.0 Hz, 2H, –CH*CH*$_2$OH), 4.70 (t, *J* = 5.3 Hz, 1H, OH, exchanges with D_2O), 5.06 (s, 2H, –O*CH*$_2$Ph), 6.78–6.85 (m, 2H, ArH), 6.86–6.90 (m, 1H, ArH), 7.17 (t, *J* = 7.9 Hz, 1H, ArH), 7.30–7.36 (m, 1H, ArH), 7.40 (t, *J* = 7.2 Hz, 2H, ArH), 7.45 (d, *J* = 7.2 Hz, 2H, ArH). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz), a mixture of rotamers, δ 13.9, 15.2, 35.8 (t), 40.2 (t), 42.1 (t), 45.4, 65.9 (t), 69.9 (t), 112.9, 115.5, 121.4, 128.5, 128.6, 129.3, 129.8, 138.1 (s), 145.9 (s), 159.0 (s), 170.8 (s). t_R: 3.08 min, λ: 273 nm. MS (ESI): *m/z* 342.31 $[M+H]^+$, *m/z* 682.51 $[2M+ H]^+$.

*3-Biphenyl-4-yl-N,N-diethyl-4-hydroxybutyramide (**8**).*

Compound **8** was synthesized according to the general procedure using **33** (0.1 g, 0.37 mmol) to yield 0.034 g (27%), white powder, mp 100.6-101.7 °C. IR (ν_{max}/cm^{-1}): 1490 (m C-N), 1617 (n C=O), 2870 and 2918 (m NH), 3349 (m b OH). ^{1}H-NMR (DMSO-*d*$_6$, 400 MHz), a mixture of rotamers, *δ* 0.92 (t, *J* = 7.0 Hz, 3H, –NCH$_2$*CH*$_3$), 1.05 (t, *J* = 7.0 Hz, 3H, –NCH$_2$*CH*$_3$), 2.58 (dd, *J* = 8.3, 15.6 Hz, 1H, –CH*CH*$_2$CO), 2.76 (dd, *J* = 5.8, 15.6 Hz, 1H, –CH*CH*$_2$CO), 3.11–3.32 (m, 5H, –N(*CH*$_2$CH$_3$)$_2$ + –*CH*CH$_2$CO), 3.56 (t, *J* = 6.1 Hz, 2H, –CH*CH*$_2$OH), 4.76 (t, *J* = 5.3 Hz, 1H, OH, exchanges with D_2O), 7.29–7.38 (m, 3H, ArH), 7.45 (t, *J* = 7.6 Hz, 2H, ArH), 7.55 (d, *J* = 8.1 Hz, 2H, ArH), 7.63 (t, *J* = 7.4 Hz, 2H, ArH). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz), a mixture of rotamers, *δ* 13.8, 15.1, 35.8 (t), 40.2 (t), 42.2 (t), 45.0 (2C), 65.8 (t), 65.9 (t), 127.1, 127.3, 128.0, 129.4, 129.8, 138.8 (s), 141.0 (s), 143.4 (s), 170.9 (s). t_R: 3.11 min, λ: 254 nm, MS (ESI): *m/z* 312.28 $[M+H]^+$, *m/z* 622.89 $[2M+H]^+$.

*3-(4-Benzyloxyphenyl)-4-hydroxybut-2-enoic acid N-benzyl-N-methylamide (**9**).*

