Deglycase-Activity Oriented Screening to Identify DJ-1 Inhibitors

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KEYWORDS. DJ-1/Park7, glycation, medicinal chemistry, anti-cancer, enzymology

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A. Supplementary Figures

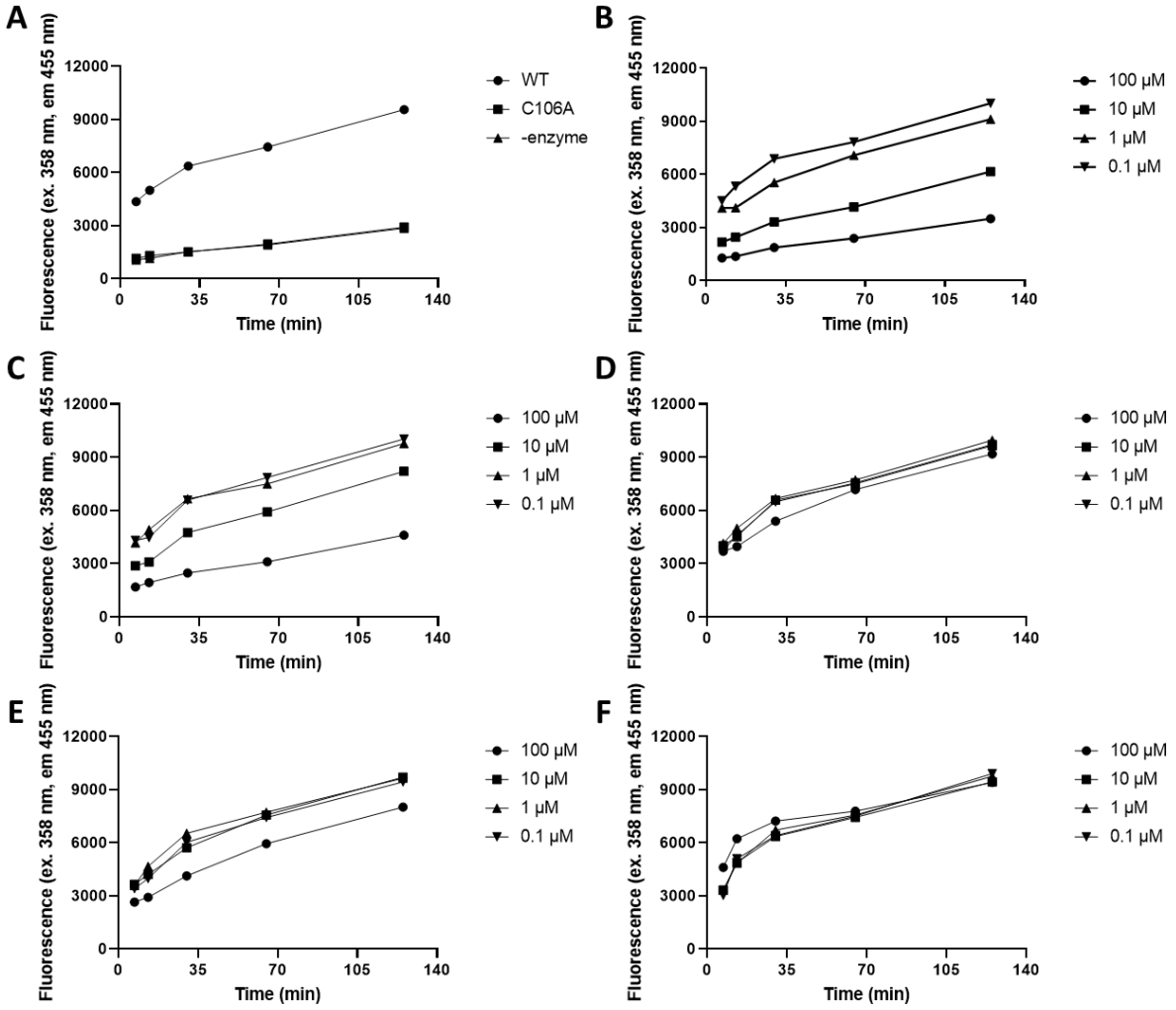


Figure S1. Isatin evaluation by lactate assay. (**A**) DJ-1 WT vs C106A, no compound (**B**) Compound **1** (**C**) Compound **2** (**D**) Compound **3** (**E**) Compound **4** (**F**) Compound **5**.

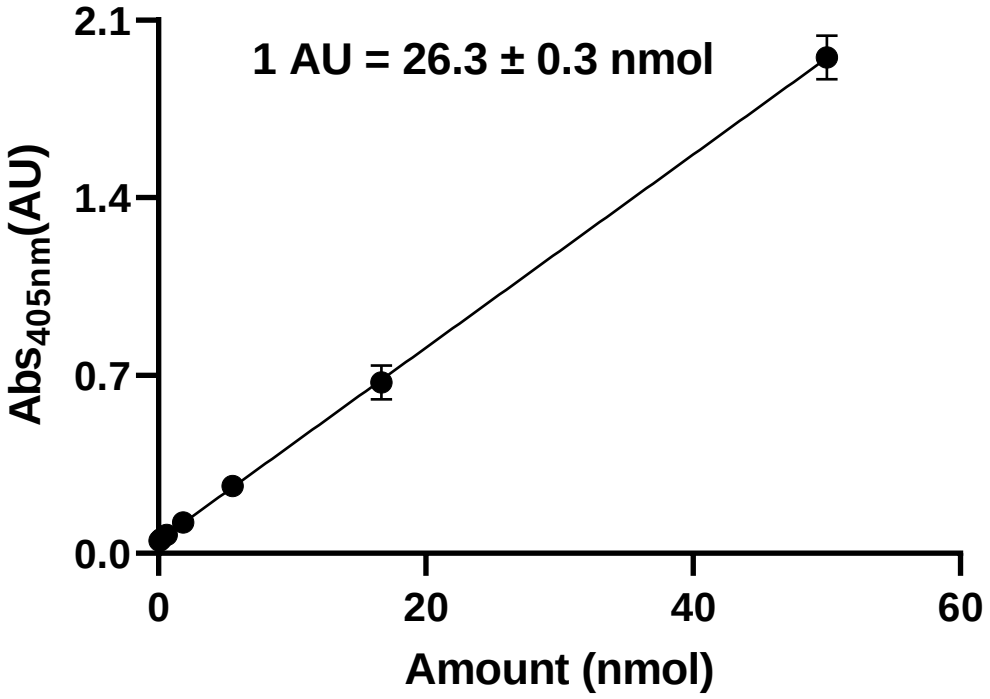


Figure S2. Standard absorbance curve of titrated PNPAc colorimetric hydrolysis product, *para*-nitrophenol (PNP), N = 3.

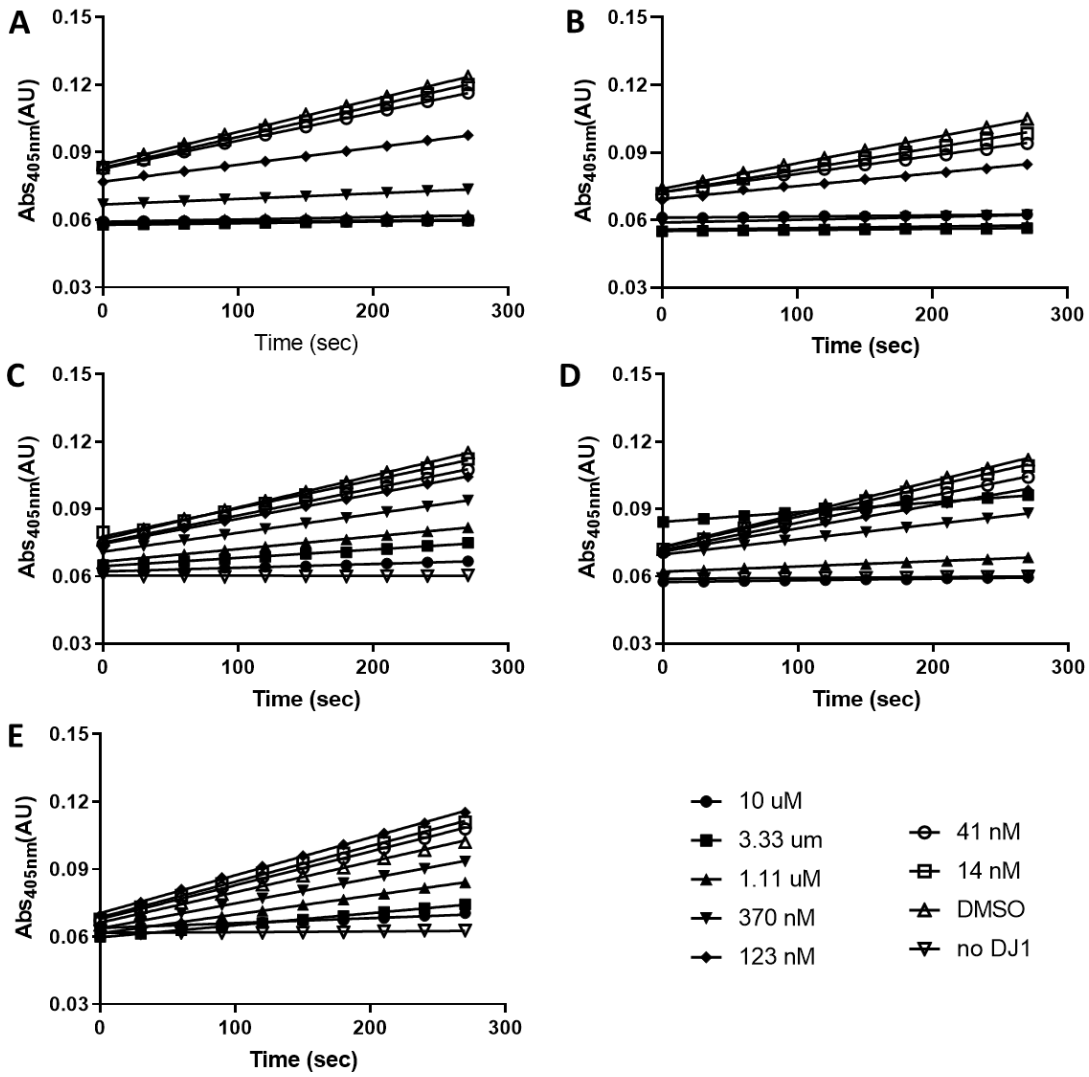


Figure S3. Isatin evaluation by PNPAc assay. (**A**) Compound **1** (**B**) Compound **2** (**C**) Compound **3** (**D**) Compound **4** and (**E**) Compound **5**.

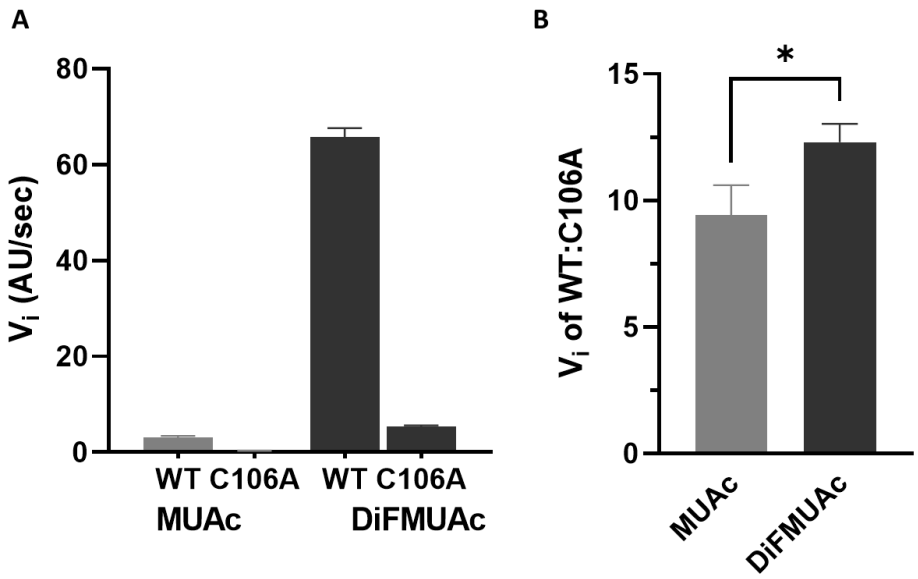


Figure S4. Comparison of DiFMUAc and MUAc assays. After adding 10 µL of 3 mM substrate to 90 µL of assay mix to yield a final DJ-1 wild type or C106A mutant concentration of 550 nM, triplicate wells were scanned at excitation 358 nm and emission 455 nm for 10 minutes, every 30 seconds. **(A)** Line of best fit was used to determine initial velocity at each condition **(B)** Ratios of initial velocity of wild type: C106A i.e. signal:noise ratios were determined and are shown; N = 3, unpaired two-tailed *t*-test, $p = 0.0226$.

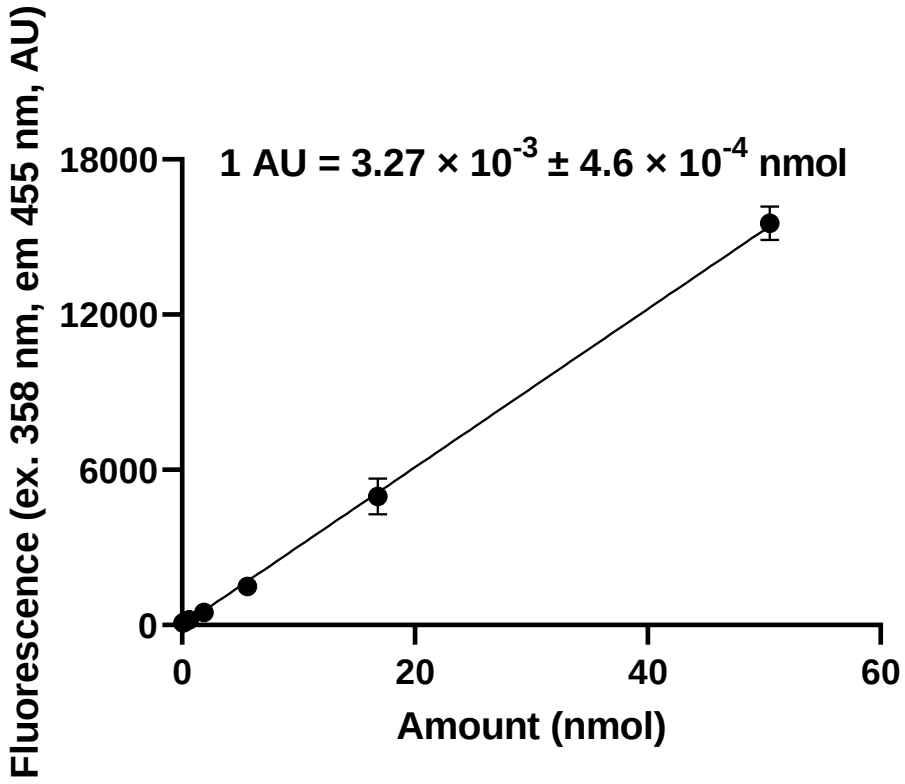


Figure S5. DiFMU standard curve. Standard fluorescence curve of titrated DiFMUAc after hydrolysis with 0.1 *N* NaOH, N = 3.

