Supporting Information

Indolactam-V is Involved in the CH/π Interaction with Pro-11 of the PKCδ C1B Domain: Application for the Structural Optimization of the PKCδ Ligand

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I. Experimental Procedures

*General remarks.* The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-2200A; Digital Polarimeter, Jasco DIP-1000; \(^1\)H NMR, Bruker ARX500 (ref. TMS); \(^19\)F NMR, Brucker AVANCE400 (ref. TFT as \(–64\) ppm); HPLC, Waters Model 600E with a Model 2487 UV detector; (HR) EI-MS, JOEL JMS-600H. HPLC was carried out on a YMC packed SH-342-5 (ODS, 20 mm i.d. \(\times\) 250 mm) column (Yamamura Chemical Laboratory). Wako gel C-200 (silica gel, Wako Pure Chemical Industries) and YMC A60-350/250 gel (ODS, Yamamura Chemical Laboratory) were used for column chromatography. \([\text{H}]\)PDBu (19.4 Ci/mol) was purchased from PerkinElmer Life Sciences Research Products. All other chemicals and reagents were purchased from chemical companies and used without further purification.

*Synthesis of Fmoc-4,4-difluoro-L-proline.* Fmoc-4,4-difluoro-L-proline was synthesized by the method of Demange *et al.* This compound existed in the mixture of two conformers in a ratio of 1 : 0.6 at room temperature. Fmoc-4,4-difluoro-L-proline: [\(\alpha\)]\(D\) \(–30.9^\circ\) (c = 0.137, MeOH, 28.4 °C); \(^1\)H NMR \(\delta\) (CDCl\(_3\), 0.0014 M, 500 MHz, 294 K) ppm for the major conformer: 2.64 (2H, m), 3.83 (2H, m), 4.26 (1H, t, \(J = 6.7\) Hz), 4.49 (2H, m), 4.64 (1H, dd, \(J = 8.8, 4.6\) Hz), 4.97 (1H, br.s), 7.30 (2H, m), 7.39 (2H, m), 7.55 (1H, d, \(J = 7.5\) Hz), 7.56 (1H, d, \(J = 7.5\) Hz), 7.77 (2H, d, \(J = 7.5\) Hz), for the minor conformer: 2.19 (1H, m), 2.64 (1H, m), 3.83 (2H, m), 4.16 (1H, t, \(J = 5.8\) Hz), 4.36 (1H, dd, \(J = 9.6, 4.0\) Hz), 4.49 (2H, m), 4.97 (1H, br.s), 7.30 (2H, m), 7.39 (2H, m), 7.51 (2H, d, \(J = 7.3\) Hz), 7.72 (1H, d, \(J = 7.3\) Hz), 7.73 (1H, d, \(J = 7.4\) Hz); \(^19\)F NMR \(\delta\) (CDCl\(_3\), 0.0014 M, 376.5 MHz, 294 K) ppm for the major conformer: \(–100.9\) (m), for the minor conformer: \(–99.6\) (d, \(J = 237\) Hz), \(–101.7\) (d, \(J = 237\) Hz); HR-FAB-MS \(m/z\): 374.1233 (MH\(^+\), calcd for \(\text{C}_{25}\text{H}_{18}\text{NO}_2\text{F}_2\), 374.1204).

*Synthesis of 2.* A solution of 2-methyl-1-nitronaphthalene (1, 3.35 g, 17.9 mmol) and dimethylformamide dimethylacetal (4.8 ml, 35.8 mmol) in anhydrous DMF (5.5 ml) was refluxed for 20 h. After cooling to room temperature, the solvent was removed *in vacuo* to give the crude enamine. To a solution of the enamine in MeOH (77 ml) was added camphor
sulfonic acid (12.5 g, 53.7 mmol). After another MeOH (13 ml) was added, the reaction mixture was refluxed for 3 h. The solvent was removed in vacuo, and the residue was dissolved in EtOAc. The solution was washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, and concentrated. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of EtOAc to give 2 (4.02 g, 15.4 mmol, 86% for two steps). Compound 2: ¹H NMR δ (CDCl₃, 0.103 M, 500 MHz, 300 K) ppm: 3.07 (2H, d, J = 5.4 Hz), 3.36 (6H, s), 4.60 (1H, t, J = 5.4 Hz), 7.49 (1H, d, J = 8.0 Hz), 7.56 (1H, t, J = 8.0 Hz), 7.61 (1H, t, J = 8.0 Hz), 7.73 (1H, d, J = 8.0 Hz), 7.89 (1H, d, J = 8.9 Hz), 7.91 (1H, d, J = 8.9 Hz); HR-MS of 2 could not be measured because of its instability.