Compound **9** was synthesized according to the general procedure using **34** (0.05 g, 0.19 mmol) to yield 0.039 g (54%), yellow oil. IR (ν_{max}/cm^{-1}): 1238 (w C-N-H), 1609 (n C=O$_{amide}$), 2922 and 3030 (m N-H), 3405 (m b O-H). ^{1}H NMR (DMSO-d_6, 400 MHz), a mixture of rotamers, δ 2.58–2.71 (m, 1H, –CH*CH$_2$*CO), 2.72 (s, 1H, –N*CH$_3$*), 2.78 (dd, *J* = 5.5, 15.6 Hz, 1H, –CH*CH$_2$*CO), 2.85 (s, 2H, –N*CH$_3$*), 3.14–3.24 (m, 1H, –*CH*CH$_2$CO), 3.37–3.57 (m, 2H, –CH*CH$_2$*OH), 4.34 (d, *J* = 15.0 Hz, 0.65H, –N*CH$_2$*Ph), 4.51 (d, *J* = 15.0 Hz, 0.65H, –N*CH$_2$*Ph), 4.53–4.57 (m, 0.7H, –N*CH$_2$*Ph), 4.65 (t, *J* = 5.3 Hz, 0.35 H, OH, exchanges with D$_2$O), 4.70 (t, *J* = 5.3 Hz, 0.65 H, OH, exchanges with D$_2$O), 5.02–5.12 (m, 2H, –O*CH$_2$*Ph), 6.86–6.94 (m, 2H, ArH), 6.94–7.00 (m, 1.4H, ArH), 7.05–7.12 (m, 1.4H, ArH), 7.14–7.30 (m, 3.7H, ArH), 7.33 (t, *J* = 7.1 Hz, 1.6 H, ArH), 7.40 (t, J= 7.1 Hz, 2H, ArH), 7.45 (d, J= 7.8 Hz, 1.9H, ArH). ^{13}C NMR (DMSO-d_6, 100 MHz). a mixture of rotamers, δ 34.3, 35.8, 35.9 (t), 36.1 (t), 44.5, 44.8, 50.7 (t), 53.2 (t), 66.2 (t), 66.4 (t), 70.0 (t), 115.2, 127.3, 127.7, 128.0, 128.1, 128.5, 128.6, 129.1, 129.3, 129.5, 129.7, 129.8, 136.0 (s), 136.1 (s), 138.2 (s), 138.4 (s), 138.6 (s), 157.6 (s), 157.7 (s), 172.2 (s), 172.3 (s). t_R: 3.26 min, λ: 273 nm, MS (ESI): *m/z* 390.30 [M+H]$^+$, *m/z* 779.01 [2M+H]$^+$.

*3-(3-Benzyloxyphenyl)-4-hydroxybut-2-enoic acid N-benzyl-N-methylamide (**10**).*

Compound **10** was synthesized according to the general procedure using **35** (0.05 g, 0.19 mmol) to yield 0.046 g (63%), yellow oil. IR (ν_{max}/cm^{-1}): 1255 (w C-N-H), 1607 (n C=O$_{amide}$), 2867 and 2921 (m N-H), 3374 (m b O-H). ^{1}H NMR (DMSO-d_6, 120 °C, 400 MHz), a mixture of rotamers, δ 2.67 (dd, *J* = 7.8, 15.5 Hz, 1H, –CH*CH$_2$*CO), 2.79–2.86 (m, 4H, –CH*CH$_2$*CO + –N*CH$_3$*), 3.29 (quint, *J* = 6.3 Hz, 1H, –*CH*CH$_2$CO), 3.57–3.67 (m, 2H, –CH*CH$_2$*OH), 4.15–4.25 (br s, 1H, OH, exchanges with D$_2$O), 4.47 (d, *J* = 15.5 Hz, 1H, –N*CH$_2$*Ph), 4.52 (d, *J* = 15.5 Hz, 1H, –N*CH$_2$*Ph), 5.09 (s, 2H, –O*CH$_2$*Ph), 6.81–6.88 (m, 2H, ArH), 6.90–6.95 (m, 1H, ArH), 7.11 (d, *J* = 7.2 Hz, 2H, ArH), 7.18 (t, *J* = 7.9 Hz, 1H, ArH), 7.20–7.34 (m, 4H, ArH), 7.35–7.41 (m, 2H, ArH), 7.42–7.47 (m, 2H, ArH).^{13}C NMR (DMSO-d_6, 100 MHz), a mixture of rotamers, δ 34.3, 35.6 (t), 35.7, 35.8 (t), 45.3, 45.6, 50.7 (t), 53.2 (t), 66.0 (t), 66.2 (t), 70.0 (t, 2C), 112.9, 113.0, 115.4, 115.5, 121.5, 121.6, 127.3, 127.7, 128.0, 128.1, 128.6 (2C), 128.7, 129.2, 129.3, 129.5, 129.8, 129.9, 136.0 (s), 138.1 (s), 138.4 (s), 138.6 (s), 145.6 (s), 145.7 (s), 159.1 (s, 2C), 172.1 (s), 172.2 (s). t_R: 3.13 min, λ: 273 nm, MS (ESI): *m/z* 390.30 [M+H]$^+$, *m/z* 779.01 [2M+H]$^+$.

