Discovery of new protein kinase CK2 inhibitors with 1,3-dioxo-2,3-dihydro-1*H*-indene core

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Experimental methods

1. Measurement of CK2 inhibitory effects of the screened-out compounds.

Measurement of CK2 activity treated with various inhibitors was carried out by method previously reported,[57] employing a CK2 assay kit (MBL International, Woburn, MA). Briefly, in a 96-well recombinant full-length p53-coated plate, 10 μL the positive control containing 20 milliunits/10 μL CK2 was incubated with 10 μL of tested samples and 80 μL of kinase reaction buffer (containing 20X adenosine triphosphate, ATP) for 30 min at 30 ºC. Then, the wells were thoroughly washed with Washing Buffer. 100 μL horseradish peroxidase (HRP) conjugated Detection Antibody TK-4D4 was added into each well. After incubation at room temperature for 60 min, 100 μL Substrate Reagent was added to each well and incubated at room temperature for 15 minutes. Finally, 100 μL Stop Solution containing 3,3',5,5'-tetramethylbenzidine (TMB) was added to each well, and the absorbance was read by Varioskan spectrofluorometer and spectrophotometer (Thermo, Waltham, MA) at dual wavelengths of 450/540 nm.

2. Molecular docking

The docking study was performed by CDOCKER module implemented in Discovery Studio 3.0. The principle of CDOCKER can be breifly summarized as follow: CDOCKER generates ligand "seeds" to populate the binding pocket. Each seed is then subjected to high temperature molecular dynamics (MD) using a modified version of CHARMm force field. The structure after MD run is then fully minimized under the forcefield. The solutions are then clustered according to position and conformation and ranked by energy. The cocrystal structure of protein kinase CK2 bound with CX-4945 (PDB id: 3PE1) was used for molecular docking. The binding sites were defined by residues around CX-4945 in the ATP binding site (in 6 Å radius). The heating step, cooling steps, and cooling temperature were set to 5000, 5000, and 310, respectively. Other parameters were kept as default.

3. ADMET and Toxicity prediction

Compound was firstly sketched using discovery studio 3.0 and then saved as sd format. It was then imported into ADMET predictor 7.0 (Simulation plus, USA) for ADMET and toxicity prediction. Calculation was performed under pH = 7.4. Other parameters were set as default. The results were exported into a sd file for further reading.

Table S1. The physicochemical properties, ADMET and toxicity prediction of selected compounds.[1, 2]

Properties	**SL-15**	**SL-16**
Toxicity[a]	fu, SG, Hp	fu, SG, Hp

[a]fu=fraction unbound, SG=SGOT and SGPT evaluation, Hp=hepatotoxicity.

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Chemistry

*1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**02**)* 1,2,4-Benzenetricarboxylic anhydride (10.00 g, 52.05 mmol) and ethyl acetoacetate (7.3 mL, 57.25 mmol) were added to acetic anhydride (26 mL). With ice cooling, triethylamine (29.0 mL, 208.4 mmol) was added to the reaction mixture at a dropwise rate. After finish, the reaction mixture was warmed to 40°C and then stirred for 12h. After cooling, the reaction mixture was slowly poured into 1 M HCl aqueous solution (200 mL). Yellow crude product (9.52 g) was obtained via filtration and washing with water under reduce pressure. To a stirred solution of the crude product in acetonitrile (60 mL) was added trifluoroacetic acid (8 mL). The mixture was heated to reflux and stirred for 2h. The reaction was cooled to rt and then precipitated yellow solid. After filtration, washing with acetonitrile and drying under reduce pressure, brown solid (2.25 g, 22.7%) was obtained. ^{1}H NMR (300 MHz, DMSO) δ 13.63 (s, 1 H, -COOH), 8.39 (dd, J = 7.9, 1.4 Hz, 1 H, Ar-H), 8.30 (d, J = 0.6 Hz, 1 H, Ar-H), 8.01 (d, J = 7.9 Hz, 1 H, Ar-H), 3.40 (s, 2 H, -CH_2).

*(Z)-2-(4-fluorobenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-02**)* The solution of intermediate **02** (200 mg ,1.05 mmol) and 4-fluorobenzaldehyde (0.200 mL, 1.16 mmol) in acetic acid (5 mL) was heated to 123 °C and stirred for 0.5 h. After cooling to room temperature, the reaction mixture was slowly poured into water (20 mL). After filtration, washing with water and drying under reduce pressure, yellow powder (152 mg, 46.8%) was obtained. m.p. 265-266 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.72 (s, 1 H, -COOH), 8.62 (ddd, J = 8.9, 5.6, 3.4 Hz, 2 H, Ar-H), 8.40 (ddd, J = 7.8, 2.8, 1.4 Hz, 1 H, Ar-H), 8.33 (d, J = 2.3 Hz, 1 H, Ar-H), 8.05 (d, J = 8.0 Hz, 1 H, Ar-H), 7.88 (s, 1 H, =CH), 7.41 (t, J = 8.8 Hz, 2 H, Ar-H); IR (cm^{-1}, KBr film): 1763 (-CO), 1684 (-COOH); HRMS (ESI): calcd. for $C_{17}H_8FO_4$, $[M-H]^+$ 295.0412, found 295.0417; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 9.376 min, 98.34%.

