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Supporting Information

Design and Synthesis of Novel 1,3,5-Triphenyl Pyrazolines as Potential Anti-inflammatory Agents Through Allosteric Inhibition of Protein Kinase Czeta (PKCζ)

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EXPERIMENTAL SECTION

I. Chemistry. Solvents and reagents were obtained from commercial suppliers and used as received. A Bruker DRX 500 spectrometer was used to obtain ^{1}H NMR and ^{13}C NMR spectra. The chemical shifts are referenced to the residual protonated solvent signals. At least 95% purity in all the tested compounds was obtained by means of HPLC coupled with mass spectrometry. Mass spectra (HPLC-ESI-MS) were obtained using a TSQ quantum (Thermo Electron Corporation) instrument prepared with a triple quadrupole mass detector (Thermo Finnigan) and an ESI source. All samples were inserted using an autosampler (Surveyor, Thermo Finnigan) by an injection volume of 10 μL. The MS detection was determined using a source CID of 10 V and carried out at a spray voltage of 4.2 kV, a nitrogen sheath gas pressure of 4.0 X 10^5Pa, a capillary temperature of 400 ^{o}C , a capillary voltage of 35 V and an auxiliary gas pressure of 1.0 X 10^5 Pa. The stationary phase used was an RP C18 NUCLEODUR 100-3 (125 X 3 mm) column (Macherey-Nagel). The solvent system consisted of water containing 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). HPLC-Method: flow rate 400 μL/min. The percentage of B started at an initial of 5%, was increased up to 100% during 16 min, kept at 100% for 2 min, and flushed back to 5% in 2 min. Melting points were determined using a BuchiB-545 melting point apparatus and are uncorrected.

General procedure for chalcone synthesis. To a solution of the appropriate acetophenone (5 mmol) in EtOH (40 mL) 10 % KOH aq. solution (20 mL) was added, followed by gradual addition of the corresponding benzaldehyde derivative (5 mmol). The mixture was left to stir at room temperature overnight and the solid obtained was

filtered and washed thoroughly with EtOH/H_2O mixture (1:1) then left to dry. In case of oily chalcones the reaction mixture was extracted with CH_2Cl_2 (3 x 30 mL) and the combined organic layers were washed with water, dried over anhydrous $MgSO_4$, evaporated under reduced pressure and used without further purification.

***(E)*-3-(4-Chlorophenyl)-1-(3-methoxyphenyl)prop-2-en-1-one (C1).** The compound was synthesized according to the general procedure for chalcone synthesis using 3'-methoxyacetophenone and 4-chlorobenzaldehyde; yellow oil; yield: 75 %.[1]

***(E)*-3-(4-Chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C2).** The compound was synthesized according to the general procedure for chalcone synthesis using 4'-methoxyacetophenone and 4-chlorobenzaldehyde; yellow solid; yield: 96 %; mp 125-126 °C.[1]

***(E)*-3-(3-Chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C3).** The compound was synthesized according to the general procedure for chalcone synthesis using 4'-methoxyacetophenone and 3-chlorobenzaldehyde; white creamy solid; yield: 85 %; mp 97-99 °C.[1]

***(E)*-3-(2-Chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C4).** The compound was synthesized according to the general procedure for chalcone synthesis using 4'-methoxyacetophenone and 2-chlorobenzaldehyde; yellow solid; yield: 2.48 g (74 %); mp 79-80 °C.[1]

***(E)*-3-(4-Chlorophenyl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (C5).** The compound was synthesized according to the general procedure for chalcone synthesis using 2',4'-

dimethoxyacetophenone and 4-chlorobenzaldehyde; yellow solid; yield: 91 %; mp 123-125 °C.[2]

***(E)*-3-(4-Chlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (C6).** The compound was synthesized according to the general procedure for chalcone synthesis using 3',4'-dimethoxyacetophenone and 4-chlorobenzaldehyde; yellow solid; yield: 89 %; mp 101-103 °C.[2]

***(E)*-1-(4-Aminophenyl)-3-(2-chlorophenyl)prop-2-en-1-one (C7).** The compound was synthesized according to the general procedure for chalcone synthesis using 4'-aminoacetophenone and 2-chlorobenzaldehyde; yellow solid; yield: 84 %; mp 105-107 °C.[2]

General Procedure for Pyrazoline Synthesis. A mixture of the chalcone derivative (1 mmol) and the corresponding chlorophenylhydrazine hydrochloride (1.2 mmol) in 10 mL of dry DMF was heated to 85 °C for 5 h under inert atmosphere. The reaction solution was left to attain room temperature and partitioned between 50 mL of diethyl ether and 20 mL of water. The organic layer was separated and washed with three 20-mL portions of water. The aqueous layers were combined and extracted with three 20-mL portions of diethyl ether. The organic layers were combined, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The remaining residue was purified by column chromatography.

