

Supplementary information

Isoindole-1,3-dione Derivatives as RSK2 Inhibitors: Synthesis, Molecular Docking Simulation and SAR Analysis

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Molecular Docking

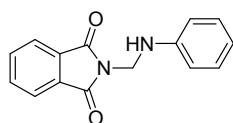
To investigate the binding patterns of the compounds in this series as well as facilitate the SAR analysis, compounds **7** and **19** were selected for the docking studies. The two inhibitors and the RSK2 NTKD (PDB code: 4NW6) were prepared respectively by the Ligprep module and Protein Preparation Wizard in Maestro (Schrödinger Inc, version 9.0). Glide in extra-precision (XP) mode was employed to do the molecular docking simulations with default parameter sets. The grid-enclosing box was centered on the ligand in 4NW6 and defined so as to include residues located within 20 Å around the ATP binding site, and a scaling factor of 1.0 was set to van der Waals (VDW) radii of those receptor atoms with the partial atomic charge less than 0.25. The top 10 docked poses of corresponding compounds ranked by GlideScore were remained for further analysis.

Synthetic Procedures and Characterizations of Compounds

Reagents and General Methods. All chemical reagents and solvents were purchased from commercial suppliers and used without further purification. Thin-layer

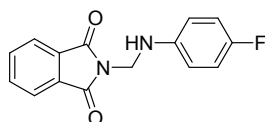
chromatography (TLC) was performed on silica gel plates. Column chromatography was performed using 200–300 mesh silica gel (Hailang, Qingdao). The ^1H and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with chemical shifts expressed in parts per million (ppm) units (in $\text{DMSO}-d_6$ or CDCl_3 , using Me_4Si as the internal standard). The low or high resolution of ESI-MS was measured at the Institute of Fine Chemistry of ECUST. Melting points were determined using an WRS-1B digital melting point apparatus.

2-((phenylamino)methyl)isoindoline-1,3-dione (**1**)



To a solution of phthalimide (300 mg, 2.04 mmol) in 80% ethanol (3.5 mL) was added formaldehyde (0.2 mL, 2.65 mmol). The reaction mixture was stirred at reflux until all of the phthalimide had dissolved. Next a solution of phenylamine (209 mg, 2.24 mmol) dissolved in 80% ethanol (1.5 mL) was added. The reaction mixture was stirred at reflux for 3 h. After cooling overnight the precipitate was filtered and washed with cold ethanol. The crude product was purified by flash silica gel chromatography (petroleum ether/ethylacetate = 5:1, v/v) to obtain the title product as a pale yellow solid (320 mg, yield 62%). mp 145.8-146.3 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.88-7.83 (m, 4H), 7.08 (t, $J = 8.0$ Hz, 2H), 6.83 (d, $J = 8.0$ Hz, 2H), 6.63-6.57 (m, 2H), 5.01 (d, $J = 5.2$ Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 168.46, 146.57, 135.10, 131.87, 129.41, 123.67, 117.65, 112.93, 47.47. HRMS (ESI) (m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{13}\text{N}_2\text{O}_2$, 253.0977; found, 253.0976.

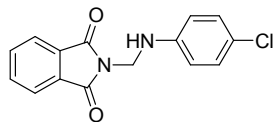
2-(((4-fluorophenyl)amino)methyl)isoindoline-1,3-dione (**2**)



The similar procedure of **1** was repeated to obtain the compound **2** (pale yellow solid, yield 63%). mp 168.3-169.2 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 7.90-7.83 (m, 4H), 6.95 (t, $J = 8.8$ Hz, 2H), 6.87-6.84 (m, 2H), 6.62 (t, $J = 7.2$ Hz, 1H), 5.00 (d, $J = 7.2$ Hz, 2H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 168.00, 156.18, 153.87, 142.71, 134.59,

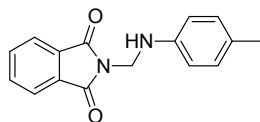
131.35, 123.17, 115.40, 115.18, 113.46, 113.38, 47.40. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{15}H_{12}N_2O_2F$, 271.0883; found, 271.0883.

2-(((4-chlorophenyl)amino)methyl)isoindoline-1,3-dione (**3**)



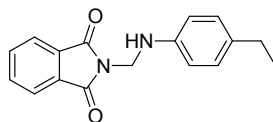
The similar procedure of **1** was repeated to obtain the compound **3** (yellow solid, yield 50%). mp 210.9-211.8 °C. 1H NMR (400 MHz, $DMSO-d_6$): δ 7.90-7.84 (m, 4H), 7.12 (d, $J = 8.8$ Hz, 2H), 6.85 (d, $J = 8.8$ Hz, 2H), 5.00 (d, $J = 5.2$ Hz, 2H). ^{13}C NMR (100 MHz, $DMSO-d_6$): δ 167.98, 145.17, 134.69, 131.39, 128.65, 123.26, 120.62, 113.95, 46.87. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{15}H_{12}N_2O_2Cl$, 287.0587; found, 287.0589.