Compound **SL-03**~**SL-17** were synthesized using the same method with varied substituted benzaldehyde.

*(Z)-2-(3-fluorobenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-03**)* Yield: 46.6%. m.p. 258-259 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.75 (s, 1 H, -COOH), 8.50 (d, J = 10.6 Hz, 1 H, Ar-H), 8.41 (ddd, J = 7.9, 2.7, 1.4 Hz, 1 H, Ar-H), 8.35 (d, J = 3.5 Hz, 1 H, Ar-H), 8.24 - 8.16 (m, 1 H, Ar-H), 8.08 (d, J = 7.9 Hz, 1 H, Ar-H), 7.88 (s, 1 H, =CH), 7.61 (dd, J = 14.2, 8.0 Hz, 1 H, Ar-H), 7.48 (td, J = 8.4, 2.1 Hz, 1 H, Ar-H); IR (cm^{-1}, KBr film): 1763 (-CO), 1684 (-COOH); HRMS (ESI): calcd. for $C_{17}H_8FO_4$, $[M-H]^+$ 295.0412, found 295.0417; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 8.951 min, 98.21%.

*(Z)-2-(3,4-difluorobenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-04**)* Yield: 35.2%. m.p. 260-261 °C; ^{1}H NMR (300 MHz, DMSO) δ 14.00 - 13.49 (m, 1 H, -COOH), 8.94 (dt, J = 15.8, 7.9 Hz, 1 H, Ar-H), 8.44 - 8.37 (m, 1 H, Ar-H), 8.35 (s, 1 H, =CH), 8.12 - 8.03 (m, 1 H, Ar-H), 7.89 (s, 1 H, Ar-H), 7.56 - 7.42 (m, 1 H, Ar-H), 7.34 (t, J = 8.6 Hz, 1 H, Ar-H); IR (cm^{-1}, KBr film): 3037 (-HO), 1678 (-CO), 1585 (-COOH); HRMS (ESI): calcd. for $C_{17}H_7F_2O_4$, $[M-H]^-$ 313.0318, found 313.0317; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 10.380 min, 91.23%.

*(Z)-1,3-dioxo-2-(3,4,5-trimethoxybenzylidene)-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-05**)* Yield: 51.7%. m.p. 241-242 °C; 1H NMR (300 MHz, DMSO) δ 13.70 (s, 1 H, -COOH), 8.35 (d, J = 7.8 Hz, 1 H, Ar-H), 8.28 (d, J = 4.6 Hz, 1 H, Ar-H), 8.06 (s, 2 H, Ar-H), 8.00 (dd, J = 7.8, 4.1 Hz, 1 H, =CH), 7.77 (d, J = 2.1 Hz, 1 H, Ar-H), 3.86 (s, 6 H, -CH_3), 3.80 (s, 3 H, -CH_3); IR (cm^{-1}, KBr film): 3482 (-HO), 1681 (-CO); HRMS (ESI): calcd. for $C_{20}H_{17}O_7$, $[M+H]^+$ 369.0969, found 369.0967; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 8.533 min, 92.48%.

*(Z)-2-(4-ethoxybenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-06**)* Yield:

39.1%. m.p. 257-258 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.69 (s, 1 H, -COOH), 8.62 - 8.41 (m, 2 H, Ar-H), 8.40 - 8.36 (m, 2 H, Ar-H), 8.31 (d, J = 6 Hz, 2 H, Ar-H), 8.02 (d, J = 7.8 Hz, 2 H, Ar-H), 7.98 (d, J = 7.83Hz, 2 H, Ar-H), 7.82 (s, 1 H, =CH), 7.10 (q, J = 7.6 Hz, 2 H, Ar-H), 4.17 (q, J = 6.93 Hz, 2 H, -CH_2), 1.37 (t, J = 6.93 Hz, 3 H, -CH_3); IR (cm^{-1}, KBr film): 1685 (-CO), 1577 (-COOH); HRMS (ESI): calcd. for $C_{19}H_{15}O_5$, $[M+H]^+$ 323.0914, found 323.0909; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 12.422 min, 95.15%.