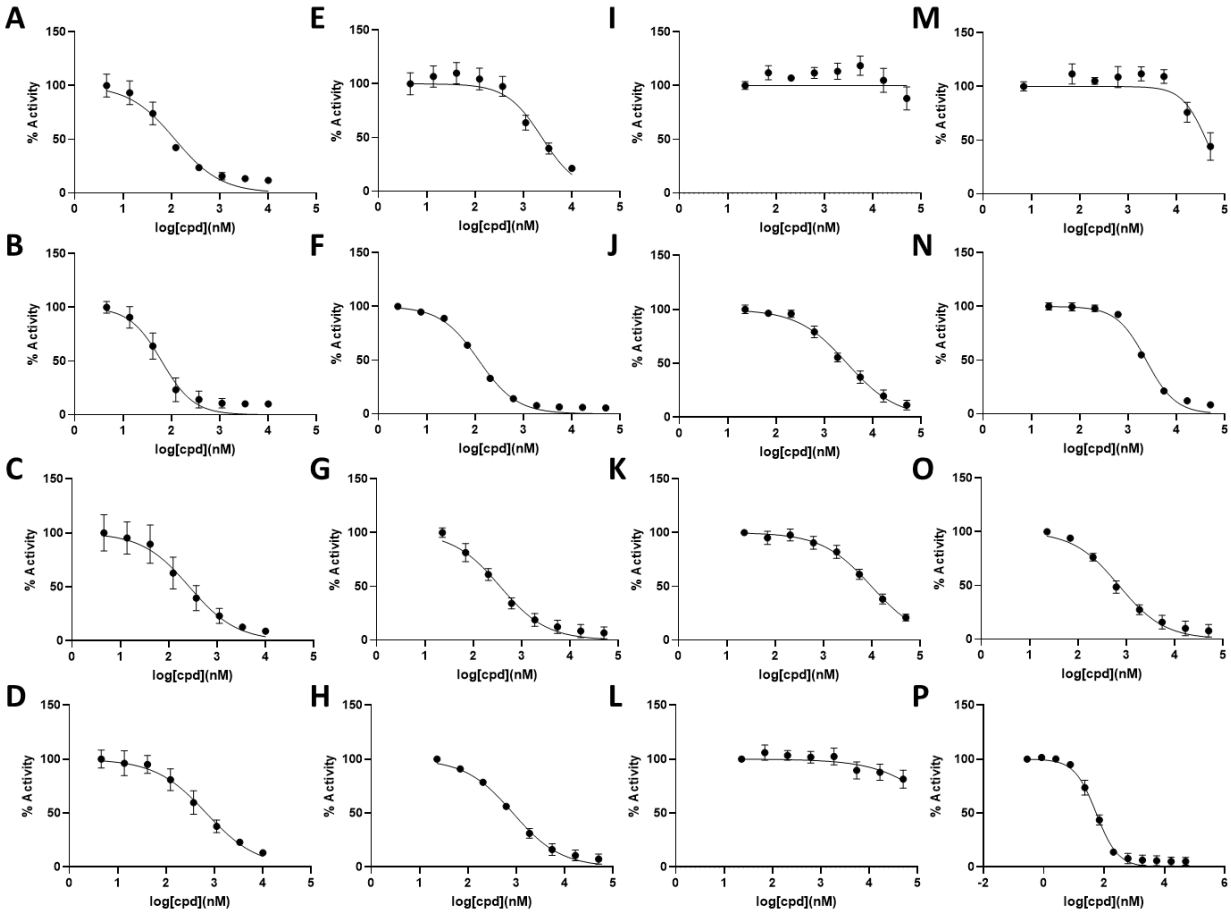


Figure S6. DiFMUAc assay evaluation of **Table 2** compounds. (**A**) compound **1**, N = 3. (**B**) Compound **2**, N = 3 . (**C**) Compound **3**, N = 3. (**D**) Compound **4**, N = 3. (**E**) Compound **5**, N = 3. (**F**) Compound **11**, N = 1. (**G**) Compound **12**, N = 2. (**H**) Compound **13**, N = 2. (**I**) Compound **14**, N = 2. (**J**) Compound **15**, N = 2. (**K**) Compound **16**, N = 2. (**L**) Compound **17**, N = 2. (**M**) Compound **18**, N = 2. (**N**) Compound **19**, N = 1. (**O**) Compound **20**, N =2. (**P**) Compound **21**, N = 1 for first 4 data points and N = 3 for remainder.

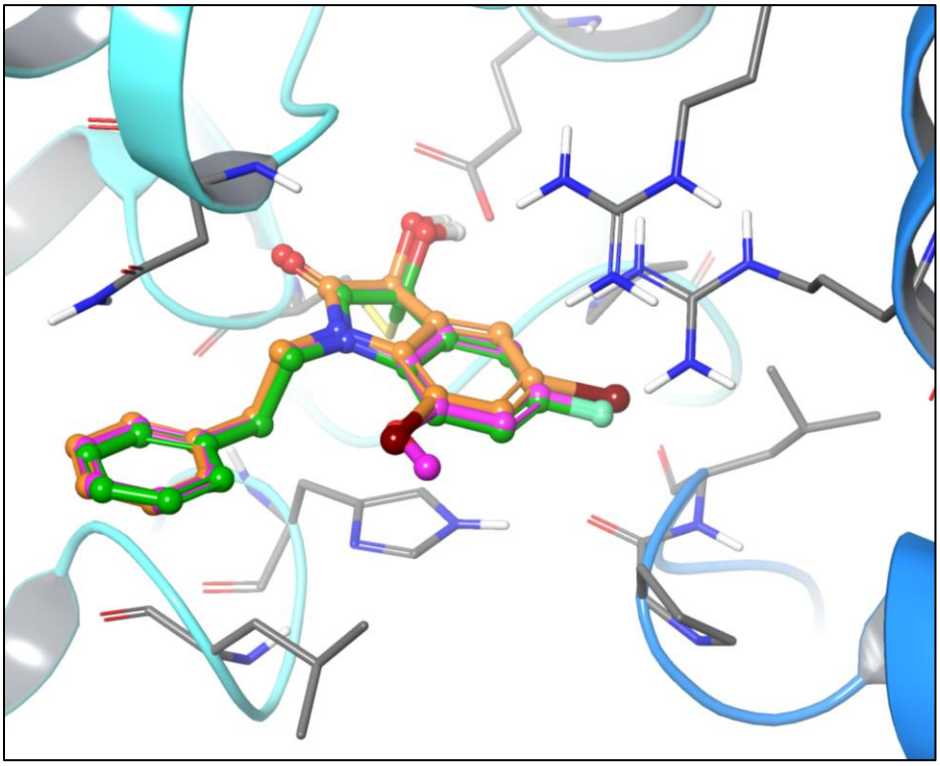


Figure S7. Covalent docking poses of isatin-based compounds **2** (orange) and **21** (magenta) against **1** (green).

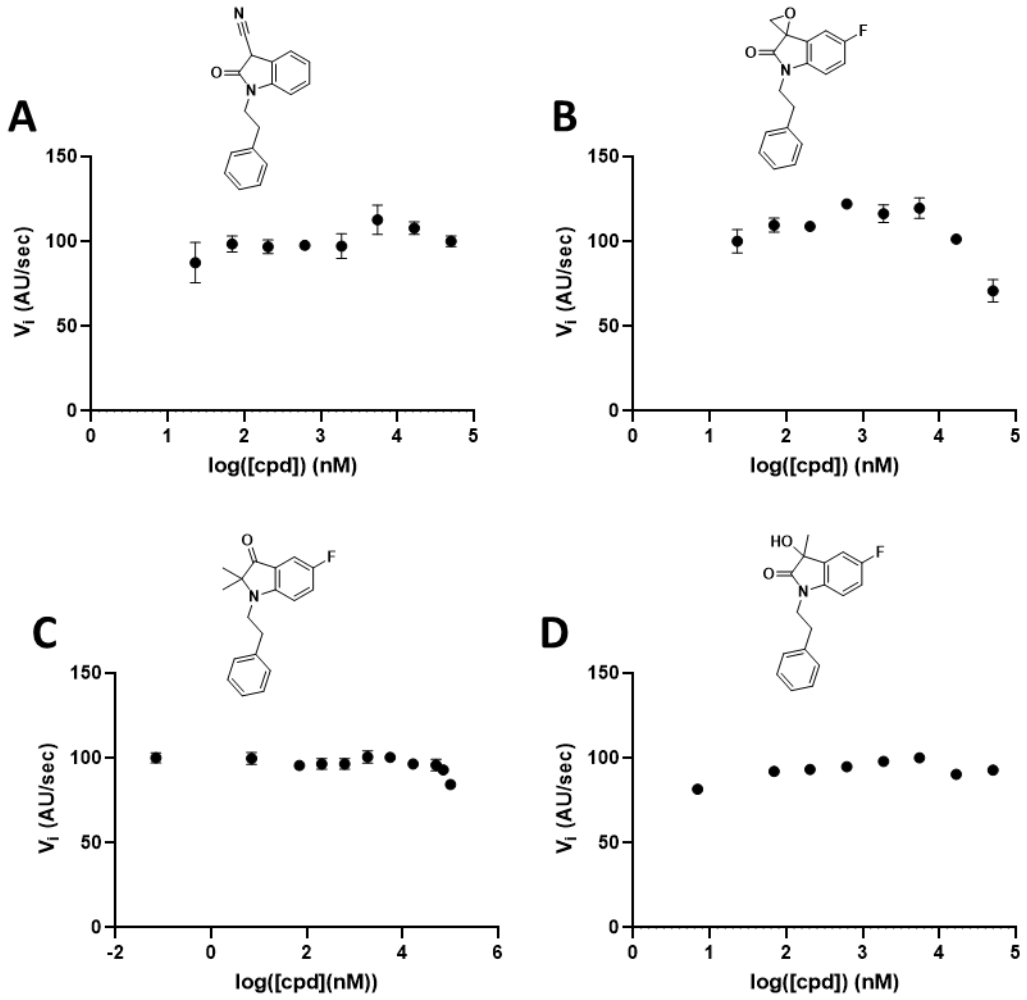


Figure S8. DiFMUAc assay evaluation of novel scaffolds. (**A**) Compound **22**, N = 2. (**B**) Compound **23**, N = 2. (**C**) Compound **24**, N = 2. (**D**) Compound **25**, N = 2.

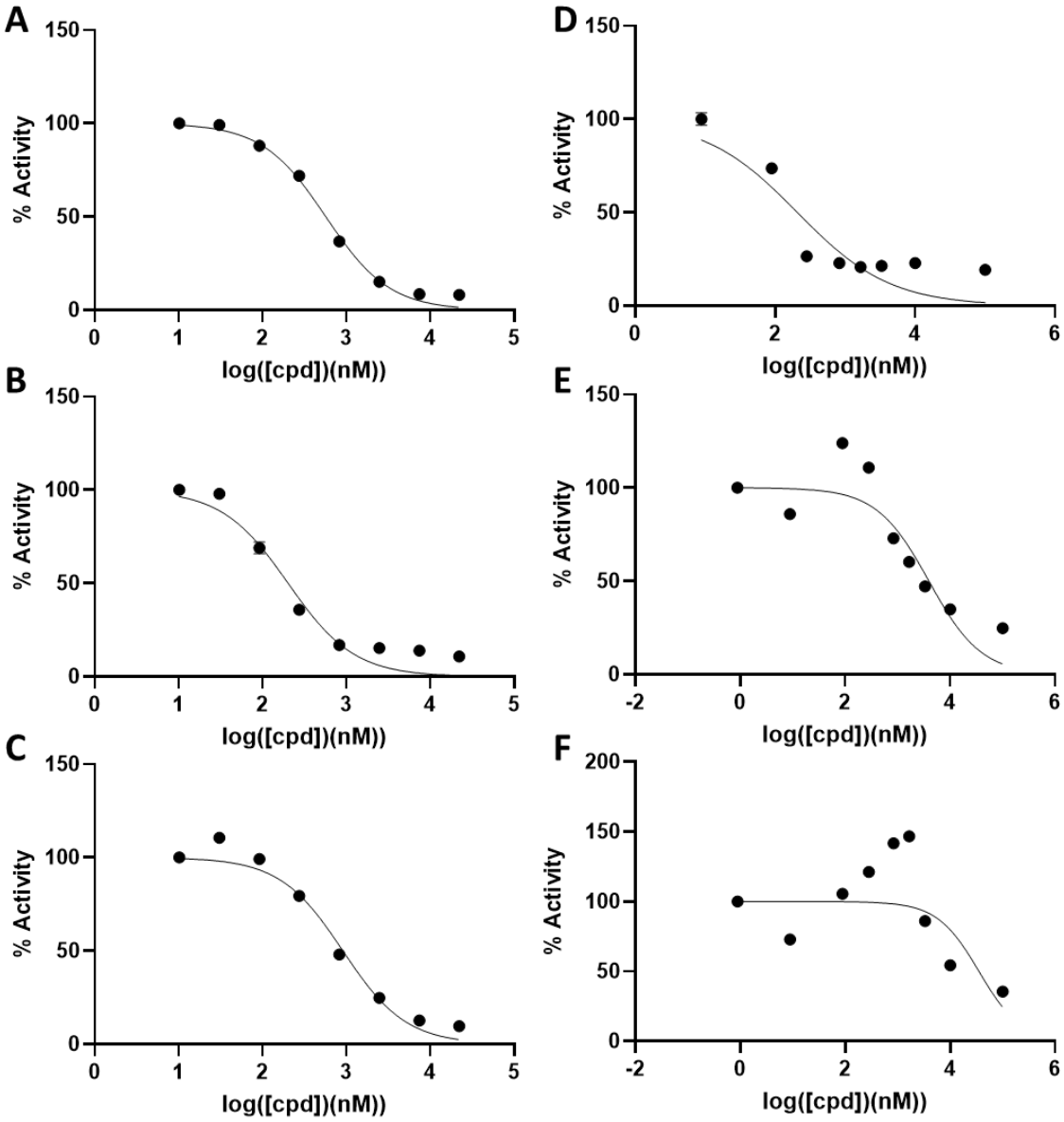


Figure S9. Time-dependent evaluation of non-isatin compound hits. Compounds preincubated with DJ-1 for 1 hr (A-C) or 1 hr 15 min (D-F) before addition of DiFMUAc substrate and fluorescence collection. (**A**) Compound **8**, N = 2. (**B**) Compound **9**, N = 2. (**C**) Compound **10**, N = 2. (**D**) Compound **26**, N = 1. (**E**) Compound **27**, N = 1. (**F**) Compound **28**, N = 1.

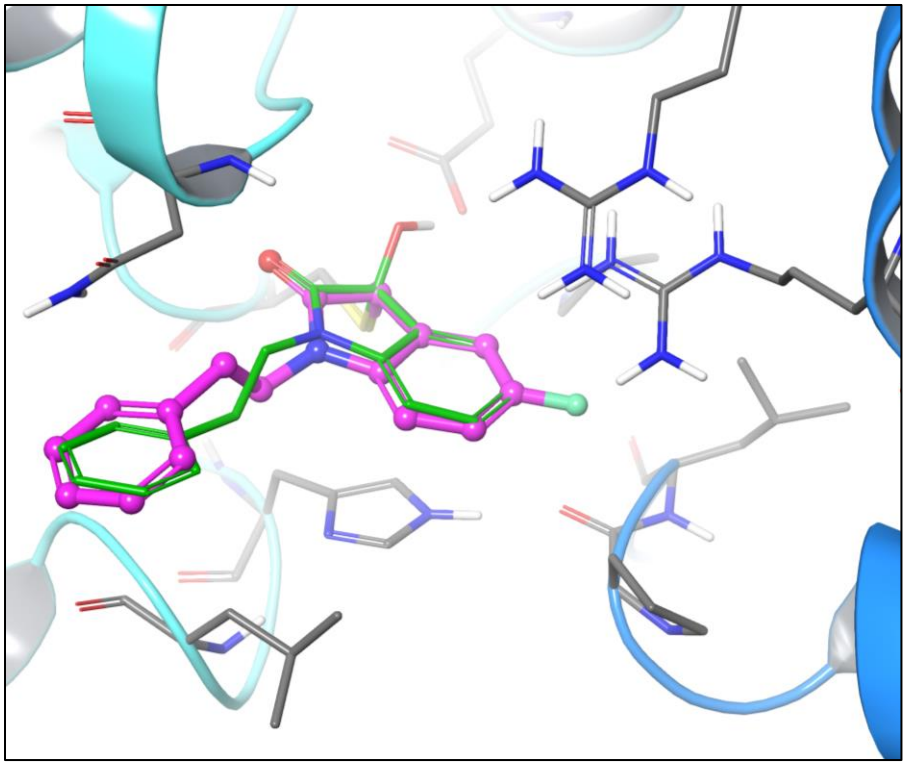


Figure S10. Covalent docking poses of non-isatin compound **26** (magenta) against **1** (green).