2-((*p*-tolylamino)methyl)isoindoline-1,3-dione (**4**)



The similar procedure of **1** was repeated to obtain the compound **4** (yellow solid, yield 85%). mp 174.7-175.4 °C. 1H NMR (400 MHz, $DMSO-d_6$): δ 7.89-7.82 (m, 4H), 6.89 (d, $J = 8.0$ Hz, 2H), 6.73 (d, $J = 8.0$ Hz, 2H), 4.98 (d, $J = 6.0$ Hz, 2H), 2.12 (s, 3H). ^{13}C NMR (100 MHz, $DMSO-d_6$): δ 168.00, 143.66, 134.62, 131.40, 129.35, 125.68, 123.18, 112.68, 47.33, 20.01. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{16}H_{15}N_2O_2$, 267.1134; found, 267.1132.

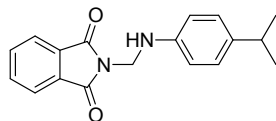
2-(((4-ethylphenyl)amino)methyl)isoindoline-1,3-dione (**5**)



The similar procedure of **1** was repeated to obtain the compound **5** (yellow solid, yield 73%). mp 114.2-115.0 °C. 1H NMR (400 MHz, $DMSO-d_6$): δ 7.88-7.80 (m, 4H), 6.93 (d, $J = 8.4$ Hz, 2H), 6.78 (d, $J = 8.4$ Hz, 2H), 6.44 (t, $J = 7.2$ Hz, 1H), 5.01 (d, $J = 7.2$ Hz, 2H), 2.42 (q, $J = 7.6$ Hz, 2H), 1.08 (t, $J = 7.6$ Hz, 3H). ^{13}C NMR (100 MHz, $DMSO-d_6$): δ 167.96, 143.85, 134.54, 132.37, 131.37, 128.14, 123.11, 112.57, 47.25, 27.23, 15.91. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{17}H_{17}N_2O_2$, 281.1290; found,

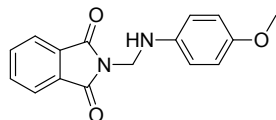
281.1292.

2-(((4-isopropylphenyl)amino)methyl)isoindoline-1,3-dione (6)



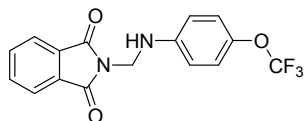
The similar procedure of **1** was repeated to obtain the compound **6** (yellow solid, yield 77%). mp 90.3-90.5 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.87-7.80 (m, 4H), 6.96 (d, *J* = 8.4 Hz, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 6.43 (t, *J* = 7.2 Hz, 1H), 5.02 (d, *J* = 7.2 Hz, 2H), 2.73-2.66 (m, 1H), 1.10 (d, *J* = 7.2 Hz, 6H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 167.96, 143.91, 137.08, 134.53, 131.38, 126.62, 123.11, 112.44, 47.22, 32.42, 24.08. HRMS (ESI) (*m/z*): [M + Na]⁺calcd for C₁₈H₁₈N₂O₂Na, 317.1266; found, 317.1267.

2-(((4-methoxyphenyl)amino)methyl)isoindoline-1,3-dione (7)



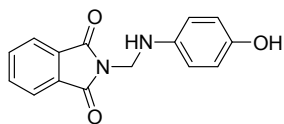
The similar procedure of **1** was repeated to obtain the compound **7** (yellow solid, yield 65%). mp 146.4-147.0 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.88-7.83 (m, 4H), 6.78 (d, *J* = 8.8 Hz, 2H), 6.71 (d, *J* = 8.8 Hz, 2H), 4.98-4.96 (m, 2H), 3.61 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.01, 151.62, 139.83, 134.56, 131.36, 123.12, 114.47, 113.76, 55.13, 47.85. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₁₆H₁₅N₂O₃, 283.1083; found, 283.1084.