1,5-bis(4-Chlorophenyl)-3-(3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (1a). The compound was prepared by reaction of (*E*)-3-(4-chlorophenyl)-1-(3-methoxyphenyl)prop-2-en-1-one (C1) and 4-chlorophenylhydrazine hydrochloride

according to the general procedure for pyrazoline synthesis; light brown solid; yield: 37%; 156-158 °C.[1]

1,5-bis(4-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (1b). The compound was prepared by reaction of *(E)*-3-(4-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C2) and 4-chlorophenylhydrazine hydrochloride according to the general procedure for pyrazoline synthesis; beige solid; yield: 51%; mp 111-112 °C.[1]

5-(3-Chlorophenyl)-1-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (1c). The compound was prepared by reaction of *(E)*-3-(3-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C3) and 4-chlorophenylhydrazine hydrochloride according to the general procedure for pyrazoline synthesis; brown solid ; yield: 39%; mp 106-108 °C.[1]

1-(3-Chlorophenyl)-5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (1d). The compound was prepared by reaction of *(E)*-3-(4-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C2) and 3-chlorophenylhydrazine hydrochloride according to the general procedure for pyrazoline synthesis; brown solid; yield: 0.28 g (36%); mp 121-122 °C.[1]

1,5-bis(3-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (1e). The compound was prepared by reaction of *(E)*-3-(3-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C3) and 3-chlorophenylhydrazine hydrochloride according to the general procedure for pyrazoline synthesis; brown solid; yield: 42%; mp 146-147 °C.[1]

1,5-bis(2-Chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole (1f). The compound was prepared by reaction of (*E*)-3-(2-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (C4) and 2-chlorophenylhydrazine hydrochloride according to the general procedure for pyrazoline synthesis; yellowish white solid; yield: 69%; mp 142-143 ^{o}C.[1]

1,5-bis(4-Chlorophenyl)-3-(2,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazole (1g). The compound was prepared by reaction of *(E)*-3-(4-chlorophenyl)-1-(2,4-dimethoxyphenyl)prop-2-en-1-one (C5) and 4-chlorophenylhydrazine hydrochloride according to the general procedure for pyrazoline synthesis; yellowish white solid; yield: 51%; mp 172-174^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.7 Hz, 1H), 7.33 – 7.28 (m, 2H), 7.27 – 7.21 (m, 2H), 7.14 – 7.06 (m, 2H), 6.96 – 6.89 (m, 2H), 6.56 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.45 (d, *J* = 2.4 Hz, 1H), 5.08 (dd, *J* = 12.1, 7.4 Hz, 1H), 3.93 (dd, *J* = 18, 12.3 Hz, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.23 (dd, *J* = 18.0, 7.4 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.75, 158.76, 147.47, 143.71, 141.13, 133.18, 129.70, 129.25, 128.72, 127.34, 123.45, 114.39, 114.32, 105.40, 98.61, 63.86, 55.46, 55.37, 46.89; MS (ESI): *m/z* = 427.1 $(M+H)^{+}$.

1,5-bis(4-Chlorophenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole (1h). The compound was prepared by reaction of *(E)*-3-(4-chlorophenyl)-1-(3,4-dimethoxyphenyl)prop-2-en-1-one (C6) and 4-chlorophenylhydrazine hydrochloride according to the general procedure for pyrazoline synthesis; beige solid; yield: 57%; mp 167-168 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.45 – 7.36 (m, 3H), 7.32 – 7.24 (m, 2H), 7.23 – 7.16 (m, 3H), 7.02 – 6.93 (m, 3H), 5.49 (dd, *J* =12.0, 5.8 Hz, 1H), 3.89 (dd, *J* = 17.5, 12.1 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 3.13 (dd, *J* = 17.5, 5.9 Hz, 1H); ^{13}C NMR (126 MHz,

DMSO-d_6) δ 149.93, 148.80, 148.37, 143.01, 141.07, 131.96, 129.00, 128.66, 127.79, 124.61, 121.90, 119.37, 114.18, 111.46, 108.54, 62.16, 55.54, 55.46, 43.09; MS (ESI): *m/z* = 426.93 $(M+H)^+$.

