Supporting information

Optimizing the Physicochemical Properties of Raf/MEK Inhibitors by Nitrogen Scanning

Toshihiro Aoki,† Ikumi Hyoudoh,† Noriyuki Furuichi,† Sawako Ozawa,† Fumio Watanabe,† Masayuki Matsushita,† Masahiro Sakaitani,† Kenji Morikami,‡ Kenji Takanashi,† Naoki Harada,† Yasushi Tomii,† Koji Shiraki,‡ Kentaro Furumoto,‡ Mitsuyasu Tabo,‡ Kiyoshi Yoshinari,† Kazutomo Ori,† Yuko Aoki,† Nobuo Shimma,† and Hitoshi Iikura*,†,‡

Research Division, Chugai Pharmaceutical Co., Ltd, †200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan.

‡1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan.

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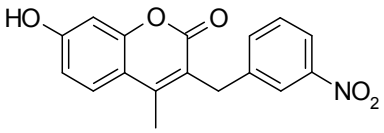
General.

Unless otherwise noted, all commercially available solvents and reagents were used as received. All reactions involving reagents or intermediates sensitive to air or moisture were performed under inert atmosphere of nitrogen in glassware. Removal of solvent or concentration under reduced pressure indicates rotary evaporation under 5 mmHg at around 40 °C. Column chromatography was performed using a Biotage cartridge-based FLASH+ system using KP-Sil silica cartridges. Preparative TLC was performed using Merck silica gel 60 F_{254} PLC plates (1 mm). Analytical TLC was performed using Merck silica gel 60 F_{254} TLC plates (250 μm) or Merck silica gel 60 NH_2 F_{254} TLC plates (250 μm).

Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were determined on a JEOL JNM-EX270 (270 MHz), Bruker ARX300 (300 MHz) or Varian 400-MR (400 MHz) spectrometer. Chemical shifts (δ) were expressed in parts per million (ppm) relative to internal chloroform (δ 7.26 for ^{1}H and δ 77.0 for ^{13}C), methanol (δ 3.30 for ^{1}H and δ 49.0 for ^{13}C) or DMSO (δ 2.49 for ^{1}H and δ 39.5 for ^{13}C). Splitting patterns were designated as follows: s, singlet; br, broad; d, doublet; t, triplet; q, quartet; m, multiplet. High-resolution mass spectra (HRMS) were recorded on a QSTAR XL (AB Sciex) equipped with gradient high performance liquid chromatography Agilent 1100 (Agilent Technologies Inc.), or a LTQ Orbitrap XL (Thermo Fisher Scientific) equipped with a Waters ACQUITY UPLC system. The purities of all the compounds tested in biological systems were assessed as being ≥95% using analytical LC/MS. LC/MS was performed using a Waters Micromass ZQ detector (ESI mode) or a Micromass SQ detector (ESI mode) equipped with binary solvent manager, PDA detector, column manager and sample manager. Elution was done with a gradient of 5–95% solvent B in solvent A (solvent A: 0.05% TFA in water, solvent B: 0.05% TFA in acetonitrile) over 4.5 minutes through Waters SunFire C18 5 μm (50 mm × 4.6 mm) reversed-phase column at 4 mL min^{-1}, or a gradient of 10–95% solvent D in solvent C (solvent C: 0.1% formic acid in water, solvent D: 0.1% formic acid in acetonitrile) over 1.4 minutes through Supelco Ascentis C18 2.7 μm (50 mm × 2.1 mm) reversed-phase column at 1 mL min^{-1}. Purity was determined at 254 nm. Preparative HPLC was performed using a Waters 2545 binary gradient module, Waters auto-sampler, Waters 2998 UV detector. Elution was done with a gradient of 10-98% solvent B in solvent A over 16 minutes through Tosoh ODS-80Ts 5 μm (25 cm × 20 mm) reversed-phase column at 15 mL min^{-1}.

Experimental Procedure.

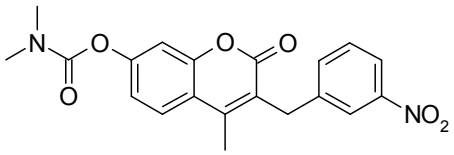
7-Hydroxy-4-methyl-3-(3-nitrobenzyl)-2*H*-chromen-2-one (**6a**)



Resorcinol (15.3 g, 139 mmol) and 2-(3-nitrobenzyl)-3-oxo-butyric acid ethyl ester[1] (36.9 g, 139 mmol) were dissolved in 70% sulfuric acid aqueous solution (160 mL). After stirring at room temperature for 18 h, the mixture was poured into water. The solid was collected by filtration, washed with water, and recrystallized from EtOH to give **6a** (26.2 g, 84.2 mmol, 60%) as a yellow solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.08–8.05 (m, 2H), 7.72–7.66 (m, 2H), 7.58 (dd, J = 7.8, 7.8 Hz, 1H), 6.82 (dd, J = 8.6, 1.9 Hz, 1H), 6.72 (d, J = 1.9 Hz, 1H), 4.08 (s, 2H), 2.44 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.3, 160.7, 153.6, 149.4, 147.8, 141.9, 134.9, 129.9, 127.0, 122.7, 121.2, 119.1, 113.0, 112.3, 102.0, 31.8, 15.2; ESIMS *m/z* 312 [M + H]$^+$; HRMS (ESI) *m/z* 312.0866 [(M + H)$^+$; calcd for $C_{17}H_{14}NO_5$: 312.0866].

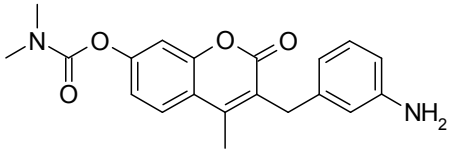
Dimethyl-carbamic acid 4-methyl-3-(3-nitrobenzyl)-2-oxo-2*H*-chromen-7-yl ester (**16a**)



To a solution of **6a** (300 mg, 0.964 mmol) in anhydrous DMF (4.5 mL) was added NaH (60% in oil, 50 mg, 1.25 mmol) at 0 °C. After stirring for 15 min, *N*,*N*-dimethylcarbamoyl chloride (115 μL, 1.25 mmol) was added to the mixture. The mixture was stirred at room temperature for 1 h, then poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (MeOH/CH_2Cl_2) to give **16a** (304 mg, 0.795 mmol, 82%) as a light yellow solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.12 (s, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.72 (d, J = 7.7 Hz, 1H), 7.58 (t, J = 7.9 Hz, 1H), 7.26 (d, J = 2.3 Hz, 1H), 7.19 (dd, J = 8.7, 2.5 Hz, 1H), 4.14 (s, 2H), 3.07 (s, 3H), 2.93 (s, 3H), 2.51 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.8, 153.4, 153.3, 152.2, 148.8, 147.9, 141.4, 134.9, 129.9, 126.4, 122.8, 122.3, 121.3, 118.5, 117.3, 109.7, 36.4, 36.2, 32.0, 15.4; ESIMS *m/z* 383 [M + H]$^+$; HRMS (ESI) *m/z* 383.1236 [(M + H)$^+$; calcd for $C_{20}H_{19}N_2O_6$: 383.1238].

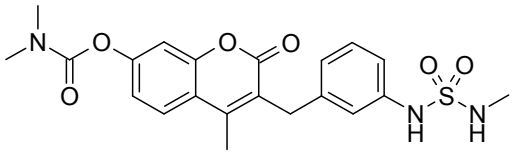
Dimethyl-carbamic acid 3-(3-aminobenzyl)-4-methyl-2-oxo-2*H*-chromen-7-yl ester (**17a**)



To a solution of **16a** (292 mg, 0.764 mmol) in anhydrous DMF (10 mL) was added $SnCl_2 \cdot 2H_2O$ (862 mg, 3.82 mmol) at room temperature. After stirring for 21 h, saturated $NaHCO_3$ aqueous solution was added to the mixture, and then extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel chromatography ($MeOH/CH_2Cl_2$) to give **17a** (246 mg, 0.698 mmol, 91%) as a light yellow solid.

^1H NMR ($CDCl_3$, 270 MHz) δ 7.59 (d, J = 8.9 Hz, 1H), 7.02–7.12 (m, 3H), 6.64 (d, J = 7.7 Hz, 1H), 6.58 (s, 1H), 6.51 (dd, J = 7.7, 2.0 Hz, 1H), 3.97 (s, 2H), 3.60 (brs, 2H), 3.13 (s, 3H), 3.03 (s, 3H), 2.42 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.8, 153.4, 153.1, 152.1, 148.8, 147.7, 139.4, 128.9, 126.2, 123.6, 118.5, 117.4, 115.7, 113.2, 111.8, 109.7, 36.4, 36.2, 32.3, 15.3.

4-methyl-3-(3-(N-methylsulfamoylamino)benzyl)-2-oxo-2*H*-chromen-7-yl dimethylcarbamate (**11a**)

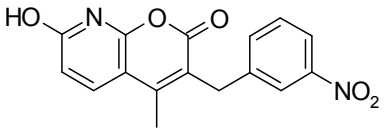


To a solution of **17a** (100 mg, 0.284 mmol) in anhydrous MeCN (3 mL) were added *N*-methyl-2-oxooxazolidine-3-sulfonamide[2] (102 mg, 0.568 mmol) and Et_3N (118 μL, 0.852 mmol) at room temperature. The reaction mixture was heated to 80 °C for 6 h. The mixture was cooled to room temperature and diluted with EtOAc. The mixture was washed with 1.0 M aqueous HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was recrystallized from EtOH to give **11a** (91.1 mg, 0.204 mmol, 72%) as a white solid.

^1H NMR ($CDCl_3$, 270 MHz) δ 7.59 (d, J = 5.4 Hz, 1H), 7.30–6.54 (m, 6H), 4.65–4.55 (m, 1H), 4.01 (s, 2H), 3.17 (s, 3H), 3.08 (s, 3H), 2.64 (d, J = 5.1 Hz, 3H), 2.44 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.8, 153.4, 153.2, 152.2, 148.2, 139.7, 139.0, 129.0, 126.3, 123.0, 122.2, 118.5, 118.0, 117.4, 115.9, 109.7, 36.4, 36.2, 32.2, 28.3, 15.4; ESIMS *m/z*

446 [M + H]$^+$; HRMS (ESI) *m/z* 446.1384 [(M + H)$^+$; calcd for $C_{21}H_{24}N_3O6S$: 446.1380].

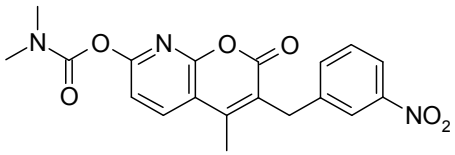
7-Hydroxy-4-methyl-3-(3-nitro-benzyl)-pyrano[2,3-*b*]pyridin-2-one (**6b**)



To a solution of 2-(3-Nitrobenzyl)-3-oxo-butyric acid ethyl ester[1] (488 mg, 1.84 mmol) and 2,6-dihydroxypyridine (204 mg, 1.84 mmol) in anhydrous methanol (10 mL) was added $Zn(OTf)_2$ (669 mg, 1.84 mmol). The mixture was heated to reflux for 30 h, then cooled to room temperature and diluted with EtOAc. The mixture was washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (EtOAc/hexane) to give **6b** (330 mg, 1.06 mmol, 58%) as a white solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.14–8.05 (m, 3H), 7.71 (d, J = 7.7 Hz, 1H), 7.57 (dd, J = 7.9, 7.7 Hz, 1H), 6.67 (d, J = 8.6 Hz, 1H), 4.07 (s, 2H), 2.44 (s, 3H); ESIMS *m/z* 313 [M + H]$^+$; HRMS (ESI) *m/z* 311.0668 [(M − H)$^-$; calcd for $C_{16}H_{11}N_2O_5$: 311.0673].

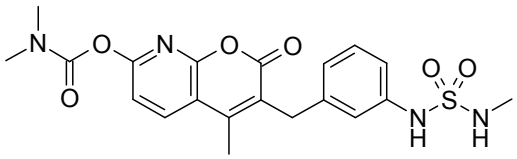
Dimethyl-carbamic acid 4-methyl-3-(3-nitro-benzyl)-2-oxo-2*H*-pyrano[2,3-*b*]pyridin-7-yl ester (**16b**)



To a solution of **6b** (330 mg, 1.06 mmol) in anhydrous DMF (4 mL) was added NaH (60% in oil, 47.0 mg, 1.17 mmol) at room temperature. After stirring for 15 min, *N*,*N*-dimethylcarbamoyl chloride (108 μL, 1.17 mmol) was added to the mixture. The mixture was heated to 100 °C for 2 h. After cooling to room temperature, water was added to the mixture. The solid was collected by filtration and washed with water and MeOH to give **16b** (721 μmol, 68%) as a gray solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.43 (d, J = 8.4 Hz, 1H), 8.14–8.05 (m, 2H), 7.73 (d, J = 7.7 Hz, 1H), 7.58 (dd, J = 8.1, 7.7 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 4.14 (s, 2H), 3.07 (s, 3H), 2.95 (s, 3H), The CH_3 peak was overlapped with the DMSO peak; ESIMS *m/z* 384 [M + H]$^+$.