2-(((4-(trifluoromethoxy)phenyl)amino)methyl)isoindoline-1,3-dione (8)



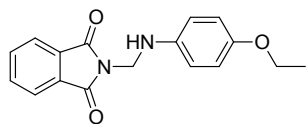
The similar procedure of **1** was repeated to obtain the compound **8** (grey solid, yield 48%). mp 115.3-116.1 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.91-7.83 (m, 4H), 7.11 (d, *J* = 8.8 Hz, 2H), 6.99 (t, *J* = 7.2 Hz, 1H), 6.95-6.91 (m, 2H), 5.03 (d, *J* = 7.2 Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 167.95, 145.51, 139.42, 134.61, 131.37, 123.20, 122.05, 118.98, 112.92, 46.83. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₁₆H₁₂N₂O₃F₃, 337.0800; found, 337.0793.

2-(((4-hydroxyphenyl)amino)methyl)isoindoline-1,3-dione (9)



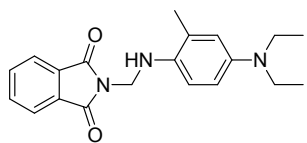
The similar procedure of **1** was repeated to obtain the compound **9** (pale yellow solid, yield 61%). mp 158.0-158.7 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.54 (s, 1H), 7.87-7.80 (m, 4H), 6.69 (d, *J* = 8.8 Hz, 2H), 6.55 (d, *J* = 8.8 Hz, 2H), 5.95 (t, *J* = 7.6 Hz, 1H), 4.95 (d, *J* = 7.6 Hz, 2H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.52, 149.86, 138.86, 135.06, 131.87, 123.62, 116.08, 114.64, 48.72. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₁₅H₁₃N₂O₃, 269.0926; found, 269.0924.

2-(((4-ethoxyphenyl)amino)methyl)isoindoline-1,3-dione (**10**)



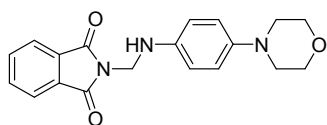
The similar procedure of **1** was repeated to obtain the compound **10** (yellow solid, yield 64%). mp 154.0-154.8 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.87-7.80 (m, 4H), 6.80 (d, *J* = 9.2 Hz, 2H), 6.71 (d, *J* = 9.2 Hz, 2H), 6.20 (t, *J* = 7.2 Hz, 1H), 4.99 (d, *J* = 7.2 Hz, 2H), 3.86 (q, *J* = 6.8 Hz, 2H), 1.25 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 167.99, 150.84, 139.80, 134.52, 131.35, 123.09, 115.16, 113.75, 63.10, 47.86, 14.75. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₁₇H₁₇N₂O₃, 297.1239; found, 297.1234.

2-(((4-(diethylamino)-2-methylphenyl)amino)methyl)isoindoline-1,3-dione (**11**)



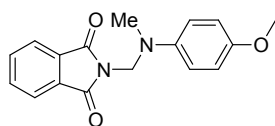
The similar procedure of **1** was repeated to obtain the compound **11** (yellow solid, yield 40%). mp 153.5-153.8 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.82-7.78 (m, 2H), 7.70-7.66 (m, 2H), 6.94 (d, *J* = 8.8 Hz, 1H), 6.56 (d, *J* = 8.8 Hz, 1H), 6.53 (s, 1H), 5.19 (d, *J* = 5.2 Hz, 2H), 4.43 (br, 1H), 3.18 (q, *J* = 7.2 Hz, 4H), 2.15 (s, 3H), 1.05 (t, *J* = 7.2 Hz, 6H). HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₂₀H₂₄N₃O₂, 338.1869; found, 338.1855.

2-(((4-morpholinophenyl)amino)methyl)isoindoline-1,3-dione (**12**)



The similar procedure of **1** was repeated to obtain the compound **12** (pale yellow solid, yield 55%). mp 182.2-183.0 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.81-7.80 (m, 2H), 7.70-7.67 (m, 2H), 6.83-6.78 (m, 4H), 5.14 (d, *J* = 8.0 Hz, 2H), 4.62 (t, *J* = 8.0 Hz, 1H), 3.81 (t, *J* = 4.8 Hz, 4H), 2.99 (t, *J* = 4.8 Hz, 4H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.01, 143.40, 139.49, 134.59, 131.38, 123.14, 117.18, 113.50, 66.20, 50.06, 47.66. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₁₉H₂₀N₃O₃, 338.1505; found, 338.1506.

