SUPPORTING INFORMATION

Small-Molecule Inhibitors of Cytokine-Mediated STAT1 Signal Transduction in β-Cells with Improved Aqueous Solubility

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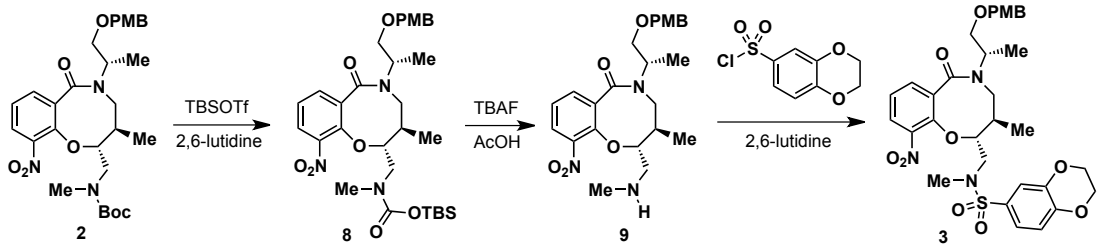
I. Synthesis and Characterization of Compounds

General information. Oxygen and/or moisture sensitive reactions were carried out in oven or flamed dried glassware under nitrogen atmosphere. All reagents and solvents were purchased and used as received from commercial vendors or synthesized according to cited procedures. Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Flash chromatography was performed using 20-40 μm silica gel (60Å mesh) on a Teldyne Isco Combiflash R_f. Analytical thin layer chromatography (TLC) was performed on 0.25 mm silica gel 60-F plates and visualized by UV light (254 nm). NMR spectra were recorded on a Bruker 300 (^{1}H, 300 MHz, ^{13}C, 75 MHz) at ambient temperature with $CDCl_3$ or DMSO-*d*6 (Cambridge Isotope Laboratories, Inc.) as the solvent. Chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ (^{1}H, 7.26; ^{13}C, 77.00) or DMSO-*d*6 (^{1}H, 2.50; ^{13}C, 39.52). Data for ^{1}H NMR are reported as follows: chemical shift, multiplicity (br = broad, ob = obstructed, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, m = multiplet), coupling constants, and integration. Tandem liquid chromatography/mass spectrometry (LCMS) was performed on a Waters 2795 separations module and 3100 mass detector. High resolution/ mass spectrometry (HRMS) was performed on an Agilent 1290 Infinity separations module and 6230

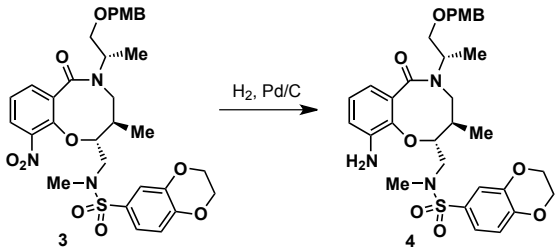
time-of-flight (TOF) mass detector. Melting points were recorded on a Fisher-Johns melting point apparatus (Fisher Scientific).

Preparation of sulfonamide 3.



TBSOTf (1.98 mL, 8.61 mmol) was added dropwise to a solution of *N*-Boc-protected amine **2**[1] (1.6 g, 2.87 mmol) and 2,6-lutidine (1.35 mL, 11.59 mmol) in CH_2Cl_2 (29 mL) at rt. The reaction mixture was stirred at rt for 1h, diluted with sat. NH_4Cl, and extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide *N*-silyl carbamate **8**, which was used directly in the next step without purification. LCMS (ESI^+) m/z: 616.39 (M+H). Crude **8** was dissolved in THF (57 mL) and cooled to 0 °C. To the resulting solution was added AcOH (0.181 mL, 3.16 mmol) followed by dropwise addition of TBAF (1.0 M in THF, 3.16 mL, 3.16 mmol). The reaction mixture was further stirred for 30 min at 0 °C, diluted with sat. NH_4Cl, and extracted into EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide secondary amine **9**, which was used directly in the next step without purification. LCMS (ESI^+) m/z: 458.41 (M+H). Crude **9** and 2,6-lutidine (1.67 mL, 14.35 mmol) were dissolved in CH_2Cl_2 (29 mL), and 1,4-Benzodioxan-6-sulfonyl chloride (808 mg, 3.44 mmol) was added to the resulting solution at rt. The reaction mixture was further stirred for 20 h at rt, diluted with with sat. NH_4Cl, and extracted into CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification on silica gel (gradient of 10-50% EtOAc in hexanes) provided 1.39 g (2.12 mmol, 74% yield for 3 steps) of **3** as a white foam. ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.70 (dd, *J* = 7.5, 1.5 Hz, 1H), 7.30-7.21 (ovrlp m, 5H), 6.95 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 8.7 Hz, 2H), 4.55 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.39 (m, 1H), 4.29 (m, 4H), 3.90-3.77 (ovrlp m, 3H), 3.77 (ovrlp s, 3H), 3.66 (dd, *J* = 9.9, 4.5 Hz, 1H), 3.38-3.20 (ovrlp m, 2H), 3.11 (d, *J* = 12.6 Hz, 1H), 2.81 (s, 3H) 2.70 (m, 1H), 1.38 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 166.2, 159.2, 149.1, 147.6, 143.8, 143.1, 135.2, 134.5, 130.6, 129.7, 129.5, 127.36, 125.42, 121.2, 118.0, 117.0, 113.8, 90.3, 72.9, 72.0, 64.6, 64.3, 55.4, 53.8, 52.6, 52.1, 37.8, 33.5, 16.5, 15.0; LCMS (ESI^+) m/z: 656.38 (M+H).