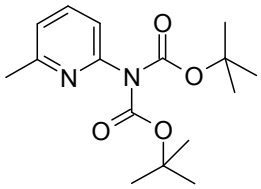
Dimethyl-carbamic acid 3-[3-(*N*-methylsulfamoylamino)-benzyl]-4-methyl-2-oxo-2*H*-pyrano[2,3-*b*]pyridin-7-yl ester (**11b**)



To a solution of **16b** (141.8 μmol) in anhydrous EtOAc (2.2 mL) was added $SnCl_2 \cdot H_2O$ (169 mg, 0.754 mmol) at room temperature. The reaction mixture was heated to reflux for 4 h. After cooling to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography on silica gel (KP-NH silica cartridge) eluting with MeOH and CH_2Cl_2 to give an aniline (49.0 mg, 139 μmol). The purity of the aniline was checked by LC/MS (ESIMS *m/z* 354 $[M + H]^+$). To a solution of the aniline (49.0 mg, 139 μmol) in anhydrous CH_3CN (2 mL) were added *N*-methyl-2-oxooxazolidine-3-sulfonamide[2] (50.0 mg, 278 μmol) and Et_3N (58.0 μL, 417 μmol) at room temperature. The reaction mixture was heated to 80 °C for 2.5 h. After cooling to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography on silica gel (KP-NH silica cartridge) eluting with MeOH and CH_2Cl_2 to give **11b** (31.0 mg, 69.4 μmol, 49%) as an amorphous.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 9.53 (br s, 1H), 8.43 (d, J = 8.2 Hz, 1H), 7.27 (d, J = 8.2 Hz, 1H), 7.20–7.14 (m, 2H), 7.02 (d, J = 7.1 Hz, 1H), 7.01 (s, 1H), 6.87 (d, J = 7.3 Hz, 1H), 3.94 (s, 2H), 3.06 (s, 3H), 2.95 (s, 3H), 2.47 (s, 3H), 2.42 (s, 3H); ESIMS *m/z* 447 $[M + H]^+$; HRMS (ESI) *m/z* 447.1329 $[(M + H)^+$; calcd for $C_{20}H_{23}N_4O_6S$: 447.1333].

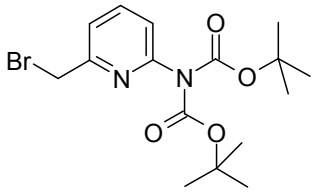
2-[Bis(*tert*-butoxycarbonyl)amino]-6-methylpyridine (**18c**)



6-Methyl-pyridin-2-ylamine (15.0 g, 139 mmol) was dissolved in Boc_2O (41.4 mL, 180 mmol). The mixture was heated to 60 °C for 15 h. After cooling to room temperature, the resulting mixture was dissolved in anhydrous THF (100 mL), and added to a solution of

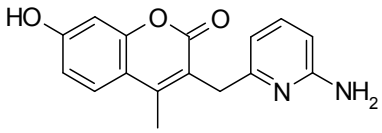
Boc_2O (95.6 mL, 416 mmol) and DMAP (59.3 g, 486 mmol) in anhydrous THF (650 mL) over 30 min. After stirring for 5h, the mixture was diluted with EtOAc, and washed with 1.0 M aqueous NH_4Cl twice, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was recrystallized from EtOH and hexane to give **18c** (34.8 g, 112 mmol, 82%) as a white solid.
^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.74 (dd, J = 7.6 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 7.14 (d, J = 7.6 Hz, 1H), 2.42 (s, 3H), 1.39–1.31 (m, 18H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 157.0, 151.0, 151.0 (×2), 138.5, 121.5, 117.9, 82.4 (×2), 27.4 (×6), 23.5; ESIMS m/z 309 $[M + H]^+$; HRMS (ESI) m/z 309.1806 $[(M + H)^+$; calcd for $C_{16}H_{25}N_2O_4$: 309.1809].

2-[Bis(*tert*-butoxycarbonyl)amino]-6-(bromomethyl)pyridine (**3c**)



To a solution of **18c** (6.00 g, 19.5 mmol) and *N*-bromosuccinimide (4.50 g, 25.4 mmol) in CCl_4 (90 mL) was added benzoylperoxide (wetted with 30% water, 675 mg, 1.95 mmol). The mixture was heated to reflux for 4 h, then cooled to room temperature and filtered. The filtrate was concentrated in vacuo and purified by silica gel chromatography (EtOAc/hexane=1/7) to give **3c** (4.34 g, 11.2 mmol, 58%) as a yellow solid.
^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.73 (dd, J = 8.1 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.20 (d, J = 8.1 Hz, 1H), 4.51 (s, 2H), 1.39–1.31 (m, 18H).

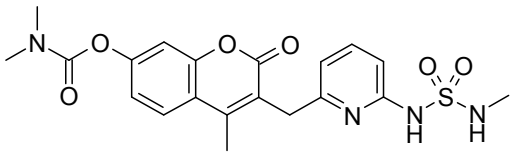
3-(6-Amino-pyridin-2-ylmethyl)-7-hydroxy-4-methyl-chromen-2-one (**6c**)



To a solution of ethyl acetoacetate (223 μL, 1.75 mmol) in anhydrous THF (5 mL) was added NaH (60% in oil, 79.2 mg, 1.98 mmol) at 0 °C. After stirring for 15 min, **3c** (450 mg, 1.17 mmol) was added to the mixture at 0 °C and then stirred at room temperature for 5 h. The mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over K_2CO_3 and

evaporated. The residue was purified by silica gel chromatography (EtOAc/hexane =1/5) to give an ester. The ester and resorcinol (230 mg, 2.09 mmol) were dissolved in sulfuric acid (222 μL, 4.17 mmol). After stirring at room temperature for 6 h, the mixture was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (MeOH/CH_2Cl_2=1/10) to give **6c** (98.0 mg, 347 μmol, 30%) as a solid. ^{1}H NMR (DMSO-d_6, 270 MHz) δ 7.64 (d, J = 8.1 Hz, 1H), 7.23 (dd, J = 8.1 Hz, 1H), 6.78 (d, J = 8.1 Hz, 1H), 6.70 (br s, 1H), 6.20–6.10 (m, 2H), 5.85–5.75 (m, 2H), 3.82 (s, 2H), 2.42 (s, 3H); ESIMS *m/z* 283 $[M + H]^+$.

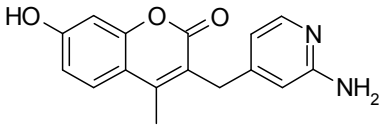
Dimethyl-carbamic acid 3-[6-(*N*-methylsulfamoylamino)-pyridin-2-ylmethyl]-4-methyl-2-oxo-2*H*-chromen-7-yl ester (**11c**)



To a solution of **6c** (53.5 mg, 190 μmol) in anhydrous DMF (1.6 mL) was added NaH (60% in oil, 8.4 mg, 209 μmol) at 0 °C. After stirring for 10 min, *N,N*-dimethylcarbamoyl chloride (22.8 μL, 285 μmol) was added to the mixture. After stirring at 0 °C for 1.5 h, the mixture was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (MeOH/CH_2Cl_2 = 1/20) to give an aminopyridine (23.7 mg, 67.1 μmol). The purity of the aminopyridine was checked by LC/MS (ESIMS *m/z* 354 $[M + H]^+$). To a solution of the aminopyridine (23.7 mg, 67.1 μmol) in anhydrous CH_3CN (0.8 mL) were added *N*-methyl-2-oxooxazolidine-3-sulfonamide (30.2 mg, 168 μmol) and Et_3N (46.6 μL, 336 μmol) at room temperature. The reaction mixture was heated to 80 °C for 12 h. The mixture was purified by preparative HPLC to give **11c** (23.3 mg, 52.2 μmol, 27%) as a white solid.

HRMS (ESI) *m/z* 447.1330 $[(M + H)^+$; calcd for $C_{20}H_{23}N_4O_6S$: 447.1333]

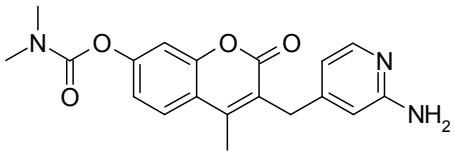
3-(2-Amino-pyridin-4-ylmethyl)-7-hydroxy-4-methyl-chromen-2-one (**6d**)



Compound **6d** was prepared by the procedures previously described for compound **6c** using 2-[bis(*tert*-butoxycarbonyl)amino]-4-(bromomethyl)pyridine[3] (46.2 mg, 573 μmol, 29%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.46 (br s, 1H), 7.75 (d, J = 5.34 Hz, 1H), 7.65 (d, J = 8.77 Hz, 1H), 6.82 (dd, J = 8.77, 2.67 Hz, 1H), 6.72 (d, J = 2.29 Hz, 1H), 6.35 (d, J = 5.34 Hz, 1H), 6.20 (s, 1H), 5.76 (br s, 2H), 3.76 (s, 2H), 2.35 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.3, 160.6, 160.0, 153.5, 149.1, 149.0, 147.5, 126.8, 118.8, 113.0, 112.4, 112.3, 106.6, 102.0, 31.6, 15.2; HRMS (ESI)*m/z* 283.1077 [(M + H)$^+$; calcd for $C_{16}H_{15}N_2O_3$: 283.1077].

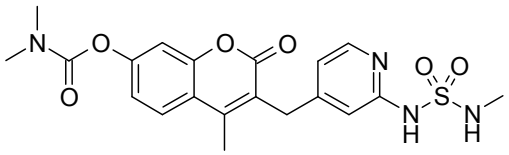
Dimethyl-carbamic acid 3-(2-amino-pyridin-4-ylmethyl)-4-methyl-2-oxo-2*H*-chromen-7-yl ester (**17d**)



Compound **17d** was prepared by the procedures previously described for compound **16a** using **6d** (68.2 mg, 193 μmol, 78%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.84 (d, J = 8.77 Hz, 1H), 7.76 (d, J = 5.34 Hz, 1H), 7.25 (d, J = 2.28 Hz, 1H), 7.18 (dd, J = 8.77, 2.28 Hz, 1H), 6.37 (dd, J = 5.34, 1.52 Hz, 1H), 6.19 (s, 1H), 5.75 (br s, 2H), 3.82 (s, 2H), 3.06 (s, 3H), 2.93 (s, 3H), 2.42 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.7, 160.1, 153.4, 153.3, 152.2, 148.6, 148.4, 147.7, 126.3, 122.1, 118.6, 117.3, 112.2, 109.7, 106.5, 36.4, 36.2, 31.7, 15.4; ESIMS *m/z* 354 [M + H]$^+$; HRMS (ESI)*m/z* 354.1442 [(M + H)$^+$; calcd for $C_{19}H_{20}N_3O_4$: 354.1448]..

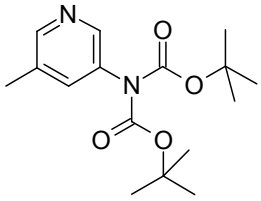
Dimethyl-carbamic acid 3-[2-(*N*-methylsulfamoylamino)-pyridin-4-ylmethyl]-4-methyl-2-oxo-2*H*-chromen-7-yl ester (**11d**)



Compound **11d** was prepared by the procedures previously described for compound **11a** using **17d** (26.8 mg, 60.0 μmol, 71%).

^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.10–8.04 (m, 1H), 7.87 (d, J = 8.6 Hz, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.18 (dd, J = 8.9, 2.4 Hz, 1H), 7.10-6.85 (br s, 1H), 6.84–6.75 (m, 2H), 3.96 (s, 2H), 3.07 (s, 3H), 2.93 (s, 3H), 2.60–2.40 (m, 6H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 162.9, 155.6, 155.5, 155.0, 154.4, 152.4, 151.2, 149.3, 128.6, 123.8, 120.8, 119.4, 119.2, 112.6, 111.9, 38.6, 38.4, 34.1, 31.0, 17.6; ESIMS *m/z* 447 [M + H]$^+$; HRMS (ESI)*m/z* 447.1332 [(M + H)$^+$; calcd for $C_{20}H_{23}N_4O_6S$: 447.1333].

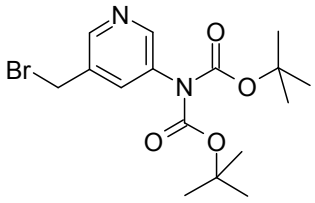
3-[Bis(*tert*-butoxycarbonyl)amino]-5-methylpyridine (**18e**)



Compound **18e** was prepared by the procedures previously described for compound **18c** using 5-methyl-pyridin-3-ylamine (3.78 g, 12.3 mmol, 33%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.37 (s, 1H), 8.24 (s, 1H), 7.54 (s, 1H), 2.33 (s, 3H), 1.46–1.35 (m. 18H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 151.0 (×2), 148.5, 145.9, 135.7, 135.3, 133.2, 82.7 (×2), 27.5 (×6), 17.5; ESIMS *m/z* 309 [M + H]$^+$; HRMS (ESI)*m/z* 309.1806 [(M + H)$^+$; calcd for $C_{16}H_{25}N_2O_4$: 309.1809].

3-[Bis(*tert*-butoxycarbonyl)amino]-5-(bromomethyl)pyridine (**3e**)

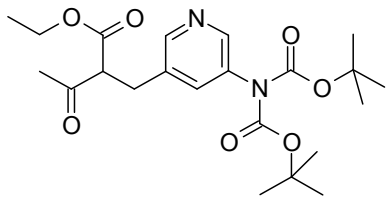


To a solution of **18e** (762 mg, 2.47 mmol) and *N*-bromosuccinimide (1.76 g, 9.88 mmol) in

CCl_4 (15 mL) was added AIBN (406 mg, 2.47 mmol). The mixture was heated to reflux for 2 h, then cooled to room temperature and filtered. The filtrate was concentrated in vacuo and purified by silica gel chromatography (EtOAc/hexane) to give **3e** (275 mg, 709 μmol, 29%) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.60 (s, 1H), 8.40 (s, 1H), 7.82 (s, 1H), 4.79 (s, 2H), 1.46–1.33 (m. 18H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 150.5 (×2), 148.5, 148.4, 136.1, 135.6, 134.4, 82.9 (×2), 30.1, 27.5 (×6); ESIMS *m/z* 387 $[M + H]^+$; HRMS (ESI)*m/z* 387.0914 $[(M + H)^+$; calcd for $C_{16}H_{24}BrN_2O_4$: 387.0914].