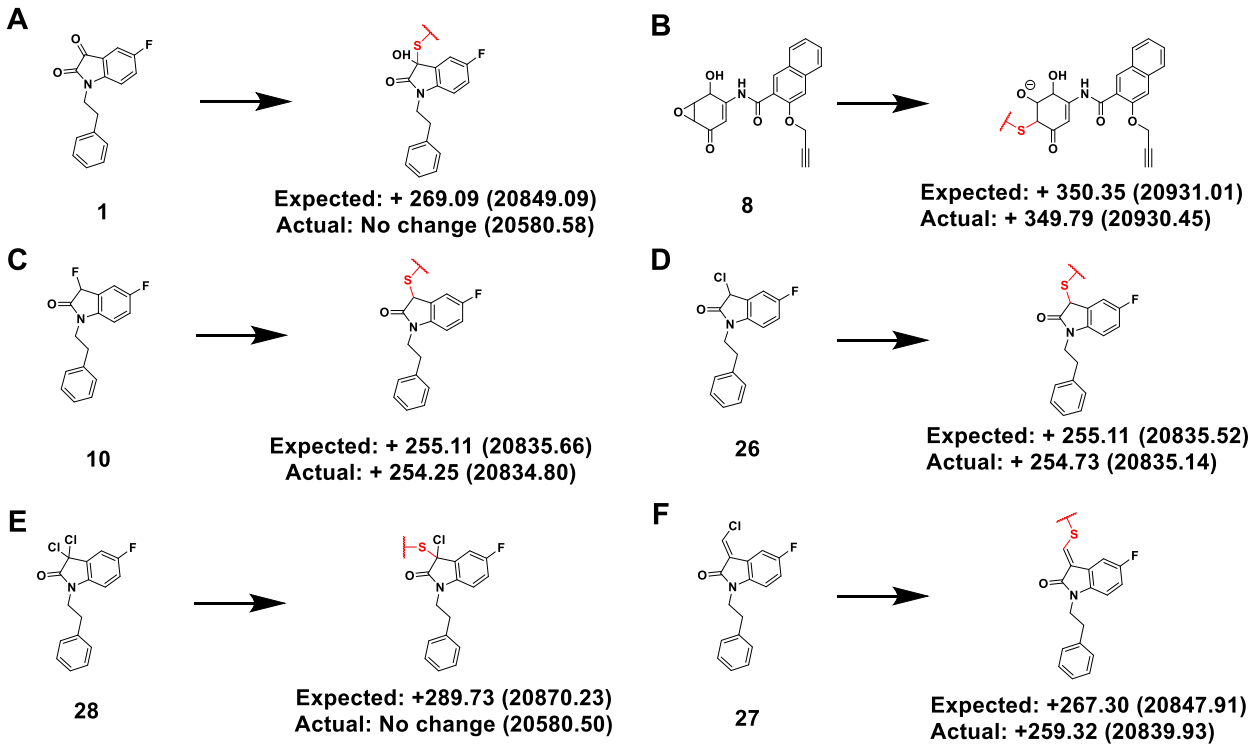


Figure S11. Evaluation of compound irreversibility, expected attachment to DJ-1 (Cys106) shown in red. (**A**) Negative control isatin, **1**. (**B**) Positive control epoxycyclohexenone, **8**. (**C**) Compound **10**. (**D**) Compound **26**. (**E**) Compound **28**. (**F**) Compound **27**.

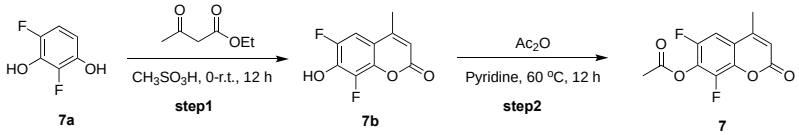
B. Materials and Methods

a. Reagents and Instrumentation

Reagents were obtained from Aldrich Chemical, Acros Organics, or Fisher Scientific unless otherwise stated and used without further purification. NMR spectra were recorded on Bruker AVIII 400MHz instruments and were calibrated using residual undeuterated solvent as an internal reference ($CHCl_3$ at 7.26 ppm 1H NMR, 77.16 ppm 13C NMR; CH_3OH at 3.31 ppm 1H NMR, 49.0 ppm 13C NMR; CH_3CN at 1.94 ppm 1 H NMR, 1.32 ppm 13C NMR). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t =triplet, q = quartet, sept= septet, m = multiplet, br = broad). Biochemical reagents were purchased from Fisher Scientific or Sigma Aldrich Corporation. Colorimetric and fluorescence data was collected on a SPECTRAmax M series microplate reader through the SoftMax Pro software. Semi-Preparative HPLC was performed using the following general HPLC Column: Phenomenex Luna C18 75*40mm*3um; Waters X-bridge C18 150*40mm*10um; Gemini NX-C18 75*40mm*3um; unless otherwise noted. Size exclusion chromatography was performed on an AKTA FPLC system from GE Healthcare equipped with a P-920 pump and UPC-900 monitor. LCMS were recorded on Agilent 1200, 6110B and 6120. General Method for 5_95AB_6min-220: The gradient was 5%B in 0.40 min and 5-95% B at 0.4-3.0min, hold on 95% B for 1.00 min, and then 95-5%B in 0.01min, the flow rate was 1.0 ml/min. (Mobile phase A was 0.04% Trifluoroacetic Acid in water, mobile phase B was 0.02% Trifluoroacetic Acid in acetonitrile). The column used for chromatography was a Kinetex C18 50*2.1mm column (5um particles). Detection methods are diode array (DAD) as well as positive electrospray ionization.MS range was 100-1000. Sephacryl S-200 columns were obtained from GE Healthcare. Graphpad Prism was used for the analysis and representation of data. Compounds **1**-**5** were synthesized according to previously published protocols (doi:10.1021/acschembio.8b00701) and compound **6** was purchased commercially. The remaining compounds (**7**-**28**) were synthesized as described below.

b. Synthesis of Compounds 7-28

Synthesis of 7



Step 1:

6,8-difluoro-7-hydroxy-4-methyl-chromen-2-one (7b)

2,4-difluorobenzene-1,3-diol (500 mg, 3.42 mmol, 1 *eq*) and ethyl 3-oxobutanoate (445 mg, 3.42 mmol, 432 uL, 1 *eq*) was add to CH_3SO_3H (5 mL) at 0 °C. The reaction was warmed to 25 °C and stirred for 12 h. The reaction was poured into H_2O (70 mL) and stirred for 20 min. The mixture was filtered and the filter cake was washed with H_2O (30 mL). The filter cake was concentrated in vacuo to obtain 6,8-difluoro-7-hydroxy-4-methyl-chromen-2-one (650 mg, crude) as a light yellow solid.

^{1}H NMR: (400 MHz, DMSO-d6) δ 11.57-11.44 (brs, 1H), 7.51-7.48 (m, 1H), 6.31 (s, 1H), 2.36 (s, 3H).

Step 2:

(6,8-difluoro-4-methyl-2-oxo-chromen-7-yl) acetate (7)

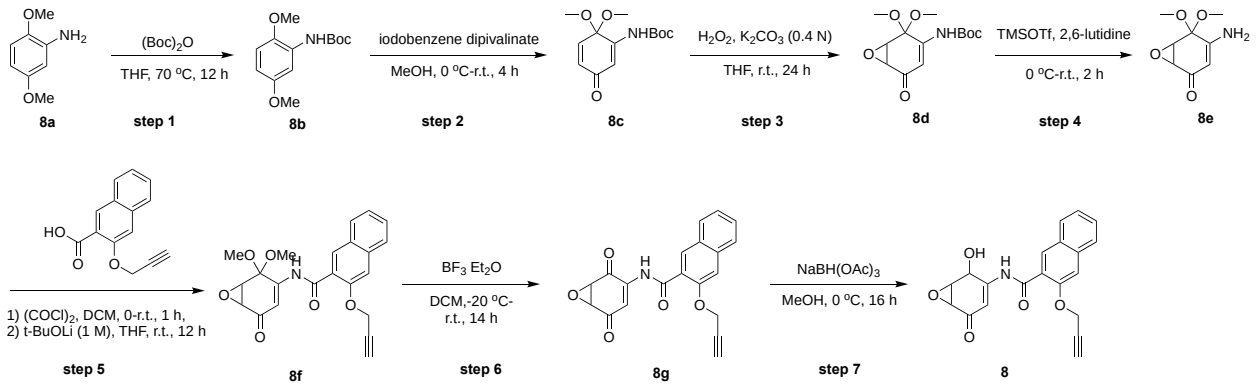
To a solution of 6,8-difluoro-7-hydroxy-4-methyl-chromen-2-one (200 mg, 942 umol, 1 *eq*) in Pyridine (5 mL) was added Ac_2O (144 mg, 1.41 mmol, 132 uL, 1.5 *eq*). Then the reaction was heated to 60 °C and stirred for 12 h. The reaction was concentrated in vacuo to get a residue. The residue was purified by prep-HPLC (HCl) (column: Luna C18 150*25 5u; mobile phase: [water (0.04%HCl) - ACN]; 30%-65%,10 min) to obtain (6,8-difluoro-4

-methyl-2-oxo-chromen-7-yl) acetate (70 mg) as a white solid.

LCMS: ($M+H^+$): 255.0 @ 2.687 min (5-95% ACN in H_2O, 4.5 min).

^{1}H NMR: (400MHz, $CDCl_3$) δ 7.21-7.18 (m, 1H), 6.37 (s, 1H), 2.43-2.42 (m, 6H).

Synthesis of 8



Step 1:

Tert-butyl N-(2,5-dimethoxyphenyl)carbamate (8b)

To a solution of 2,5-dimethoxyaniline (10.0 g, 65.3 mmol, 1 eq) in THF (100 mL) was added $(Boc)_2O$ (18.5 g, 84.9 mmol, 19.5 mL, 1.3 eq). The mixture was stirred at 70 °C for 12 hr. The reaction was concentrated in vacuo and the residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=10/1 to 1/1). Compound tert-butyl N-(2,5-dimethoxyphenyl)carbamate (16 g, 97% yield) was obtained as yellow oil.

Step 2:

Tert-butyl N-(6,6-dimethoxy-3-oxo-cyclohexa-1,4-dien-1-yl)carbamate (8c)

To a solution of tert-butyl N-(2,5-dimethoxyphenyl)carbamate (15.0 g, 59.2 mmol, 1 eq) in MeOH (180 mL) was added [acetoxy(phenyl)-iodanyl] acetate (21.0 g, 65.1 mmol, 1.1 eq) at 0 °C. After addition, the mixture was stirred at 25 °C for 4 hr. The mixture was diluted with EtOAc (300 mL) at 20 °C , washed with saturated aq. $NaHCO_3$ (300 mL*3), H_2O (300 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash silica gel chromatography (ISCO®; 220 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethylacetate/Petroleum ethergradient @ 100 mL/min). Compound tert-butyl N-(6,6-dimethoxy-3-oxo-cyclohexa-1,4-dien-1-yl) carbamate (9 g, 56% yield) was obtained as yellow solid.

Step 3:

Tert-butyl N-(5,5-dimethoxy-2-oxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl)carbamate (8d)

To a solution of tert-butyl N-(6,6-dimethoxy-3-oxo-cyclohexa-1,4-dien-1-yl)carbamate (4.50 g, 16.7 mmol, 1 eq) in THF (180 mL) was added H_2O_2 (70.8 g, 625 mmol, 60 mL, 30% purity, 37 eq) and K_2CO_3 (0.4 M, 83.6 mL, 2 eq). The mixture was stirred at 25 °C for 18 hr. The mixture was diluted with EtOAc (400 mL) at 20 °C, washed with H_2O (200 mL), saturated aq. Na_2SO_3 (400 mL*2), brine (400 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash silica gel chromatography (ISCO®; 120 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethylacetate/Petroleum ether gradient @ 100 mL/min). Compound tert-butyl N-(5,5-dimethoxy-2-oxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl)carbamate (8.5 g, 58.3% purity, crude) was obtained as white solid.

Step 4:

4-amino-5,5-dimethoxy-7-oxabicyclo[4.1.0]hept-3-en-2-one (8e)

To a solution of tert-butyl N-(5,5-dimethoxy-2-oxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl) carbamate (1.00 g, 3.51 mmol, 1 *eq*) in DCM (10 mL) was added TFA (4.85 g, 42.5 mmol, 3.15 mL, 12 *eq*) drop-wise slowly. The mixture was stirred at 25 °C for 2 hr. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with EtOAc (30 mL), $NaHCO_3$ was added to the solution and stirred for 0.5 hr and then filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~10% DCM/MeOH @ 45 mL/min). Compound 4-amino-5,5-dimethoxy-7-oxabicyclo[4.1.0]hept-3-en-2-one (0.17 g, crude) was obtained as brown solid.

Step 5:

N-(5,5-dimethoxy-2-oxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl)-3-prop-2-ynoxy-naphthalene-2-carboxamide (8f)

To a solution of 3-prop-2-ynoxynaphthalene-2-carboxylic acid (0.36 g, 1.59 mmol, 1 eq) in DCM (5 mL) was added $(COCl)_2$ (404 mg, 3.18 mmol, 279 uL, 2 eq) drop-wise slowly and a drop of DMF at 0 °C. After addition, the mixture was stirred at 25 °C for 2 hr. The reaction mixture was concentrated directly under reduced pressure and the residue was dissolved in THF (10 mL). To a solution of 4-amino-5,5-dimethoxy-7-oxabicyclo [4.1.0]hept-3-en-2-one (0.29 g, 1.57 mmol, 1 eq) in THF (10 mL) was added t-BuOLi (1 M, 4.70 mL, 3 eq) at 0 °C and stirred for 0.5 hr. And then the previous solution was added over 0.5 hr at 0 °C. The mixture was stirred at 25 °C for 12 hr. The mixture was quenched by addition of H_2O (25 mL) at 0 °C, extracted with EtOAc (20 mL*2). The combined organic layers were washed with H_2O (20 mL), brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ether gradient @ 50 mL/min). Compound N-(5,5-dimethoxy-2-oxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl)-3-prop-2-ynoxy-naphthalene-2-carboxamide (0.31 g, 66.4% purity) was obtained as yellow solid.

Step 6:

N-(2,5-dioxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl)-3-prop-2-ynoxy-naphthalene-2-carboxamide (8g)

To a solution of N-(5,5-dimethoxy-2-oxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl)-3-prop-2- ynoxy-naphthalene-2-carboxamide (0.26 g, 439 umol, 1 *eq*) in DCM (6 mL) was added $BF_3.Et_2O$ (187 mg, 1.32 mmol, 162 uL, 3 *eq*) drop-wise slowly at -20 °C. After addition, the mixture was stirred at 25 °C for 8 hr. The mixture was quenched by addition of H_2O (10 mL) at 0 °C, extracted with DCM (15 mL*2). The combined organic layers were washed with brine (20 mL), dried over Na2SO4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by flash silica gel chromatography (ISCO®; 12 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ether gradient @ 36 mL/min). Compound N-(2,5-dioxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl)-3-prop-2-ynoxy-naphthalene -2-carboxamide (0.085 g) was obtained as light-yellow solid.

^{1}H NMR: ($CDCl_3$, 400 MHz) δ 10.72 (brs, 1H), 8.78 (s, 1H), 7.93-7.95 (m, 1H), 7.79-7.82 (m, 2H), 7.60-7.62 (m, 1H), 7.44-7.49 (m, 2H), 5.12 (s, 2H), 3.96-3.97 (m, 1H), 3.87-3.88 (m, 1H), 2.68 (s, 1 H).

Step 7:

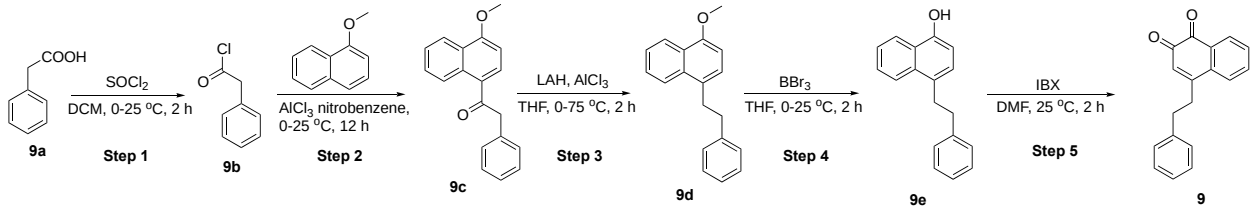
N-(2-hydroxy-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-3-yl)-3-prop-2-ynoxy-naphthalene-2-carboxamide (8)

To a solution of N-(2,5-dioxo-7-oxabicyclo[4.1.0]hept-3-en-4-yl)-3-prop-2-ynoxy- naphthalene-2-carboxamide (0.075 g, 216 umol, 1 *eq*) in MeOH (3 mL)/THF (2 mL) was added $NaBH(OAc)_3$ (68.7 mg, 324 umol, 1.5 *eq*) at 0 °C. The mixture was stirred at 0 °C for 12 hr and warmed to 25 °C for 8 hr. Then the mixture was cooled to 0 °C and additional $NaBH(OAc)_3$ (45.8 mg, 216 umol, 1 *eq*) was added. The mixture was stirred at 0 °C for additional 10 hr. The mixture was quenched by addition H_2O (15 mL) at 0 °C, extracted with EtOAc (20 mL*2). The combined organic layers were washed with H_2O (20 mL), brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-HPLC (FA condition; column: Phenomenex Luna C18 200*40mm*10um; mobile phase: [water(0.2%FA)-ACN]; 40%-80%, 10 min). Compound N-(2-hydroxy-5-oxo-7-oxabicyclo[4.1.0]hept-3-en-3-yl)-3-prop-2-ynoxy-naphthalene-2-carboxamide (34.2 mg) was obtained as white solid.