2-(((4-methoxyphenyl)(methyl)amino)methyl)isoindoline-1,3-dione (**13**)

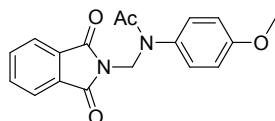


To a suspension of NaOMe (2.18 g, 40.6 mmol) in MeOH (12 mL) was added p-anisidin (1.00 g, 8.12 mmol), the resulting brown solution was poured into a suspension of paraformaldehyde (340 mg, 11.36 mmol) in MeOH (8 mL). The reaction mixture was stirred for 5 h at room temperature and then NaBH₄ (306 mg, 8.12 mmol) was added. The solution was heated to reflux for 2 h. The reaction mixture was treated with 1 M KOH after evaporating part of the solvent. After extraction with ethylacetate, the organic layer was dried over Na₂SO₄. The solvent was removed in vacuo, and the crude product was purified by flash silica gel chromatography (petroleum ether/ethylacetate =30:1, v/v) to obtain p-methoxy-N-methylaniline (681 mg, 61%). ¹H NMR (400 MHz, CDCl₃): δ 6.80 (d, *J* = 8.8 Hz, 2H), 6.58 (d, *J* = 8.8 Hz, 2H), 3.74 (s, 3H), 3.26 (br, 1H), 2.79 (s, 3H).

To a solution of phthalimide (200 mg, 1.36 mmol) in 80% ethanol (3.5 mL) was added formaldehyde (0.13 mL, 1.77 mmol). The reaction mixture was stirred at reflux until all of the phthalimide had dissolved. Next a solution of p-methoxy-N-methylaniline (187 mg, 1.36 mmol) dissolved in 80% ethanol (1.5 mL) was added. The reaction mixture was stirred at reflux for 3 h. After cooling overnight the precipitate was filtered and washed with cold ethanol. The crude product was recrystallized from ethanol to obtain the title product as yellow crystals (50 mg, 12%).

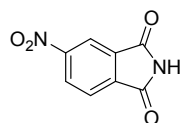
mp 146.1-147.2 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.88-7.83 (m, 4H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.81 (d, *J* = 8.8 Hz, 2H), 5.12 (s, 2H), 3.66 (s, 3H), 2.94 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.80, 152.75, 142.19, 135.12, 131.89, 123.71, 116.13, 114.70, 57.44, 55.64, 39.13. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₁₇H₁₇N₂O₃ [M+H]⁺, 297.1239, found 297.1239.

***N*-((1,3-dioxoisindolin-2-yl)methyl)-*N*-(4-methoxyphenyl)acetamide (14)**



To a solution of compound **7** (200 mg, 0.71 mmol) in triethylamine (108 mg, 1.06mmol) and dichloromethane (10 mL) was added a solution of acetyl chloride (556 mg, 7.08mmol) in dichloromethane (5 mL) at 0 °C. The reaction mixture was stirred for 12 h at room temperature. The solvent was removed in vacuo, and the crude product was purified by flash silica gel chromatography (petroleum ether/ethylacetate = 2:1, v/v) to obtain the title product as a white solid (158 mg, 69%). mp 149.9-150.4 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.83 (br, 4H), 7.14 (d, *J* = 8.8 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.50 (s, 2H), 3.71 (s, 3H), 1.70 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 169.92, 167.27, 159.17, 135.20, 133.29, 131.57, 130.20, 123.77, 115.04, 55.68, 49.59, 22.91. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₁₈H₁₆N₂O₄ [M+Na]⁺, 347.1008, found 347.1004.

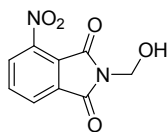
5-nitroisindoline-1,3-dione (26)



To a mixture of concentrated sulfuric acid (50 mL) and nitric acid (13 mL) at 5 °C was added phthalimide (10 g, 67.97 mmol) in portions over a 10-min interval with stirring. The temperature of the reaction mixture was raised slowly to 35 °C and held for 40 min. The reaction mixture was cooled to 0 °C and slowly stirred into ice water; the precipitate was filtered and washed with cold water. The product was recrystallized from ethanol to obtain off-white plate-like crystals (8.52 g, 65%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.82 (s, 1H), 8.61 (dd, *J* = 8.0, 2.0 Hz, 1H), 8.44 (d,

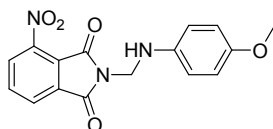
$J = 2.0$ Hz, 1H), 8.08 (d, $J = 8.0$ Hz, 1H).

2-(hydroxymethyl)-4-nitroisindoline-1,3-dione (27)



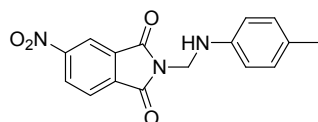
To a mixed solvent of water (2 mL) and 1,4-dioxane (7 mL) was added 4-nitroisindoline-1,3-dione (2 g, 10.41 mmol) and formaldehyde aqueous solution (2 mL, 26 mmol). The reaction mixture was stirred at reflux for 7 h. After cooling to room temperature, the reaction mixture was poured into ice-cold water. The precipitate was filtered and washed with cold water to obtain the title product as a white solid (860 mg, 37%). $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 8.32 (d, $J = 8.4$ Hz, 1H), 8.22 (d, $J = 7.2$ Hz, 1H), 8.09 (t, $J = 8.0$ Hz, 1H), 6.50 (t, $J = 7.2$ Hz, 1H), 4.97 (d, $J = 7.2$ Hz, 2H).