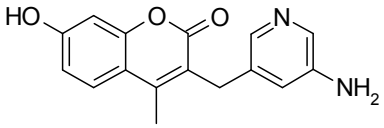
Ethyl 2-({5-[bis(*tert*-butoxycarbonyl)amino]pyridine-3-yl}methyl)-3-oxobutanonate (**4e**)



To a solution of ethyl acetoacetate (234 uL, 1.84 mmol) in anhydrous THF (8 mL) was added NaH (60% in oil, 40.4 mg, 1.01 mmol) at 0 °C. After stirring for 15 min, **3e** (178 mg, 460 μmol) in anhydrous THF (2 mL) was added to the mixture at 0 °C and then stirred at room temperature for 24 h. The mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (EtOAc/hexane) to give **4e** (61.0 mg, 140 μmol, 30%) as a light yellow oil.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.37 (d, J = 1.6 Hz, 1H), 8.27 (d, J = 1.6 Hz, 1H), 7.59 (dd, J = 1.6, 1.6 Hz, 1H), 4.13 (dd, J = 8.8, 6.6 Hz, 1H), 4.10–3.98 (m, 2H), 3.09 (dd, J = 14.1, 6.6 Hz, 1H), 3.02 (dd, J = 14.1, 8.8 Hz, 1H), 2.20 (s, 3H), 1.46–1.33 (m. 18H), 1.11 (dd, J = 7.1, 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 202.3, 168.5, 150.7 (×2), 148.6, 146.9, 135.4, 135.4, 134.2, 82.7 (×2), 61.0, 59.5, 29.7, 29.3, 27.4 (×6), 13.8; HRMS (ESI)*m/z* 437.2277 $[(M + H)^+$; calcd for $C_{22}H_{33}N_2O_7$: 437.2282].

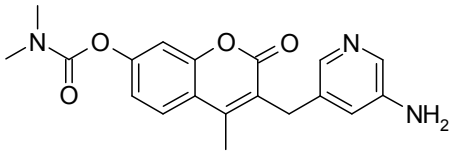
3-(5-Amino-pyridin-3-ylmethyl)-7-hydroxy-4-methyl-chromen-2-one (**6e**)



Resorcinol (27.3 mg, 248 μmol) and **4e** (54.2 mg, 124 μmol) were dissolved in sulfuric acid (19.9 μL, 373 μmol) at 0 °C. After stirring at room temperature for 4 h, saturated aqueous $NaHCO_3$ was added to the mixture. The aqueous mixture was extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by preparative HPLC to give **6e** (30.3 mg, 107 μmol, 86%) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.56 (s, 1H), 7.95 (br s, 1H), 7.85 (br s, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.38 (br s, 1H), 6.84 (dd, J = 8.8, 2.2 Hz, 1H), 6.73 (d, J = 2.2 Hz, 1H), 6.36 (br s, 2H), 3.96 (s, 2H), 2.44 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.2, 160.9, 153.6, 150.0, 147.6, 139.4, 128.2, 127.1, 126.3, 124.5, 117.9, 113.1, 112.3, 102.0, 29.3, 15.2; ESIMS *m/z* 283 $[M + H]^+$; HRMS (ESI) *m/z* 283.1074 $[(M + H)^+$; calcd for $C_{16}H_{15}N_2O_3$: 283.1077].

Dimethyl-carbamic acid 3-(5-amino-pyridin-3-ylmethyl)-4-methyl-2-oxo-2*H*-chromen-7-yl ester (**17e**)



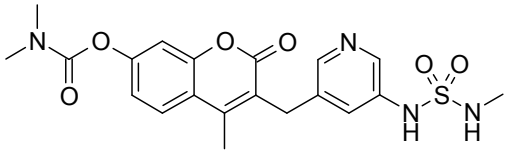
To a solution of **6e** (17.4 mg, 62.0 μmol) in anhydrous DMF (1 mL) was added K_2CO_3 (8.5 mg, 62.0 μmol) at room temperature. After stirring for 5 min, *N*,*N*-dimethylcarbamoyl chloride (7.4 μL, 80.0 μmol) was added to the mixture. The mixture was stirred at room temperature for 7 h, then poured into brine, and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by preparative HPLC to give **17e** (10.1 mg, 28.6 μmol, 46%) as a solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.84 (d, J = 8.8 Hz, 1H), 7.74 (br s, 1H), 7.69 (br s, 1H), 7.25 (d, J = 2.2 Hz, 1H), 7.18 (dd, J = 8.8, 2.2 Hz, 1H), 6.70 (br s, 1H), 5.22 (br s, 2H), 3.85 (s, 2H), 3.07 (s, 3H), 2.93 (s, 3H), 2.47 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.8, 153.4, 153.3, 152.2, 148.1, 144.7, 136.9, 134.4, 134.3, 126.3, 122.9, 118.9, 118.5, 117.3, 109.7, 36.4, 36.2, 29.5, 15.3; ESIMS *m/z* 354 $[M + H]^+$; HRMS (ESI) *m/z* 354.1451

[(M + H)$^+$; calcd for $C_{19}H_{20}N_3O_4$: 354.1448].

Dimethyl-carbamic acid 3-[5-(*N*-methylsulfamoylamino)-pyridin-3-ylmethyl]-4-methyl-2-oxo-2*H*-chromen-7-yl ester

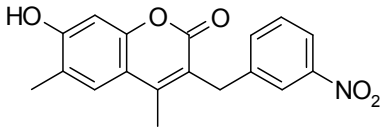
(**11e**)



Compound **11e** was prepared by the procedures previously described for compound **11a** using **17e** (2.2 mg, 4.9 μmol, 56%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.96 (s, 1H), 8.29 (br s., 1H), 8.23 (br s, 1H), 7.87 (d, J= 8.4 Hz, 1H), 7.51 (q, J= 4.9 Hz, 1H), 7.49 (br s, 1H), 7.26 (d, J= 2.2 Hz, 1H), 7.19 (dd, J= 8.4, 2.2 Hz, 1H), 4.00 (s, 2H), 3.07 (s, 3H), 2.93 (s, 3H), 2.44 (d, J= 4.9 Hz, 3H), The CH_3 peak was overlapped with the DMSO peak; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.7, 153.4, 153.3, 152.2, 148.7, 142.0, 136.0, 135.9, 135.7, 126.4, 126.1, 122.0, 118.6, 117.3, 109.7, 36.4, 36.2, 29.6, 28.3, 15.5; ESIMS *m/z* 447 [M + H]$^+$; HRMS (ESI)*m/z* 447.1331 [(M + H)$^+$; calcd for $C_{20}H_{23}N_4O_6S$: 447.1333].

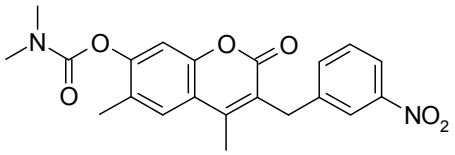
7-Hydroxy-4,6-dimethyl-3-(3-nitro-benzyl)-chromen-2-one (**6a'**)



Compound **6a'** was prepared by the procedures previously described for compound **6a** using 4-methylresorciol (1.45 g, 4.46 mmol, 45%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.49 (s, 1H), 8.07 (s, 1H), 8.05 (d, J= 7.5 Hz, 1H), 7.69 (d, J= 7.5 Hz, 1H), 7.57 (dd, J= 7.5, 7.5 Hz, 1H), 7.56 (s, 1H), 6.74 (s, 1H), 4.07 (s, 2H), 2.44 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.5, 158.8, 151.7, 149.5, 147.9, 142.0, 134.9, 129.9, 126.9, 122.6, 121.9, 121.2, 118.9, 111.9, 101.1, 31.8, 15.7, 15.2; ESIMS *m/z* 326 [M + H]$^+$; HRMS (ESI)*m/z* 326.1024 [(M + H)$^+$; calcd for $C_{18}H_{16}NO_5$: 326.1023].

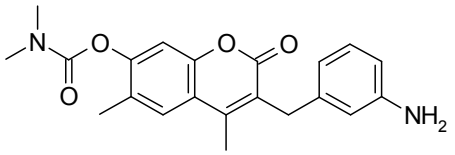
Dimethyl-carbamic acid 4,6-dimethyl-3-(3-nitro-benzyl)-2-oxo-2*H*-chromen-7-yl ester (**16a'**)



Compound **16a'** was prepared by the procedures previously described for compound **16a** using **6a'** (2.35 g, 5.93 mmol, 84%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.11 (s, 1H), 8.07 (d, J = 7.5 Hz, 1H), 7.75 (s, 1H), 7.70 (d, J = 7.5 Hz, 1H), 7.57 (dd, J = 7.5, 7.5 Hz, 1H), 7.22 (s, 1H), 4.12 (s, 2H), 3.10, (s, 3H), 2.94 (s, 3H), 2.23 (s, 3H), The CH_3 peak was overlapped with the DMSO peak; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.9, 153.1, 151.9, 150.4, 148.7, 147.9, 141.5, 134.9, 129.9, 127.1, 126.9, 122.8, 122.4, 121.3, 117.4, 110.2, 36.4, 36.2, 32.0, 15.5, 15.4; ESIMS *m/z* 397 $[M + H]^+$; HRMS (ESI) *m/z* 397.1391 $[(M + H)^+$; calcd for $C_{21}H_{21}N_2O_6$: 397.1394].

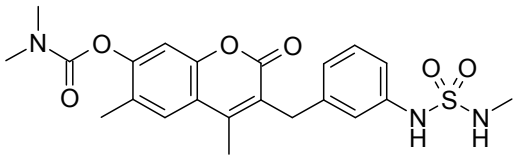
Dimethyl-carbamic acid 3-(3-amino-benzyl)-4,6-dimethyl-2-oxo-2*H*-chromen-7-yl ester (**17a'**)



To a solution of **16a'** (507 mg, 1.28 mmol) in anhydrous EtOAc (25 mL) was added $SnCl_2 \cdot 2H_2O$ (1.16 g, 5.12 mmol) at room temperature. The mixture was heated to 80 °C for 4 h. The mixture was cooled to room temperature, poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with water and then brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was washed with MeOH to give **17a'** (294 mg, 0.802 mmol, 62%) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.73 (s, 1H), 7.20 (s, 1H), 6.90 (dd, J = 7.5, 7.5 Hz, 1H), 6.38–6.35 (m, 3H), 4.96 (br s, 2H), 3.83 (s, 2H), 3.10, (s, 3H), 2.94 (s, 3H), 2.42 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.9, 153.1, 151.6, 150.3, 148.8, 147.7, 139.4, 128.9, 126.8, 126.8, 123.6, 117.5, 115.7, 113.1, 111.8, 110.1, 36.4, 36.2, 32.3, 15.5, 15.3; ESIMS *m/z* 367 $[M + H]^+$; HRMS (ESI) *m/z* 367.1647 $[(M + H)^+$; calcd for $C_{21}H_{23}N_2O_4$: 367.1652].

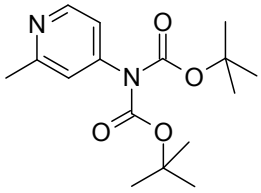
4,6-dimethyl-3-[3-(N-methylsulfamoylamino)benzyl]-2-oxo-2*H*-chromen-7-yl dimethylcarbamate (**12a**)



Compound **12a** was prepared by the procedures previously described for compound **11a** using **17a'** (192 mg, 0.418 mmol, 76%).

^{1}H NMR (DMSO-d_6, 270 MHz) δ 7.75 (s, 1H), 7.22–7.14 (m, 3H), 7.04–7.00 (m, 2H), 6.86 (d, J = 8.1 Hz, 1H), 3.93 (s, 2H), 3.10 (s, 3H), 2.94 (s, 3H), 2.46 (s, 3H), 2.42 (d, J = 5.4 Hz, 3H), 2.23 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.9, 153.1, 151.7, 150.3, 148.1, 139.8, 139.0, 129.0, 126.9, 126.9, 123.1, 122.2, 118.0, 117.5, 115.9, 110.1, 36.5, 36.2, 32.2, 28.3, 15.5, 15.4; ESIMS *m/z* 460[M + H]$^+$; HRMS (ESI)*m/z* 460.1537 [(M + H)$^+$; calcd for $C_{22}H_{26}N_3O_6S$: 460.1537].

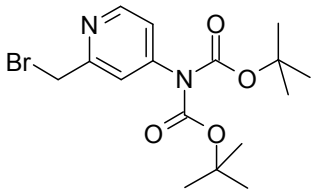
4-[Bis(*tert*-butoxycarbonyl)amino]-6-methylpyridine (**18f**)



Compound **18f** was prepared by the procedures previously described for compound **18c** using 2-methyl-pyridin-4-ylamine (9.61 g, 31.1 mmol, 84%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.39 (d, J = 5.3 Hz, 1H), 7.05 (d, J = 2.2 Hz, 1H), 7.00 (dd, J = 5.3, 2.2 Hz, 1H), 2.42 (s, 3H), 1.46–1.30 (m. 18H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.0, 150.6 (×2), 149.7, 146.5, 121.0, 119.1, 83.1 (×2), 27.4 (×6), 23.9; ESIMS *m/z* 309 [M + H]$^+$; HRMS (ESI)*m/z* 309.1806 [(M + H)$^+$; calcd for $C_{16}H_{25}N_2O_4$: 309.1809].

4-[Bis(*tert*-butoxycarbonyl)amino]-6-(bromomethyl)pyridine (**3f**)

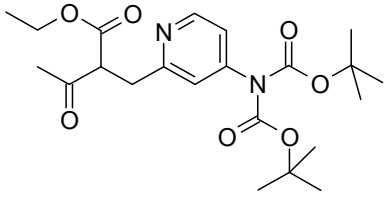


Compound **3f** was prepared by the procedures previously described for compound **3e**

using **18f** (1.05 g, 2.71 mmol, 42%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.55 (d, J = 5.7 Hz, 1H), 7.44 (br s, 1H), 7.22 (d, J = 5.7 Hz, 1H), 4.71 (s, 2H), 1.50–1.32 (m. 18H); ESIMS *m/z* 387 $[M + H]^+$.