^{1}H NMR: (DMSO-d6, 400 MHz) δ 10.58 (brs, 1H), 8.63 (s, 1H), 8.08 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.70 (s, 1H), 7.61-7.65 (m, 1H), 7.47-7.51 (m, 1H), 6.91 (s, 1H), 6.78-6.80 (m, 1H), 5.17 (s, 2H), 4.87 (d, *J* = 7.2 Hz 1H), 3.87-3.89 (m, 1H), 3.69-.70 (m, 1H), 3.45-3.46 (m, 1 H).

LCMS: ($M+H^+$): 282.1 @ 2.925 min, WUXIAB10.M, 4.5 min).

Synthesis of 9



Step 1 :

2-phenylacetyl chloride (9b)

To a solution of 2-phenylacetic acid (5.00 g, 36.7 mmol, 4.63 mL, 1 *eq*) in DCM (50 mL) was added thionyl chloride (16.4 g, 138 mmol, 10.0 mL, 3.75 *eq*) at 0 °C. Then the mixture was stirred at 25 °C for 2 h. The mixture was

concentrated under reduced pressure to give 2-phenylacetyl chloride (6 g, crude) as yellow liquid. The crude product will be used directly in the next step.

Step 2：

1-(4-methoxy-1-naphthyl)-2-phenyl-ethanone (9c)

To a solution of 1-methoxynaphthalene (2.00 g, 12.6 mmol, 1.83 mL, 1 *eq*) and 2-phenylacetyl chloride (1.95 g, 12.6 mmol, 1.68 mL, 1 *eq*) in nitrobenzene (17 mL) was added $AlCl_3$ (1.69 g, 12.6 mmol, 691 uL, 1 *eq*) at 0 °C. Then the solution was stirred at 25 °C for 12 h under N_2. The reaction mixture was diluted with H_2O (200 mL) and extracted with EtOAc (100 mL*3). The organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 10~50% Ethyl acetate/Petroleum ether gradient @100 mL/min) to give 1-(4-methoxy-1-napht

hyl)-2-phenyl-ethanone (2.3 g, 66% yield) as green solid.

^{1}H NMR: (400MHz, DMSO-d6) δ 8.71 (d, *J* = 8.4 Hz, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.21 (d, *J* = 7.7 Hz, 1H), 7.60 (ddd, *J* = 1.4, 6.9, 8.4 Hz, 1H), 7.56-7.50 (m, 1H), 7.28 (d, *J* = 4.4 Hz, 4H), 7.19 (qd, *J* = 4.2, 8.5 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 4.41 (s, 2H), 4.04 (s, 3H).

Step 3：

1-methoxy-4-(2-phenylethyl)naphthalene (9d)

To a solution of $LiAlH_4$ (395 mg, 10.4 mmol, 1.25 *eq*) in THF (25 mL) was added $AlCl_3$ (2.50 g, 18.7 mmol, 1.02 mL, 2.25 *eq*) at 0 °C. Then the solution was added 1-(4-methoxy-1-naphthyl)-2-phenyl-ethanone (2.30 g, 8.32 mmol, 1 *eq*) in THF (12 mL) at 0 °C. Then the mixture was stirred at 75 °C for 2 h. The reaction mixture was diluted with water (100 mL) and the mixture was adjusted to pH=3 with aq HCl solution (2M). Then the mixture was extracted with EtOAc (50 mL*3) and the organic layer was washed with brine (50 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 200 g SepaFlash® Silica Flash Column, Eluent of 0% Ethyl acetate/Petroleum ether gradient @ 75 mL/min) to give 1-methoxy-4-(2-phenylethyl)naphthalene (1.90 g, 87% yield) as yellow solid.

^{1}H NMR: (400MHz, DMSO-d6) δ 8.22 (dd, *J* = 0.7, 8.2 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.60 (ddd, *J* = 1.3, 6.9, 8.3 Hz, 1H), 7.55-7.50 (m, 1H), 7.33-7.24 (m, 5H), 7.21 (dt, *J* = 2.7, 5.9 Hz, 1H), 6.86 (d, *J* = 7.9 Hz, 1H), 3.94 (s, 3H), 3.28-3.22 (m, 2H), 2.98-2.92 (m, 2H).

Step 4:

4-(2-phenylethyl)naphthalen-1-ol (9e)

To a solution of 1-methoxy-4-(2-phenylethyl)naphthalene (1.90 g, 7.24 mmol, 1 *eq*) in DCM (20 mL) was added BBr_3 (5.44 g, 21.7 mmol, 2.09 mL, 3 *eq*) at 0 °C. Then the solution was stirred at 25 °C for 2 h. MeOH (2 mL) was added into the mixture dropwise under N_2 and the mixture was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~10% Ethyl acetate/Petroleum ether gradient @ 75 mL/min) to give 4-(2-phenylethyl)naphthalen-1-ol (1.3 g, 65% yield, 90% purity) as yellow solid.

^{1}H NMR: (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 8.17 (d, *J* = 8.3 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 1H), 7.57-7.50 (m, 1H), 7.48-7.42 (m, 1H), 7.32-7.24 (m, 4H), 7.19 (dt, *J* = 2.6, 6.0 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 6.76 (d, *J* = 7.8 Hz, 1H), 3.23-3.16 (m, 2H), 2.96-2.88 (m, 2H).

Step 5 :

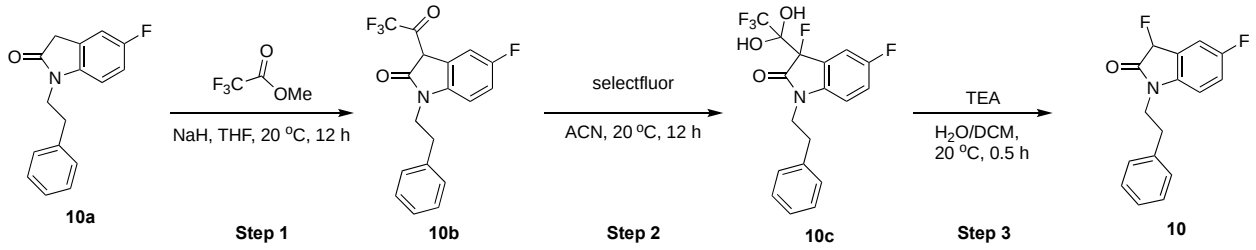
4-(2-phenylethyl)naphthalene-1,2-dione (9)

To a solution of 4-(2-phenylethyl)naphthalen-1-ol (300 mg, 1.21 mmol, 1 *eq*) in DMF (3 mL) was added IBX (372 mg, 1.33 mmol, 1.1 *eq*). Then the solution was stirred at 25 °C for 2 h. The reaction mixture was diluted with H_2O (80 mL) and extracted with EtOAc (100 mL*3), the organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered and the filter cake was concentrated under reduced pressure. The mixture was purified by prep-HPLC (column: Welch Xtimate C18 150*25mm*5um; mobile phase: [water(0.04%HCl)-ACN]; 50%-80%,10 min) followed by re-purification by prep-HPLC (column: Nano-micro Kromasil C18 80*25mm 3um;mobile phase: [water(0.04%HCl)-ACN]; 55%-62%,7min) to give 4-(2-phenylethyl)naphthalene-1,2-dione (44.1 mg) as yellow solid.

LCMS: ($M+H^+$): 263.2 @ 3.024 min (5-95% ACN in H_2O, 4.5 min).

^{1}H NMR: (400MHz, DMSO-d6) δ 8.02-7.97 (m, 1H), 7.84-7.76 (m, 2H), 7.63-7.59 (m, 1H), 7.36-7.29 (m, 4H), 7.24-7.19 (m, 1H), 6.35 (s, 1H), 3.07-3.00 (m, 2H), 2.97-2.91 (m, 2H).

Synthesis of 10



Step 1:

(3E)-5-fluoro-1-(2-phenylethyl)-3-(2,2,2-trifluoro-1-hydroxy-ethylidene)indolin-2-one (10b)

To a stirred solution of NaH (94.0 mg, 2.35 mmol, 60% purity, 2 *eq*) in THF (5 mL) was added drop wise the solution of CF_3CO_2Et (175 mg, 1.23 mmol, 170 uL, 1.05 *eq*) and 5-fluoro-1-(2-phenylethyl)indolin-2-one (0.3 g, 1.18 mmol, 1 *eq*) in THF (2 mL), the mixture stirred for 12 h at 20°C. The mixture was diluted with 1 M HCl solution (PH = 3) and extracted with EtOAc (10 mL*3), the combined organic phase was washed with brine (10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 20/1 to 1/1). (3E)-5-fluoro-1-(2-phenylethyl)-3-(2,2,2-trifluoro-1-hydroxy-ethylidene)indolin-2-one (400 mg, crude) was obtained as yellow solid. The crude product was used into the next step without further purification.

Step 2:

3,5-difluoro-1-(2-phenylethyl)-3-(2,2,2-trifluoro-1,1-dihydroxy-ethyl)indolin-2-one (10c)

A solution of (3E)-5-fluoro-1-(2-phenylethyl)-3-(2,2,2-trifluoro-1-hydroxy-ethylidene) indolin-2-one (400 mg, 1.14 mmol, 1 *eq*) in ACN (6 mL) was treated with 1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane;ditetrafluoroborate (484 mg, 1.37 mmol, 1.2 *eq*) at 20 °C for 12 h. The mixture was poured into water (10 mL) and extracted with ethyl acetate (10 mL * 3). The combined organic phase was washed with brine (20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. 3,5-difluoro-1-(2-phenylethyl)-3-

(2,2,2-trifluoro-1,1-dihydroxy-ethyl)indolin-2-one (400 mg, crude) was obtained as yellow solid. The crude product was used into the next step without further purification.

Step 3:

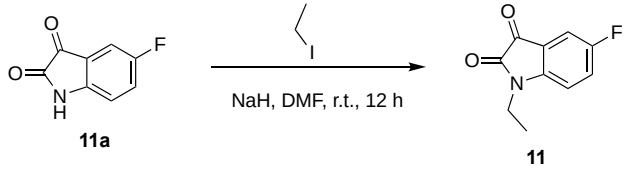
3,5-difluoro-1-(2-phenylethyl)indolin-2-one (10)

To a solution of 3,5-difluoro-1-(2-phenylethyl)-3-(2,2,2-trifluoro-1,1-dihydroxy-ethyl) indolin-2-one (0.4 g, 1.03 mmol, 1 *eq*) in DCM (3 mL) and H_2O (0.3 mL) was added TEA (313.52 mg, 3.10 mmol, 431 uL, 3 *eq*) at 20 °C and the mixture was stirred for 0.5 h. The reaction mixture was concentrated under reduced pressure to remove solvents. The residue was purified by prep-HPLC (neutral condition). 3,5-difluoro-1-(2-phenylethyl) indolin-2-one (114 mg, 40% yield) was obtained as light yellow solid.

LCMS: (M-1): 272.1 @ 2.992min (5-95 % ACN in H_2O, 4.5 min).

^{1}H NMR: (400 MHz, DMSO-d_6) δ 7.44 (br d, *J* = 7.70 Hz, 1H), 7.32-7.17 (m, 6H), 7.10 (dd, *J* = 8.50, 3.97 Hz, 1H), 6.04-5.79 (m, 1H), 3.92-3.80 (m, 2H), 2.87 (t, *J* = 7.40 Hz, 2H).

Synthesis of 11



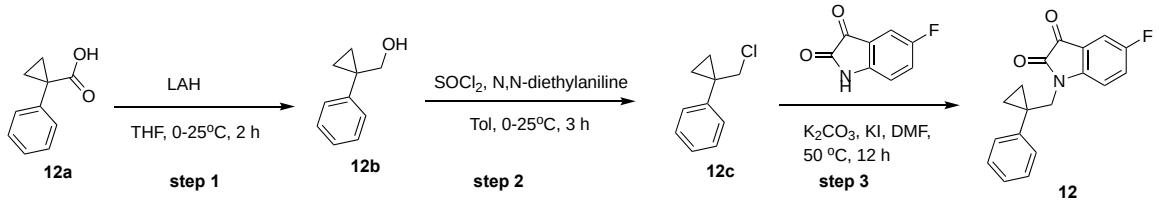
1-ethyl-5-fluoro-indoline-2,3-dione (11)

To a solution of 5-fluoroindoline-2,3-dione (0.2 g, 1.2 mmol, 1 *eq*) in DMF (2 mL) was added NaH (72.6 mg, 1.81 mmol, 60% purity, 1.5 *eq*) at 0 °C . The mixture was stirred at 0 °C for 0.5 hr and then iodoethane (283 mg, 1.81 mmol, 145 uL, 1.5 *eq*) was added. The mixture was warmed to 15 °C for 11.5 hours. The mixture was quenched by addition of ice-H_2O (5 mL) at 0 °C, extracted with EtOAc (10 mL*2). The combined organic layers were washed with H_2O (20 mL*2), brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by prep-HPLC (HPLC: neutral condition; column: Welch Xtimate C18 250*50mm*10um; mobile phase: [water(10mM NH4HCO3)-ACN]; 25%-55%,10 min) to afford the compound 1-ethyl-5-fluoro-indoline-2,3-dione (0.175 g, 73% yield) as red solid.

LCMS: ($M+H^+$): 194.1 @ 3.022 min, WUXIAB10.M, 4.5 min).

^{1}H NMR: (DMSO-d6, 400 MHz) δ 7.52-7.53 (m, 1H), 7.43-7.45 (m, 1H), 7.20-7.23 (m, 1H), 3.67-3.72 (m, 2H),1.15-1.18 (m, 3H).

Synthesis of 12



Step 1:

(1-phenylcyclopropyl)methanol (12b)

To a solution of 1-phenylcyclopropanecarboxylic acid (2 g, 12.3 mmol, 1 *eq*) in THF (20 mL) was added LAH (562 mg, 14.8 mmol, 1.2 *eq*) at 0°C. Then the reaction was warmed to 25°C and stirred for 2 hours at 25°C. H_2O (0.5 mL), 15%NaOH (0.5 mL), H_2O (1.5 mL) was added at 0°C and stirred for 30 min. The mixture was filtered and washed with DCM (20 mL). The filtrate was concentrated in vacuum to obtain (1-phenylcyclopropyl)methanol (2 g, crude) as light yellow oil.

^{1}H NMR: (400MHz, $CDCl_3$) δ 7.40-7.25 (m, 5H), 3.69 (s, 2H), 0.94-0.86 (m, 4H).

Step 2:

[1-(chloromethyl)cyclopropyl]benzene (12c)

To a solution of (1-phenylcyclopropyl)methanol (1 g, 6.8 mmol, 1 *eq*) in Toluene (10 mL) was added N,N-diethylaniline (1.21 g, 8.10 mmol, 1.30 mL, 1.2 *eq*), $SOCl_2$ (1.61 g, 13.5 mmol, 979 uL, 2 *eq*) at 0°C and stirred for 1 hour. The mixture was washed with HCl (10 mL, 4M), NaOH (10 mL, 15%), brine (10 mL). Then the organic layer was concentrated in vacuum to get a residue. The residue was purified by column (SiO2) with PE/EA=1:0 to 20:1 to obtain [1-(chloromethyl)cyclopropyl]benzene (500 mg) as light yellow oil. The material was directory used in the next step.