*N-Benzyl-3-biphenyl-4-yl-4-hydroxy-N-methylbutyramide (**11**).*

Compound **11** was synthesized according to the general procedure using **33** (0.05 g, 0.21 mmol) to yield 0.032 g (42 %), yellow powder, mp 81.6-83.7 °C. IR (ν_{max}/cm^{-1}): 1263 (w C-N-H), 1415 (m C-N), 1617(n C=O$_{amide}$), 2854 and 2921 (m NH), 3326 (m b OH). ^{1}H NMR (DMSO-d_6, 120°C, 400

MHz), a mixture of rotamers, δ 2.73 (dd, *J* = 7.9, 15.5 Hz, 1H, –CH*CH*$_2$CO), 2.86–2.94 (m, 4H, –CH*CH*$_2$CO + –N*CH*$_3$), 3.36 (quint, *J* = 6.5 Hz, 1H, –*CH*CH$_2$CO), 3.67 (br s, 2H, –CH*CH*$_2$OH), 4.26 (br s, 1H, OH, exchanges with D_2O), 4.49 (d, *J* = 15.5 Hz, 1H, –*CH*$_2$Ph), 4.54 (d, *J* = 15.5 Hz, 1H, –*CH*$_2$Ph), 7.12 (d, *J* = 7.1 Hz, 2H, ArH), 7.19–7.38 (m, 6H, ArH), 7.45 (t, *J* = 7.5 Hz, 2H, ArH), 7.54 (d, *J* = 8.1 Hz, 2H, ArH), 7.63 (d, *J* = 8.1 Hz, 2H, ArH). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz), a mixture of rotamers, *δ* 34.3, 35.6 (t), 35.8, 35.9 (t), 45.0, 45.3, 50.7 (t), 53.2 (t), 66.1 (t), 66.2 (t), 127.2, 127.3, 127.4, 127.7, 128.0, 128.1, 129.2, 129.4, 129.5, 129.8, 138.4 (s), 138.6 (s), 138.9 (s, 2C), 141.1 (s), 143.3 (s), 143.4 (s), 172.1 (s, 2C). *t*$_R$*:* 3.23 min, λ: 254 nm, MS (ESI): *m/z* 360.30 $[M+H]^+$, *m/z* 718.85 $[2M+H]^+$.

*3-(4-Benzyloxyphenyl)-4-hydroxy-1-morpholin-4-ylbut-2-en-1-one (**12**).*

Compound **12** was synthesized according to the general procedure using **34** (0.05 g, 0.19 mmol) to yield 0.038 g (57.1%), white powder, mp 134.5-135.8 °C. IR (ν_{max}/cm^{-1}) 1240 (w C-N-H), 1604 (n C=O$_{amide}$), 2862, 2925 and 3259 (m N-H), 3462 (m b O-H); ^{1}H NMR (DMSO-*d*$_6$, 400 MHz), a mixture of rotamers, *δ* 2.55 (dd, *J* = 8.7, 15.3 Hz, 1H, –CH*CH*$_2$CO), 2.72 (dd, *J* = 5.5, 15.3 Hz, 1H, –CH*CH*$_2$CO), 3.10 (quint, *J* = 6.7 Hz, 1H, –*CH*CH$_2$CO), 3.24–3.43 (m, 6H, MorH), 3.43–3.53 (m, 4H, –CH*CH*$_2$OH + MorH), 4.68 (t, *J* = 5.3 Hz, 1H, OH, exchanges with D_2O), 5.07 (s, 2H, –O*CH*$_2$Ph), 6.90 (d, *J* = 8.6 Hz, 2H, ArH), 7.14 (d, *J* = 8.6 Hz, 2H, ArH), 7.22–7.48 (m, 5H, ArH). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz), a mixture of rotamers, *δ* 35.8 (t), 42.3 (t), 44.7, 46.5 (t), 66.2 (t), 66.9 (t), 67.0 (t), 70.0 (t), 115.2, 128.4, 128.6, 129.3, 129.8, 136.0 (s), 138.2 (s), 157.6 (s), 170.8 (s). *t*$_R$*:* 2.75 min, λ: 273 nm, MS (ESI): *m/z* 356.27 $[M+H]^+$, *m/z* 710.89 $[2M+H]^+$.