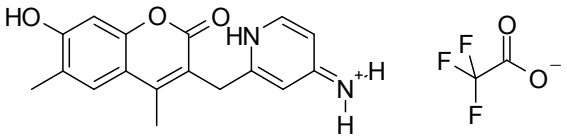
Ethyl 2-({4-[bis(*tert*-butoxycarbonyl)amino]pyridine-2-yl}methyl)-3-oxobutanonate (**4f**)



Compound **4f** was prepared by the procedures previously described for compound **4e** using **3f** (970 mg, 2.22 mmol, 82%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.44 (d, J = 5.3 Hz, 1H), 7.17 (d, J = 1.8 Hz, 1H), 7.09 (dd, J = 5.3, 1.8 Hz, 1H), 4.27 (dd, J = 7.5, 7.5 Hz, 1H), 4.12–4.00 (m, 2H), 3.29 (dd, J = 15.9, 7.5 Hz, 1H), 3.20 (dd, J = 15.9, 7.5 Hz, 1H), 2.25 (s, 3H), 1.50–1.32 (m. 18H), 1.13 (dd, J = 7.5, 7.5 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 202.8, 168.9, 159.1, 150.5 (×2), 149.5, 146.6, 121.1, 119.8, 83.2 (×2), 60.8, 57.2, 34.8, 29.5, 27.4 (×6), 13.9; ESIMS *m/z* 437 $[M + H]^+$; HRMS (ESI) *m/z* 437.2280 $[(M + H)^+$; calcd for $C_{22}H_{33}N_2O_7$: 437.2282].

Trifluoro-acetate 2-(7-hydroxy-4,6-dimethyl-2-oxo-2*H*-chromen-3-ylmethyl)-1*H*-pyridin-4-ylidene-ammonium (**6f**)

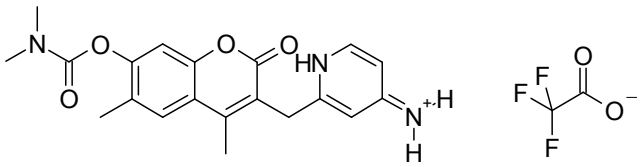


4-Methylresorcinol (684 mg, 5.51 mmol) and **4f** (481 mg, 1.10 mmol) were dissolved in sulfuric acid (353 μL, 6.62 mmol) at 0 °C. After stirring at room temperature for 18 h, 5.0 M aqueous NaOH (2.64 mL, 13.2 mmol) and DMSO were added to the mixture at 0 °C. The mixture was purified by preparative HPLC to give **6f** (221 mg, 538 μmol, 49%) as an oil.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.15 (br s, 1H), 10.65 (br s, 1H), 8.05–8.02 (m, 1H), 7.92 (br s, 1H), 7.76 (br s, 1H), 7.63 (s, 1H), 6.77 (s, 1H), 6.70–6.65 (m, 1H), 6.45 (s, 1H), 4.02 (s, 2H), 2.43 (s, 3H), 2.21 (s, 3H)); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 161.2, 160.1, 159.4, 158.4 (q, $J_{C\text{-}F}$ = 31.7 Hz), 152.0, 152.0, 151.4, 139.8, 127.1, 122.2, 117.3 (q, $J_{C\text{-}F}$ =

301.8 Hz), 115.0, 111.7, 107.5, 106.2, 101.3, 30.2, 15.7, 15.3; ESIMS *m/z* 297 [M + H − TFA]$^+$; HRMS (ESI) *m/z* 297.1229 [(M + H − TFA)$^+$; calcd for $C_{17}H_{17}N_2O_3$: 297.1234].

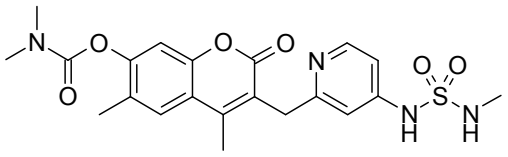
Dimethyl-carbamic acid Trifluoro-acetate 2-(7-dimethylcarbamoyloxy-4,6-dimethyl-2-oxo-2*H*-chromen-3-ylmethyl)-1*H*-pyridin-4-ylidene-ammonium (**17f**)



To a solution of **6f** (19.3 mg, 47.0 μmol) in anhydrous DMF (0.5 mL) was added K_2CO_3 (13.0 mg, 94.1 μmol) at 0 °C. After stirring for 5 min, *N*,*N*-dimethylcarbamoyl chloride (4.3 μL, 47 μmol) was added to the mixture. After stirring at room temperature for 5 h, the mixture was filtered. The filtrate was purified by preparative HPLC to give **17f** (11.5 mg, 23.9 μmol, 51%) as an oil.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.13 (br s, 1H), 8.05 (dd, *J* = 7.0, 7.0 Hz, 1H), 7.92 (br s, 1H), 7.84 (s, 1H), 7.74 (br s, 1H), 7.28 (s, 1H), 6.68 (dd, *J* = 7.0, 2.2 Hz, 1H), 6.45 (d, *J* = 2.2 Hz, 1H), 4.09 (s, 2H), 3.10 (s, 3H), 2.94 (s, 3H), 2.24 (s, 3H), The CH_3 peak was overlapped with the DMSO peak; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.6, 160.0, 158.0 (q, $J_{C\text{-}F}$ = 31.7 Hz), 153.1, 152.3, 151.5, 150.7, 150.6, 139.9, 127.3, 127.3, 118.7, 117.3, 117.1 (q, $J_{C\text{-}F}$ = 298.4 Hz), 110.4, 107.5, 106.3, 36.5, 36.3, 30.3, 15.5, 15.4; ESIMS *m/z* 368 [M + H − TFA]$^+$; HRMS (ESI) *m/z* 368.1600 [(M + H − TFA)$^+$; calcd for $C_{20}H_{22}N_3O_4$: 368.1605].

Dimethyl-carbamic acid 3-[5-(*N*-methylsulfamoylamino)-pyridin-3-ylmethyl]-4,6-dimethyl-2-oxo-2*H*-chromen-7-yl ester (**12f**)

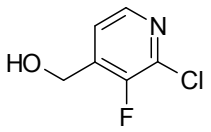


Compound **12f** was prepared by the procedures previously described for compound **11a** using **17f** (16.4 mg, 35.6 μmol, 69%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.20 (br s, 1H), 8.21 (d, *J* = 4.8 Hz, 1H), 7.74 (s, 1H), 7.64 (br s, 1H), 7.21 (s, 1H), 7.00–6.85 (m, 2H), 4.04 (s, 2H), 3.10 (s, 3H), 2.94 (s, 3H), 2.45–2.40 (m, 6H), 2.23 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.7, 159.2, 153.0,

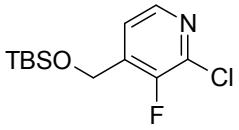
151.5, 150.3, 149.5, 148.3, 146.1, 126.8, 126.7, 122.1, 117.4, 110.0, 109.6, 109.0, 36.3, 36.1, 35.0, 28.1, 15.3 (×2); ESIMS *m/z* 461 $[M + H]^+$; HRMS (ESI)*m/z* 461.1485 $[(M + H)^+$; calcd for $C_{21}H_{25}N_4O_6S$: 461.1489].

(2-Chloro-3-fluoro-pyridin-4-yl)-methanol (**19g**)



To a solution of 0.47 M lithium diisopropylamide in anhydrous THF (41.4 mL, 14.9 mmol) was slowly added 2-chloro-3-fluoropyridine (1.51 mL, 14.9 mmol) at −78 °C. After stirring for 2 h, anhydrous DMF (11.4 mL, 149 mmol) was added to the mixture. The mixture was warmed up to room temperature. After stirring for 2 h, $NaBH_4$ (731 mg, 19.3 mmol) was added to the mixture at 0 °C. The mixture was stirred at 0 °C for 1 h, then poured into water, and extracted with EtOAc. The extract was washed with 1.0 M aqueous HCl, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography ($MeOH/CH_2Cl_2$) to give **19g** (1.40 g, 8.67 mmol, 58%) as a light yellow solid.
^1H NMR (DMSO-d_6, 270 MHz) δ 8.27 (d, J = 4.6 Hz, 1H), 7.56 (dd, J = 4.6 Hz, 1H), 5.70 (t, J = 5.8 Hz, 1H), 4.65 (d, J = 5.8 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 151.2 (d, $J_{C\text{-}F}$ = 257.7 Hz), 145.1 (d, $J_{C\text{-}F}$ = 6.3 Hz), 141.2 (d, $J_{C\text{-}F}$ = 13.1 Hz), 136.6 (d, $J_{C\text{-}F}$ = 18.7 Hz), 123.0, 56.1 (d, $J_{C\text{-}F}$ = 3.0 Hz); ESIMS *m/z* 162 $[M + H]^+$; HRMS (ESI)*m/z* 162.0116 $[(M + H)^+$; calcd for C_6H_6ClFNO: 162.0116].

4-(*tert*-Butyl-dimethyl-silanyloxymethyl)-2-chloro-3-fluoro-pyridine (**8g**)

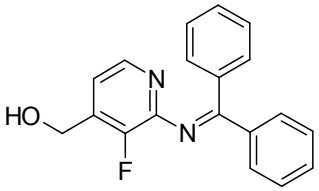


To a solution of **19g** (200 mg, 1.24 mmol) in anhydrous DMF (4 mL) were added imidazole (253 mg, 3.72 mmol) and TBSCl (373 mg, 2.48 mmol) at room temperature. After stirring for 2 h, the mixture was diluted with CH_2Cl_2, and washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (CH_2Cl_2) to give **8g** (319 mg, 1.16 mmol, 93%) as a light yellow

solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.30 (d, J = 4.9 Hz, 1H), 7.51 (dd, J = 4.9 Hz, 1H), 4.87 (s, 2H), 0.86 (s, 9H), 0.12 (s, 6H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 151.1 (d, $J_{C\text{-}F}$ = 258.4 Hz), 145.3 (d, $J_{C\text{-}F}$ = 6.8 Hz), 139.9 (d, $J_{C\text{-}F}$ = 12.7 Hz), 136.7 (d, $J_{C\text{-}F}$ = 18.7 Hz), 122.5 (d, $J_{C\text{-}F}$ = 1.1 Hz), 57.9 (d, $J_{C\text{-}F}$ = 3.7 Hz), 25.7 (×3), 18.0, -5.5 (×2); ESIMS *m/z* 276 [M + H]$^+$; HRMS (ESI) *m/z* 276.0979 [(M + H)$^+$; calcd for $C_{12}H_{20}ClFNOSi$: 276.0981].

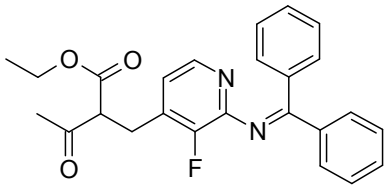
[2-(Benzhydrylidene-amino)-3-fluoro-pyridin-4-yl]-methanol (**3g**)



Compound **8g** (80.0 g, 290 mmol), benzophenone imine (58.4 mL, 348 mmol), sodium *tert*-butoxide (33.4 g, 348 mmol), $Pd_2(dba)_3$ (7.97 g, 8.70 mmol) and *rac*-BINAP (12.6g, 20.3 mmol) were dissolved in anhydrous toluene (970 mL). N_2 gas was bubbled into the mixture for 10 min, and the mixture was heated to 60 °C for 4 h. After cooling to room temperature, the mixture was filtered through Celite, and then poured into saturated aqueous $NaHCO_3$. The aqueous mixture was extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and evaporated to give a crude aminopyridine. The purity of the aminopyridine was checked by LC/MS (ESIMS *m/z* 421 [M + H]$^+$). The crude aminopyridine was dissolved in anhydrous THF (480 mL), and 1.0 M TBAF in THF (305 mL, 305 mmol) was slowly added at room temperature. After stirring for 2 h, the mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was recrystallized from CH_2Cl_2 and hexane to give **3g** (67.0 g, 219 mmol, 76%) as a light yellow solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.04 (d, J = 5.0 Hz, 1H), 7.75–7.45 (m, 5H), 7.40–7.25 (m, 3H), 7.20–7.04 (m, 3H), 5.47 (t, J = 5.6 Hz, 1H), 4.47 (d, J = 5.6 Hz, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.7 (d, $J_{C\text{-}F}$ = 1.1 Hz), 151.2 (d, $J_{C\text{-}F}$ = 12.3 Hz), 145.1 (d, $J_{C\text{-}F}$ = 252.4 Hz), 143.4 (d, $J_{C\text{-}F}$ = 6.7 Hz), 138.6 (d, $J_{C\text{-}F}$ = 12.0 Hz), 137.7, 135.7, 131.9, 129.3, 129.2 (×2), 128.6 (×2), 128.1 (×2), 128.1 (×2), 118.4, 55.8 (d, $J_{C\text{-}F}$ = 4.5 Hz); ESIMS *m/z* 307 [M + H]$^+$; HRMS (ESI) *m/z* 307.1239 [(M + H)$^+$; calcd for $C_{19}H_{16}FN_2O$: 307.1241].