2-(((4-methoxyphenyl)amino)methyl)-4-nitroisindoline-1,3-dione (28)



To a solution of compound **27** (300 mg, 1.35 mmol) in ethanol (4 mL) was added 4-methoxyaniline (166 mg, 1.35 mmol). The reaction mixture was stirred at reflux for 3 h. The precipitate was filtered and washed with cold ethanol to obtain the title product as a red solid (330 mg, 75%). $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 8.28 (d, $J = 7.2$ Hz, 1H), 8.17 (d, $J = 7.2$ Hz, 1H), 8.05 (t, $J = 8.0$ Hz, 1H), 6.78-6.70 (m, 4H), 6.22 (t, $J = 7.2$ Hz, 1H), 4.97 (d, $J = 7.2$ Hz, 2H), 3.62 (s, 3H).

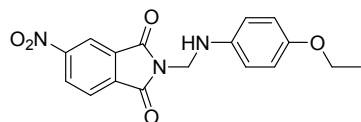
5-nitro-2-((p-tolylamino)methyl)isindoline-1,3-dione (15)



The similar procedure of **28** was repeated to obtain the compound **15** (yellow solid, yield 82%). mp 154.2-154.3 °C. $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 8.60 (dd, $J = 8.0, 2.0$ Hz, 1H), 8.48 (d, $J = 2.0$ Hz, 1H), 8.12 (d, $J = 8.0$ Hz, 1H), 6.76 (d, $J = 8.4$ Hz, 2H), 6.71 (d, $J = 8.4$ Hz, 2H), 6.43 (t, $J = 7.0$ Hz, 1H), 2.10 (s, 3H). $^{13}\text{C NMR}$ (100

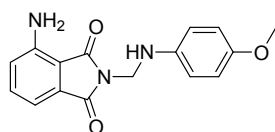
MHz, DMSO-*d*₆): δ 166.90, 166.62, 151.96, 144.00, 136.48, 133.26, 130.24, 129.82, 126.26, 125.16, 118.53, 113.11, 48.47, 20.46. MS (ESI⁺): m/z = 312.1 [M + H]⁺.

2-(((4-ethoxyphenyl)amino)methyl)-5-nitroisoindoline-1,3-dione (16)



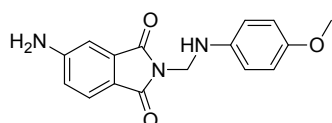
The similar procedure of **28** was repeated to obtain the compound **16** (cardinal red solid, yield 78%). mp 155.0-155.3 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.61 (d, J = 2.0 Hz, 1H), 8.56 (dd, J = 8.0, 2.0 Hz, 1H), 7.99 (d, J = 8.4 Hz, 1H), 6.77 (m, 4H), 5.18 (br, 2H), 4.58 (s, 1H), 3.90 (q, J = 7.2 Hz, 2H), 1.33 (t, J = 7.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 166.47, 166.20, 152.81, 151.80, 137.84, 136.33, 133.35, 129.36, 124.63, 118.84, 115.69, 115.28, 63.86, 49.66, 14.92. HRMS (ESI) (m/z): [M + H]⁺calcd for C₁₇H₁₆N₃O₅, 342.1090; found, 342.1089.

4-amino-2-(((4-methoxyphenyl)amino)methyl)isoindoline-1,3-dione (17)



A solution of compound **28** (100 mg, 0.31 mmol) in ethanol (20 mL) was treated with 10% palladium on charcoal (10 wt %) and hydrogenated at 30 psi for 6 h. The catalyst was filtered through Celite, and the filtrate was concentrated under reduced pressure to give the title product as a yellow solid (90 mg, 99%). mp 174.2-174.8 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.42 (dd, J = 8.0, 7.2 Hz, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.95 (d, J = 7.2 Hz, 1H), 6.77 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H), 6.48 (s, 2H), 6.07 (t, J = 7.2 Hz, 1H), 4.88 (d, J = 7.2 Hz, 2H), 3.61 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 170.04, 168.70, 152.06, 147.06, 140.50, 135.73, 132.65, 121.97, 114.97, 114.26, 111.21, 109.20, 55.67, 47.74. HRMS (ESI) (m/z): [M + H]⁺calcd for C₁₆H₁₆N₃O₃ [M+H]⁺, 298.1192, found 298.1190.