*3-(3-Benzyloxyphenyl)-4-hydroxy-1-morpholin-4-ylbut-2-en-1-one (**13**).*

Compound **13** was synthesized according to the general procedure using **35** (0.1 g, 0.38 mmol) to yield 0.058 g (44%), white powder, mp 119.8-120.8 °C. IR (ν_{max}/cm^{-1}): 1247 (w C-N-H) , 1601(n C=O$_{amide}$) , 2855 and 2926 (m N-H), 3257(m b O-H). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz), a mixture of rotamers, *δ* 2.60 (dd, *J* = 8.5, 15.4 Hz, 1H, –CH*CH*$_2$CO), 2.73 (dd, *J* = 5.7, 15.4 Hz, 1H, –CH*CH*$_2$CO), 3.09–3.19 (m, 1H, –*CH*CH$_2$CO), 3.25–3.45 (m, 6H, MorH), 3.45–3.57 (m, 4H,–CH*CH*$_2$OH + MorH), 4.72 (t, *J* = 5.3 Hz, 1H, OH, exchanges with D_2O), 5.07 (s, 2H, –O*CH*$_2$Ph), 6.79–6.86 (m, 2H, ArH), 6.87–6.91 (m, 1H, ArH), 7.18 (t, *J* = 7.9 Hz, 1H, ArH), 7.30–7.36 (m, 1H, ArH), 7.37–7.43 (m, 2H, ArH), 7.43–7.49 (m, 2H, ArH). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz), a mixture of rotamers, *δ* 35.2 (t), 42.3 (t), 45.6, 46.5 (t), 66.0 (t), 66.9 (t), 67.0 (t), 70.0 (t), 113.0, 115.5, 121.5, 128.6, 128.7, 129.3, 129.9, 138.1 (s), 145.6 (s), 159.1 (s), 170.7 (s). *t*$_R$: 2.74 min, λ: 273 nm, MS (ESI): *m/z* 356.28 $[M+H]^+$, *m/z* 710.86 $[2M+H]^+$.

*3-Biphenyl-4-yl-4-hydroxy-1-morpholin-4-ylbutan-1-one (**14**).*

Compound **14** was synthesized according to the general procedure using **33** (0.05 g, 0.21 mmol) to yield 0.035 g (51%), white powder, mp 135-136.4 °C. IR (ν_{max}/cm^{-1}): 1262 (w C-N-H), 1609 (n C=O$_{amide}$), 2856 and 2964 (m NH), 3361(m b OH). ^{1}H NMR (DMSO-d_6, 400 MHz), a mixture of rotamers, δ 2.66 (dd, J = 8.6, 15.5 Hz, 1H, –CH*CH$_2$*CO), 2.79 (dd, J = 5.5, 15.5 Hz, 1H, –CH*CH$_2$*CO), 3.17–3.27 (m, 1H, –*CH*CH$_2$CO), 3.29–3.54 (m, 8H, MorH), 3.57 (app t, J = 5.9 Hz, 2H, –CH*CH$_2$*OH), 4.76 (t, J = 5.3 Hz, 1H, OH, exchanges with D_2O), 7.30–7.38 (m, 3H, ArH), 7.46 (t, J = 7.6 Hz, 2H, ArH), 7.56 (d, J = 8.1 Hz, 2H, ArH), 7.63 (t, J = 7.7 Hz, 2H, ArH). ^{13}C NMR (DMSO-d_6, 100 MHz), a mixture of rotamers, δ 35.6 (t), 42.4 (t), 45.1, 46.4 (t), 66.0 (t), 66.9 (t), 67.0 (t), 127.2, 127.4, 128.0, 129.5, 129.8, 139.0 (s), 141.1 (s), 143.2 (s), 170.7 (s). t_R: 2.73 min, λ: 254 nm, MS (ESI): *m/z* 326.28 $[M+H]^+$, *m/z* 650.91 $[2M+H]^+$.

2.4 Chiral chromatography

In order to identify the optimal experimental conditions for the enantioresolution of **1** and **2**, a standard screening protocol [10] was applied to both Chiralcel OJ-H (0.46 cm diameter × 15 cm length, 5 μm) and Chiralpak IC (0.46 cm diameter × 25 cm length, 5 μm) columns produced by Daicel Industries Ltd. (Tokyo, Japan). Elution conditions experimented include mixtures of *n*-hexane and polar modifiers (ethanol or 2-propanol) as well as alcohols (methanol, ethanol and 2-propanol). Results of the screening protocol are reported in Table 1 as capacity factor (*k*), selectivity (α) and resolution (*Rs*) factors. The retention factor (*k*) was calculated using the equation

$$k = (t_R - t_0)/ t_0$$

where t_R is the retention time and t_0 the dead time (t_0 was considered to be equal to the peak of the solvent front and was taken from each particular run). The enantioselectivity (α) and the resolution factor (R_s) were calculated as follows:

$$\alpha = k_2 / k_1 \text{ and } R_s = 2\,(t_{R2} - t_{R1}) / (w_1 + w_2)$$

where t_{R2} and t_{R1} are the retention times of the second and the first eluted enantiomers, and w_1 and w_2 are the corresponding base peak widths.