(Z)-2-(4-isopropylbenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid ***(SL-07)*** Yield: 51.2%. m.p. 240-241 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.75 (s, 1 H, -COOH), 8.50 - 8.41 (m, 4 H, Ar-H), 8.08 (d, J = 7.83Hz, 1 H, Ar-H), 7.88 (s, 1 H, =CH), 7.47 (q, J = 7.6 Hz, 2 H, Ar-H), 3.01 (q, J = 6.8 Hz, 1 H, -CH), 1.25 (d, J = 6.8 Hz, 6 H, -CH_3); IR (cm^{-1}, KBr film): 1689 (-CO), 1584 (-COOH); HRMS (ESI): calcd. for $C_{20}H_{17}O_4$, $[M+H]^+$ 321.1121, found 321.1126; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 21.04 min, 98.99%.

(Z)-2-(4-nitrobenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid ***(SL-08)*** Yield: 34.2%. m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.76 (s, 1 H, -COOH), 8.59 (d, J = 5.2 Hz, 2 H, Ar-H), 8.42 (d, J = 7.9 Hz, 1 H, Ar-H), 8.34 (d, J = 8.6 Hz, 3 H, Ar-H), 8.15 - 8.04 (m, 1 H, Ar-H), 7.97 (s, 1 H, =CH); IR (cm^{-1}, KBr film): 1686 (-CO); HRMS (ESI): calcd. for $C_{17}H_{10}NO_6$, $[M+H]^+$ 324.0503, found 324.0495; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 4.862 min, 96.07%.

(Z)-2-(3-nitrobenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid ***(SL-09)*** Yield: 33.9%. m.p. 285-286 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.76 (s, 1 H, -COOH), 9.51 (d, J = 1.7 Hz, 1 H, Ar-H), 8.67 (t, J = 7.1 Hz, 1 H, Ar-H), 8.46 - 8.38 (m, 2 H, Ar-H), 8.36 (d, J = 6.3 Hz, 1 H, Ar-H), 8.09 (dd, J = 7.7, 2.8 Hz, 1 H, Ar-H), 8.00 (s, 1 H, =CH), 7.83 (t, J = 8.1 Hz, 1 H, Ar-H); IR (cm^{-1}, KBr film): 1737 (-CO), 1602 (-COOH); HRMS (ESI): calcd. for $C_{17}H_{10}NO_6$, $[M+H]^+$ 324.0503, found 324.0495; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 4.862 min, 96.07%.

(Z)-2-(4-cyano benzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid ***(SL-10)*** Yield: 35.2%. m.p. >300 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.77 (s, 1 H, -COOH), 8.54 (dd, J = 8.3, 3.6 Hz, 2 H, Ar-H), 8.42 (d, J = 7.8 Hz, 1 H, Ar-H), 8.35 (s, 1 H, Ar-H), 8.09 (dd, J = 7.8, 5.7 Hz, 1 H, Ar-H), 8.01 (d, J = 8.3 Hz, 2 H, Ar-H), 7.92 (s, 1 H, =CH); IR (cm^{-1}, KBr film): 1690 (-CO), 1612 (-COOH); HRMS (ESI): calcd. for $C_{18}H_9NNaO_4$, $[M+Na]^+$ 326.0424, found 326.0427; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 10.380 min, 95.53%.

(Z)-2-(3-cyano benzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid ***(SL-11)*** Yield: 52.8%. m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.75 (s, 1 H, -COOH), 8.88 (s, 1 H, Ar-H), 8.66 (t, J = 7.1 Hz, 1 H, Ar-H), 8.40 (ddd, J = 7.8, 2.7, 1.4 Hz, 1 H, Ar-H), 8.35 - 8.28 (m, 1 H, Ar-H), 8.10 - 7.99 (m, 2 H, Ar-H), 7.88 (s, 1H, =CH), 7.75 (t, J = 7.9 Hz, 1 H, Ar-H); IR (cm^{-1}, KBr film): 1731 (-CO), 1608 (-COOH); HRMS (ESI): calcd. for $C_{18}H_{10}NO_4$, $[M+H]^+$ 304.0604, found 304.0608; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 8.193 min, 96.81%.

(Z)-2-(4-cyano benzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid ***(SL-12)*** Yield: 57.8%. m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.78 (s, 1 H, -COOH), 8.59 (dd, J = 8.5, 2.1 Hz, 2 H, Ar-H), 8.43 (d, J = 7.9 Hz, 1 H, Ar-H), 8.37 (s, 1 H, Ar-H), 8.09 (dd, J = 12.1, 6.5 Hz, 3 H, Ar-H), 7.96 (s, 1 H, =CH), 3.03 (s, 3 H, -CH_3); IR (cm^{-1}, KBr film): 1771 (-CO), 1584 (-COOH); HRMS (ESI): calcd. for $C_{18}H_{12}NaO_6S$, $[M+Na]^+$ 379.0247, found 379.0242; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 5.171 min, 94.74%.