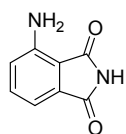
5-amino-2-(((4-methoxyphenyl)amino)methyl)isoindoline-1,3-dione (18)



The similar procedure of **17** was repeated to obtain the compound **18** (yellow solid,

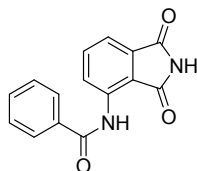
yield 99%). mp 250.3-250.8 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.47(d, *J* = 8.4 Hz, 1H), 6.90 (br, 1H), 6.79-6.76 (m, 2H), 6.74 (m, 1H), 6.68 (d, *J* = 8.8 Hz, 2H), 6.48 (s, 2H), 6.05 (t, *J* = 7.2 Hz, 1H), 4.86 (d, *J* = 7.2 Hz, 2H), 3.61 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.93, 168.56, 155.56, 152.03, 140.50, 134.81, 125.46, 117.29, 116.87, 114.96, 114.26, 107.39, 55.66, 47.81. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₁₆H₁₆N₃O₃ [M+H]⁺, 298.1192, found 298.1192.

4-aminoisoindoline-1,3-dione (30)



A solution of 4-nitroisoindoline-1,3-dione (4 g, 20.82 mmol) in ethanol (350 mL) was treated with 10% palladium on charcoal (10 wt %) and hydrogenated at 30 psi for 7 h. The catalyst was filtered through Celite, and the filtrate was concentrated under reduced pressure to give the title product as a yellow solid (3.3 g, 98%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.87 (s, 1H), 7.44-7.40 (m, 1H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 6.8 Hz, 1H), 6.38 (s, 2H).

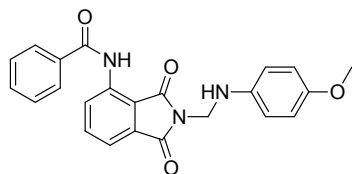
N-(1,3-dioxisoindolin-4-yl)benzamide (31)



To a 10-mL three neck flask, compound **30** (300 mg, 1.85 mmol) and 6 mL of 1-methyl-2-pyrrolidinone were added. While stirring the resultant at 0 °C, a solution of benzoyl chloride (0.3 mL, 2.41 mmol) in acetonitrile (3 mL) was dropped down slowly through a dropping funnel. After stirring the reaction mixture at 0 °C for 30 min, it was further stirring at room temperature for 4 h. Water (15 mL) was added to the reaction mixture, and the precipitate was filtered and washed with acetonitrile. The filter cake was dried in a vacuum oven to obtain the title compound as a white solid (460 mg, 93%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.52 (s, 1H), 10.47 (s, 1H), 8.53 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 7.2 Hz, 2H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.70 (t, *J*

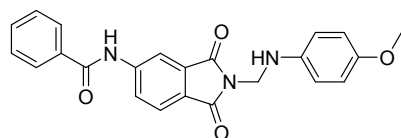
= 7.2 Hz, 1H), 7.63 (t, $J = 7.2$ Hz, 2H), 7.56 (d, $J = 7.2$ Hz, 1H).

***N*-(2-(((4-methoxyphenyl)amino)methyl)-1,3-dioxoisindolin-4-yl)benzamide (19)**



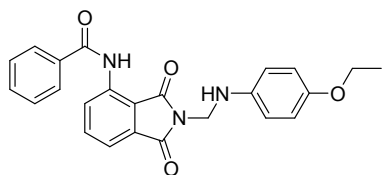
The similar procedure of **28** was repeated to obtain the compound **19** (pale yellow solid, yield 63%). mp 196.1-196.7 °C. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 10.46 (s, 1H), 8.60 (t, $J = 7.6$ Hz, 1H), 8.00 (d, $J = 7.6$ Hz, 2H), 7.85 (t, $J = 7.6$ Hz, 1H), 7.71 (t, $J = 7.2$ Hz, 1H), 7.66-7.62 (m, 3H), 6.78 (d, $J = 8.4$ Hz, 2H), 6.72 (d, $J = 8.4$ Hz, 2H), 6.23 (t, $J = 6.4$ Hz, 1H), 4.98 (d, $J = 6.4$ Hz, 2H), 3.61 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 169.81, 168.02, 165.34, 152.16, 140.29, 137.01, 136.56, 133.81, 133.14, 132.02, 129.54, 127.68, 126.04, 118.90, 118.20, 115.05, 114.17, 55.68, 48.51. HRMS (ESI) (m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{20}\text{N}_3\text{O}_4$, 402.1454; found, 402.1453.