4-(1,5-bis(2-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)aniline (1i). The compound was prepared by reaction of *(E)*-1-(4-minophenyl)-3-(2-chlorophenyl)prop-2-en-1-one (C7) and 2-chlorophenylhydrazine hydrochloride according to the general procedure for pyrazoline synthesis; brown solid; yield: 54%; mp 108-109 ^oC; ^1H NMR (500 MHz, DMSO-d_6) δ 7.49 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.47 – 7.43 (m, 2H), 7.41 (ddd, *J* = 7.6, 1.2, 0.7 Hz, 1H), 7.26 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.24 – 7.13 (m, 4H), 6.90 (ddd, *J* = 7.9, 7.3, 1.6 Hz, 1H), 6.63 – 6.56 (m, 2H), 6.07 (dd, *J* = 11.2, 5.7 Hz, 1H), 5.69 (s, *2*H), 3.83 (dd, *J* = 16.9, 11.2 Hz, 1H), 3.04 (dd, *J* = 16.9, 5.7 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 150.77, 149.89, 142.88, 138.78, 131.19, 130.53, 129.65, 128.94, 127.61, 127.47, 127.30, 127.08, 123.03, 122.59, 121.40, 119.34, 113.59, 62.14, 41.76; MS (ESI): *m/z* = 382.23 $(M+H)^+$.

General procedure for methoxy group deprotection. A 1 M BBr_3 solution in CH_2Cl_2 (9 equiv) was added dropwise via syringe under inert atmosphere to a stirred solution of the methyl ether derivative (1 mmol, 1 equiv) in CH_2Cl_2 at -78 °C. Then the reaction was kept at -78 °C for 1 hour, after that allowed to reach room temperature and left to stir overnight. The mixture was cooled to 0 °C and H_2O was carefully added (15-25 mL). The product was then repeatedly extracted with EtOAc and the organic layers were dried over anhydrous Na_2SO_4. Upon solvent removal the residue was purified by column chromatography.

3-(1,5-bis(4-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (2a). The compound was prepared by demethylation of 1,5-bis(4-chlorophenyl)-3-(3-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole **(1a)** according to the general procedure for the methoxy group deprotection; beige solid; yield: 38%; mp 86-88 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.57 (s, 1H), 7.45 – 7.36 (m, 2H), 7.31 – 7.25 (m, 2H), 7.25 – 7.16 (m, 4H), 7.17 – 7.08 (m, 1H), 7.02 – 6.90 (m, 2H), 6.79 (ddd, *J* = 8.1, 2.5, 0.9 Hz, 1H), 5.50 (dd, *J* = 12.1, 6.0 Hz, 1H), 3.88 (dd, *J* = 17.5, 12.2 Hz, 1H), 3.07 (dd, *J* = 17.5, 6.0 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.45, 148.26, 142.82, 140.89, 133.09, 132.01, 129.71, 129.01, 128.72, 127.83, 122.23, 117.02, 116.32, 114.30, 112.13, 62.24, 42.92; MS (ESI): *m/z* = 383.03 $(M+H)^{+}$.

4-(1,5-bis(4-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (2b). The compound was prepared by demethylation of 1,5-bis(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole **(1b)** according to the general procedure for the methoxy group deprotection; yellowish brown solid; yield: 43%; mp 93-95 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.81 (s, 1H), 7.57 (t, *J* = 11.9 Hz, 2H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.27 (d, *J* = 8.5 Hz, 2H), 7.20 – 7.14 (m, 2H), 6.92 (dd, *J* = 13.1, 11.2 Hz, 2H), 6.81 (d, *J* = 8.7 Hz, 2H), 5.44 (dd, *J* = 12.0, 5.9 Hz, 1H), 3.86 (dd, *J* = 17.4, 12.0 Hz, 1H), 3.06 (dd, *J* = 17.4, 5.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.53, 148.55, 143.20, 141.13, 131.92, 128.97, 128.63, 127.82, 127.58, 122.90, 121.71, 115.49, 114.07, 62.08, 43.16; MS (ESI): *m/z* = 383.18 $(M+H)^{+}$.