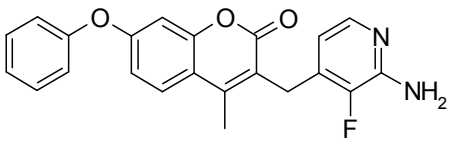
2-[2-(Benzhydrylidene-amino)-3-fluoro-pyridin-4-ylmethyl]-3-oxo-butyric acid ethyl ester (**4g**)



To a solution of **3g** (10.0 g, 32.6 mmol) in anhydrous THF (80 mL) was added 1.0 M lithium *tert*-butoxide in THF (35.9 mL, 35.9 mmol) and MsCl (3.03 mL, 39.2 mmol) at 0 °C. After stirring for 1h, the resulting mixture was added to a solution of ethyl acetoacetate (8.32 mL, 65.3 mmol), 1.0 M lithium *tert*-butoxide in THF (39.2 mL, 39.2 mmol), NaI (4.89 g, 32.6 mmol) and anhydrous THF (36 mL) at 0 °C over 30 min. The mixture was warmed up to 50 °C for 3 h. After cooling to room temperature, the mixture was diluted with EtOAc, and washed with 0.2 M aqueous LiOH 4 times and brine. The organic layer was dried over $MgSO_4$ and evaporated to give **4g** (13.0 g, 31.0 mmol, 95%) as a light yellow oil.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 7.93 (d, J = 4.3 Hz, 1H), 7.75–7.30 (m, 8H), 7.15–7.05 (m, 2H), 6.91 (dd, J = 4.3 Hz, 1H), 4.05 (q, J = 7.0 Hz, 2H), 3.94 (t, J = 7.7, 1H), 3.05–2.90 (m, 2H), 2.13 (s, 3H), 1.10 (t, J = 7.0, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 201.8, 171.8, 168.2, 151.8 (d, $J_{C\text{-}F}$ = 13.1 Hz), 146.5 (d, $J_{C\text{-}F}$ = 256.2 Hz), 143.2 (d, $J_{C\text{-}F}$ = 8.6 Hz), 137.6, 135.6, 134.3 (d, $J_{C\text{-}F}$ = 13.1 Hz), 131.9, 129.3, 129.3 (×2), 128.6 (×2), 128.1 (×2), 128.1 (×2), 121.1, 61.2, 57.6, 29.2, 25.6, 13.8; ESIMS *m/z* 419 [M + H]$^+$; HRMS (ESI) *m/z* 419.1761 [(M + H)$^+$; calcd for $C_{25}H_{24}FN_2O_3$: 419.1765].

3-(2-Amino-3-fluoro-pyridin-4-ylmethyl)-4-methyl-7-phenoxy-chromen-2-one (**10g**)

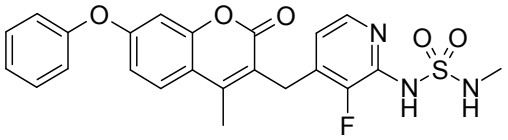


Compound **10g** was prepared by the procedures previously described for compound **6e** using **4g** and 3-phenoxyphenol (6.0 mg, 16 μmol, 24%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.88 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 5.3 Hz, 1H), 7.50 (ddd, J = 7.5, 7.5, 1.3 Hz, 2H), 7.30 (tt, J = 7.5, 1.3 Hz, 1H), 7.11 (ddd, J = 7.5, 1.3, 1.3 Hz, 2H), 7.03 (dd, J = 8.8, 2.2 Hz 1H), 6.99 (d, J = 2.2 Hz, 1H), 6.29 (dd, J = 5.3 Hz, $J_{H\text{-}F}$ = 5.3 Hz, 1H), 6.13 (br s, 2H), 3.94 (s, 2H), 2.46 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.6,

159.6, 155.1, 153.1, 149.2 (d, $J_{C\text{-}F}$ = 13.8 Hz), 149.0, 144.2 (d, $J_{C\text{-}F}$ = 248.7 Hz), 142.5 (d, $J_{C\text{-}F}$ = 8.2 Hz), 132.3 (d, $J_{C\text{-}F}$ = 11.6 Hz), 130.4 (×2), 127.4, 124.8, 120.1, 119.8 (×2), 115.5, 114.3, 112.0, 104.9, 25.0, 15.2; ESIMS *m/z* 377 $[M + H]^+$; HRMS (ESI) *m/z* 377.1294 $[(M + H)^+$; calcd for $C_{22}H_{18}FN_2O_3$: 377.1296].

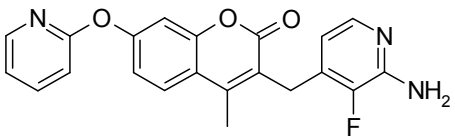
3-[2-(*N*-Methylsulfamoylamino)-3-fluoro-pyridin-4-ylmethyl]-4-methyl-7-phenoxy-chromen-2-one (**13g**)



To a solution of **10g** (30.0 mg, 79.7 μmol) in anhydrous DMF (2.0 mL) were added pyridine (64.5 μL, 797 μmol) and 7wt% *N*-methylsulfamoyl chloride[4] in CH_3CN (1.54 mL, 598 μmol) at room temperature. After stirring for 2 h, the mixture was diluted with EtOAc, and washed with brine twice. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by preparative HPLC to give **13g** (30.3 mg, 64.5 μmol, 81%) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.33 (br s, 1H), 7.94–7.92 (m, 1H), 7.87 (d, J = 8.8 Hz, 1H), 7.48 (ddd, J = 7.5, 7.5, 1.3 Hz, 2H), 7.26 (tt, J = 7.5, 1.3 Hz, 1H), 7.15 (ddd, J = 7.5, 1.3, 1.3 Hz, 2H), 7.01 (dd, J = 8.8, 2.2 Hz 1H), 6.99–6.96 (m, 1H), 6.96 (d, J = 2.2 Hz 1H), 6.85–6.75 (m, 1H), 3.99 (s, 2H), 2.45 (s, 3H), The CH_3 peak was overlapped with the DMSO peak; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.6, 159.8, 155.1, 153.2, 149.6, 145.8 (d, $J_{C\text{-}F}$ = 255.8 Hz), 142.7, 141.1 (d, $J_{C\text{-}F}$ = 18.4 Hz), 135.1 (d, $J_{C\text{-}F}$ = 11.3 Hz), 130.4 (×2), 127.6, 124.9, 119.8 (×2), 119.5, 118.6, 115.4, 114.4, 104.9, 29.0, 25.4, 15.3; ESIMS *m/z* 470 $[M + H]^+$; HRMS (ESI) *m/z* 470.1178 $[(M + H)^+$; calcd for $C_{23}H_{21}FN_3O_5S$: 470.1180].

3-(2-Amino-3-fluoro-pyridin-4-ylmethyl)-4-methyl-7-(pyridin-2-yloxy)-chromen-2-one (**10h**)

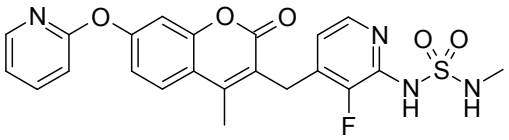


To a solution of compound **4g** (182 mg, 435 μmol) in 2,2,2-trifluoroethanol (1.5 mL) were added resorcinol (57.4 mg, 522 μmol) and MsOH (216 μL, 2.16 mmol) at room

temperature. The reaction mixture was heated to 60 °C for 4 h. After cooling to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$. The solid was collected by filtration, and washed with water, and EtOH to give **6h** (107 mg, 357 μmol). The purity of **6h** was checked by LC/MS (ESIMS *m/z* 301 $[M + H]^+$). To a solution of **6h** (107 mg, 357 μmol) in anhydrous DMF (1.0 mL) were added 2-bromopyridine (39 μL, 409 μ mol), Cs_2CO_3 (136 mg, 417 μ mol), CuI (68.0 mg, 357 μ mol) and *N*,*N*'-dimethylethylenediamine (43.0 μL, 400 μmol). The reaction mixture was heated to reflux for 5 h. After cooling to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by preparative TLC (EtOAc) to give **10h** (21.3 mg, 56.4 μmol, 13%).

^{1}H NMR ($CDCl_3$, 270 MHz) δ 8.21 (d, *J* = 3.6 Hz, 1H), 7.80–7.60 (m, 3H), 7.20–7.00 (m, 4H), 6.49 (dd, *J* = 4.8, 4.8 Hz, 1H), 4.60 (br s, 2H), 4.04 (s, 2H), 2.42 (s, 3H); ESIMS *m/z* 378 $[M + H]^+$.

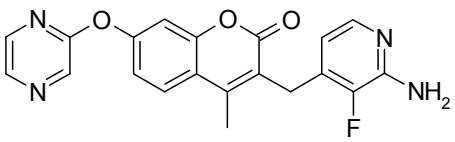
3-[2-(*N*-Methylsulfamoylamino)-3-fluoro-pyridin-4-ylmethyl]-4-methyl-7-(pyridin-2-yloxy)-chromen-2-one (**13h**)



Compound **13h** was prepared by the procedures previously described for compound **13g** using **10h** (1.4 mg, 2.5 μmol, 6%).

^{1}H NMR (CD_3OD, 270 MHz) δ 8.21 (dd, *J* = 4.9, 1.2 Hz, 1H), 7.95–7.83 (m, 2H), 7.73 (m, 1H), 7.28–7.07 (m, 4H), 6.75 (br t, *J* = 5.3 Hz, 1H), 4.09 (2H), 2.60 (s, 3H), 2.52 (s, 3H); ESIMS *m/z* 471 $[M + H]^+$; HRMS (ESI) *m/z* 471.1133 $[(M + H)^+$; calcd for $C_{22}H_{20}FN_4O_5S$: 471.1133].

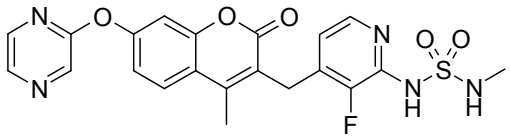
3-(2-Amino-3-fluoro-pyridin-4-ylmethyl)-4-methyl-7-(pyrazin-2-yloxy)-chromen-2-one (**10i**)



To a solution of **6h** (90.0 mg, 303 μmol) in anhydrous DMF (0.9 mL) were added Cs_2CO_3 (128 mg, 393 μmol) and 2-chloropyrazine (54.0 μL, 605 μmol). The reaction mixture was heated to 100 °C for 8 h. After cooling to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$, and extracted with a 1:9 of MeOH and CH_2Cl_2. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (EtOAc) to give **10i** (70.7 mg, 187 μmol, 62%).

^{1}H NMR ($CDCl_3$, 270 MHz) δ 8.52 (s, 1H), 8.35 (s, 1H), 8.13 (s, 1H), 7.71 (br s, 1H), 7.69 (d, J = 8.8 Hz, 1H), 7.21 (s, 1H), 7.15 (d, J = 8.8 Hz, 1H), 6.50 (br s, 1H), 4.57 (br s, 2H), 4.05 (s, 2H), 2.45 (s, 3H); ESIMS *m/z* 379 $[M + H]^+$.

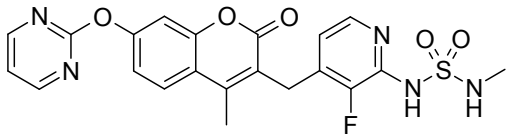
3-[2-(*N*-Methylsulfamoylamino)-3-fluoro-pyridin-4-ylmethyl]-4-methyl-7-(pyrazin-2-yloxy)-chromen-2-one (**13i**)



Compound **13i** was prepared by the procedures previously described for compound **13g** using **10i** (11.8 mg, 25.1 μmol, 31%).

HRMS (ESI) *m/z* 472.1082 [(M + H)$^+$; calcd for $C_{21}H_{19}FN_5O_5S$: 472.1085].

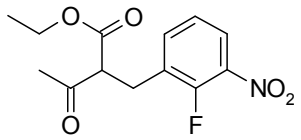
3-[2-(*N*-Methylsulfamoylamino)-3-fluoro-pyridin-4-ylmethyl]-4-methyl-7-(pyrimidin-2-yloxy)-chromen-2-one (**1**)



To a solution of **6h** (9.00 g, 30.0 mmol) in anhydrous DMF (80 mL) were added Cs_2CO_3 (11.7 g, 36.0 mmol) and 2-bromopyrimidine (9.53 g, 59.9 mmol). The reaction mixture was heated to 100 °C for 2 h. After cooling to room temperature, water was added to the mixture. The solid was collected by filtration, and washed with water to give a crude pyrimidine (9.81 g). To a solution of the crude pyrimidine in anhydrous DMF (120 mL) were added pyridine (4.41 mL, 54.3 mmol) and 7.3wt% *N*-methylsulfamoyl chloride in CH_3CN (91.5 g, 51.7 mmol) at −10 °C. After stirring at room temperature for 1 h, THF

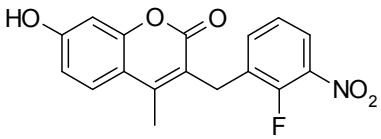
was added to the mixture. The solid was collected by filtration, and washed with THF to give **1** (10.4 g, 22.0 mmol, 73%, 2 steps) as a white solid.
^{1}H NMR (CD_3OD, 270 MHz) δ 8.63 (d, J = 4.9 Hz, 2H), 7.95–7.80 (m, 2H), 7.30–7.20 (m, 3H), 6.84 (dd, J = 5.4 Hz, 1H), 4.22 (s, 2H), 2.62 (s, 3H), 2.54 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.3, 160.5, 160.2 (×2), 155.0, 152.7, 149.5, 145.8 (d, $J_{C\text{-}F}$ = 255.8 Hz), 142.7(d, $J_{C\text{-}F}$ = 6.4 Hz), 141.0 (d, $J_{C\text{-}F}$ = 13.1 Hz), 135.0 (d, $J_{C\text{-}F}$ = 11.6 Hz), 127.0, 120.5, 118.6, 118.4, 117.6, 117.4, 109.6, 29.0, 25.5, 15.4; ESIMS *m/z* 472 $[M + H]^+$; HRMS (ESI) *m/z* 472.1081 $[(M + H)^+$; calcd for $C_{21}H_{19}FN_5O_5S$: 472.1085].

2-(2-Fluoro-3-nitro-benzyl)-3-oxo-butyric acid ethyl ester (**4k**)



To a solution of ethyl acetoacetate (37.4 mL, 294 mmol) in anhydrous THF (600 mL) was added NaH (65% in oil, 10.6 g, 294 mmol) at 0 °C. After stirring for 30 min, 1-bromomethyl-2-fluoro-3-nitorobenzene (68.7 g, 294 mmol) in anhydrous THF (400 mL) was added to the mixture at 0 °C and then stirred at room temperature for 12 h. The mixture was poured into 0.5 M aqueous HCl, and extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (EtOAc/hexane) to give **4k** (52.2 g, 185 mmol, 42%) as an oil.
^{1}H NMR (DMSO-d_6,, 270 MHz) δ 8.01 (td, J = 7.6, 1.9 Hz, 1H), 7.72 (td, J = 7.2, 1.9 Hz, 1H), 7.37 (td, J = 7.5, 1.1 Hz, 1H), 4.11 (m, 1H), 4.07 (qd, J = 7.0, 1.0 Hz, 2H), 3.16 (t, J = 7.3 Hz, 2H), 2.23 (s, 3H), 1.10 (t, J = 7.0 Hz, 3H); ESIMS *m/z* 284 $[M + H]^+$.