Synthesis of 3. To a solution of 2 (2.61 g, 10.0 mmol) in CHCl₃ (10 ml) were added TFA (3 ml) and H₂O (3 ml). The reaction mixture was stirred at room temperature for 4 h, then warmed to 40 °C and stirred for another 2 h. The reaction was quenched by the addition of saturated aqueous NaHCO₃ and the mixture was extracted with CHCl₃. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of EtOAc to give the aldehyde quantitatively. To a solution of the aldehyde (2.15 g, 10.0 mmol) in MeOH (19 ml) was added (R)-phenylglycinol (1.51 g, 11.0 mmol). After stirring at 40 °C for 0.5 h, TMSCN (2.0 ml, 15.0 mmol) was added and the reaction mixture was stirred at 40 °C for another 1.5 h, and then partitioned between EtOAc and brine. The organic layer was dried over Na₂SO₄ and concentrated. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of EtOAc to give the (R)-amino nitrile (3.48 g, 9.64 mmol, 92%). A solution of the amino nitrile (3.48 g, 9.64 mmol) in HCl-saturated MeOH (25 ml) was stirred at room temperature for 24 h under an Ar atmosphere. The reaction mixture was neutralized with 1 N NaOH, and then partitioned between EtOAc and brine. The organic layer was dried over Na₂SO₄ and concentrated. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of
EtOAc to give 3 (3.58 g, 9.09 mmol, 94%). Compound 3: [α]D +29.8° (c = 0.460, EtOH, 32.4 °C); 1H NMR δ (CDCl3, 0.056 M, 500 MHz, 300 K) ppm: 2.07 (1H, br.s), 2.41 (1H, br.s), 3.00 (1H, dd, J = 14.0, 9.2 Hz), 3.13 (1H, dd, J = 14.0, 5.2 Hz), 3.45 (1H, dd, J = 9.2, 5.2 Hz), 3.47 (1H, dd, J = 10.8, 8.5 Hz), 3.63 (1H, dd, J = 10.8, 4.2 Hz), 3.72 (1H, dd, J = 8.5, 4.2 Hz), 3.72 (3H, s), 6.92 (2H, d, J = 7.3 Hz), 7.00 (2H, t, J = 7.3 Hz), 7.11 (1H, t, J = 7.3 Hz), 7.27 (1H, d, J = 7.7 Hz), 7.62 (2H, m), 7.72 (1H, d, J = 8.5 Hz), 7.85 (1H, d, J = 8.5 Hz), 7.90 (1H, d, J = 7.7 Hz); HR-FAB-MS m/z: 395.1622 (MH⁺, calcld for C22H23N2O6, 395.1607).

**Synthesis of 4.** To a solution of 3 (3.58 g, 9.09 mmol) in THF (25 ml) was added LiBH4 (498 mg) at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h, then warmed to room temperature and stirred for another 3 h. After neutralizing with 1 N HCl, the mixture was partitioned between EtOAc and brine. The organic layer was dried over Na2SO4 and concentrated. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of EtOAc to give 4 (2.95 g, 8.06 mmol, 89%). Compound 4: [α]D +37.7° (c = 0.765, EtOH, 32.4 °C); 1H NMR δ (CDCl3, 0.070 M, 500 MHz, 300 K) ppm: 2.51 (2H, br.s), 2.82 (1H, dd, J = 13.9, 8.6 Hz), 2.89 (1H, dd, J = 13.9, 5.8 Hz), 2.96 (1H, m), 3.48 (1H, dd, J = 11.2, 3.6 Hz), 3.51 (1H, dd, J = 10.9, 8.5 Hz), 3.64 (1H, dd, J = 10.9, 4.3 Hz), 3.75 (1H, dd, J = 11.2, 3.8 Hz), 3.80 (1H, dd, J = 8.5, 4.3 Hz), 6.92 (2H, d, J = 7.5 Hz), 6.99 (2H, t, J = 7.5 Hz), 7.09 (1H, t, J = 7.5 Hz), 7.20 (1H, d, J = 8.5 Hz), 7.62 (3H, m), 7.76 (1H, d, J = 8.5 Hz), 7.87 (1H, d, J = 8.5 Hz); HR-FAB-MS m/z: 367.1669 (MH⁺, calcld for C21H23N2O4, 367.1658).