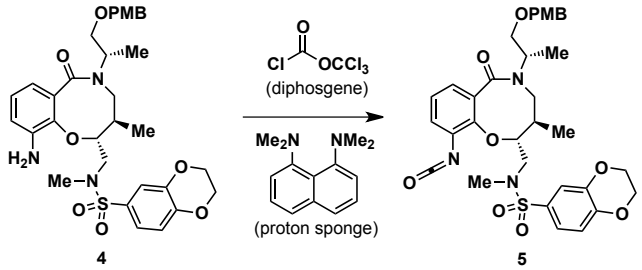
Preparation of aniline 4.



Under hydrogen (H_2) atmosphere, a solution of **3** (1.39 g, 2.12 mmol) and palladium (10% on activated carbon, 226 mg, 0.212 mmol) in EtOH (42 mL) was stirred at 35°C for 3.5 h. The reaction mixture was

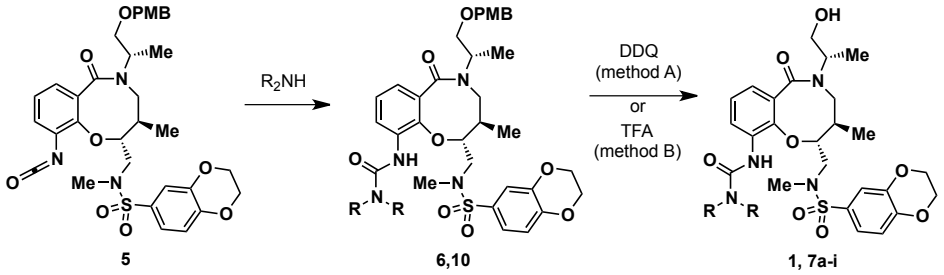
cooled, filtered and washed through a pad of celite, and concentrated *in vacuo*. Purification on silica gel (gradient of 10-60% EtOAc in hexanes) provided 1.21 g (1.93 mmol, 91% yield) of **4** as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.26 (ovrlp m, 4H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 6.79 (d, *J* = 7.8 Hz, 2H), 4.67 (m, 1H), 4.54 (d, *J* = 11.4 Hz, 1H), 4.47-4.43 (ovrlp m, 3H), 4.36-4.33 (ovrlp m, 4H), 3.81-3.74 (ovrlp m, 2H), 3.79 (ovrlp s, 3H), 3.67 (dd, *J* = 10.2, 4.5 Hz, 1H), 3.49 (dd, *J* = 15.6, 10.5 Hz, 1H), 3.30 (d, *J* = 13.8 Hz, 1H), 3.06 (d, *J* = 15.0 Hz, 1H), 2.94-2.86 (ovrlp m, 1H), 2.90 (ovrlp s, 3H), 2.01 (m, 1H), 1.32 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 159.3, 147.9, 143.8, 141.5, 139.7, 131.8, 130.3, 129.5, 128.8, 125.6, 121.4, 118.0, 117.8, 117.2, 113.8, 84.6, 72.7, 72.0, 64.6, 64.3, 55.4, 55.3, 51.4, 50.3, 38.8, 35.5, 16.8, 14.9; LCMS (ESI$^+$) m/z: 626.47 (M+H); HRMS (ESI$^+$) m/z calculated for $C_{32}H_{39}N_3O_8SNa$ (M+Na): 648.2356, found 648.2352.

Preparation of isocyanate 5.



To a solution of disphosgene (11 µL, 0.095 mmol) in CH_2Cl_2 (0.5 mL) at 0°C was added dropwise a solution of aniline **4** (100 mg, 0.160 mmol) and proton sponge (67 mg, 0.314 mmol) in CH_2Cl_2 (0.5 mL). The reaction mixture was warmed to rt, stirred for 15 min, and concentrated *in vacuo*. The residue was redissolved in CH_2Cl_2, washed with 1M HCl (4X's) and 1M NaOH (1X), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to provide 120 mg (0.184 mmol, 96% yield) of crude **5** that did not require further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32-7.26 (ovrlp m, 5H), 7.156 (m, 2H), 7.00 (d, *J* = 13.2 Hz, 1H), 6.87 (d, *J* = 9.3 Hz, 2H), 4.57 (d, *J* = 11.7 Hz, 1H), 4.49(d, *J* = 11.7 Hz, 1H), 4.44 (ovrlp m, 2H), 3.86 (dd, *J* = 9.6 Hz, 7.2 Hz, 1H), 3.80 (s, 3H), 3.77 (ovrlp m, 1H), 3.63 (m, 3H), 3.36-3.15 (ovrlp m, 5H), 2.89 (s, 3H), 2.53 (m, 1H), 1.38 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.9 Hz, 3H); LCMS (ESI$^+$) m/z: 652.25 (M+H).

Preparation of BRD0476 (1) and urea analogs 7.