4-(5-(3-Chlorophenyl)-1-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (2c). The compound was prepared by demethylation of 5-(3-chlorophenyl)-1-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole **(1c)** according to the general procedure for

the methoxy group deprotection; brown solid; yield: 36.5%; mp 82-84 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.83 (s, 1H), 7.61 – 7.55 (m, 2H), 7.40 – 7.34 (m, 1H), 7.34 – 7.28 (m, 2H), 7.22 – 7.15 (m, 3H), 6.97 – 6.91 (m, 2H), 6.84 – 6.78 (m, 2H), 5.45 (dd, *J* = 12.0, 5.9 Hz, 1H), 3.86 (dd, *J* = 17.5, 12.0 Hz, 1H), 3.10 (dd, *J* = 17.5, 6.0 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.57, 148.63, 144.70, 143.19, 133.48, 131.01, 128.70, 127.62, 127.48, 125.76, 124.48, 122.83, 121.80, 115.50, 114.06, 62.16, 43.18; MS (ESI): *m/z* = 383.03 $(M+H)^+$.

4-(1-(3-Chlorophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (2d). The compound was prepared by demethylation of 1-(3-chlorophenyl)-5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole **(1d)** according to the general procedure for the methoxy group deprotection; brown solid; yield: 29%; mp 97-98 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.77 (s, 1H), 7.53 (d, *J* = 8.5 Hz, 2H), 7.33 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.06 (t, *J* = 8.1 Hz, 1H), 6.96 (s, 1H), 6.76 (t, *J* = 9.4 Hz, 2H), 6.71 (d, *J* = 8.3 Hz, 1H), 6.63 (d, *J* = 7.9 Hz, 1H), 5.41 (dd, *J* = 11.9, 5.5 Hz, 1H), 3.80 (dd, *J* = 17.4, 12.1 Hz, 1H), 3.01 (dd, *J* = 17.6, 5.5 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.65, 149.07, 145.48, 141.04, 133.58, 131.98, 130.46, 129.01, 127.78, 127.72, 122.76, 117.52, 115.51, 112.06, 110.99, 61.82, 43.11; MS (ESI): *m/z* = 383.1 $(M+H)^+$.

4-(1,5-bis(3-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (2e). The compound was prepared by demethylation of 1,5-bis(3-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole **(1e)** according to the general procedure for the methoxy group deprotection; beige solid; yield: 58%; mp 80-81 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.84 (s, 1H), 7.64 – 7.57 (m, 2H), 7.39 – 7.35 (m, 1H), 7.33 (dd, *J* = 7.3, 1.2 Hz, 2H), 7.21 (d, *J* =

7.5 Hz, 1H), 7.14 (t, *J* = 8.1 Hz, 1H), 7.05 (t, *J* = 2.1 Hz, 1H), 6.84 – 6.80 (m, 2H), 6.78 (dd, *J* = 8.4, 1.5 Hz, 1H), 6.73 – 6.68 (m, 1H), 5.49 (dd, *J* = 12.0, 5.7 Hz, 1H), 3.87 (dd, *J* = 17.5, 12.0 Hz, 1H), 3.12 (dd, *J* = 17.5, 5.7 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.69, 149.17, 145.47, 144.61, 133.62, 133.50, 131.04, 130.52, 127.77, 127.54, 125.73, 124.45, 122.70, 117.61, 115.51, 112.07, 110.95, 61.89, 43.11; MS (ESI): *m/z* = 383.08 $(M+H)^+$.

4-(1,5-bis(2-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)phenol (2f). The compound was prepared by demethylation of 1,5-bis(2-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole **(1f)** according to the general procedure for the methoxy group deprotection; beige solid; yield: 45%; mp 86-87 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.83 (s, 1H), 7.67 – 7.54 (m, 2H), 7.48 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.41 (td, *J* = 3.0, 1.5 Hz, 1H), 7.28 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.26 – 7.12 (m, 4H), 6.92 (ddd, *J* = 7.9, 7.4, 1.6 Hz, 1H), 6.86 – 6.77 (m, 2H), 6.11 (dd, *J* = 11.3, 6.1 Hz, 1H), 3.88 (dd, *J* = 17.0, 11.3 Hz, 1H), 3.10 (dd, *J* = 17.0, 6.1 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.64, 150.07, 142.52, 138.50, 131.27, 130.57, 129.69, 129.05, 127.73, 127.49, 127.36, 123.42, 123.00, 122.92, 121.62, 115.47, 62.37, 41.68; MS (ESI): *m/z* = 383.04 $(M+H)^+$.