**Synthesis of 5.** A mixture of 4 (1.30 g, 3.55 mmol) and 10% Pd-C (260 mg) in MeOH (17 ml) was vigorously stirred under 1 atom of H2 at room temperature for 7 h. The reaction mixture was filtered, and the filtrate was concentrated to give the amine quantitatively. A mixture of the amine (630 mg, 1.90 mmol) and 10% Pd-C (630 mg) in MeOH (10 ml) was vigorously stirred under 1 atom of H2 at room temperature for 64 h, and then filtered. The
filtrate was concentrated to give the crude diamine. To the mixture of the diamine and Et₃N (529 µl, 3.80 mmol) in 1,4-dioxane (1.1 ml) and water (1.1 ml) was added Boc-ON (514 mg, 2.10 mmol) at 0 °C. After stirring for 1.5 h at 0 °C, the reaction mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over Na₂SO₄, and concentrated. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of EtOAc to give 5 (293 mg, 0.930 mmol, 49% for two steps). Compound 5: [α]₀ –29.9° (c = 0.215, EtOH, 32.4 °C); ¹H NMR δ (CDCl₃, 0.024 M, 500 MHz, 300 K) ppm: 1.48 (9H, s), 2.88 (1H, dd, J = 13.6, 10.6 Hz), 3.07 (1H, br.d, J = 13.6 Hz), 3.57 (1H, dd, J = 10.8, 3.3 Hz), 3.64 (1H, br.d, J = 10.8 Hz), 3.83 (1H, m), 4.81 (2H, br.s), 5.18 (1H, br.d, J = 6.1 Hz), 7.17 (1H, d, J = 8.2 Hz), 7.25 (1H, d, J = 8.2 Hz), 7.44 (2H, m), 7.77 (1H, d, J = 8.2 Hz), 7.81 (1H, d, J = 8.2 Hz); HR-EI-MS m/z: 316.1784 (M⁺, calcd for C₁₈H₂₄N₂O₃, 316.1787).

Synthesis of 6. To a solution of 5 (430 mg, 1.36 mmol) in 1,2-dichloroethane (5.4 ml) were added 8 (925 mg, 2.72 mmol) and 2,6-lutidine (316 µl, 2.72 mmol), and the mixture was refluxed for 17 h. After cooling to room temperature, the solvent was removed in vacuo. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of EtOAc to give 6 (547 mg, 1.08 mmol, 79%). Compound 6: [α]₀ –35.3° (c = 0.300, EtOH, 32.4 °C); ¹H NMR δ (CDCl₃, 0.021 M, 500 MHz, 300 K) ppm: 1.10 (3H, d, J = 6.8 Hz), 1.22 (3H, d, J = 6.8 Hz), 1.46 (9H, s), 2.33 (1H, m), 2.95 (1H, dd, J = 13.4, 10.4 Hz), 3.21 (1H, dd, J = 13.4, 4.1 Hz), 3.28 (1H, br.d, J = 9.4 Hz), 3.43 (1H, br.d, J = 10.3 Hz), 3.79 (1H, m), 3.90 (1H, d, J = 6.0 Hz), 4.80 (1H, br.s), 4.86 (2H, s), 6.96 (2H, d, J = 6.8 Hz), 7.24 (4H, m), 7.43 (2H, m), 7.51 (1H, d, J = 8.3 Hz), 7.78 (1H, d, J = 8.1 Hz), 8.09 (1H, d, J = 8.1 Hz); HR-EI-MS m/z: 506.2783 (M⁺, calcd for C₃₀H₃₈N₂O₅, 506.2780).

Synthesis of 7. A mixture of 6 (530 mg, 1.05 mmol) and 10% Pd-C (53 mg) in CH₂CN (8 ml) was vigorously stirred under 1 atom of H₂ at room temperature for 0.5 h. The reaction
mixture was filtered, and the filtrate was concentrated to give the crude carboxylic acid. To a mixture of the carboxylic acid and N-hydroxysuccimide (242 mg, 2.10 mmol) in CH$_3$CN (6.5 ml) was added a solution of DCC (324 mg, 1.58 mmol) in CH$_3$CN (6.5 ml) at 0 °C. After stirring for 1.5 h at 0 °C, the reaction mixture was filtered and then concentrated. The residue was dissolved in CHCl$_3$ and the solution was washed with water. The organic layer was dried over Na$_2$SO$_4$ and concentrated. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of EtOAc to give the activated ester. A mixture of the activated ester and TFA (7 ml) in CH$_2$Cl$_2$ (7 ml) was stirred for 1 h at 0 °C. The solvent was then removed in vacuo to give the crude amine. To a solution of the amine in EtOAc (60 ml) was added saturated aqueous NaHCO$_3$ (10 ml). The reaction mixture was refluxed for 1 h. After cooling to room temperature, the organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated. The residue was purified by chromatography on Wako gel C-200 using hexane and increasing amounts of EtOAc to give 7 (207 mg, 0.696 mmol, 66% for four steps). Compound 7: [α]$_D$ = -152.0° (c = 1.395, EtOH, 32.4 °C); $^1$H NMR δ (CDCl$_3$, 0.242 M, 500 MHz, 300 K) ppm: 1.18 (3H, d, $J$ = 6.8 Hz), 1.28 (3H, d, $J$ = 6.8 Hz), 2.31 (1H, m), 2.94 (1H, dd, $J$ = 16.2, 11.4 Hz), 3.08 (1H, dd, $J$ = 16.2, 5.4 Hz), 3.45 (2H, m), 3.72 (1H, br.d, $J$ = 5.2 Hz), 3.83 (1H, t, $J$ = 5.2 Hz), 4.98 (1H, m), 7.05 (1H, d, $J$ = 8.1 Hz), 7.06 (1H, d, $J$ = 8.4 Hz), 7.42 (1H, t, $J$ = 8.1 Hz), 7.48 (1H, t, $J$ = 8.1 Hz), 7.49 (1H, d, $J$ = 8.4 Hz), 7.77 (1H, d, $J$ = 8.1 Hz), 7.86 (1H, d, $J$ = 8.1 Hz); HR-ESI-MS $m/z$: 298.1687 (M$^+$, calcd for C$_{18}$H$_{22}$N$_2$O$_5$, 298.1681).