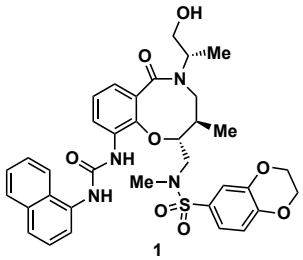
<u>General procedure for urea formation:</u>

Isocyanate **5** (1.0 equiv) and aryl or alkyl amine (5.0 equiv) were dissolved in toluene (0.06 M) and stirred at rt for 1-6 h. The reaction mixture was concentrated *in vacuo* and directly purified on silica gel (gradient of EtOAc in hexanes or MeOH in CH_2Cl_2) to provide the desired urea products.

General procedures for PMB deprotection:

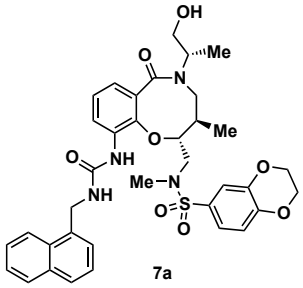
Method A. The corresponding PMB-protected alcohol (1.0 equiv) was dissolved in a solution of CH_2Cl_2 and pH 7.4 buffer (0.46 M, 14:1). The mixture was cooled to 0 °C and DDQ (1.5 equiv) was added. The reaction mixture was stirred with warming to rt for 3-4.5 h, diluted with water, and extracted into CH_2Cl_2. The combined organic layers were washed with sat. $NaHCO_3$ before activated carbon was added. The mixture was filtered with a pad of celite and the filter cake was washed several times with CH_2Cl_2. The filtrate was concentrated *in vacuo* and purification on silica gel (gradient of EtOAc in hexanes or MeOH in CH_2Cl_2) provided the free alcohol products.

Method B. The corresponding PMB-protected alcohol (1.0 equiv) was dissolved in CH_2Cl_2 and TFA (0.02 M, 2:1 (final volume)) was added dropwise at rt. The reaction mixture was further stirred for 15-30 min, concentrated *in vacuo*, and directly purified on silica gel (gradient of EtOAc in hexanes or MeOH in CH_2Cl_2) to provide the desired free alcohol products.



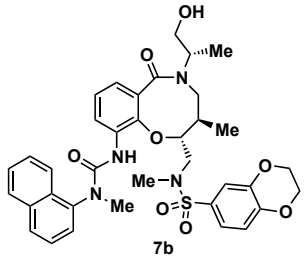
1

BRD0476 (1). Following the general procedure for urea formation, 110 mg (0.138 mmol, 99% yield) of PMB-protected BRD0476 (**10**) was prepared from isocyanate **5** (91 mg, 0.140 mmol) and 1-naphthylamine (100 mg, 0.698 mmol). LCMS (ESI^+) m/z: 796.39 (M+H). Following method A for PMB deprotection, 56 mg of **1** (0.083 mmol, 63% yield) was prepared from **8** (110 mg, 0.138 mmol) and DDQ (45 mg, 0.198 mmol). M.p. = 170-172 °C; ^1H NMR and MS spectra were identical to previous reported spectra for **1**.[1]



7a

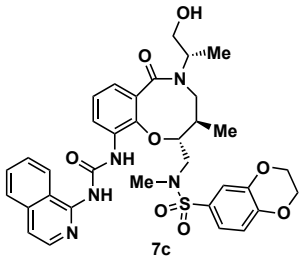
1-Naphthylmethyl urea 7a. Following the general procedure for urea formation, 24 mg (0.030 mmol, 88% yield) of **6a** was prepared from isocyanate **5** (22 mg, 0.034 mmol) and 1-naphthylmethylamine (27 mg, 0.170 mmol). LCMS (ESI^+) m/z: 809.46 (M+H). Following method B for PMB deprotection, 12 mg of **7a** (0.017 mmol, 59% yield) was prepared from **6a** (24 mg, 0.030 mmol). ^1H NMR (300 MHz, $CDCl_3$) δ 8.44 (dd, J = 8.1 Hz, 1.2 Hz, 1H), 8.23 (d, J = 8.4 Hz, 1H), 7.88-7.76 (ovrlp m, 3H), 7.61 (d, J = 6.9 Hz, 1H), 7.56-7.45 (ovrlp m, 3H), 7.18 (t, J = 8.0 Hz, 1H), 7.05 (ovrlp m, 2H), 6.94 (s, 2H), 6.55 (br s, 1H), 5.04 (br d, J = 13.5 Hz, 1H), 4.91 (br d, J = 14.1 Hz, 1H), 4.31 (br s, 4H), 4.06 (m, 1H), 3.85 (dd, J = 11.6 Hz, 2.9 Hz, 1H), 3.72 (d, J = 11.4 Hz, 6.6 Hz, 1H), 3.53 (m, 2H), 3.29-2.88 (ovrlp m, 4H), 2.33 (s, 3H), 1.94 (m, 1H), 1.37 (d, J = 7.2 Hz, 3H), 0.78 (d, J = 6.6 Hz, 3H); LCMS (ESI^+) m/z: 689.37 (M+H); HRMS (ESI^+) m/z calculated for $C_{36}H_{40}N_4O_8SNa$ (M+Na): 711.2465, found 711.2466.