Step 3:

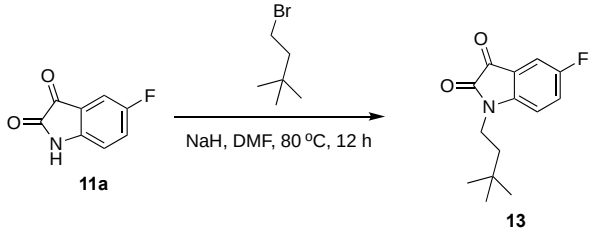
5-fluoro-1-[(1-phenylcyclopropyl)methyl]indoline-2,3-dione (12)

To a solution of [1-(chloromethyl)cyclopropyl]benzene (400 mg, 1.20 mmol, 1 *eq*) in DMF (10 mL) was added K_2CO_3 (332 mg, 2.40 mmol, 2 *eq*), KI (199 mg, 1.20 mmol, 1 *eq*), 5-fluoroindoline-2,3-dione (198 mg, 1.20 mmol, 1 *eq*). Then the reaction was heated to 50°C and stirred for 12 hours. The mixture was poured into H_2O (30 mL), extracted with EA (20mL*3). The combined organic layer was washed with brine (20 mL *2), dried with Na_2SO_4, filtered and concentrated in vacuo to get a residue**.** The residue was purified by prep -HPLC (HCl) (column: Welch Xtimate C18 250*50mm*10um;mobile phase: [water(0.05%HCl)-ACN]; 45%-65%,10min) to obtain 5-fluoro-1-[(1-phenylcyclopropyl)methyl]indoline-2,3-dione (160 mg) as yellow solid.

^{1}H NMR: (400MHz, DMSO-*d6*) δ 7.37-7.32 (m, 4H), 7.28-7.16 (m, 3H), 6.59-6.57 (m, 1H), 3.87 (s, 2H), 1.04 (s, 2H), 0.79 (s, 2H).

LCMS: ($M+H^+$): 296.0 @ 3.102 min (5-95% ACN in H_2O, 4.5 min).

Synthesis of 13



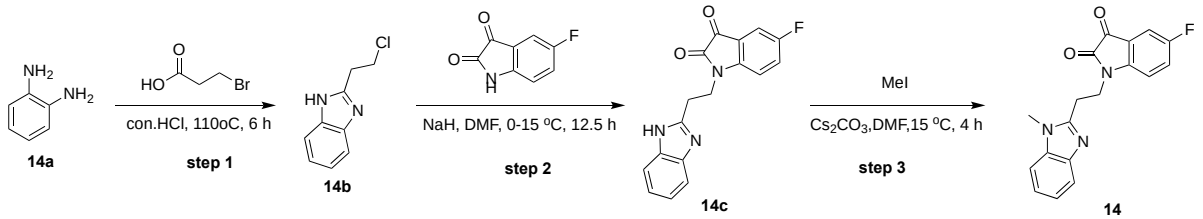
1-(3,3-dimethylbutyl)-5-fluoro-indoline-2,3-dione (13)

To a solution of 5-fluoroindoline-2,3-dione (0.2 g, 1.2 mmol, 1 *eq*) in DMF (2 mL) was added Cs_2CO_3 (1.18 g, 3.63 mmol, 3 *eq*) and1-bromo-3,3-dimethyl-butane (240 mg, 1.45 mmol, 1.2 *eq*). The mixture was heated to 80 °C and stirred for 12 hours. The mixture was quenched by addition of H_2O (10 mL) at 0 °C , extracted with EtOAc (10 mL*2). The combined organic layers were washed with H_2O (20 mL*2), brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by prep-HPLC (HCl condition; column: Welch Xtimate C18 250*50mm*10um; mobile phase: [water(0.05%HCl)-ACN]; 40%-70%,10min) to afford the compound 1-(3,3-dimethylbutyl)-5-fluoro-indoline-2,3-dione (9.9 mg, 3.1% yield).

LCMS: (M+H+): 250.1 @ 3.114 min, WUXIAB10.M, 4.5 min).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ 7.29-7.33 (m, 2H), 6.81-6.84 (m, 1H), 3.71-3.75 (m, 2H), 1.56-1.59 (m, 2H), 1.03(s, 9H).

Synthesis of 14



Step 1 :

2-(2-chloroethyl)-1H-benzimidazole (14b)

A mixture of benzene-1,2-diamine (3 g, 28 mmol, 1 *eq*) and 3-bromopropanoic acid (8.49 g, 55.5 mmol, 5.73 mL, 2 *eq*) in conc.HCl (150 mL) was stirred at 110 °C for 6 hr. The solution was adjusted with NaOH (solid) to pH =7 and extracted with 2-methyltetrahydrofuran (70 mL*5). The organic layer was washed with brine and concentrated to give the crude residue. The residue was purified by MPLC (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 50

~100% Ethyl Acetate/Petroleum ether and 0~20% MeOH/EtOAc gradient @ 100 mL/min) to afford crude 2-(2-chloroethyl)- 1H-benzimidazole and 2-(1H-benzo[d]imidazol-2-yl)ethanol (1.3 g) as orange solid. And the crude material was used into next step directly.

Step 2 :

1-[2-(1H-benzimidazol-2-yl)ethyl]-5-fluoro-indoline-2,3-dione (14c)

To a solution of 5-fluoroindoline-2,3-dione (450 mg, 2.73 mmol, 1 *eq*) in DMF (5 mL) was added NaH (218 mg, 5.45 mmol, 60% purity, 2 *eq*) at 0 °C. The reaction was stirred at 0 °C for 0.5 h. A solution of 2-(2-chloroethyl)-1H-benzimidazole (747 mg, 4.09 mmol, 1.5 *eq*) in DMF (5 mL) was added. The resulting mixture was stirred at 15 °C for 12 h. The reaction was quenched with NH_4Cl (sat., aq., 20 mL) and extracted with 2-Me THF (50 mL*5). The aqueous was concentrated to give the residue. The residue was purified by reverse-phase HPLC (HCl condition, MeOH/H_2O) to give crude 1-[2-(1H-benzimidazol-2-yl)ethyl]-5-fluoro-indoline-2,3-dione (20 mg, 1.8% yield, 77% purity) as red solid, which was used in the next step without further purifications.

Step 3:

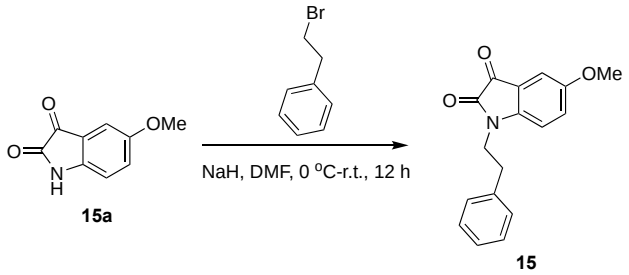
5-fluoro-1-[2-(1-methylbenzimidazol-2-yl)ethyl]indoline-2,3-dione (14)

To a solution of 1-[2-(1H-benzimidazol-2-yl)ethyl]-5-fluoro-indoline-2,3-dione (15 mg, 48.5 umol, 1 *eq*) in DMF (2 mL) was added Cs_2CO_3 (47.4 mg, 146 umol, 3 *eq*) and MeI (10.3 mg, 72.8 umol, 4.53 uL, 1.5 *eq*). The reaction was stirred at 15 °C for 4 hr. The reaction was diluted with MeOH (2 mL) and purified by prep-HPLC (column: Phenomenex Luna C18 200*40mm*10um; mobile phase: [water (0.225%FA)-ACN]; 1%-40%,12min) to give 5-fluoro-1-[2-(1-methylbenzimidazol-2-yl)ethyl]indoline-2,3-dione (6.4 mg) as red solid.

^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.29 (d, *J* = 8.2 Hz, 1H), 8.18 (d, *J* = 8.8 Hz, 1H), 8.00 (dd, *J* = 2.5, 8.0 Hz, 1H), 7.89 (t, *J* = 7.9 Hz, 1H), 7.68-7.61 (m, 2H), 7.52 (dt, *J* = 2.6, 9.0 Hz, 1H), 5.09 (br t, *J* = 7.7 Hz, 2H), 3.61 (s, 3H), 3.00 (br t, *J* = 7.7 Hz, 2H).

LCMS: ($M+H^+$): 324.1 @ 2.129min (10-100%, 4.5 min).

Synthesis of 15



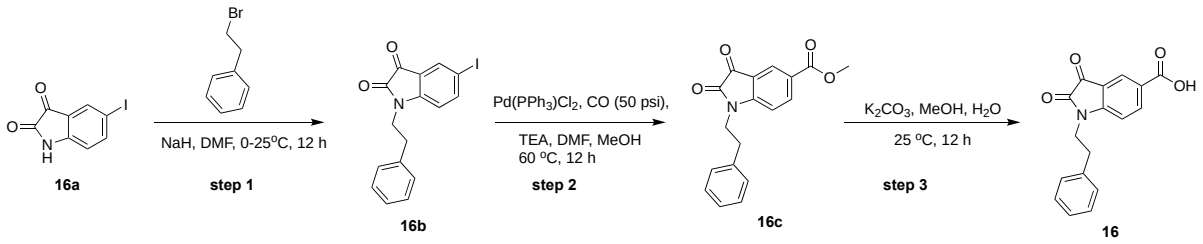
5-methoxy-1-(2-phenylethyl)indoline-2,3-dione (15)

To a solution of 5-methoxyindoline-2,3-dione (0.2 g, 1.13 mmol, 1 *eq*) in DMF (2 mL) was added NaH (67.7 mg, 1.69 mmol, 60% purity, 1.5 *eq*) at 0 °C. The mixture was stirred at 0 °C for 0.5 hr and then 2-bromoethylbenzene (313 mg, 1.69 mmol, 229 uL, 1.5 *eq*) was added. The mixture was warmed to 15 °C for 11.5 hours. The mixture was quenched by addition of ice-H_2O (5 mL) at 0 °C, extracted with EtOAc (10 mL*2). The combined organic layers were washed with H_2O (20 mL*2), brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by prep-HPLC (HCl condition; column: Phenomenex Luna C8 250*50mm*10um; mobile phase: [water(0.05%HCl)-ACN]; 50%-80%,15min) to afford the compound 5-methoxy-1-(2-phenylethyl)indoline-2,3-dione (0.1 g, 31% yield) as red solid.

LCMS: ($M+H^+$): 282.1 @ 2.925 min, WUXIAB10.M, 4.5 min).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ 7.15-7.28 (m, 6H),7.09 (m, 2H), 6.66 (m, 1H), 3.82-3.95 (m, 5H), 3.02 (m, 2H).

Synthesis of 16



Step 1:

5-iodo-1-(2-phenylethyl)indoline-2,3-dione (16b)

To a solution of 5-iodoindoline-2,3-dione (2 g, 7.3 mmol, 1 *eq*) in DMF (20 mL) was added NaH (439 mg, 11.0 mmol, 60% purity, 1.5 *eq*) at 0 °C and stirred for 1hour. 2-bromoethylbenzene (1.63 g, 8.79 mmol, 1.2 *eq*) was added at 0°C. The reaction was warmed to 25 °C and stirred for 11 hours. The mixture was poured into H_2O (60 mL) and extracted with EA (50 mL*3). The combined organic layer was washed with brine (50 mL *2), dried by Na_2SO_4, filtered and concentrated in vacuum to get a residue. The residue was purified by column (SiO_2) with PE: EA = 10: 1 to 2: 1 to obtain 5-iodo-1-(2-phenylethyl)indoline-2,3-dione (1 g, crude) as a yellow solid.

^{1}H NMR: (400MHz, DMSO-*d*6) δ 7.93-7.91 (m, 2H), 7.79-7.78 (m, 1H), 7.27-7.26 (m, 4H), 7.22-7.18 (m, 1H), 7.01-6.99 (m, 1H), 3.88-3.85 (m, 2H), 2.90-2.86 (m, 2H).

Step 2:

Methyl 2,3-dioxo-1-(2-phenylethyl)indoline-5-carboxylate (16c)

To a solution of 5-iodo-1-(2-phenylethyl)indoline-2,3-dione (0.5 g, 1.3 mmol, 1 *eq*) in DMF (5 mL), MeOH (10 mL) was added $Pd(PPh_3)_2Cl_2$ (46.5 mg, 66.3 umol, 0.05 *eq*), TEA (429 mg, 4.24 mmol, 590 uL, 3.2 *eq*) under CO (50psi) at 60 °C and stirred for 12 hours. The mixture was poured into H_2O (50 mL) and extracted by EA (20 mL*3). The combined organic layer was washed by brine (20 mL *3), dried by Na_2SO_4, filtered and concentrated in vacuum to get methyl 2,3-dioxo-1-(2-phenylethyl)indoline-5-carboxylate (400 mg, crude) as a yellow solid.

Step 3:

2,3-dioxo-1-(2-phenylethyl)indoline-5-carboxylic acid (16)

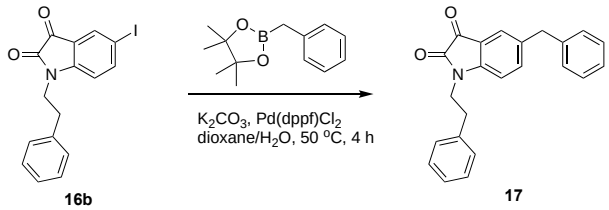
To a solution of methyl 2,3-dioxo-1-(2-phenylethyl)indoline-5-carboxylate (50 mg, 160 umol, 1 *eq*) in H_2O (0.5 mL), MeOH (2 mL) was added K_2CO_3 (335 mg, 2.42 mmol, 15 *eq*) at 25°C and stirred for 12 hours. The mixture was poured into HCl (2N, 10 mL) and extracted with DCM (10 mL *3). The combined organic layer was washed with brine (10 mL), dried with Na_2SO_4, filtered and concentrated in vacuum to get a residue. The residue was purified by prep-HPLC (HCl) (column: Phenomenex Luna C18 150*30mm*5um; mobile phase: [water(0.04%HCl)-ACN]; 30%-65%,10min) to obtain 2,3-dioxo-1-(2-phenylethyl)indoli

ne-5-carboxylic acid (18 mg) as yellow solid.

^{1}H NMR: (400MHz, DMSO- *d*6) δ 13.13-13.06 (m, 1H), 8.15-8.13 (m ,1H), 7.29 (s, 1H), 7.28-7.23 (m ,6H), 3.94-3.91 (m, 2H), 2.93-2.90 (m ,2H).

LCMS: ($M+H^+$): 313.1 @ 1.949 min (5-95% ACN in H_2O, 4.5 min).

Synthesis of 17



5-benzyl-1-(2-phenylethyl)indoline-2,3-dione (17)

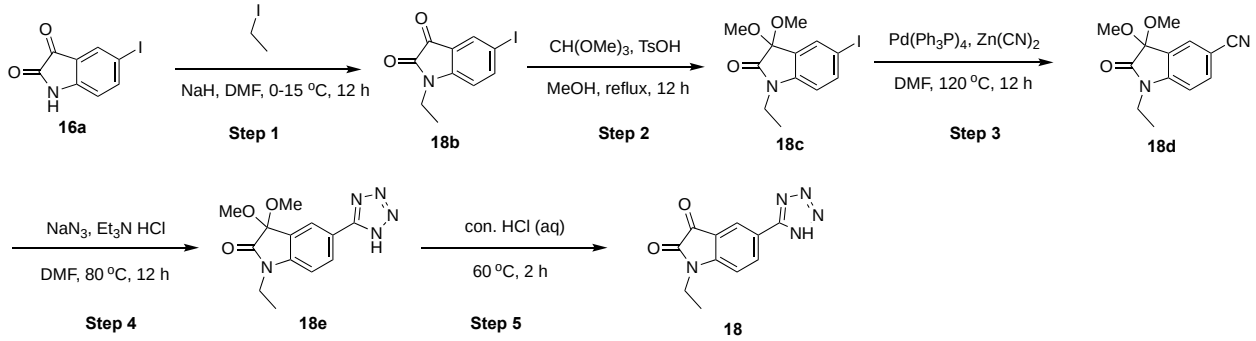
To a solution of 5-iodo-1-(2-phenylethyl)indoline-2,3-dione (400 mg, 1.06 mmol, 1 *eq*) in dioxane (15 mL) H_2O (3 mL) was added 2-benzyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (578 mg, 2.65 mmol, 2.5 *eq*), K_2CO_3 (220 mg, 1.59 mmol, 1.5 *eq*), $Pd(dppf)Cl_2$ (77.6 mg, 106 umol, 0.1 *eq*). Then the mixture was heated to 50°C and stirred for 4 hours. The mixture was poured into H_2O (50 mL), extracted with EA (50 mL*3). The combined organic layer was washed by

brine (30 mL*2), dried with Na_2SO_4, filtered and concentrated in vacuum to get crude product. The crude was purified by prep-HPLC (HCl) to get a residue (column: Phenomenex Luna C18 150*30mm*5um; mobile phase: [water(0.04%HCl)-ACN]; 45%-75%,10min). The residue was purified by prep-TLC (PE: EA = 2: 1) to get a crude product. The crude was purified by prep-HPLC (HCl) (column: Phenomenex Luna C18 150*30mm*5um;mobile phase: [water(0.04%HCl)-ACN]; 45%-75%,10min) to obtain 5-benzyl-1-(2-phenylethyl)indoline-2,3-dione (9 mg) as yellow solid.