***N*-(2-(((4-methoxyphenyl)amino)methyl)-1,3-dioxoisindolin-5-yl)benzamide (20)**



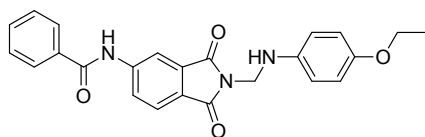
The similar procedure of **28** was repeated to obtain the compound **20** (pale yellow solid, yield 61%). mp 285.3-286.3 °C. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ 10.81 (s, 1H), 8.39 (s, 1H), 8.14 (d, $J = 8.0$ Hz, 1H), 7.98 (d, $J = 7.6$ Hz, 2H), 7.88 (d, $J = 8.0$ Hz, 1H), 7.64 (t, $J = 7.6$ Hz, 1H), 7.57 (d, $J = 7.6$ Hz, 2H), 6.78 (d, $J = 8.8$ Hz, 2H), 6.71 (d, $J = 8.8$ Hz, 2H), 6.17 (t, $J = 6.8$ Hz, 1H), 4.96 (d, $J = 6.8$ Hz, 2H), 3.62 (s, 3H). ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$): δ 168.37, 168.16, 166.72, 152.13, 145.40, 140.39, 134.67, 133.26, 132.62, 129.00, 128.34, 126.02, 125.03, 124.74, 115.00, 114.38, 114.28, 55.66, 48.38. HRMS (ESI) (m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{20}\text{N}_3\text{O}_4$, 402.1454; found, 402.1452.

***N*-(2-(((4-ethoxyphenyl)amino)methyl)-1,3-dioxoisindolin-4-yl)benzamide (21)**



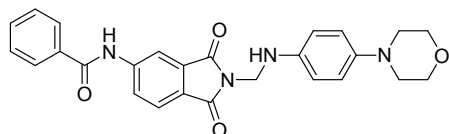
The similar procedure of **28** was repeated to obtain the compound **21** (pale yellow solid, yield 77%). mp 171.0-171.7 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.44 (s, 1H), 8.59 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 7.2 Hz, 2H), 7.84 (t, *J* = 8.0 Hz, 1H), 7.70 (t, *J* = 7.2 Hz, 1H), 7.65-7.60 (m, 3H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.70 (d, *J* = 8.8 Hz, 2H), 6.22 (t, *J* = 7.2 Hz, 1H), 4.97 (d, *J* = 7.2 Hz, 2H), 3.85 (q, *J* = 7.2 Hz, 2H), 1.23 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 169.80, 168.02, 165.36, 151.36, 140.27, 137.01, 136.56, 133.83, 133.14, 132.04, 129.54, 127.69, 126.08, 118.92, 118.25, 115.78, 114.18, 63.66, 48.54, 15.27. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₂₄H₂₂N₃O₄ [M+H]⁺, 416.1610, found 416.1609.

***N*-(2-(((4-ethoxyphenyl)amino)methyl)-1,3-dioxoisindolin-5-yl)benzamide (22)**



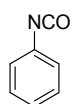
The similar procedure of **28** was repeated to obtain the compound **22** (pale yellow solid, yield 51%). mp 272.1-272.9 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.79 (s, 1H), 8.38 (s, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 7.6 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.64 (t, *J* = 7.2 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 2H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 6.14 (t, *J* = 7.2 Hz, 1H), 4.95 (d, *J* = 7.2 Hz, 2H), 3.86 (q, *J* = 6.8 Hz, 2H), 1.24 (t, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.35, 168.15, 166.71, 151.35, 145.41, 140.38, 134.68, 133.27, 132.60, 128.99, 128.34, 126.04, 125.04, 124.71, 115.74, 114.40, 114.31, 63.67, 48.43, 15.28. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₂₄H₂₂N₃O₄ [M+H]⁺, 416.1610, found 416.1608.

***N*-(2-(((4-morpholinophenyl)amino)methyl)-1,3-dioxoisindolin-5-yl)benzamide (23)**



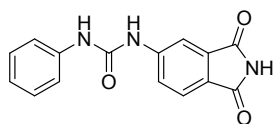
The similar procedure of **28** was repeated to obtain the compound **23** (yellow solid, yield 57%). mp 255.4-256.4 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.80 (s, 1H), 8.39(s, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 7.2 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.64 (t, *J* = 7.2 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 2H), 6.76 (br, 4H), 6.15 (br, 1H), 4.95 (br, 2H), 3.68 (t, *J* = 4.4 Hz, 4H), 2.89 (br, 4H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.35, 168.15, 166.72, 145.40, 143.90, 140.14, 134.68, 133.27, 132.62, 129.00, 128.34, 126.05, 125.03, 124.73, 117.72, 114.38, 114.03, 66.72, 50.60, 48.27. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₂₆H₂₅N₄O₄ [M+H]⁺, 457.1876, found 457.1878.