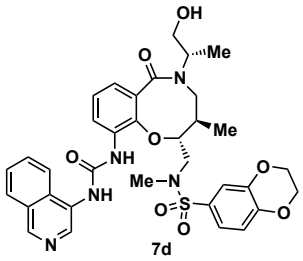
7b

***N*-Methyl-*N*-1-naphthyl urea 7b.** Following the general procedure for urea formation, 7 mg (0.0087 mmol, 36% yield) of **6b** was prepared from isocyanate **5** (16 mg, 0.024 mmol) and *N*-methyl-*N*-1-naphthylamine[2] (19 mg, 0.120 mmol). LCMS (ESI^+) m/z: 809.31 (M+H). Following method A for PMB deprotection, 3 mg of **7b** (0.0044 mmol, 50% yield) was prepared from **6b** (7 mg, 0.0087 mmol) and DDQ (3 mg, 0.013 mmol). M.p. = 144-146 °C; ^1H NMR (300 MHz, $CDCl_3$, 1:1 mixture of *N*-methyl rotamers) δ 8.54 (d, J = 7.8 Hz, 1H), 8.42 (d, J = 7.2 Hz, 1H), 7.90 (m, 1H), 7.79 (m, 2H), 7.68 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 7.2 Hz, 1H), 7.06-7.49 (ovrlp m, 3H), 7.44 (d, J = 7.5 Hz, 1H), 7.39-7.33 (ovrlp m, 3H), 7.26-7.04 (ovrlp m, 10H), 6.31 (s, 1H), 6.14 (s, 1H), 4.47 (ovrlp s, 3H), 4.45 (ovrlp s, 3H), 3.80 (ovrlp m, 5H), 3.44 (ovrlp s, 3H), 3.42 (ovrlp m, 3H), 3.41 (ovrlp s, 3H), 3.17 (m, 2H), 2.90 (m, 3H), 2.72 (m, 1H),

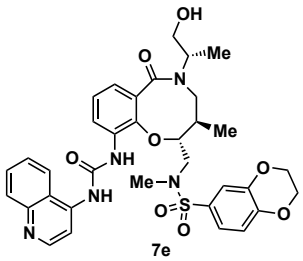
2.53 (m, 2H), 2.29 (m, 4H), 1.43 (d, J = 6.0 Hz, 3H), 1.37 (d, J = 5.7 Hz, 3H), 0.79 (d, J = 6.6 Hz, 3H), 0.52 (d, J = 6.9 Hz, 3H); LCMS (ESI^+) m/z: 689.23 (M+H).



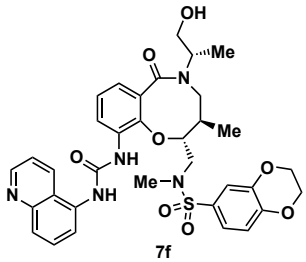
1-Isoquinoline urea 7c. Following the general procedure for urea formation, 12 mg (0.015 mmol, 98% yield) of **6c** was prepared from isocyanate **5** (10 mg, 0.015 mmol) and 1-aminoisoquinoline (11 mg, 0.077 mmol). LCMS (ESI^+) m/z: 796.06 (M+H). Following method B for PMB deprotection, 3 mg of **7c** (0.0044 mmol, 29% yield) was prepared from **6c** (12 mg, 0.015 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.12 (br s, 1H), 9.51 (br s, 1H), 8.05-7.89 (ovrlp m, 3H), 7.59 (d, J = 7.2 Hz, 1H), 7.53-7.40 (ovrlp m 3H), 7.30-7.08 (ob ovrlp m, 4H), 6.77 (d, J = 7.2 Hz, 1H), 4.21 (ovrlp m, 4H), 3.92 (m, 1H), 3.83-3.71 (ovrlp m, 3H), 3.51 (m, 2H), 3.04-2.84 (ovrlp m, 2H), 2.91 (ovrlp s, 3H), 2.32 (m, 1H), 1.50 (d, J = 6.6 Hz, 3H), 0.84 (dd, J = 6.6 Hz, 3H); LCMS (ESI^+) m/z: 676.27 (M+H).



4-Isoquinolyl urea 7d. Following the general procedure for urea formation, 14 mg (0.018 mmol, 73% yield) of **6d** was prepared from isocyanate **5** (16 mg, 0.024 mmol) and 4-aminoisoquinoline (17 mg, 0.120 mmol). LCMS (ESI^+) m/z: 796.29 (M+H). Following method B for PMB deprotection, 3 mg of **7d** (0.0044 mmol, 44% yield) was prepared from **6d** (8 mg, 0.010 mmol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.16 (s, 1H), 9.13 (s, 1H), 8.91 (s, 1H), 8.23 (ovrlp m, 2H), 8.11 (d, J = 8.4 Hz, 1H), 7.91 (t, J = 7.2 Hz, 1H), 7.81 (ovrlp m, 2H), 7.21 (ovrlp m, 3H), 7.10 (dd, J = 7.7 Hz, 1.7 Hz, 1H), 8.88 (d, J = 9.0 Hz, 1H), 4.20 (ovrlp m, 5H), 3.89 (dd, J = 14.0 Hz, 4.1 Hz, 1H), 3.80-3.32 (ovrlp m, 5H), 3.11 (d, J = 11.7 Hz, 1H), 2.83 (s, 3H), 2.38 (m, 1H), 1.27 (d, J = 6.9 Hz, 3H), 0.85 (d, J = 6.3 Hz, 3H); LCMS (ESI^+) m/z: 676.30 (M+H).