^{1}H NMR: (400MHz, $CDCl_3$) δ 7.42-7.41 (m, 1H), 7.32-7.22 (s, 9H), 7.17-7.15 (m, 2H), 6.69-6.67 (m, 1H), 3.94-3.91 (m, 4H), 3.00-2.97 (m,2H).

LCMS: ($M+H^+$): 342.1 @ 3.330 min (5-95% ACN in H_2O, 4.5 min).

Synthesis of 18



Step 1:

1-ethyl-5-iodo-indoline-2,3-dione (18b)

To a solution of 5-iodoindoline-2,3-dione (2 g, 7.3 mmol, 1.0 *eq*) in DMF (20 mL) was added NaH (439 mg, 11.0 mmol, 60% purity, 1.5 *eq*) at 0 °C. The mixture was stirred at 0 °C for 0.5 hours and then iodoethane (1.71 g, 11.0 mmol, 1.5 *eq*) was added. The mixture was warmed to 15 °C for 11.5 hours. The mixture was quenched by addition of ice-H_2O (20 mL) at 0 °C, extracted with EtOAc (20 mLx2). The combined organic layers were washed with H_2O (30 mLx2), brine (30 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethylacetate/Petroleum ether gradient @ 50 mL/min) to afford the compound 1-ethyl-5-iodo-indoline-2,3-dione (1.7 g, 59% yield,) as red solid with purity 76.96% on LCMS.

Step 2:

1-ethyl-5-iodo-3,3-dimethoxy-indolin-2-one (18c)

To a solution of 1-ethyl-5-iodo-indoline-2,3-dione (0.5 g, 1.7 mmol, 1.0 *eq*) in MeOH (10 mL) was added $TsOH.H_2O$ (63 mg, 330 umol, 0.2 *eq*) and trimethoxymethane (264 mg, 2.49 mmol, 1.5 *eq*). The mixture was stirred at 80 °C for 12 hours. The mixture was concentrated directly under reduced pressure to afford a residue. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethylacetate/Petroleum ether gradient @ 50 mL/min) to afford the compound 1-ethyl-5-iodo-3,3-dimethoxy-indolin-2-one (0.42 g, 73% yield) as light-yellow solid.

^{1}H NMR: ($CDCl_3$, 400 MHz) δ 7.67-7.69 (m, 2H), 6.66 (d, *J* = 8.0 Hz, 1H), 3.67-3.72 (q, *J* = 7.2 Hz, 2H), 3.56 (s, 6H), 1.22-1.26 (t, *J* = 7.2 Hz, 3H).

Step 3:

1-ethyl-3,3-dimethoxy-2-oxo-indoline-5-carbonitrile (18d)

A mixture of 1-ethyl-5-iodo-3,3-dimethoxy-indolin-2-one (0.37 g, 1.07 mmol, 1.0 *eq*), $Zn(CN)_2$ (626 mg, 5.33 mmol, 5.0 *eq*) and $Pd(PPh_3)_4$ (123 mg, 107 umol, 0.1 *eq*) in DMF (5 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 120 °C for 12 hours under N_2 atmosphere. The mixture was cooled to 15 °C and quenched by addition of H_2O (25 mL) at 0 °C, and filtered. The filtrate was concentrated under reduced pressure to afford a residue. The product was further purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ether gradient @ 50 mL/min) to give compound 1-ethyl-3,3-dimethoxy-2-oxo-indoline-5-carbonitrile (0.21 g, 80% yield) as colorless solid.

^{1}H NMR: ($CDCl_3$, 400 MHz) δ 6.67-6.71 (m, 2H), 6.92 (d, *J* = 8.0 Hz 1H), 3.72-3.78 (q, *J* = 7.2 Hz, 2H), 3.58 (s, 6H),1.26-1.29 (t, *J* = 7.2 Hz, 2H).

Step 4:

1-ethyl-3,3-dimethoxy-5-(1H-tetrazol-5-yl)indolin-2-one (18e)

To a solution of 1-ethyl-3,3-dimethoxy-2-oxo-indoline-5-carbonitrile (0.16 g, 650 umol, 1.0 *eq*) in DMF (1.6 mL) was added NaN_3 (0.66 g, 10.2 mmol, 16 *eq*) and N,N-diethylethanamine;hydrochloride (268 mg, 1.95 mmol, 3.0 *eq*). The mixture was stirred at 80 °C for 6 hours. LC-MS showed 34.18% of Reactant 1 remained. One new peak was shown on LC-MS and ~54.88% of desired compound was detected. The mixture was stirred for additional 6 hours. The residue was triturated with EtOAc and filtered. The filtrate was concentrated under reduced pressure to afford the crude product 1-ethyl-3,3-dimethoxy-5-(2H-tetrazol-5-yl)indolin-2-one (0.22 g) as light-yellow solid which was used into the next step without further purification.

Step 5:

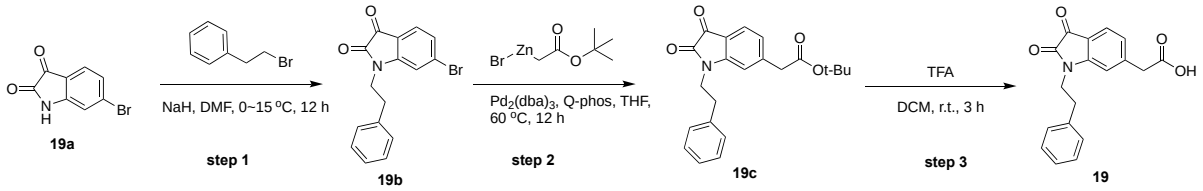
1-ethyl-5-(1H-tetrazol-5-yl)indoline-2,3-dione (18)

A solution of 1-ethyl-3,3-dimethoxy-5-(2H-tetrazol-5-yl)indolin-2-one (0.2 g, 690 umol, 1 *eq*) in THF (4 mL) was added HCl (12 M, 2 mL, 35 *eq*) drop-wise slowly. The mixture was stirred at 60 °C for 2 hours. The mixture was concentrated directly under reduced pressure to afford a residue. The residue was purified by prep-HPLC (HPLC: HCl condition; column: Phenomenex Luna C18;100*30mm*5um; mobile phase: [water(0.04%HCl)-ACN]; 5%-35%, 10 min) to afford the compound 1-ethyl-5-(2H-tetrazol-5-yl)indoline-2,3-dione (0.092 g, 327 umol, 47% yield) as yellow solid with purity 86.5%. The residue was purified by prep-HPLC (HCl condition; column: Phenomenex Luna C18:150*30mm*5um; mobile phase: [water(0.04%HCl)-ACN]; 5%-40%,10min) to afford the compound 1-ethyl-5-(2H-tetrazol-5-yl)indoline-2,3-dione hydrochloride salt (0.026 g, 28% yield) as yellow solid.

LCMS: ($M+H^+$): 244.0 @ 2.161 min, WUXICD00.M).

^{1}H NMR: (MeOD, 400 MHz) δ 8.30-8.33 (m, 1H), 8.12 (s, 1H), 7.42-7.46 (m, 1H), 3.74-3.79 (q, *J* = 7.2 Hz, 2H), 1.20-1.23 (t, *J* = 7.2 Hz, 3H).

Synthesis of 19



Step 1:

6-bromo-1-(2-phenylethyl)indoline-2,3-dione (19b)

To a solution of 6-bromoindoline-2,3-dione (5 g, 22.1 mmol, 1 eq) in DMF (50 mL) was added NaH (885 mg, 354 mmol, 60% purity, 16 eq) at 0 °C, the mixture was stirred for 0.5 hr and 2-bromoethylbenzene (5.32 g, 28.8 mmol, 3.88 mL, 1.3 eq) was added. The mixture was warmed to 15 °C and stirred for 11.5 hr. The mixture was poured into ice (100 g), extracted with EtOAc (60 mL*2). The combined organic layers were washed with H_2O (100 mL*2), brine (100 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; X80g SepaFlash® Silica Flash Column, Eluent of 0~50% Ethyl acetate/Petroleum ether gradient @ 100 mL/min) to give compound 6-bromo-1-(2-phenylethyl)indoline-2,3-dione (3.2 g, 35% yield) was obtained as orange solid. The material was used in the next step without further purifications.

Step 2:

Tert-butyl 2-[2,3-dioxo-1-(2-phenylethyl)indolin-6-yl]acetate (19c)

To a solution of 6-bromo-1-(2-phenylethyl)indoline-2,3-dione (0.5 g, 1.5 mmol, 1 eq) and Q-phos (129 mg, 182 umol, 0.12 eq) in THF (5 mL) was added $Pd_2(dba)_3$ (139 mg, 151 umol, 0.1 eq) and bromo-(2-tert-butoxy-2-oxo-ethyl)zinc (0.5 M, 3.33 mL, 1.1 eq). After addition, the mixture was heated to 70 °C and stirred for 12 hr under N2 atmosphere. The mixture was quenched by addition of saturated aq NH_4Cl (20 mL) at 0 °C, extracted with EtOAc (20 mL*2). The combined organic layers were washed with H_2O (20 mL), brine (20 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (ISCO®; 20 g

SepaFlash® Silica Flash Column, Eluent of 0~10% Ethyl acetate/Petroleum ether gradient @ 60 mL/min) to give crude compound tert-butyl 2-[2,3-dioxo-1-(2-phenylethyl)indolin-6-yl]acetate (0.35 g), which was directory used in the next step.

Step 3:

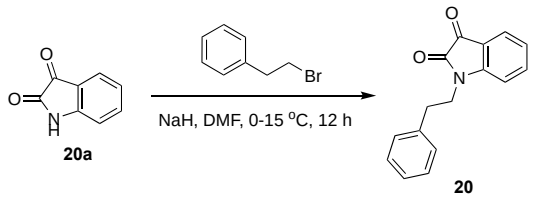
2-[2,3-dioxo-1-(2-phenylethyl)indolin-6-yl]acetic acid (19)

To a solution of tert-butyl 2-[2,3-dioxo-1-(2-phenylethyl)indolin-6-yl]acetate (0.3 g, 820 umol, 1 eq) in DCM (4 mL) was added TFA (3.08 g, 27.0 mmol, 2 mL, 33 eq) drop-wise slowly. After addition, the mixture was stirred at 15 °C for 2 hr under N_2 atmosphere. The mixture was concentrated under reduced pressure. The residue was purified by prep-HPLC (HCl condition; column: Phenomenex Luna C18 150*30mm*5um;mobile phase: [water(0.04%HCl)-ACN]; 20%-55%,10min) to give compound 2-[2,3-dioxo-1-(2-phenylethyl)indolin-6-yl]acetic acid (53.2 mg) as orange solid.

LCMS: ($M+H^+$): 310.1 @ 2.755 min, WUXIAB01.M, 4.5 min).

^{1}H NMR: (DMSO-d6, 400 MHz) δ 12.54 (brs, 1H), 7.49 (d, J = 7.6 Hz, 1H), 7.20-7.28 (m, 5H), 7.10 (s, 1H), 7.00-7.02 (m, 1H), 3.84-3.88 (t, J = 7.6 Hz, 2H), 3.68 (s.2 H), 2.89-2.93 (t, J = 7.6 Hz, 2H).

Synthesis of 20



1-(2-phenylethyl)indoline-2,3-dione (20)

To a solution of indoline-2,3-dione (2 g, 13.6 mmol, 1 eq) in DMF (20 mL) was added NaH (815 mg, 20.4 mmol, 60% purity, 1.5 eq) at 0 °C and 2-bromoethylbenzene (3.02 g, 16.3 mmol, 2.20 mL, 1.2 eq). After addition, the mixture was warmed to 15 °C and stirred at 15 for 12 hr. The mixture was quenched by addition of H_2O (25 mL) at 0 °C, extracted with EtOAc (30 mL*2). The combined organic layers were washed with H_2O (50 mL), brine (50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to afford a residue. The residue was purified by flash silica gel chromatography (ISCO®; 40 g SepaFlash® Silica Flash Column, Eluent of 0~100% Ethyl acetate/Petroleum ether gradient @ 75 mL/min) to afford the crude product (2.4 g). 100 mg of the crude product was further purified by prep-HPLC (HPLC: ET20595-387-P1B, HCl condition; column: Phenomenex LunaC18 100*30mm*5um; mobile phase: [water(0.04%HCl)-ACN]; 35%-65%,10 min) to give Compound 1-(2-phenylethyl)indoline-2,3-dione (40 mg) as red solid.

^{1}H NMR: (400MHz, $CDCl_3$) δ 7.58-7.60 (m, 1H),7.52-7.53 (m, 1H), 7.23-7.30 (m, 5H), 709 (m, 1H), 6.74-6.76 (m, 1H), 3.94-3.98 (m, 2H), 2.99-3.03 (m, 2H).

LCMS: ($M+H^+$): 252.1 @ 2.886 min (5-95% ACN in H_2O, 4.5 min).

Synthesis of 21

Step 1:

(2E)-N-(4-fluoro-2-methoxy-phenyl)-2-hydroxyimino-acetamide (21b)

To a solution of HCl (12 M, 2.63 mL, 2.2 *eq*), 2,2,2-trichloroethane-1,1-diol (2.58 g, 15.6 mmol, 2.03 mL, 1.1 *eq*), in H_2O (66 mL) was added Na_2SO_4 (13.4 g, 94.5 mmol, 6.7 *eq*), hydroxylamine;hydrochloride (3.15 g, 45.3 mmol, 3.2 *eq*), 4-fluoro-2-methoxy-aniline (2 g, 14 mmol, 1 *eq*). The mixture was heated to 45°C and stirred for 1.5 hours. Then heated to 90°C and stirred for 2.5 hours. The reaction was cooled to 0°C and stirred for 30 min. The mixture was filtered and washed with PE (100 mL) and H_2O (100 mL). The filter cake was concentrated in vacuo to obtain crude (2E)-N-(4-fluoro-2-methoxy-phenyl)-2-hydroxy imino-acetamide (2.3 g) as gray solid.

^{1}H NMR: (400MHz, DMSO-*d*6) δ 12.33 (s, 1H), 9.14 (S, 1H), 8.06-8.02 (s, 1H), 7.66 (s, 1H), 7.04-7.01 (s, 1H), 6.80-6.76 (s, 1H), 3.87 (s, 3H).

Step 2:

5-fluoro-7-methoxy-indoline-2,3-dione (21c)

To a solution of $MeSO_3H$ (10 mL) was added (2E)-N-(4-fluoro-2-methoxy-phenyl)-2-hydroxyimino-acetamide (1.0 g, 4.7 mmol, 1 *eq*). Then the reaction was heated to 80 °C and stirred for 5 hours. The mixture was poured into H_2O (50 mL) and extracted with EA (50 mL *3). The combined organic layer was washed with brine (50 mL*2), dried with Na_2SO_4, filtered and concentrated in vacuum to obtain crude 5-fluoro-7-methoxy-indoline-2,3-dione (0.9 g) as yellow oil. The crude material was directory used in the next step.