The enantiomers of **1** and **2** were then completely resolved by a (semi)-preparative process using a Chiralpak^{TM} IC (1cm diameter × 25 cm length, 5 μm), eluting with *n*-hexane/2-propanol (90/10, v/v) at room temperature with a flow rate of 3 mL min^{-1} and 2 mL min^{-1} for **1** and **2**, respectively. The eluate was properly partitioned according with UV profile (detection preformed at 274 nm for compound **1** and 254 nm for compound **2**). Analytical control of collected fractions was performed on a Daicel Chiralpak IC (0.46 cm diameter × 25 cm length, 5 μm) column eluting with *n*-hexane/2-

propanol (90/10, v/v), at room temperature at a flow rate of 1 mL min^{-1} and UV detection at 274 nm for compound **1** and 254 nm for compound **2**. The fractions obtained containing the enantiomers were evaporated at reduced pressure.

*(+)-2-[4-(Benzyloxy)pheny]-3-hydroxypropyl pivalate (**1A**).*

White solid, mp 49.7-53.0 ºC; *e.e.* 99.9% determined by analytical chiral HPLC: t_R: 19.9 min; $[\alpha]_{405}^{20}$: +37.7 (c 0.6 in methanol). Spectroscopic properties comply with those reported for compound **1**.

*(-)-2-[4-(Benzyloxy)phenyl]-3-hydroxypropyl pivalate (**1B**)*

White solid, mp 49.7-53.0 ºC; *e.e.* 99.9% determined by analytical chiral HPLC: t_R: 22.5 min; $[\alpha]_{405}^{20}$: -37.2 (c 0.6 in methanol). Spectroscopic properties comply with those reported for compound **1**.

*(+)-2-[4-(Benzyloxy)phenyl]-3-hydroxypropyl 2-phenylacetate (**2A**)*

White solid, mp 42.6-44.5 ºC; *e.e.* 99.9% determined by analytical chiral HPLC: t_R: 36.7 min; $[\alpha]_{405}^{20}$: +37.5 (c 0.4 in methanol). Spectroscopic properties comply with those reported for compound **2**.

*(-)-2-[4-(Benzyloxy)phenyl]-3-hydroxypropyl 2-phenylacetate (**2B**)*

White solid**,** mp 42.6-44.5 ºC; *e.e.* 99.9% determined by analytical chiral HPLC: t_R: 47.1 min min; $[\alpha]_{405}^{20}$: -37.4 (c 0.4 in methanol). Spectroscopic properties comply with those reported for compound **2**.

Table 2 Screening results for the enantiomer separation of 1 and 2

1

	Chiracel® OJ-H[b]				Chiralpak® IC[b]			
Eluent[a]	k_1	k_2	α	R_s	k_1	k_2	α	R_s
A	1.37		-	-	n.t.	n.t.	n.t.	n.t.
B	0.90		-	-	n.t.	n.t.	n.t.	n.t.
C	0.80		-	-	0.09		-	-
D[c]	0.80		-	-	0.46		-	-
E	7.14	7.45	-	-	2.16		-	-
F	n.t.	n.t.	n.t.	n.t.	8.15	8.33	-	-
G	n.t	n.t	n.t	n.t	n.t.	n.t.	n.t.	n.t.
H	n.t	n.t	n.t	n.t	n.t.	n.t.	n.t.	n.t
I	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t
L	3.19	3.43	-	-	n.t	n.t	n.t	n.t
M	7.65	8.27	1.08	0.75	**5.18**	**6.00**	**1.16**	**2.00**
N	19.06	20.75	1.09	1.06	13.25	15.37	1.16	2.50
O	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t

2

	Chiralcel® OJ-H[b]				Chiralpak® IC[b]			
Eluent[a]	k_1	k_2	α	R_s	k_1	k_2	α	R_s
A	19.67		-	-	n.t.	n.t.	n.t.	n.t.
B	12.05		-	-	n.t.	n.t.	n.t.	n.t.
C	8.12	9.01	1.11	1.00	0.17		-	-
D[c]	7.85	8.94	1.14	0.93	0.76	0.91	-	-
E	n.t.	n.t	n.t	n.t	3.95	4.60	-	-
F	n.t.	n.t.	n.t.	n.t.	9.57	11.36	1.19	3.09
G	n.t	n.t	n.t	n.t	5.13	5.96	1.16	1.50
H	8.34	9.27	1.11	0.92	n.t	n.t	n.t	n.t
I	8.37.	9.33	1.12	0.92	n.t.	n.t.	n.t.	n.t.
L	-	-	-	-	n.t	n.t	n.t	n.t
M	n.t.	n.t.	n.t.	n.t.	**10.36**	**13.59**	**1.31**	**4.16**
N	n.t	n.t	n.t	n.t	n.t	n.t	n.t	n.t
O	6.63	7.29	-	-	n.t	n.t	n.t	n.t