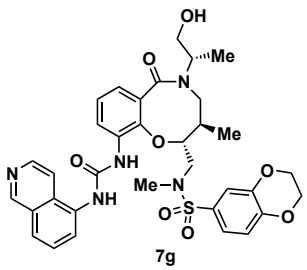


4-Quinolyl urea 7e. Following the general procedure for urea formation, 4 mg (0.0051 mmol, 47% yield) of **6e** was prepared from isocyanate **5** (7 mg, 0.011 mmol) and 4-aminoquinoline (7 mg, 0.054 mmol). LCMS (ESI^+) m/z: 796.29 (M+H). Following method B for PMB deprotection, 3 mg of **7e** (0.0044 mmol, 71% yield) was prepared from **6e** (5 mg, 0.0063 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.47 (s, 1H), 8.73 (d, J = 8.1 Hz, 1H), 8.45 (s, 1H), 8.15 (ovrlp m, 2H), 8.02 (dd, J = 8.4 Hz, 0.6 Hz, 1H), 7.76 (ovrlp m, 2H), 7.65 (t, J = 7.8 Hz, 1H), 7.25 (ovrlp m, 3H), 6.82 (d, J = 8.1 Hz, 1H), 4.90 (m, 1H), 4.32-4.13 (ovrlp m, 5H), 3.89 (m, 1H), 3.77 (m, 1H), 3.65 (m, 1H), 3.56 (m, 1H), 3.11 (m, 1H), 2.80 (s, 3H), 2.73 (m, 1H), 2.27 (m, 1H), 1.27 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 6.6 Hz, 3H); LCMS (ESI^+) m/z: 676.24 (M+H).



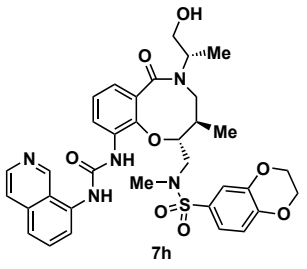
5-Quinolyl urea 7f. Following the general procedure for urea formation, 82 mg (0.103 mmol, 64% yield) of **6f** was prepared from isocyanate **5** (104 mg, 0.160 mmol) and 5-aminoquinoline (115 mg, 0.80 mmol). LCMS (ESI^+) m/z: 796.39 (M+H). Following method B for PMB deprotection, 52 mg of **7f** (0.077 mmol, 82% yield) was prepared from **6f** (75 mg, 0.094 mmol). M.p. = 164-166 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (br s, 1H), 8.55 (d, J = 8.4 Hz, 1H), 8.47 (dd, J = 8.3 Hz, 1.4 Hz, 1H), 8.32 (s, 1H), 8.25 (s, 1H), 8.03 (t, J = 4.8 Hz, 1H), 7.73 (ovrlp m, 2H), 7.46 (dd, J = 8.1 Hz, 4.2 Hz, 1H), 7.27 (ob m, 2H), 7.22-7.12 (ovrlp m, 2H), 7.01 (d, J = 8.1 Hz, 1H), 4.34 (ovrlp d, J = 4.2 Hz, 2H), 4.31 (ovrlp d, J = 4.5 Hz, 2H), 4.15 (m, 1H), 3.95-3.74 (ovrlp m, 4H), 3.62 (dd, J = 15.6 Hz, 10.8 Hz, 1H), 3.06 (ovrlp dd, J = 15.5 Hz, 0.8 Hz,

1H), 3.00 (ovrlp s, 3H), 2.02 (ob m, 1H), 1.43 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.6 Hz, 3H), 3.08 (d, J = 15.9 Hz, 2H), 2.88 (s, 3H), 2.68 (s, 1H); LCMS (ESI^+) m/z: 676.40 (M+H).



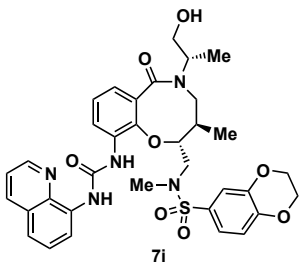
7g

5-Isoquinolyl urea 7g. Following the general procedure for urea formation, 15 mg (0.019 mmol, 82% yield) of **6g** was prepared from isocyanate **5** (15 mg, 0.023 mmol) and 5-aminoisoquinoline (17 mg, 0.115 mmol). LCMS (ESI^+) m/z: 796.54 (M+H). Following method B for PMB deprotection, 5 mg of **7g** (0.0074 mmol, 45% yield) was prepared from **6g** (13 mg, 0.016 mmol). ^1H NMR (300 MHz, DMSO-d_6) δ 9.38 (s, 1H), 9.09 (s, 1H), 8.55 (d, *J* = 5.7 Hz, 1H), 8.16 (ovrlp m, 2H), 7.91 (ovrlp m, 2H), 7.81 (d, *J* = 6.0 Hz, 1H), 7.67 (t, *J* = 7.7 Hz, 1H), 7.21 (ovrlp m, 3H), 7.09 (m, 1H), 6.89 (d, *J* = 8.7 Hz, 1H), 4.20 (ovrlp m, 4H), 3.93-3.11 (ovrlp m, 8H), 2.82 (s, 3H), 2.27 (m, 1H), 1.26 (d, *J* = 7.2 Hz, 3H), 0.84 (d, *J* = 5.7 Hz, 3H); LCMS (ESI^+) m/z: 676.19 (M+H).