4-(1,5-bis(4-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)benzene-1,3-diol (2g). The compound was prepared by demethylation of 1,5-bis(4-chlorophenyl)-3-(2,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole **(1g)** according to the general procedure for the methoxy group deprotection; grey solid; yield: 62%; mp 108-110 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.42 (s, 1H), 9.90 (s, 1H), 7.42 – 7.38 (m, 2H), 7.33 – 7.29 (m, 2H), 7.24 (t, *J* = 5.5 Hz, 1H), 7.23 – 7.19 (m, 2H), 6.91 – 6.79 (m, 2H), 6.36 (dt, *J* = 8.4, 2.3 Hz, 2H), 5.42 (dd, *J* = 11.9, 6.2 Hz, 1H), 3.99 (dd, *J* = 17.6, 11.9 Hz, 1H), 3.21 (dd, *J* = 17.7, 6.2

Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.09, 158.03, 151.33, 142.69, 140.75, 132.07, 129.51, 129.02, 128.85, 127.92, 122.35, 114.13, 108.23, 107.69, 102.54, 61.04, 43.98; MS (ESI): *m/z* = 399.20 $(M+H)^+$.

4-(1,5-bis(4-Chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl)benzene-1,2-diol (2h). The compound was prepared by demethylation of 1,5-bis(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole **(1h)** according to the general procedure for the methoxy group deprotection; yellowish brown solid; yield: 55%; mp 80-82 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33 (s, 1H), 9.15 (s, 1H), 7.42 – 7.36 (m, 2H), 7.29 – 7.23 (m, 3H), 7.20 – 7.14 (m, 2H), 6.95 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.93 – 6.87 (m, 2H), 6.76 (d, *J* = 8.2 Hz, 1H), 5.42 (dd, *J* = 11.9, 5.9 Hz, 1H), 3.83 (dd, *J* = 17.3, 12.0 Hz, 1H), 3.01 (dd, *J* = 17.3, 5.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 148.70, 147.04, 145.40, 143.21, 141.11, 131.92, 128.97, 128.65, 127.83, 123.29, 121.67, 118.29, 115.48, 114.04, 112.68, 62.10, 43.16; MS (ESI): *m/z* = 399.14 $(M+H)^+$.

II. Biological assays.

Expression plasmids and proteins. The plasmids used to express GST-fused PKCζ were previously constructed by cloning of the full coding sequence of the atypical PKCs into the pEBG-2T vector,[3] as described by Balendran et al.[4] The GST fusion proteins were expressed in HEK293 cells (ATCC® CRL-1573™) and purified as described previously.[5]

Protein Kinase Assays. PKCζ, PKCι and mutant PKCζ assays were done as described before. [5, 6] In brief, the inhibition assays were performed using the GST-tagged, recombinant PKCζ (0.1-0.2 μM) or PKCι (0.04 μM) with myelin basic protein (Millipore) as an artificial substrate in the presence of 100 μM γ^{32}P-ATP/Mg^{2+}. After an incubation of 40 min, the reaction mixtures were spotted on P81 phosphocellulose paper (Whatman), the paper washed and the incorporated ^{32}P measured in a phosphoimager.

Nitrite Assay (Griess Assay). The assay was done using RAW 264.7 cells (ATCC® TIB-71™) as previously reported.[6]

Reporter Gene Assay. The assay was done using U937 cells (ATCC® CRL-1593™) transfected with the reporter gene plasmid (pGL4.32[luc2P/NF-kBRE/Hygro], Promega) as previously described.[5]

III. Molecular modeling. The docking was basically done as previously described,[4] using Amber99 as a force field in a pharmacophore-guided docking, using the positions of 1- and 5-phenyl as in the previous docking model for the pharmacophore definition. Side chains within a distance of 8 Å were defined as flexible. The docking pose with the highest score was refined using the ligX routine with the ligand strength set to 5000.