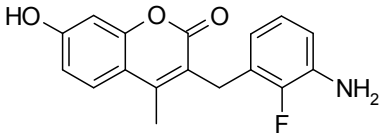
3-(2-Fluoro-3-nitro-benzyl)-7-hydroxy-4-methyl-chromen-2-one (**20k**)



Resorcinol (14.8 g, 135 mmol) and **4k** (38.2 g, 135 mmol) were dissolved in conc. sulfuric acid (22 mL) at 0 °C. After stirring at room temperature for 12 h, the mixture was poured into water. The solid was collected by filtration, washed with water and MeOH

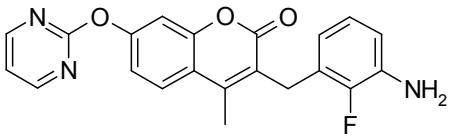
to give **20k** (28.3 g, 85.9 mmol, 64%) as a light yellow solid.
^{1}H NMR (DMSO-d_6, 270 MHz) δ 7.98 (td, J = 8.9, 1.6 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.58 (td, J = 6.2, 4.3 Hz, 1H), 7.32 (td, J = 8.9, 1.1 Hz, 1H), 6.83 (dd, J = 8.9, 2.4 Hz, 1H), 6.72 (d, J = 2.4 Hz, 1H), 4.02 (s, 2H), 2.43 (s, 3H); ESIMS *m/z* 330 $[M + H]^+$.

3-(3-Amino-2-fluorobenzyl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (**6k**)



To a solution of **20k** (5.00 g, 15.2 mmol) in EtOAc (115 mL) was added $SnCl_2 \cdot 2H_2O$ (17.1 g, 75.8 mmol) at room temperature. The mixture was refluxed for 1.5 h. The mixture was cooled to room temperature, poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated to give **6k** (3.25 g, 10.9 mmol, 71%) as a solid.
^{1}H NMR (DMSO-d_6, 270 MHz) δ 10.47 (br s, 1H), 7.64 (d, J = 2.4 Hz, 1H), 6.81 (dd, J = 8.7, 2.4 Hz, 1H), 6.75-6.67 (m, 2H), 6.59 (ddd, J = 8.2, 1.5 Hz, $J_{H\text{-}F}$ = 8.2 Hz, 1H), 6.20 (ddd, J = 7.6, 1.5 Hz, $J_{H\text{-}F}$ = 7.6 Hz, 1H), 5.06 (br s, 2H), 3.85 (s, 2H), 2.35 (s, 3H); ESIMS *m/z* 300 $[M + H]^+$.

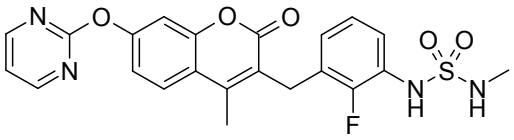
3-(3-Amino-2-fluoro-benzyl)-4-methyl-7-(pyrimidin-2-yloxy)-chromen-2-one (**10k**)



To a solution of **6k** (38.5 g, 129 mmol) in anhydrous DMF (500 mL) were added Cs_2CO_3 (50.3 g, 154 mmol) and 2-bromopyrimidine (81.8 g, 515 mmol). The reaction mixture was heated to 100 °C for 40 min. After cooling to room temperature, the mixture was poured into water, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was washed with EtOAc to give **10k** (37.0 g, 98.0 mmol, 76%) as a solid.
^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.68 (d, J = 4.8 Hz, 2H), 7.89 (d, J = 8.8 Hz, 1H), 7.37 (d, J = 2.4 Hz, 1H), 7.34 (t, J = 4.8 Hz, 1H), 7.26 (dd, J = 8.8, 2.4 Hz, 1H), 6.73 (dd, J = 8.2, 7.2 Hz, 1H), 6.61 (ddd, J = 8.2, 1.7 Hz, $J_{H\text{-}F}$ = 8.2 Hz, 1H), 6.25 (ddd, J = 7.2, 1.7 Hz, $J_{H\text{-}F}$

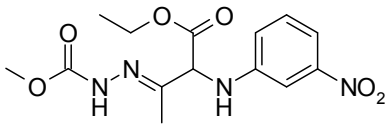
= 7.2 Hz, 1H), 5.08 (br s, 2H), 3.93 (s, 2H), 2.45 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.3, 160.6, 160.2 (×2), 154.7, 152.6, 148.8 (d, $J_{C\text{-}F}$ = 235.7 Hz), 148.3, 136.4 (d, $J_{C\text{-}F}$ = 13.5 Hz), 126.7, 125.0 (d, $J_{C\text{-}F}$ = 12.7 Hz), 123.9 (d, $J_{C\text{-}F}$ = 3.4 Hz), 122.3, 118.2, 117.5, 117.4, 115.6, 114.4 (d, $J_{C\text{-}F}$ = 3.1 Hz), 109.5, 25.6, 15.2; ESIMS *m/z* 378 [M + H]$^+$; HRMS (ESI) *m/z* 378.1246 [(M + H)$^+$; calcd for $C_{21}H_{17}FN_3O$: 378.1248].

3-(2-Fluoro-3-(N-methylsulfamoylamino)benzyl)-4-methyl-7-(pyrimidin-2-yloxy)-chromen-2-one (**14**)



To a solution of **10k** (18.5g, 49.0 mmol) in anhydrous CH_3CN (300 mL) were added *N*-methyl-2-oxooxazolidine-3-sulfonamide[2] (26.5 g, 146 mmol) and Et_3N (34.0 ml, 245 mmol) at room temperature. The reaction mixture was heated to 75 °C for 3.5 h. After cooling to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with 10% aqueous Na_2CO_3. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (EtOAc/CH_2Cl_2) to give **14** (14.0 g, 29.8 mmol, 63%) as a solid. ^{1}H NMR (DMSO-d_6, 270 MHz) δ 9.38 (br s, 1H), 8.69 (dd, *J* = 4.8, 1.2 Hz, 2H), 7.91 (d, *J* = 8.4 Hz, 1H), 7.40–7.34 (m, 4H), 7.17 (br s, 1H), 7.06–6.97 (m, 1H), 6.92–6.83 (m, 1H), 3.99 (s, 2H), 2.45 (s, 3H) ,The CH_3 peak was overlapped with the DMSO peak; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.3, 160.6, 160.2 (×2), 154.8, 152.7 (d, $J_{C\text{-}F}$ = 246.1 Hz), 152.6, 148.9, 126.9, 126.0 (d, $J_{C\text{-}F}$ = 14.9 Hz), 125.9 (d, $J_{C\text{-}F}$ = 9.7 Hz), 124.8 (d, $J_{C\text{-}F}$ = 2.6 Hz), 124.0 (d, $J_{C\text{-}F}$ = 6.2 Hz), 122.3, 121.6, 118.3, 117.6, 117.4, 109.5, 28.4, 25.8, 15.3; ESIMS *m/z* 471 [M + H]$^+$; HRMS (ESI) *m/z* 471.1130 [(M + H)$^+$; calcd for $C_{22}H_{20}FN_4O_5S$: 471.1133].

3-(Methoxycarbonyl-hydrazono)-2-(3-nitro-phenylamino)-butyric acid ethyl ester (**21j**)

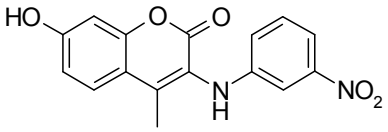


To a solution of ethyl 3-carbomethoxyazocrotonate[5] (4.20 g, 21.1 mmol) in anhydrous

THF (40 mL) was added 3-nitroaniline (2.92 g, 21.1 mmol) at room temperature. The mixture was heated to 70 °C for 12 h. After cooling to room temperature, hexane was added to the mixture. The solid was collected by filtration and washed with hexane to give **21j** (5.08 g, 15.0 mmol, 71%) as a solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 10.10 (s, 1H), 7.55 (s, 1H), 7.46–7.31 (m, 2H), 7.11 (d, J = 8.1 Hz, 1H), 6.96 (d, J = 7.4 Hz, 1H), 4.94 (d, J = 7.7 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.67 (s, 3H), 1.83 (s, 3H), 1.21 (t, J = 7.1 Hz, 3H); ESIMS *m/z* 339 $[M + H]^+$.

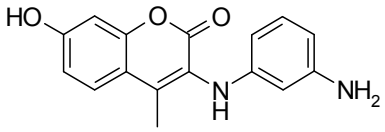
7-Hydroxy-4-methyl-3-(3-nitro-phenylamino)-chromen-2-one (**20j**)



To a solution of **21j** (3.67 g, 10.8 mmol) in acetone (37 mL) was added 10wt% $TiCl_3$ in 20-30% HCl aqueous solution (8.80 mL, 5.70 mmol) at room temperature. The mixture was stirred at room temperature for 1 h, then poured into water, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica gel chromatography (EtOAc/hexane) to give **4j** (2.75 g, 10.3 mmol) as a yellow oil. The purity was checked by LC/MS (ESIMS *m/z* 267 $[M + H]^+$). To a solution of compound **4j** (2.75 g, 10.3 mmol) in trifluoroethanol (30 mL) were added resorcinol (2.27 g, 20.7 mmol) and MsOH (3.35 mL, 51.6 mmol) at room temperature. The reaction mixture was heated to 65 °C for 12 h. After cooling to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by silica gel chromatography (EtOAc/hexane) to give **20j** (1.46 g, 4.67 mmol, 45%) as a solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 10.50 (s, 1H), 8.13 (s, 1H), 7.65 (d, J = 8.7 Hz,1H), 7.51 (d, J = 9.1 Hz, 1H), 7.42–7.34 (m, 2H), 7.01 (d, J = 7.3 Hz,1H), 6.86 (dd, J = 8.7, 2.3 Hz, 1H), 6.77 (d, J = 2.3 Hz, 1H), 2.29 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 160.4, 159.0, 152.7, 148.6, 147.4, 145.4, 130.0, 127.0, 119.9, 119.9, 113.2, 112.3, 112.1, 107.5, 102.1, 14.1; ESIMS *m/z* 313 $[M + H]^+$; HRMS (ESI) *m/z* 311.0669 $[(M - H)^-$; calcd for $C_{16}H_{11}N_2O_5$: 311.0673].

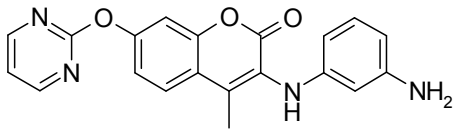
3-(3-Amino-phenylamino)-7-hydroxy-4-methyl-chromen-2-one (**6j**)



To a solution of **20j** (500 mg, 1.60 mmol) in anhydrous EtOAc (16 mL) was added $SnCl_2 \cdot H_2O$ (1.08 g, 4.80 mmol) at room temperature. The reaction mixture was heated to 75 °C for 10 h. After cooling to room temperature, the mixture was poured into saturated aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated. The solid was washed with EtOAc to give **6j** (135 mg, 478 μmol, 30%) as a brown solid.

^{1}H NMR (DMSO-d_6, 270 MHz) δ 10.37 (s, 1H), 7.59 (d, J = 8.9 Hz, 1H), 7.02 (s, 1H), 6.84 (dd, J = 8.6 , 2.3 Hz, 1H), 6.80–6.73 (m, 3H), 5.98–5.88 (m, 2H), 5.80 (m, 1H), 5.05 (br s, 1H), 2.22 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.8, 159.7, 152.2, 148.0, 146.6, 142.9, 129.2, 126.5, 121.5, 113.1, 112.7, 105.4, 104.0, 102.0, 100.1, 14.3; ESIMS *m/z* 283 $[M + H]^+$; HRMS (ESI) *m/z* 283.1079 $[(M + H)^+$; calcd for $C_{16}H_{15}N_2O_3$: 283.1077].

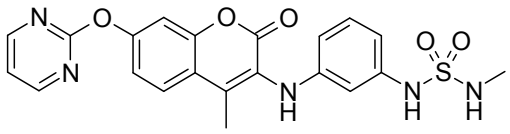
3-(3-Amino-phenylamino)-4-methyl-7-(pyrimidin-2-yloxy)-chromen-2-one (**22j**)



To a solution of **6j** (135 mg, 478 μmol) in anhydrous DMF (4 mL) were added Cs_2CO_3 (234 mg, 717 μmol) and 2-bromopyrimidine (152 mg, 956 μmol). The reaction mixture was heated to 75 °C for 50 min. After cooling to room temperature, the mixture was poured into water, and extracted with EtOAc. The extract was washed with brine. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residue was purified by silica gel chromatography (EtOAc/hexane) to give **22j** (81.7 mg, 227 μmol, 47%).

^{1}H NMR (DMSO-d_6, 270 MHz) δ 8.69 (d, J = 4.8 Hz, 2H), 7.81 (d, J = 8.6 Hz, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.33 (t, J = 4.8 Hz, 1H), 7.29–7.24 (m, 2H), 6.80 (t, J = 7.7 Hz, 1H), 6.00 (m, 2H), 5.86 (s, 1H), 4.86 (s, 2H), 2.26 (s, 3H); ESIMS *m/z* 361 $[M + H]^+$.

3-[3-(*N*-methylsulfamoylamino)-phenylamino]-4-methyl-7-(pyrimidin-2-yloxy)-chromen-2-one (**15j**)



Compound **15j** was prepared by the procedures previously described for compound **13g** using **22j** (71.0 mg, 157 μmol, 75%).

HRMS (ESI) *m/z* 454.1178 [(M + H)$^+$; calcd for $C_{21}H_{20}N_5O_5S$: 454.1180].