7h

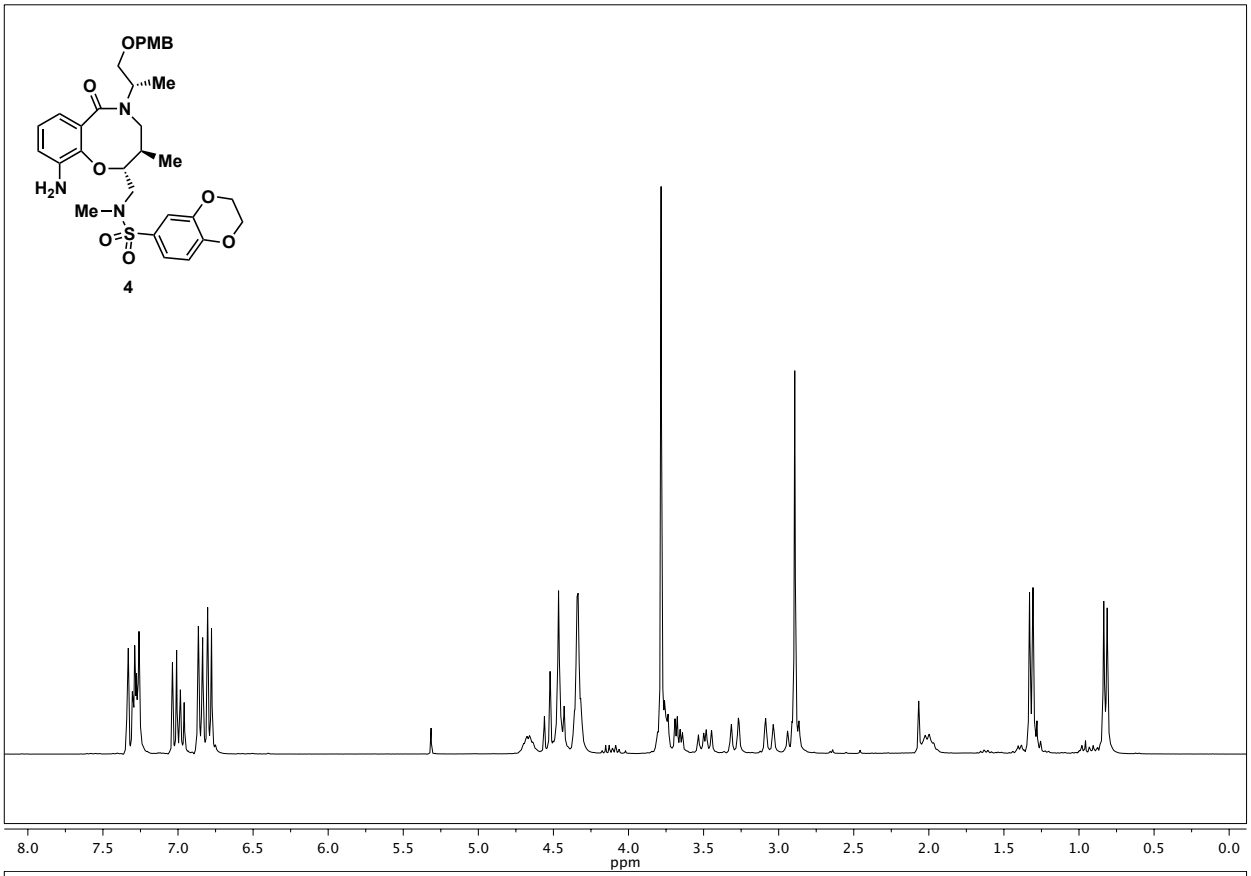
8-Isoquinolyl urea 7h. Following the general procedure for urea formation, 7 mg (0.0088 mmol, 57% yield) of **6h** was prepared from isocyanate **5** (10 mg, 0.015 mmol) and 8-aminoisoquinoline (11 mg, 0.077 mmol). LCMS (ESI^+): 796.13 (M+H). Following method B for PMB deprotection, 4 mg of **7h** (0.0059 mmol, 67% yield) was prepared from **6h** (7 mg, 0.0088 mmol). ^1H NMR (300 MHz, $CDCl_3$) δ 9.55 (s, 1H), 9.38 (s, 1H), 8.59 (d, *J* = 5.7 Hz, 1H), 8.20 (s, 1H), 8.06 (d, *J* = 8.1 Hz, 1H), 7.96 (d, *J* = 5.4 Hz, 1H), 7.83-7.72 (ovrlp m, 3H), 7.23 (ovrlp m, 3H), 7.09 (d, *J* = 8.1 Hz, 1H), 7.04 (d, *J* = 9.0 Hz, 1H), 4.25-4.12 (ovrlp m, 6H), 3.90 (d, *J* = 14.1 Hz, 1H), 3.79-3.65 (ovrlp m, 2H), 3.51 (ob m, 3H), 3.11 (d, *J* = 13.8 Hz, 1H), 2.82 (s, 3H), 1.25 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.9 Hz, 3H); LCMS (ESI^+) m/z: 676.23 (M+H).