Step 3:

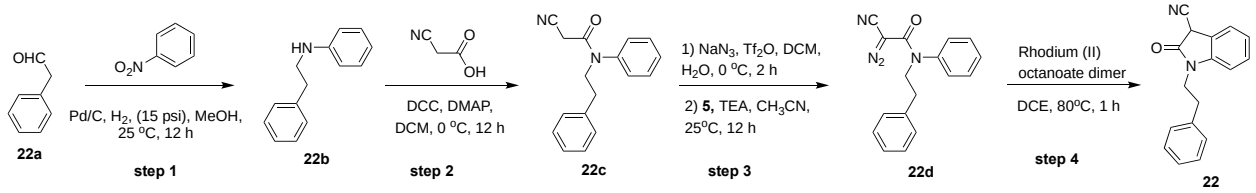
5-fluoro-7-methoxy-1-(2-phenylethyl)indoline-2,3-dione (21)

To a solution of 5-fluoro-7-methoxy-indoline-2,3-dione (0.85 g, 4.36 mmol, 1 *eq*) in DMF (20 mL) was added NaH (261 mg, 6.53 mmol, 60% purity, 1.5 *eq*), 2-bromoethylbenzene (1.21 g, 6.53 mmol, 1.5 *eq*) at 0 °C. Then the reaction was warmed to 25°C and stirred for 3 hours. The mixture was poured into H_2O (100 mL), extracted with EA (50 mL*3). The combined organic layer was washed by brine (50 mL*2), dried by Na_2SO_4, filtered and concentrated in vacuo to get a residue. The residue was purified by prep-HPLC (FA) (column: Phenomenex Luna C18 200*40mm*10um;mobile phase: [water(0.225%FA)-ACN]; 50%-70%,12min) to obtain 5-fluoro-7-methoxy-1-(2-phenylethyl)indoline-2,3-dione (78 mg) as yellow solid.

^{1}H NMR: (400MHz, $CDCl_3$) δ 7.25-7.16 (m, 5H), 6.90-6.85 (m, 2H), 4.14-4.10 (m, 2H), 3.88 (s, 3H), 2.94-2.90 (m, 2H).

LCMS: ($M+H^+$): 300.0 @ 2.252 min (5-95% ACN in H_2O, 4.5 min).

Synthesis of 22



Step 1:

N-(2-phenylethyl)aniline (22b)

To a solution of 2-phenylacetaldehyde (5 g, 41.6 mmol, 3.25 mL, 1 *eq*), nitrobenzene (5.12 g, 41.6 mmol, 4.27 mL, 1 *eq*), in MeOH (100 mL) was added Pd/C (1 g, 10% purity) at 25 °C. The suspension was degassed under vacuum and purged with H_2 several times. The mixture was stirred under H_2 (15 psi) at 25°C for 12 hours. The reaction mixture was filtered and washed with MeOH (100 mL). The filtrate was concentrated in vacuo to get a residue. The residue was purified by column (SiO_2) with petroleum ether: EtOAc = 50: 1 to 10: 1 to obtain N-(2-phenylethyl) aniline (6 g, crude) as light yellow oil.

^{1}H NMR: (400MHz, $CDCl_3$) δ 7.27-7.23 (m, 2H), 7.18-7.11 (m, 5H), 6.63-6.60 (m, 1H), 6.55-6.53 (m ,2H), 3.60 (bar, 1H), 3.35-3.31 (m, 2H), 2.86-2.83 (m, 2H).

Step 2:

2-cyano-N-phenyl-N-(2-phenylethyl)acetamide (22c)

To a solution of N-(2-phenylethyl)aniline (5.8 g, 29.4 mmol, 1 *eq*), 2-cyanoacetic acid (2.75 g, 32.3 mmol, 1.1 *eq*) in DCM (50 mL) was added a solution of DCC (6.67 g, 32.3 mmol, 6.54 mL, 1.1 *eq*), DMAP (180 mg, 1.47 mmol, 0.05 *eq*) in DCM (10 mL) at 0°C. Then the mixture was warmed to 25°C and stirred for 12 hours. The mixture was filtered and washed with DCM (50 mL). The filtrate was concentrated in vacuum to get a residue. The residue was purified with PE/EA=10:1 to 3:1 to obtain 2-cyano-N-phenyl-N-(2-phenylethyl)acetamide (7.0 g, crude) as yellow oil.

^{1}H NMR: (400MHz, $CDCl_3$) δ 7.46-7.44 (m, 3H), 7.28-7.16 (m, 5H), 7.09-7.07 (m, 2H), 3.98-3.94 (m, 2H), 3.19 (s, 2H), 2.94-2.90 (m, 2H).

Step 3:

2-cyano-2-diazo-N-phenyl-N-(2-phenylethyl)acetamide (22d)

To a solution of NaN_3 (6.15 g, 94.6 mmol, 10 *eq*) in DCM (20 mL) H_2O (40 mL) was added Tf_2O (9.34 g, 33.1 mmol, 5.46 mL, 3.5 *eq*) at 0°C. Then the reaction was stirred for 2 hours at 0 °C. The mixture was extracted with DCM (5 mL *2). The combined organic layer was washed with $NaHCO_3$ (10 mL), dried with Na_2SO_4 to get the DCM solution of $CF_3SO_2N_3$. To a solution of 2-cyano-N-phenyl-N-(2-phenylethyl)acetamide (2.50 g, 9.46 mmol, 1 *eq*) in ACN (50 mL) was added the DCM solution of $CF_3SO_2N_3$, TEA (4.31 g, 42.6 mmol, 5.92 mL, 4.5 *eq*) at 0°C. Then the reaction was warmed to 25 °C and stirred for 12 hours. The combined mixture was concentrated in vacuo to get a residue. The residue was purified by column (SiO_2) with PE/EA=10:1 to 2:1 to obtain 2-cyano-2-diazo-N-phenyl-N-(2-phenylethyl)acetamide as yellow oil.

^{1}H NMR: (400MHz, $CDCl_3$) δ 7.49-7.27 (m, 3H), 7.27-7.23 (m, 2H), 7.19-7.16 (m, 5H), 4.01-3.54 (m, 2H), 2.95-2.91 (m, 2H).

Step 4:

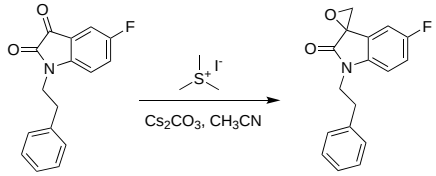
2-oxo-1-(2-phenylethyl)indoline-3-carbonitrile (22)

To a solution of di(octanoyloxy)rhodium (134 mg, 344 umol, 0.1 *eq*) in DCE (40 mL) was added dropwise 2-cyano-2-diazo-N-phenyl-N-(2-phenylethyl)acetamide (1.00 g, 3.44 mmol, 1 *eq*) in DCE (10 mL) during 1 hours under N_2 at 80 °C. The mixture was concentrated in vacuo to get a crude material. The crude material was purified by column (SiO_2) with petroleum ether: EtOAc = 10:1 to 0:1 to get a residue (1.2 g). The part of the residue (400 mg) was purified by prep-HPLC (HCl) (column: Phenomenex Luna C18 100*30mm*5um; mobile phase: [water(0.04%HCl)-ACN]; 40%-75%, 10 min) to obtain 2-oxo-1-(2-phenylethyl)indoline-3-carbonitrile (50 mg) as white solid.

^{1}H NMR: (400MHz, DMSO-*d6*) δ 7.44-7.40 (m, 2H), 7.36-7.34 (m, 2H), 7.31-7.27 (m, 1H), 6.50-6.46 (m, 2H), 6.36-6.34 (m, 1H), 6.25-6.22 (m, 1H), 4.25-4.23 (m, 1H), 3.70-3.67(m, 1H), 3.52-3.47 (m, 1H), 2.77-2.75 (m, 1H), 2.33-2.30 (m, 1H).

LCMS: ($M+H^+$): 263.1 @ 2.486 min (5-95% ACN in H_2O, 4.5 min).

Synthesis of 23



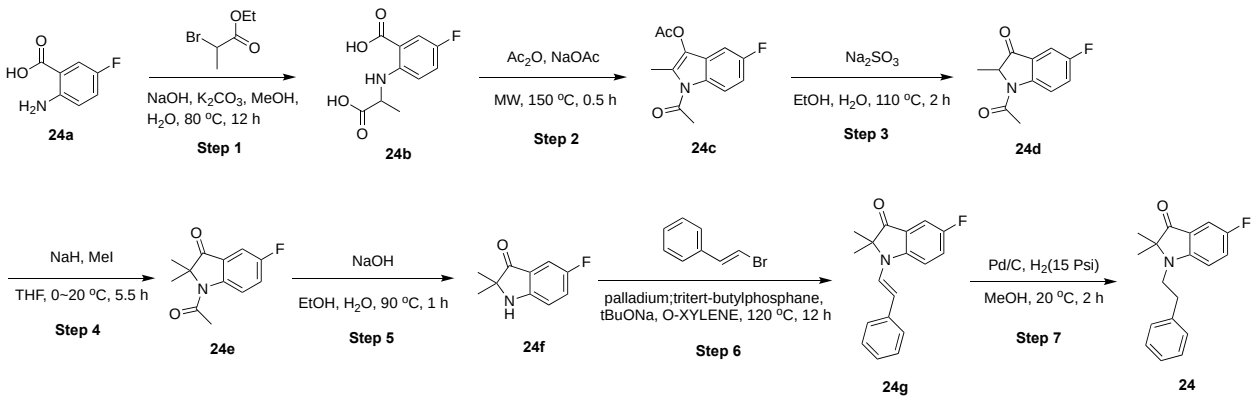
5-fluoro-1-(2-phenylethyl)spiro[indoline-3,2'-oxirane]-2-one (23)

A mixture of trimethylsulfonium;iodide (227 mg, 1.11 mmol, 2 *eq*), Cs_2CO_3 (363 mg, 1.11 mmol, 2 *eq*) in MeCN (5 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 50 °C for 1 h under N_2 atmosphere. To this, a solution of 5-fluoro-1-(2-phenylethyl)indoline-2,3-dione (150 mg, 557 umol, 1 *eq*) in MeCN (2 mL) was added slowly, then the mixture was stirred at 50 °C for 1 h under N_2 atmosphere. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (HCl condition) to give compound 5-fluoro-1-(2-phenylethyl)spiro[indoline-3,2'-oxirane]-2-one (34.5 mg, 22% yield) was obtained as white solid.

LCMS: ($M+H^+$):284.1@ 2.562 min (5-95 % ACN in H_2O, 6 min).

^{1}H NMR: (400 MHz, DMSO-d6) δ 7.29-7.16 (m, 8H), 3.95 (t, *J* = 7.52 Hz, 2H), 3.66 (d, *J* = 6.60 Hz, 1H), 3.32 (s, 2H), 2.91 (t, *J* = 7.40 Hz, 2H).

Synthesis of 24



Step 1:

2-(1-carboxyethylamino)-5-fluoro-benzoic acid (24b)

To a mixture of 2-amino-5-fluoro-benzoic acid (5 g, 32.2 mmol, 1 *eq*) in H_2O (50 mL) was added NaOH (2.58 g, 64.4 mmol, 2 *eq*) followed by K_2CO_3 (8.91 g, 64.5 mmol, 2 *eq*). A solution of ethyl 2-bromopropanoate (11.7 g, 64.5 mmol, 8.40 mL, 2 *eq*) in MeOH (50 mL) was added to the mixture and the mixture was then heated at 80 °C for 12 hr. After cooling to 20 °C, MeOH was removed under reduced pressure. The aqueous residue was extracted with ethyl acetate (30 mL). The aqueous layer was adjust pH = 2 with addition of con.HCl, and then extracted with ethyl acetate (30 mL*2). The combined organic phase was washed with brine (40 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. Compound 2-(1-carboxyethylamino)-5-fluoro-benzoic acid (10 g, crude) was obtained as a yellow solid. The crude was used in the next step without further purification.

Step 2:

(1-acetyl-5-fluoro-2-methyl-indol-3-yl) acetate (24c)

2-(1-carboxyethylamino)-5-fluoro-benzoic acid (1 g*10, 4.40 mmol, 1 *eq*), Ac_2O (18.0 g*10, 176 mmol, 16.5 mL, 40 *eq*) and NaOAc (1.44 g*10, 17.6 mmol, 4 *eq*) was heated at 150 °C for 30 min in a microwave. The reaction mixture was concentrated under reduced pressure to remove EtOH. Then the residue was poured into $NaHCO_3$ (50 mL) and extracted with ethyl acetate (30 mL). The combined organic phase was washed with brine (30 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=1/0 to 4/1). Compound (1-acetyl-5-fluoro-2-methyl-indol-3-yl) acetate (3.6 g, 13.0 mmol, 30% yield, 90% purity) was obtained as a yellow solid.

Step 3:

1-acetyl-5-fluoro-2-methyl-indolin-3-one (24d)

A mixture of (1-acetyl-5-fluoro-2-methyl-indol-3-yl) acetate (3.66 g, 14.68 mmol, 1 *eq*), Na_2SO_3 (2.22 g, 17.62 mmol, 1.2 *eq*) in EtOH (36 mL) and H_2O (36 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 110 °C for 2 hr under N_2 atmosphere. The reaction mixture (combined with ET16082-997, 40 mg) was concentrated under reduced pressure to remove EtOH. The residue was diluted with H_2O (30 mL) and extracted with EtOAc (50 mL * 3). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 50/1 to 4/1). Compound 1-acetyl-5-fluoro-2-methyl-indolin-3-one (2 g, 90% purity) was obtained as a yellow solid.

^{1}H NMR: (400 MHz, DMSO-d_6) δ 8.46 (br s, 1H), 7.64 (td, *J* = 9.05, 2.81 Hz, 1H), 7.52 (dd, *J*=7.21, 2.81 Hz, 1H), 4.67 (q, *J* = 6.48 Hz, 1H), 2.31 (br s, 3 H), 1.46 (d, *J* = 7.09 Hz, 3H).

Step 4:

1-acetyl-5-fluoro-2,2-dimethyl-indolin-3-one (24e)

To a stirred mixture of 1-acetyl-5-fluoro-2-methyl-indolin-3-one (0.8 g, 3.86 mmol, 1 eq) in THF (20 mL) at 0 °C was added NaH (308.88 mg, 7.72 mmol, 60% purity, 2 eq). After stirring for 30 min, CH_3I (657.63 mg, 4.63 mmol, 288.43 uL, 1.2 eq) was added to the mixture. The mixture was allowed to warm to 20 °C. The mixture was stirred for 5 hour at 20 °C. The mixture was poured into water (50 mL) and extracted with ethyl acetate (30 mL*2). The combined organic phase was washed with brine (30 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The

residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 5/1). Compound 1-acetyl-5-fluoro-2,2-dimethyl-indolin-3-one (0.3 g, crude) was obtained as a yellow solid.

Step 5:

5-fluoro-2,2-dimethyl-indolin-3-one (24f)

A mixture of 1-acetyl-5-fluoro-2,2-dimethyl-indolin-3-one (400 mg, 1.81 mmol, 1 *eq*), NaOH (2 M in H_2O, 1.08 mL, 1.2 *eq*) in EtOH (5 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 90 °C for 1 hr under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 3/1). Compound 5-fluoro-2,2-dimethyl-indolin-3-one (0.35 g, 1.56 mmol, 86.42% yield, 80% purity) was obtained as a yellow solid.

^{1}H NMR: (400 MHz, CHLOROFORM-d) δ 7.29 (d, *J* = 2.65 Hz, 1H), 7.23 (td, *J* = 8.71, 2.65 Hz, 1H), 6.82 (dd, *J* = 8.82, 3.75 Hz, 1H), 1.35 (s, 6H).

Step 6:

5-fluoro-2,2-dimethyl-1-[(E)-styryl]indolin-3-one (24g)

A mixture of 5-fluoro-2,2-dimethyl-indolin-3-one (50 mg, 279 umol, 1 *eq*) ,[(E)-2-bromovinyl]benzene (61.3 mg, 335 umol, 42.9 uL, 1.2 *eq*), tBuONa (67.0 mg, 698 umol, 2.5 *eq*), palladium;tritert-butylphosphane (14.3 mg, 27.9 umol, 0.1 *eq*) in O-XYLENE (2 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 120 °C for 12 hr under N_2 atmosphere. The mixture was poured into water (20 mL) and extracted with ethyl acetate (20 mL*3). The combined organic phase was washed with brine (20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by prep-TLC (SiO_2, Petroleum ether/Ethyl acetate = 3/1). Compound 5-fluoro-2,2-dimethyl-1-[(E)-styryl]indolin-3-one (50.0 mg, 164 umol, 59% yield, 92% purity) was obtained as a orange solid.

^{1}H NMR: (400 MHz, CHLOROFORM-*d*) δ 7.40-7.29 (m, 6H), 7.24-7.17 (m, 2H), 7.08 (d, *J*=14.63 Hz, 1H), 6.24 (d, *J* = 14.63 Hz, 1H), 1.54 (s, 6H).