Conformational analysis

Torsional distributions of phenyl derivatives and nitrogen-containing aromatic derivatives:

Crystal structures of phenyl derivatives and nitrogen-containing aromatic derivatives were searched in the CSD[6] version 5.34 by the Program ConQuest.[7] Filters (R-factor ≤ 0.05, not disordered, no errors, not polymeric, no ions, no powder structures and only organics) were applied to search crystal structures.

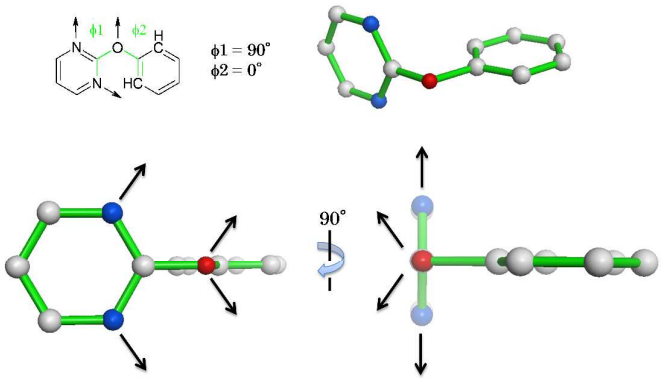


Figure S1. Schematic representation of lone pairs

Potential energy surfaces were calculated at the B3LYP/6-31G(d) level. To evaluate potential energy surfaces, 144 conformations were constructed by varying each torsional angle between 0 and 330 degrees at 30 degree intervals. These conformations were optimized by fixing each torsional angle with the program Gaussian 09.[8] The lowest energy conformations were searched by full geometry optimization (no torsional angles fixed). ΔE of each conformation was the energy difference between each conformation and the lowest energy conformations.

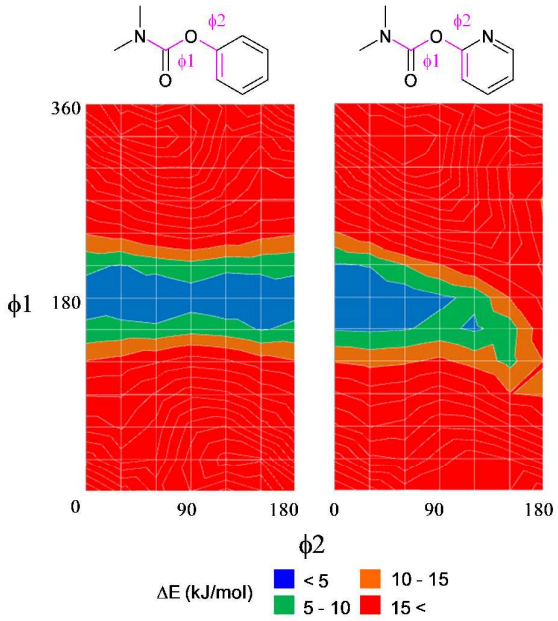


Figure S2.

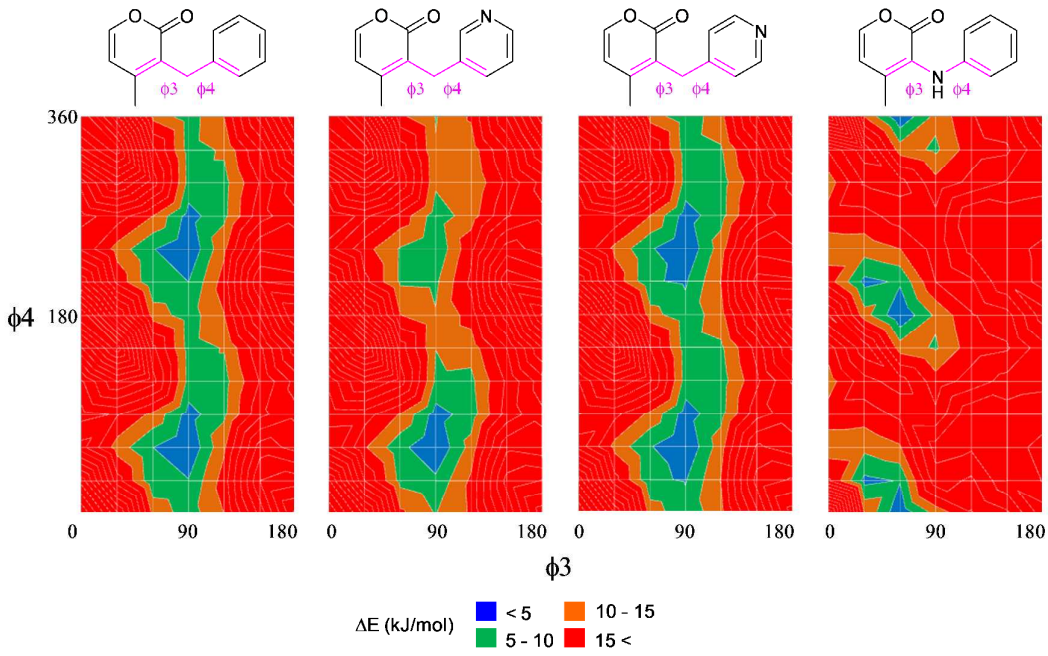


Figure S3.

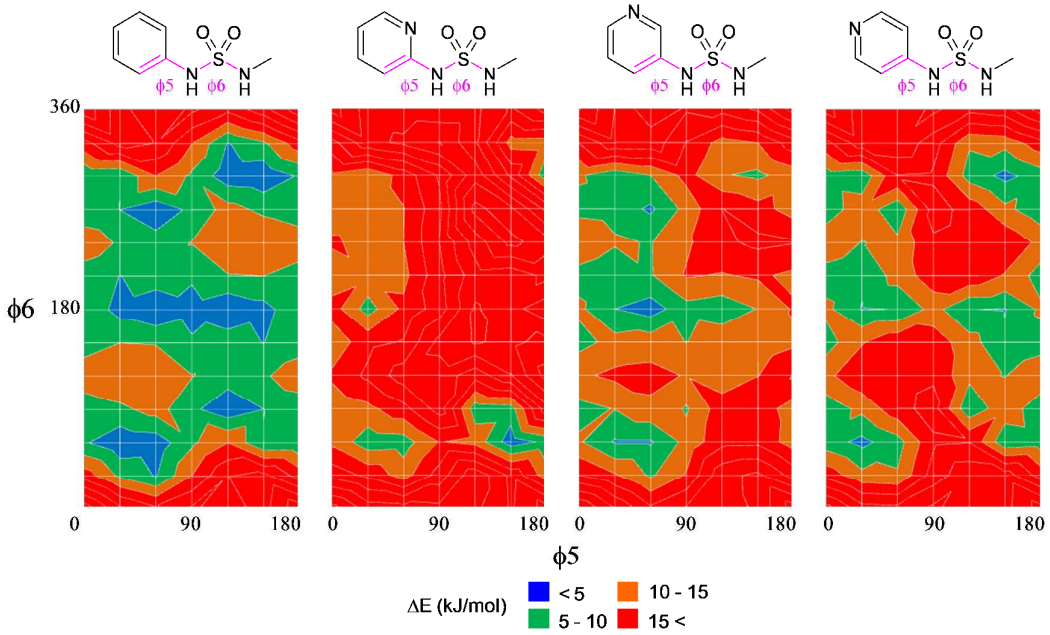


Figure S4.

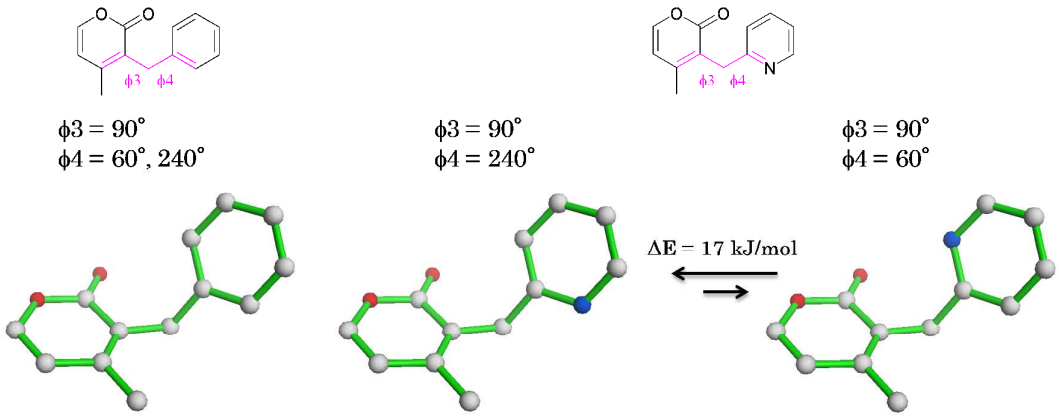


Figure S5.

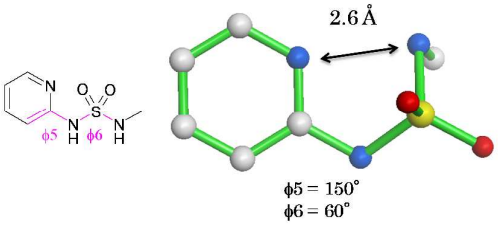


Figure S6. The most stable conformation of 2-(*N*-methylsulfamoylamino)-pyridine in potential energy surfaces.

Biological Experimental Section

C-Raf, MEK1 and cellular proliferation assays:

In vitro kinase assay

Enzymatic assays for C-Raf, MEK1 and other kinases were carried out as previously described[9]. Briefly: **C-Raf assay**: C-Raf substrate (MEK1 [K97R], inactive) was sequentially mixed with test article, C-Raf ([truncated], active) and ATP. After incubation for 45 min at 30 °C, the phosphorylation of MEK1 was qualitatively detected by europium-anti-phospho-MEK1/2 [Ser217/221] antibody. **MEK1 assay**: MEK1 (MEK1 SESE) was sequentially mixed with substrate MAP kinase 2/Erk2, unactive, test article and ATP, and incubated for 30 min at 30 °C. Then substrate FAM-Erktide was added and incubated for 60 min at 30 °C. The phosphorylation of the peptide was determined using IMAP fluorescence polarization screening kit. **Other kinases**: Test article, the substrate peptide, ATP and the enzyme were mixed sequentially and incubated for 90 min at 30 °C. Quantitative analysis of the phosphorylation of the substrate was conducted using a europium-labeled antiphospho-substrate antibody (TR-FRET assay) or an IMAP FP screening express kit (FP assay).

Cell culture and growth inhibition assay

Human colon cancer cell line HCT-116 was purchased from the American Type Culture Collection and was maintained in McCoy's 5A medium containing 1.5 mmol/L L-glutamine & 10% of FBS. Cells were seeded at 2×10^3 cells per well, and then treated with the dilution of test compounds at 37 °C for 96 h. Viable cells were detected using Counting Kit-8, and IC_{50} was calculated as half-maximum concentration for cell growth inhibition.

Physicochemical Studies

High-throughput Solubility Assay (LYSA): Samples were prepared in triplicate from 10 mM dimethylsulfoxide stock solutions. After evaporation (1 h) of dimethylsulfoxide with a centrifugal vacuum evaporator, the compounds were dissolved in FaSSIF[10], and shaken for 2 h. After one night, the solutions were filtered using a Whatman Unifilter microtiter filter plate (GE Healthcare) and the filtrate and its 1/10 dilution were analyzed by UV measurement or HPLC-UV. In addition, a four point calibration curve was prepared from the 10 mM stock solutions and used to determine the solubility of the compounds. The results were expressed in μg/mL. Starting from a 10 mM stock solution, the measurement range for MW 500 was 0–666 μg/mL.

Saturated Solubility Assay:

The saturated solubility of a crystalline free acid in FaSSIF was determined by shaking-flask method. A 96-well polypropylene plate containing 0.5 mL of FaSSIF per well was placed in the incubator. Excess amounts of compounds were added to the wells, and then shaken at 37 °C for 24 h. The aqueous samples were filtered through a MultiScreen solubility plate with a 0.4 μm polycarbonate isopore membrane (Millipore Corporation, Billerica, MA) and the concentration was measured by HPLC.

Supersaturated Solubility Assay:

The supersaturated solubility of crystalline potassium salt in FaSSIF was determined by a mini-scale dissolution test. The mini-scale dissolution tests were carried out by the paddle method (50 rpm, 50 mL) using a VK7010 dissolution station and a VK8000 dissolution sampling station (Varian Medical Systems, Inc., Palo Alto, CA) in a 100 mL glass vessel (42 mm diameter × 105 mm) (Takao Manufacturing Co., Ltd., Kyoto, Japan). The dissolution tests were carried out for 4 h. The dissolved concentration of salt in FaSSIF was measured by HPLC.

Preformulation study of compound 1 and 14

Compd	Saturated and supersaturated solubility (μg/mL)	
	Free	Salt
1	2.7	57
14	5.5	12

pK_a measurements: Spectroscopic titration (UV metric pK_a assay) was used to measure pK_a. The phosphate buffer (25 μL) and the sample (3 μL of a 10 mM DMSO stock) were dispensed into the sample vial. The method was automatically measured by a Sirius T3 (Sirius Analytical). The standard working range was pH 2.0 to pH 12.0. The pK_a values were extrapolated to zero percent organic content using the Yasuda-Shedlovsky extrapolation procedure, which yielded an extrapolated aqueous pK_a value and information about the slope that was used to ascertain the acid/basic characteristics of the ionisable groups.