Isocyanatobenzene (33)



To a 50-mL three neck flask, triphosgene (1.05 g, 3.54 mmol) and 20 mL of toluene were added. While stirring the resultant at 0 °C, a solution of phenylamine (1 g, 10.74 mmol) in toluene (5 mL) was dropped down slowly through a dropping funnel. The reaction mixture was stirred at reflux for 8 h. The resulting solution of isocyanatobenzene in toluene was concentrated and used for the next step without further purification.

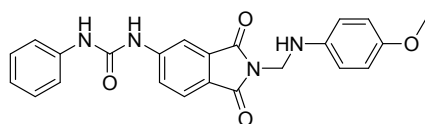
1-(1,3-dioxoisindolin-5-yl)-3-phenylurea (34)



To a solution of 5-aminoisindoline-1,3-dione (150 mg, 0.93 mmol) in toluene (3 mL) was added DMF (1 drop) and the above solution of compound **33** (220 mg, 0.93mmol) in toluene. The reaction mixture was stirred at 110 °C for 3 h. Water and ethyl acetate was added to the reaction mixture. The precipitated crystal was collected by filtration and washed with ethyl acetate to obtain the title compound as a white solid (261 mg, 48%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.13 (s, 1H), 9.36 (s, 1H), 8.88 (s, 1H), 8.05 (s, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.67 (d, *J* = 8.4 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.02 (t, *J* = 7.6 Hz, 1H).

1-(2-(((4-methoxyphenyl)amino)methyl)-1,3-dioxoisindolin-5-yl)-3-phenylurea

(24)



The similar procedure of **1** was repeated from compound **34** to obtain the compound **24** (yellow solid, yield 79%). mp 242.3-242.8 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ 9.39 (s, 1H), 8.90 (s, 1H), 8.10 (d, *J* = 1.6 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.66 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.02 (t, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.70 (d, *J* = 8.8 Hz, 2H), 6.11 (t, *J* = 7.2 Hz, 1H), 4.94 (d, *J* = 7.2 Hz, 2H), 3.61 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ 168.44, 168.23, 152.62, 152.14, 146.27, 140.40, 139.52, 133.65, 129.31, 124.86, 124.06, 122.94, 122.84, 119.13, 115.00, 114.32, 112.15, 55.68, 48.29. HRMS (ESI) (*m/z*): [M + H]⁺calcd for C₂₃H₂₁N₄O₄, 417.1563; found, 417.1558.

In vitro kinase inhibition assay

The kinase inhibition assay was performed as previously reported.¹ The ADP Quest assay (DiscoverX) was conducted in 96-well flat-bottom plates in 40 μL reaction volume according to the manufacturer's instructions. 20 ng of the kinase (Millipore) in 30 μL of assay buffer (15 mM of HEPES, pH 7.4, 20 mM of NaCl, 1 mM of EGTA, 0.02% of Tween 20, 10 mM of MgCl₂, and 0.1 mg/mL BGG) containing 25 μM of S6 peptide (AKRRRLSSLRA, Anaspec) was incubated for 20 minutes at room temperature with indicated concentrations of the compounds to be tested. Reactions were started by the addition of 10 μL of ATP to a final concentration of 10 μM, after 60 min at room temperature, 20 μL of ADP reagent A and 40 μL of ADP reagent B were added to terminate the reactions. Ro31-8220 was used as the positive control compound. Solutions of tested compound were prepared from DMSO stock and diluted with assay buffer for the inhibition assay. The amount of ADP produced was detected by the fluorescence signal, as a result of the kinase activities were recorded by Synergy 2 multimode microplate reader (BioTek) at an excitation wave length of 530 nm and an emission wavelength of 590 nm at 30 min

after the addition of ADP reagent B. All experiments were repeated at least three times. The data were analyzed using GraphPad Prism version 4.0 to determine the IC₅₀ values.

REFERENCES

1. S. L. Li, Y. Zhou, W. Q. Lu, Y. Zhong, W. L. Song, K. D. Liu, J. Huang, Z. J. Zhao, Y. F. Xu, X. F. Liu and H. L. Li, *J. Chem. Inf. Model.*, 2011, **51**, 2939-2947.