Step 7:

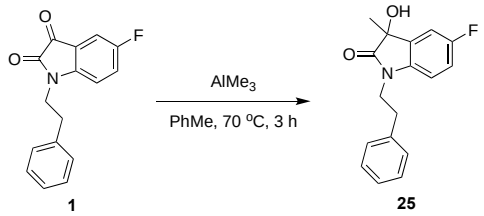
5-fluoro-2,2-dimethyl-1-(2-phenylethyl)indolin-3-one (24)

A mixture of 5-fluoro-2,2-dimethyl-1-[(E)-styryl]indolin-3-one (50.0 mg, 178 umol, 1 *eq*), Pd/C (50 mg, 180 umol, 10% purity) in MeOH (10 mL) was degassed and purged with H_2 for 3 times, and then the mixture was stirred at 20 °C for 2 h under H_2 (15Psi) atmosphere. The reaction mixture filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (HCl condition) to give compound 5-fluoro-2,2-dimethyl-1-(2-phenylethyl)indolin-3-one (44.6 mg, 157 umol, 88% yield, 99.5% purity) as yellow solid.

LCMS: ($M+H^+$):284.2@ 3.058 min (5-95 % ACN in H_2O, 6 min)

^{1}H NMR: (400 MHz, METHANOL-*d4*) δ 7.33-7.16 (m, 7H), 6.83 (dd, *J* = 9.13, 3.63 Hz, 1H), 3.62 (t, *J* = 7.50 Hz, 2 H), 2.96 (t, *J* = 7.44 Hz, 2H), 1.17 (s, 6H).

Synthesis of 25



Step 1:

5-fluoro-3-hydroxy-3-methyl-1-(2-phenylethyl)indolin-2-one (25)

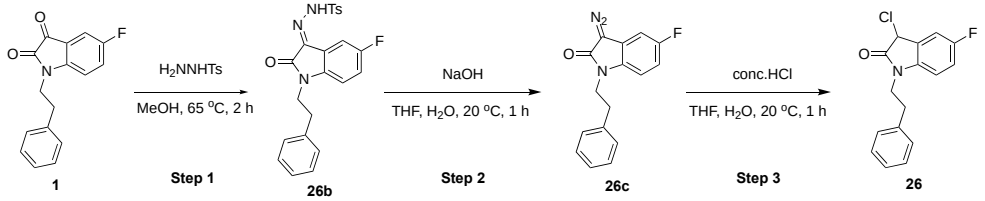
To a solution of 5-fluoro-1-(2-phenylethyl)indoline-2,3-dione (0.2 g, 743 umol, 1 *eq*) in Toluene (5 mL) was added trimethylaluminium (2 M in toluene, 743 uL, 2 *eq*). The mixture was stirred at 70 °C for 3 h. The mixture was poured into water (50 mL) and extracted with ethyl acetate (30 mL*3). The combined organic phase was washed with brine (40 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by prep-HPLC

(neutral condition) to give compound 5-fluoro-3-hydroxy-3-methyl-1- (2-phenylethyl)indolin-2-one (81.6 mg, 38% yield) as white solid.

LCMS: (M-18): 268.1@ 2.686 min (5-95 % ACN in H_2O, 6 min).

^{1}H NMR: (400 MHz, DMSO-d6) δ 7.31-7.16 (m, 6H), 7.13-6.98 (m, 2H), 6.02 (s, 1H), 3.98-3.86 (m, 1H), 3.78 (dt, *J* = 13.97, 7.02 Hz, 1H), 2.95-2.80(m, 2H), 1.28 (s, 3 H).

Synthesis of 26



Step 1:

N-[(E)-[5-fluoro-2-oxo-1-(2-phenylethyl)indolin-3-ylidene]amino]-4-methyl-benzenesulfonamide (26b)

4-methylbenzenesulfonohydrazide (152 mg, 817 umol, 1.1 *eq*) was added to a solution of 5-fluoro-1-(2-phenylethyl)indoline-2,3-dione (0.2 g, 743 umol, 1 *eq*) in MeOH (5 mL). The mixture was stirred vigorously at 65 °C for 2 h. The reaction mixture was cooled to room temperature, a lot of solids were produced, filtered and washed with MeOH (10 mL), and then the filter cake was concentrated under reduced pressure to give N-[(E)-[5-fluoro-2-oxo-1-(2-phenylethyl)indolin-3-ylidene]amino]-4-methyl-benzenesulfonamide (0.1 g, crude) as yellow solid. The crude material was directly used in the next step without further purification.

Step 2:

3-diazo-5-fluoro-1-(2-phenylethyl)indolin-2-one (26c)

NaOH (18.3 mg, 457 umol, 1.60 mL, 2 *eq*) in H_2O (1 mL) was added to a solution of the crude N-[(E)-[5-fluoro-2-oxo-1-(2-phenylethyl)indolin-3-ylidene]amino]-4-methyl-benzenesulfonamide (0.1 g, 229 umol, 1 *eq*) in THF (1 mL) and the resulting solution was stirred for 1 h at 20 °C. The volatiles were removed by evaporation to give crude 3-diazo-5-fluoro-1-(2-phenylethyl)indolin-2-one (64 mg). The crude material was used immediately in the next step.

Step 3:

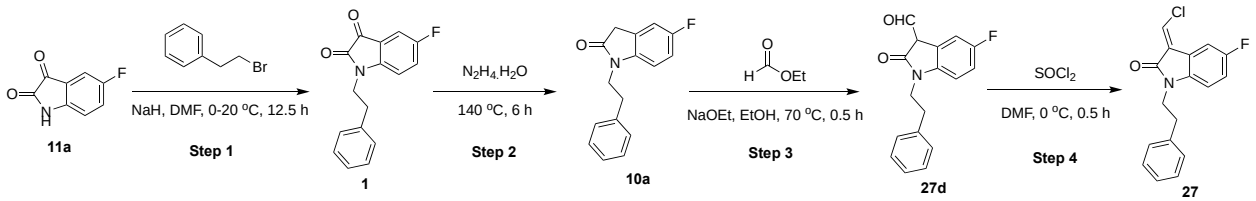
3-chloro-5-fluoro-1-(2-phenylethyl)indolin-2-one (26)

A solution of crude 3-diazo-5-fluoro-1-(2-phenylethyl)indolin-2-one (64 mg, 227 umol, 1 *eq*) in H_2O (2 mL) and THF (2 mL) was added HCl (1 mL), and then the mixture was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 20 °C for 1 h under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by prep-HPLC (HCl condition) to give compound 3-chloro-5-fluoro-1-(2-phenylethyl)indolin-2-one (5.6 mg, 8.3% yield) as yellow solid.

LCMS: ($M+H^+$): 290.0@ 3.658 min (5-95 % ACN in H_2O, 6 min).

^{1}H NMR: (400 MHz, DMSO-d6) δ 7.33 (br d, J=6.38 Hz, 1 H), 7.14 - 7.29 (m, 6 H), 7.09 (br dd, J=8.44, 4.19 Hz, 1 H), 5.64 (s, 1 H), 3.89 (br t, J=7.19 Hz, 2 H), 3.33 (s, 22 H), 2.88 (br t, J=7.25 Hz, 2 H), 2.50 (br s, 5 H).

Synthesis of 27



Step 1:

5-fluoro-1-(2-phenylethyl)indoline-2,3-dione (1)

To a solution of 5-fluoroindoline-2,3-dione (1 g, 6.06 mmol, 1 *eq*) in DMF (10 mL) was added NaH (363 mg, 9.08 mmol, 60% purity, 1.5 *eq*) at 0°C, after 0.5 h 2-bromoethylbenzene (1.34 g, 7.27 mmol, 982 uL, 1.2 *eq*) was added into

the mixture. The mixture was stirred at 20°C for 12 hr. The mixture was poured into water (50 mL) and extracted with ethyl acetate (30 mL*3). The combined organic phase was washed with brine (50 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 1/0 to 4/1). Compound 5-fluoro-1-(2-phenylethyl)indoline-2,3-dione (0.7 g, 39% yield) was obtained as brown oil.

^{1}H NMR: (400 MHz, DMSO-d6) δ 7.95 (s, 1H), 7.55-7.41 (m, 2H), 7.28 (d, J=4.25 Hz, 4H), 7.24-7.15 (m, 2H), 3.89 (t, *J* = 7.50 Hz, 2H), 2.93-2.89 (m, 2H).

Step 2:

5-fluoro-1-(2-phenylethyl)indolin-2-one (10a)

A mixture of 5-fluoro-1-(2-phenylethyl)indoline-2,3-dione (0.7 g, 2.60 mmol, 1 *eq*) and $N_2H_4.H_2O$ (6.64 g, 130 mmol, 6.45 mL, 50 *eq*) in one flask. The mixture was stirred at 140 °C for 6 h. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 50/1 to 4/1). Compound 5-fluoro-1-(2-phenylethyl)indolin-2-one (0.5 g) was obtained as yellow solid.

LCMS: ($M+H^+$): 256.1@ 1.084 min (5-95 % ACN in H_2O, 2 min).

^{1}H NMR: (400 MHz, DMSO-d6) δ 7.31-7.13 (m, 6H), 7.10-6.95 (m, 2H), 3.86 (t, *J* = 7.58 Hz, 2H), 3.54 (s, 2H), 2.85 (t, *J* = 7.52 Hz, 2H).

Step 3:

5-fluoro-2-oxo-1-(2-phenylethyl)indoline-3-carbaldehyde (27d)

A mixture of 5-fluoro-1-(2-phenylethyl)indolin-2-one (0.2 g, 783 umol, 1 *eq*), ethyl formate (81.3 mg, 1.10 mmol, 88.2 uL, 1.4 *eq*), NaOEt (74.6 mg, 1.10 mmol, 1.4 *eq*) in EtOH (0.5 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 70 °C for 0.5 hr under N_2 atmosphere. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by prep-HPLC (neutral condition). Compound 5-fluoro-2-oxo-1-(2-phenylethyl)indoline-3-carbaldehyde (80 mg, crude) was obtained as yellow solid.

LCMS: ($M+H^+$): 284.0@ 1.022 min (5-95 % ACN in H_2O, 2 min).

Step 4:

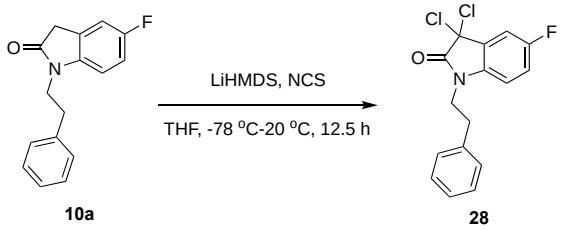
(3E)-3-(chloromethylene)-5-fluoro-1-(2-phenylethyl)indolin-2-one (27)

To a mixture of 5-fluoro-2-oxo-1-(2-phenylethyl)indoline-3-carbaldehyde (80 mg, 282 umol, 1 *eq*) in DMF (2 mL) was added $SOCl_2$ (168 mg, 1.41 mmol, 102 uL, 5 *eq*) drop-wise at 0°C under nitrogen atmosphere. The mixture was stirred at 0°C for 0.5 hr. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was purified by prep-HPLC (HCl condition) to give compound (3E)-3-(chloromethylene)-5-fluoro-1-(2-phenylethyl)indolin-2-one (26 mg, 30% yield) as yellow solid.

LCMS: ($M+H^+$): 302.0@ 3.081 min (5-95 % ACN in H_2O, 6 min).

^{1}H NMR: (400 MHz, DMSO-d6) δ 7.79-7.63 (m, 2H), 7.28-7.16 (m, 6H), 7.10 (dd, *J* = 8.50, 4.22 Hz, 1H), 3.93 (br t, *J* = 7.27 Hz, 2H), 3.34 (s, 9H), 2.89 (br t, *J* = 7.27 Hz, 2H), 2.50 (br s, 3H).

Synthesis of 28



10a **28**

3,3-dichloro-5-fluoro-1-(2-phenylethyl)indolin-2-one (28)

To a solution of 5-fluoro-1-(2-phenylethyl)indolin-2-one (30 mg, 118 umol, 1 *eq*) in THF (5 mL) was added LiHMDS (1 M, 118 uL, 1 *eq*) at -78 °C, the mixture was stirred for 30 min, and then NCS (16 mg, 118 umol, 1 *eq*) was added into the mixture, then the reaction mixture was warmed slowly to 20 °C .The mixture was stirred at 20°C for 12 hr. The mixture was poured into water (20 mL) and extracted with ethyl acetate (10 mL*2). The combined organic phase was washed with brine (20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuum. The residue was

purified by prep-HPLC (HCl condition). 3,3-dichloro-5-fluoro-1-(2-phenylethyl) indolin-2-one (9.5 mg) was obtained as white solid.

LCMS: ($M+H^+$): 324.0 @ 3.059 min (5-95 % ACN in H_2O, 6 min).

^{1}H NMR: (400 MHz, DMSO-d6) δ 7.70 (dd, *J* = 7.63, 2.50 Hz, 1H), 7.38-7.27 (m, 1 H), 7.26-7.14 (m, 6H), 3.98 (t, *J* = 7.07 Hz, 2H), 2.93 (t, *J* = 7.00 Hz, 2H).

c. Expression and Purification of DJ-1

The expression and purification of both the wild type and C106A mutant were carried out as previously described (doi.org/10.1038/s41467-019-09192-z). Briefly, pET3a-His-DJ1 and C106A plasmids were transformed into *E. coli* (BL21) cells and were grown for 5 hours at 37 °C before induction with 500 µM IPTG for 3 hours at 37 °C. The bacterial pellet was lysed (50 mM Tris pH 7.5, 100 mM NaCl, 1 mM DTT, 10% glycerol) before being pelleted at 20,000 g for 20 minutes. The His_6-tagged DJ-1 was isolated using Ni^{2+} beads and further purified via size exclusion chromatography. The protein purity was verified by SDS-PAGE and LC-MS analyses. The protein concentration was determined using 280 nm wavelength on a NanoDrop 2000c (Thermo Scientific).

d. Lactate Assay

Assay buffer MIX was prepared according to manufacturer's instructions before being added 1:1 to the glycation buffer MIX. The glycation MIX consisted of DJ-1 (50 µM), MGO (5 mM), compound and assay buffer with DJ-1 and compound being incubated for 20 min at 24 °C in assay buffer before MGO was added. Once the two mixes were combined, fluorescence time-points (excitation: 544 nm and emission: 590 nm) were taken and an endpoint of 120 min was selected. Differences in fluorescence readings at this endpoint were utilized to quantify the % activity of DJ-1.

e. PNPAc Assay

Potential inhibitor, DJ-1 (700 nM), PNPAc (250 µM), dmso (total 2.25%) were incubated in PBS and the resulting absorbance at 405 nm recorded every 30 seconds for 30 min.

f. MUAc and DiFMUAc Assay

Potential inhibitor, DJ-1 (550 nM), MUAc (300 µM), dmso (total 2%) were incubated in PBS and the resulting fluorescence (excitation: 358 nm; emission: 455 nm) recorded every 30 seconds for 30 min.

g. Binding Site Mapping

The covalently bound compound on Cys106 was removed from the DJ-1 crystal structure (PDB: 6AFL). The structure was then prepared and minimized using the Protein Preparation Wizard in the Schrödinger Suite (release 2019-1, Maestro, Schrödinger, LLC, New York, NY). The binding features were characterized using SiteMap program (release 2019-1, Maestro, Schrodinger, LLC, New York, NY) with default settings applied.

h. Covalent Docking

All covalent docking calculations were performed using the Schrodinger Suite (release 2019-1, Maestro, Schrodinger, LLC, New York, NY). In the DJ-1 structure prepared in Binding Site Mapping, the Cys106 (A chain) was selected as the reactive residue. The reaction type was chosen according to the ligand structure. For the isatin analogs and compound **8**, 'Nucleophilic Addition to a Double Bond' was used as the reaction type with a custom SMARTS string [C,c]-[C,c](=[O])-[C,c]=[O] to indicate the covalent bond position.

h. Covalent irreversibility assay

Potential inhibitor (18 µM) and DJ-1 (18 µM) were incubated for 1 hr in PBS and compounds were removed by size exclusion chromatography using Zeba ™ Micro Spin Desalting Columns, 7K MWCO, 75 uL from ThermoFisher Scientific according to manufacturer instructions. Isolated DJ-1 was diluted 1:20 with water containing 0.1% formic acid and analyzed by LC-MS.