*Synthesis of NL-V8.* A mixture of 7 (145 mg, 0.487 mmol) and 38% aqueous HCHO (0.4 ml, 5.49 mmol) in AcOH (1.5 ml) was stirred at room temperature for 1 h. To the resultant solution of the imine was then added NaBH$_4$CN (101 mg, 1.58 mmol), and the mixture was stirred for another 2 h. After neutralizing with saturated aqueous NaHCO$_3$, the mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried over Na$_2$SO$_4$, and concentrated. The residue was purified by HPLC on YMC SH-342-5 using
70% MeOH to give NL-V8 (110 mg, 0.351 mmol, 72%).  NL-V8: [α]D –190.0° (c = 0.707, MeOH, 28.6 °C); UV λmax (MeOH) nm (ε): 329 (2,600), 239 (7,800); 1H NMR δ (CD3OD, 0.056 M, 500 MHz, 300 K) ppm: 1.05 (3H, d, J = 6.6 Hz), 1.14 (3H, d, J = 6.8 Hz), 2.50 (1H, br.d, J = 17.2 Hz), 2.58 (1H, m), 2.74 (3H, s), 2.83 (1H, dd, J = 17.2, 7.2 Hz), 3.35 (1H, br.m), 3.42 (1H, br.m), 3.52 (1H, m), 3.77 (1H, br.s), 4.23 (1H, br.s), 6.92 (1H, d, J = 8.3 Hz), 7.26 (1H, t, J = 7.8 Hz), 7.33 (1H, d, J = 8.3 Hz), 7.34 (1H, d, J = 7.8 Hz), 7.66 (1H, d, J = 7.8 Hz), 7.98 (1H, br.s), 8.13 (1H, d, J = 7.8 Hz); HR-EI-MS m/z: 312.1841 (M+, calcd for C19H18N2O2, 312.1838). The NOE enhancement between the benzyl proton (δ 2.83) and the α proton of the valine unit (δ 3.77), which is characteristic of BL-V8, was observed in NL-V8.

II. Modeling Methods

The 3D structures of the cis-amide conformer of IL-V and BL-V8 used for the docking simulations were obtained by optimization of the initial structures, which were selected by the simulated annealing method, with ab initio molecular orbital (MO) schemes on the Hartree-Fock(HF) theory with a 6-31G* basis set. The program package used for ab initio MO calculations was AMOSS-H11, developed by the NEC quantum chemistry group. The X-ray coordinates of the PKCδ C1B domain (Protein Data Bank code: 1ptq)[4] were used as the receptor structure. The positions of generated hydrogen atoms were optimized with molecular modeling and Molecular Dynamics (MD) method.

Docking simulations were carried out using the FlexX program.[11] The initial docking structures selected on the basis of a consensus scoring method were optimized by MD method with a distance-dependent dielectric model for the solvent. The most stable docking structure was then selected according to consensus scoring and binding energy calculations. The program system used for the docking simulations, MD simulations, and consensus scoring calculations was “Sybyl”, developed by Tripos Inc.

S7
OB: 3895.77, Calc: 3894.89

Average molecular mass (MH)

8-CIB(111dP)

III. MALDI-TOF-MS data of δ-CIB(P11dP)

Acquired: 02:50, August 08, 2004

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Average molecular mass (MH)

Obs: 5895.57, Calcd: 5894.89

Average molecular mass (MH)

Obs: 5895.57, Calcd: 5894.89