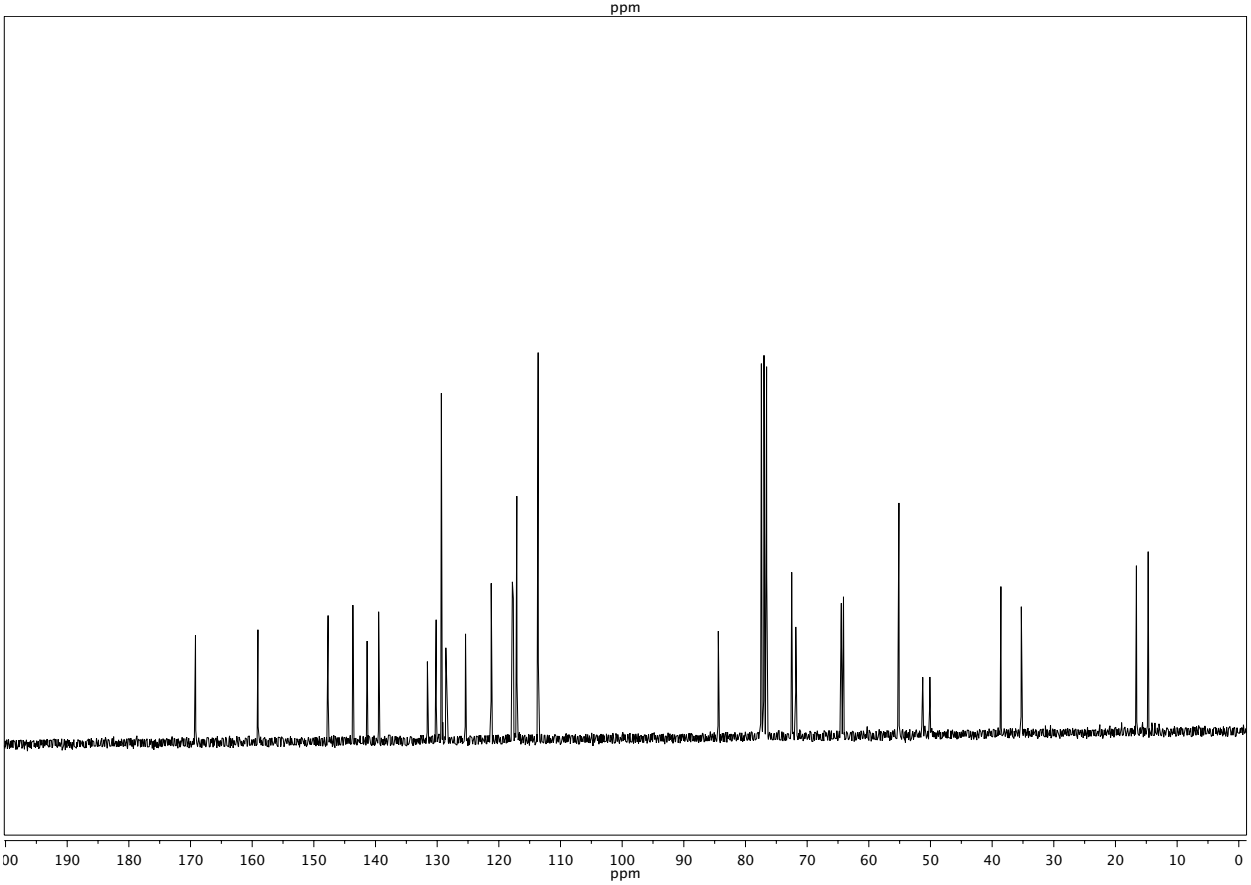


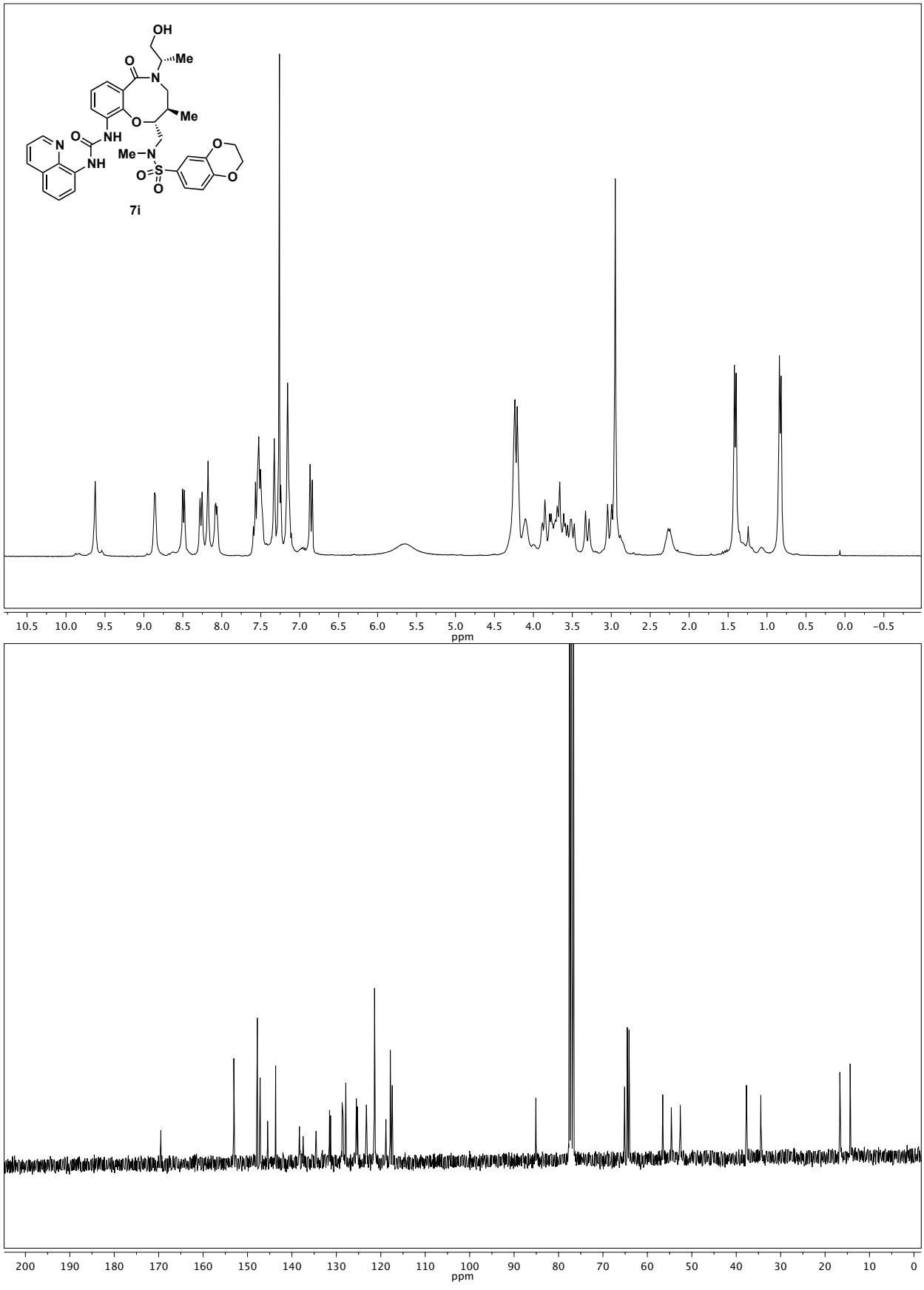
7i

8-Quinolyl urea 7i. Following the general procedure for urea formation, 88 mg (0.111 mmol, 90% yield) of **6i** was prepared from isocyanate **5** (80 mg, 0.123 mmol) and 8-aminoquinoline (89 mg, 0.615 mmol). LCMS (ESI^+) m/z: 796.37 (M+H). Following method B for PMB deprotection, 39 mg of **7i** (0.058 mmol, 87% yield) was prepared from **6i** (53 mg, 0.067 mmol). M.p. = 151-153 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 9.63 (s, 1H), 8.86 (d, *J* = 2.7 Hz, 1H), 8.49 (d, *J* = 7.2 Hz, 1H), 8.27 (d, *J* = 8.1 Hz, 1H), 8.18 (s, 1H), 8.07 (dd, *J* = 6.6 Hz, 3.0 Hz, 1H) 7.53 (ovrlp m, 3H), 7.32 (d, *J* = 1.8 Hz, 1H), 7.26 (ovrlp dd, *J* = 8.1 Hz, 1.5 Hz, 1H), 7.16 (ovrlp m, 2H), 6.85 (d, *J* = 8.4 Hz, 1H), 5.65 (br s, 1H), 4.22 (ovrlp m, 4H), 4.10 (m, 1H), 3.78 (dd, *J* = 11.1 Hz, 2.1 Hz, 1H), 3.79-3.47 (ovrlp m, 4H), 3.31 (d, *J* = 13.5 Hz, 1H), 3.02 (ovrlp d, *J* = 15.6 Hz, 1H), 2.95 (ovrlp s, 3H), 2.26 (m, 1H), 1.41 (d, *J* = 6.9 Hz, 3H), 0.83 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75.0 MHz, $CDCl_3$) δ 169.5, 153.1, 147.8, 147.2, 145.5, 143.7, 138.32, 138.29, 