(Z)-2-(4-aminobenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid ***(SL-13)*** Yield: 35.7%. m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.41 (s, 1 H, -COOH), 8.45 (d, J = 8.1 Hz, 2 H, Ar-H), 8.31 (dd, J = 7.2, 5.7 Hz, 1 H, Ar-H), 8.22 (d, J = 7.7 Hz, 1 H, Ar-H), 7.91 (dd, J = 7.8, 2.8 Hz,

1 H, Ar-H), 7.63 (s, 1 H, =CH), 7.12 (s, 2 H, -NH_2), 6.68 (d, *J* = 8.7 Hz, 2 H, Ar-H); IR (cm^{-1}, KBr film): 3420 (-NH_2), 3246 (-HO),1719 (-CO), 1602 (-COOH); HRMS (ESI): calcd. for $C_{17}H_{12}NO_4$, $[M+H]^+$ 294.0761, found 294.0759; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 8.193 min, 96.81%.

*(Z)-2-(4-acetamidobenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-14**)* Yield: 51.1%. m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.67 (s, 1 H, -COOH), 10.42 (s, 1 H, -NH-), 8.58 - 8.44 (m, 2 H, Ar-H), 8.37 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.29 (d, *J* = 5.2 Hz, 1H, Ar-H), 8.00 (d, *J* = 7.8 Hz, 1 H, =CH), 7.72 (d, *J* = 7.3 Hz, 3 H, Ar-H), 2.09 (s, 3 H, -CH_3); IR (cm^{-1}, KBr film): 1737 (-CO), 1613 (-COOH); HRMS (ESI): calcd. for $C_{19}H_{14}NO_5$, $[M+H]^+$ 336.0866, found 336.0866; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 3.882 min, 94.70%.

*(Z)-2-(4-(methylamino)benzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-15**)* Yield: 51.1%. m.p. > 300 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.77 (s, 1 H, -COOH), 8.51 - 8.42 (m, 2 H, Ar-H), 8.30 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.21 (d, *J* = 5.6 Hz, 1H, Ar-H), 8.00 (d, *J* = 7.4 Hz, 1 H, =CH), 7.62 (d, *J* = 7.5 Hz, 3 H, Ar-H), 6.47 (s, 1 H, -NH-), 2.54 (s, 3 H, -CH_3); IR (cm^{-1}, KBr film): 1730 (-CO), 1602 (-COOH); HRMS (ESI): calcd. for $C_{18}H_{13}NO_4$, $[M+H]^+$ 308.0869, found 308.0863; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 5.783 min, 96.40%.

*(Z)-2-(4-hydroxybenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-16**)* Yield: 32.4%. m.p. 299-300 °C; 1H NMR (300 MHz, DMSO) δ 13.67 (s, 1 H, -COOH), 10.97 (s, 1 H, -OH), 8.53 (dd, *J* = 8.9, 2.6 Hz, 2 H, Ar-H), 8.36 (ddd, *J* = 7.8, 4.0, 1.3 Hz, 1 H, Ar-H), 8.28 (d, *J* = 5.7 Hz, 1 H, Ar-H), 8.00 (d, *J* = 7.8 Hz, 1 H, Ar-H), 7.77 (s, 1 H, =CH), 6.93 (d, *J* = 8.5 Hz, 2 H, Ar-H); IR (cm^{-1}, KBr film): 1675 (-CO), 1550 (-COOH); HRMS (ESI): calcd. for $C_{17}H_{11}O_5$, $[M+H]^+$ 295.0601, found 295.0603; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_{Rc}= 8.193cmin, 96.81%.

*(Z)-2-(3,4-dihydroxybenzylidene)-1,3-dioxo-2,3-dihydro-1H-indene-5-carboxylic acid (**SL-17**)* Yield: 32.5%. m.p. 206-208 °C; ^{1}H NMR (300 MHz, DMSO) δ 13.54 (s, 1 H, -COOH), 11.09 (s, 1 H, -OH), 10.95 (s, 1 H, -OH), 9.11 (dd, *J* = 9.5, 5.0 Hz, 1 H, Ar-H), 8.42 - 8.27 (m, 2 H, Ar-H), 8.25 (d, *J* = 5.4 Hz, 1 H, Ar-H), 7.95 (d, *J* = 7.8 Hz, 1 H, =CH), 6.42 (s, 2 H, Ar-H); IR (cm^{-1}, KBr film): 1696 (-CO), 1545 (-COOH); HRMS (ESI): calcd. for $C_{17}H_{11}O_6$, $[M+H]^+$ 311.055, found 311.0546; HPLC (80% methanol in water with 1% trifluoroacetic acid): t_R = 4.982 min, 91.85%.

References

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