Bold type indicates the best result obtained

[a] Eluent composition: A: methanol (100); B: methanol/ethanol (50/50,v/v); C: ethanol (100); D: 2-propanol (100); E: *n*-hexane/ethanol (90/10, v/v); F: *n*-hexane/ethanol (95/5, v/v); G: *n*-hexane/ethanol (92/8, v/v); H: *n*-hexane/ethanol (10/90, v/v); I: *n*-hexane ethanol (5/95, v/v); L: *n*-hexane/ 2-propanol (80/20, v/v); M: *n*-hexane/ 2-propanol (90/10, v/v); N: *n*-hexane/ 2-propanol (95/5, v/v); O: *n*-hexane/2-propanol (10/90, v/v). Sample dissolved in 2-propanol; conc. 1mg mL^{-1}, injection volume 10 µL. Detection: λ= 274 nm (compound **1**) - 254 nm (compound **2**).

[b] Flow rate: 1 mL min^{-1}.

[c] Flow rate: 0.5 mL min^{-1}. n.t: not tested

3. Biological Assays

3.1 Materials

[20-^{3}H]Phorbol-12,13-dibutyrate ([^{3}H]PDBu) was custom-labeled by Amersham Radiolabeling Service (GE Healthcare, Little Chalfont, UK). Bovine immunoglobulin G (IgG) and phosphatidyl-L-serine (PS) were acquired from Sigma-Aldrich (Steinheim, Germany) and protease inhibitors (Complete Protease Inhibitor Cocktail Tablets) from Roche (Mannheim, Germany).

*3.2 Production of recombinant human PKCα and PKCδ in insect cells***.** The cloning of the expression vectors for full-length human recombinant PKCα and PKCδ has been described previously[11,12]. The proteins were produced in baculovirus-infected Sf9 insect cells as described. Briefly, Sf9 cells were infected with an optimized amount of recombinant baculovirus and harvested 2 days postinfection. Cells were then washed with PBS and cell pellets were frozen. Crude cell lysates were prepared by suspending the cells in buffer containing 25 mM Tris-HCl (pH 7.5), 0.5 mM EGTA, 0.1% Triton-X 100, and protease inhibitors according to the manufacturer's instructions. Suspensions were incubated on ice for 30 min and subsequently centrifuged at 16000*g* for 15 min at 4 °C, whereafter the supernatant was collected. The protein concentration was determined and the supernatant was used for [^{3}H]PDBu binding experiments.

3.3 [^{3}H]PDBu binding assay for assessment of binding affinity

The affinity of the synthesized compounds to the C1 domains of PKCα and PKCδ was determined as their ability to compete with radioactively labeled phorbol ester [^{3}H]PDBu for binding to PKC using a filtration-based method as described previously.[2] Briefly, 20 μg of protein/well from the supernatant of Sf9 cell was incubated with the test compound and [^{3}H]PDBu for 10 min at room temperature (RT) in a 96-well filter plate (Millipore, cat. no. MSHVN4B50, Bedford, MA) in a total volume of 125 μL. The reaction buffer consisted of 20 mM Tris-HCl (pH 7.5), 40 μM $CaCl_2$, 10 mM $MgCl_2$, 400 μg mL^{-1} bovine IgG, 50 nM [^{3}H]PDBu, and 0.1 mg/ml phosphatidyl-L-serine. To precipitate the proteins, 125 μL of ice-cold 20% poly(ethylene glycol) 6000 was added, and the plates were shaken rigorously for 15 min at RT, whereafter the filters were washed six times with washing buffer (20 mM Tris-HCl (pH 7.5), 100 μM $CaCl_2$, 5 mM $MgCl_2$). The plates were then dried, liquid scintillant (Optiphase SuperMix; PerkinElmer, Groningen, The Netherlands) was added, plates were equilibrated for at least two hours, and radioactivity was measured with Wallac Microbeta Trilux microplate liquid scintillation counter (PerkinElmer, Waltham, MA). All the test

compounds were diluted into DMSO so that the final DMSO concentration in the assay was 4%, and the results were calculated as percentage of control (DMSO) on the same plate.

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