Stability in liver microsome: Mouse or human microsome incubations were conducted by an automated procedure implemented on a Biomek FX (Beckman Coulter). Compounds (5 μM) were incubated in microsomes at 1.0 mg protein/mL in a 50 mM

potassium phosphate buffer, pH 7.4, at 37 °C. Cofactor (NADPH) was produced by a generating system (glucose 6-phosphate, 3.2 mM; β-NADP, 2.6 mM; $MgCl_2$, 6.5 mM). Addition of the NADPH generating system to the pre-warmed microsomes containing the test compound started the reaction. Aliquots (50 μL) were taken at four defined time points within 60 min and transferred into 100 μL of methanol containing an internal standard. Concentration of each compound was analyzed by LC/MS/MS using an ODS-3 RP 18 column (GL Sciences). Quantitative detection was achieved on an API 365 instrument (AB Sciex) using electron spray ionization. Concentrations were determined by ratio of test compound and internal standard peaks and given as a percentage of the concentration measured at the first time point (substrate depletion). Intrinsic clearance (CL, in μL/min/mg microsomal protein) is the rate constant of the first-order decay of the test compound, normalized for the protein concentration in the incubation.

Permeability assay (PAMPA): PAMPA PSR4p is an automated assay which is based on 96-well microplates. The permeation of compounds is measured using a "sandwich" construction. A filter plate is coated with phospholipids (membrane) and placed into a donor plate containing a drug/buffer solution. Finally the filter plate is filled with buffer solution (acceptor). The donor concentration is measured at t-start (reference) and compared with the donor and acceptor concentration after a certain time, t-end.
The following setup was used for the PAMPA PSR4p assay:

Donor:	0.05 M MOSPO buffer at pH 6.5 + 0.5% (w/v) Glyco Cholic Acid
Membrane:	10% (w/v) Egg Lecithin + 0.5% (w/v) Cholesterol in Dodecane
Acceptor:	0.05 M MOPO buffer at pH 6.5

The liquid handling was done with an RSP 100 pipetting robot (Tecan). The drug analysis was based on UV spectroscopy. All samples were transferred into 96-well UV plates. A SpectraMax 190 UV plate reader (Molecular Devices) was used to collect the UV spectras.

CYP Inhibition Assay

For CYP inhibition screening, incubation mixtures were prepared as a total volume of 100 μL with final component concentrations as follows: 0.1 M potassium phosphate buffer (pH 7.4), 1.0 mM NADPH, 0.1 mg/mL human liver microsomes, test drug (5 points of concentration) or positive controls and substrates. The substrates were used at concentrations less than their respective Michaelis–Menten constant (Km) values: 0.5 μM for diclofenac (CYP2C9) and 0.2 μM for midazolam (CYP3A4). NADPH was added

after (for IC_{50} (−)) or before (for IC_{50} (+)) a 30 min pre-incubation of other components in a water bath at 37 °C. Substrates were added after the 30 min pre-incubation. After a given incubation time of 5 min, the reactions were terminated by the addition of 150 μL of ice-cold 2-propanol. All samples were analyzed by LC/MS/MS, in which an Acquity UPLC (Waters)/ API4000(AB Sciex) system was used and the data acquisition and quantitative processing were performed using Analyst 1.4 software (AB Sciex).

IC_{50} was calculated according to the following equation

$$IC_{50} = \frac{(50\% - \text{Low percentage})}{(\text{High percentage} - \text{Low percentage})} (\text{High conc.} - \text{Low conc.}) + (\text{Low conc.})$$

with the following definitions applying:

Low percentage = highest percent inhibition less than 50%

Low concentration = concentration of test substance or positive control at the low percentage

High percentage = lowest percent inhibition greater than 50%

High concentration = concentration of test substance or positive control at the high percentage

hERG K^+ Channel Assay

Chinese hamster ovary (CHO) cells stably expressing the human ether a-go-go-related gene (hERG) were cultured in DMEM/F-12 medium (Gibco-BRL) containing 10% fetal bovine serum (Gibco-BRL) and 250 μg/mL Geneticin (Sigma) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The cells were placed on a recording chamber and superfused continuously at a rate of approximately 4 mL/min with an external solution consisting of 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose (pH 7.4 adjusted with NaOH). Membrane currents were recorded by the whole-cell voltage-clamp technique using a patch clamp amplifier (Axopatch 1D; Molecular Devices). Internal solution was composed of 130 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 5 mM EGTA and 5 mM MgATP (pH 7.2 adjusted with KOH). The cells were held at a holding potential of −80 mV and then depolarized to +20 mV for 1 sec to activate the hERG channels, and then repolarized to −40 mV for 0.5 sec to induce tail current. After stabilizing the tail current, test compounds were applied cumulatively at 1 and 10 μM. Data was analyzed using pCLAMP 8 software (Molecular Devices). Percent change of post-application against pre-application values in tail peak

currents was calculated. The percentages obtained for each test compound was then corrected for the respective mean vehicle rundown of hERG currents.

Pharmacokinetic Studies

In mice:

The pharmacokinetic study in female BALB-nu/nu mice (CAnN.Cg-Foxn1<nu>/CrlCrlj nu/nu) was approved by the Chugai Institutional Animal Care and Use Committee at Chugai Pharmaceutical Co., Ltd. In 24 h exposure studies, female BALB-nu/nu mice (n = 2) were orally administered test compound at a dose of 5□100 mg/kg. In pharmacokinetic studies, female BALB-nu/nu mice (n = 2–3) were administered compounds 1 and 14 orally and intravenously at a dose of 5□20 mg/kg and 2.5□10 mg/kg, respectively. Blood samples were collected from the orbital vein using a heparin-treated hematocrit tube and were immediately centrifuged to separate the plasma.

The concentration of test compound in plasma was determined by using solid-phase extraction (Oasis HLB, Waters) followed by LC/MS/MS.

In 24 h exposure studies, $AUC_{0\text{-}t}$ was calculated by linear trapezoidal rule to the last measurable plasma concentration using Microsoft Excel 2003.

In pharmacokinetic studies, PK parameters except oral bioavailability were analyzed by non-compartment analysis using WATSON ver.7.1 software (Thermo Fisher Scientific). Oral bioavailability was determined by $AUC_{inf(po)}/AUC_{inf(iv)}$.

In rat:

The pharmacokinetic study in male rats (Crlj:CD) was approved by the Chugai Institutional Animal Care and Use Committee at Chugai Pharmaceutical Co., Ltd. Male rats (n = 4) were administered compounds 1 and 14 orally and intravenously at a dose of 1□10 mg/kg and 1□5 mg/kg, respectively. Blood samples were collected from the cervical vein using a heparin-treated syringe and were immediately centrifuged to separate the plasma.

The concentration of test compound in plasma was determined by using solid-phase extraction (Oasis HLB, Waters) followed by LC/MS/MS.

PK parameters except oral bioavailability were analyzed by non-compartment analysis using WATSON ver.7.1 software. Oral bioavailability was determined by $AUC_{inf(po)}/AUC_{inf(iv)}$.

In monkey:

The pharmacokinetic study in male and female cynomolgus monkeys was approved by the Chugai Institutional Animal Care and Use Committee at Chugai Pharmaceutical Co., Ltd. Male and female cynomolgus monkeys (n = 2) were administered compounds 1 and 14 orally and intravenously at a dose of 1□5 mg/kg and 0.5□2.5 mg/kg, respectively. Blood samples were collected from the forearm cephalic vein using a heparin-treated syringe and were immediately centrifuged to separate the plasma.

The concentration of test compound in plasma was determined by using solid-phase extraction (Oasis HLB, Waters Corp.) followed by LC/MS/MS.

PK parameters except oral bioavailability were analyzed by non-compartment analysis using WATSON ver.7.1 software. Oral bioavailability was determined by $AUC_{inf(po)}/AUC_{inf(iv)}$.

PK profiles of compounds 1[ac] and 14[bc]

Species	mouse		rat		monkey	
compd	**1**	**14**	**1**	**14**	**1**	**14**
iv/po Dose (mg/kg)	2.5/5	10/20	1/1	5/10	0.5/1	2.5/5
AUC_{inf} (μM·h), po	147	75	33	43	272	108
$t_{1/2}$ (h), iv	4.5	1.3	7.9	2.9	24.6	4.8
CL (mL min^{-1} kg^{-1})	1.1	6.8	0.7	6.6	0.1	0.9
bioavailability (%)	93	75	66	84	82	59

[a] Vehicle：5% DMSO/10% HPCD/85% water. [b] Vehicle：5% DMSO/5% Cremophor EL/15% PEG400/15% HPCD/60% water. [c] Na salt was used.

In Vivo Tumor Efficacy Studies

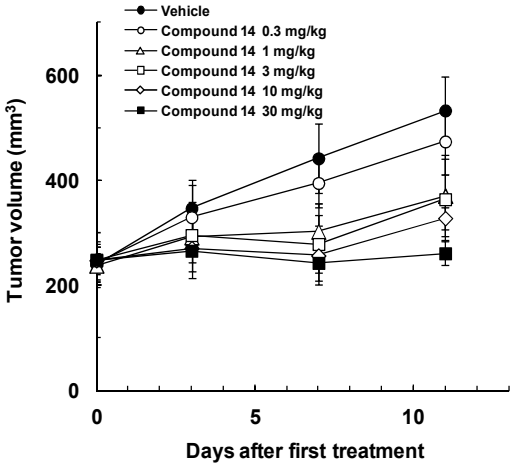
Female BALB-nu/nu mice (CAnN.Cg-Foxn1<nu>/CrlCrlj nu/nu) were purchased from Charles River Laboratories, Japan. Animals were housed in suitable cages under specified pathogen-free conditions. This study was conducted under the protocol approved by the Chugai Institutional Animal Care and Use Committee.

C32 human malignant melanoma cells were obtained from the American Type Culture Collection and were maintained in minimum essential medium Eagle (E-MEM) containing 10% fetal bovine serum, 0.1 mM MEM non-essential amino acids solution (NEAA) and 1 mM sodium pyruvate solution (SP). C32 cells were inoculated subcutaneously into the right flank of BALB-nu/nu mice. Once palpable tumors were established, animals were randomized so that all groups had similar mean tumor volumes at the start of the study.

Test articles were dissolved in distilled water containing 5% DMSO and 10% 2-hydroxypropyl-ß-cyclodextrin. The articles were administered orally, once daily for 11

days. Tumor measurements and body weight were recorded twice per week. Tumor volume (V) was calculated using the following formula: $V = ab^2/2$, where a is the length of the tumor and b is the width. Tumor growth inhibition (TGI) was calculated using the following formula: $TGI = [1 - (T - T_0)/(C - C_0)] \times 100$, where T and T_0 are the mean tumor volumes on a specific experimental day and on the first day of treatment, respectively, for the experimental groups, and likewise, where C and C_0 are the mean tumor volumes for the control group.

In vivo efficacy of 14 (Na salt) in the C32 human malignant melanoma xenograft model



	control group	p value
1 0.03 mg/kg	vehicle	0.0308 *
1 0.3 mg/kg	vehicle	0.0001 ***
1 0.75 mg/kg	vehicle	<0.0001 ***
1 1.5 mg/kg	vehicle	<0.0001 ***

	control group	p value
14 0.3 mg/kg	vehicle	0.5082
14 1 mg/kg	vehicle	0.0051 **
14 3 mg/kg	vehicle	0.0036 **
14 10 mg/kg	vehicle	0.0006 ***
14 30 mg/kg	vehicle	<0.0001 ***

Reference

(1) Görlitzer, K.; Bartke, U. 3-(Nitrobenzyliden)-2,4(3*H*,5*H*)-furandione in der Hantzsch-Pyridine-Synthese. *Pharmazie*, **2002**, *57*, 672–678.

(2) Borghese, A.; Antoine, L.; Van Hoeck, J. P.; Mockel, A.; Merschaert, A. Mild and safer preparative method for nonsymmetrical sulfamides via *N*-sulfamoyloxazolidinone derivatives: electronic effects affect the transsulfamoylation reactivity. *Org. Process Res. Dev.* **2006**, *10*, 770–775.

(3) Bisacchi, G. S.; Slusarchyk, W. A.; Bolton, S. A.; Hartl, K. S.; Jacobs, G.; Mathur, A.; Meng, W.; Ogletree, M. L.; Pi, Z.; Sutton, J. C.; Treuner, U.; Zahler, R.; Zhao, G.; Seiler, S. M. Synthesis of potent and highly selective nonguanidine azetidinone inhibitors of human tryptase. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2227–2231.

(4) Kloek, J. A.; Leschinsky, K. L. An Improved Synthesis of Sulfamoyl Chlorides. *J. Org. Chem.*, **1976**, *41*, 4028–4029.
(5) Schultz, A. G.; Hagmann, W. K. Synthesis of Indole-2-carboxylic Esters. *J. Org. Chem.*, **1978**, *43*, 3391–3393.
(6) Allen, F. H. The Cambridge Structural Database: A Quarter of a Million Crystal Structures and Rising. *Acta Crystallogr.* **2002**, *B58*, 380–388.
(7) Bruno, I. J.; Cole, J. C.; Edgington, P. R.; Kessler, M.; Macrae, C. F. McCabe, P.; Pearson, J.; Taylor, R. New software for searching the Cambridge Structural Database and visualizing crystal structures. *Acta Crystallogr., Sect. B: Struct. Sci.* **2002**, *B58*, 389–397.
(8) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 09; Gaussian Inc.: Wallingford, CT, 2009.
(9) Ishii, N.; Harada, N.; Joseph, E. W.; Ohara, K.; Miura, T.; Sakamoto, H.; Matsuda, Y.; Tomii, Y.; Tachibana-Kondo, Y.; Iikura, H.; Aoki, T.; Shimma, N.; Arisawa, M.; Sowa, Y.; Poulikakos, P. I.; Rosen, N.; Aoki, Y.; Sakai, T. Enhanced inhibition of ERK signaling by a novel allosteric MEK inhibitor, CH5126766, that suppresses feedback reactivation of RAF activity. *Cancer Res.* **2013**, *73*, 4050–4060.
(10) Galia, E.; Nicolaides, E.; Hörter, D.; Löbenberg, R.; Reppas, C.; Dressman, J. B. Evaluation of Various Dissolution Media for Predicting In Vivo Performance of Class I and II Drugs. *Pharm. Res.* **1998**, *15*, 698–705.