Figure S1

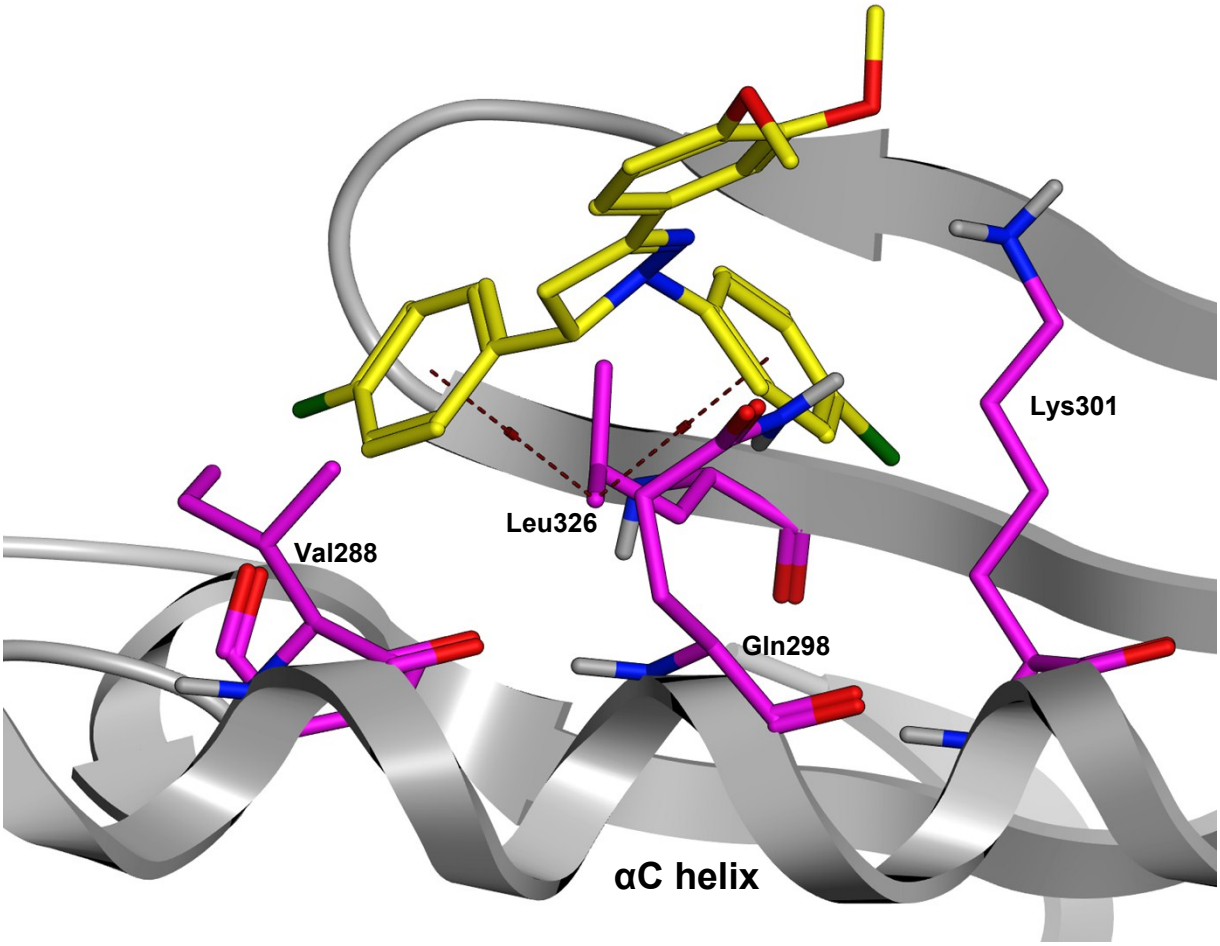

Figure S1. Model for the hypothetical interactions of the inactive compound **1h**. Compound **1h** (yellow) was docked into the PIF-pocket of PKCζ that was modeled based on the PKCι coordinates (PDB entry 3A8X) using MOE. Masking of the 4-hydroxyl function at the 3-phenyl resulted in a conformation in which both the H-bond interaction with Gln298 as well as the cation–π bond with Lys301 are lost.

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