137.5, 134.6, 131.5, 131.3, 128.7, 128.5, 127.9, 125.5, 125.3, 123.2, 121.4, 118.8, 117.9, 117.4, 85.1, 65.1, 64.5, 64.1, 56.5, 54.6, 52.6, 37.7, 34.5, 16.7, 14.4; LCMS (ESI^+) m/z: 676.23 (M+H); HRMS (ESI^+) m/z calculated for $C_{34}H_{38}N_5O_8S$ (M+H): 676.2441, found 676.2441.

II. ^{1}H NMR and ^{13}C NMR spectra of 4 and 7i






OH
Me
O
N
Me
O
NH
N
NH
Me–N
O=S=O
O
O
O
7i
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
ppm
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

III. Aqueous Solubility Assay

Solubility was determined in phosphate buffered saline (PBS) pH 7.4. Six aliquots of a 10 mM solution were plated in a 96-well deep-well plate and dried down in a Genevac centrifugal evaporator. DMSO was then added to three wells and PBS to the other three, for a final concentration of 250 uM. StirStix were added to each well and compounds were allowed to equilibrate at room temperature with a 350 rpm orbital shake for 24 hours. After equilibration, samples were centrifuged and the supernatant analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer. The DMSO samples were used to create a two-point calibration curve to which the response in PBS was fit.

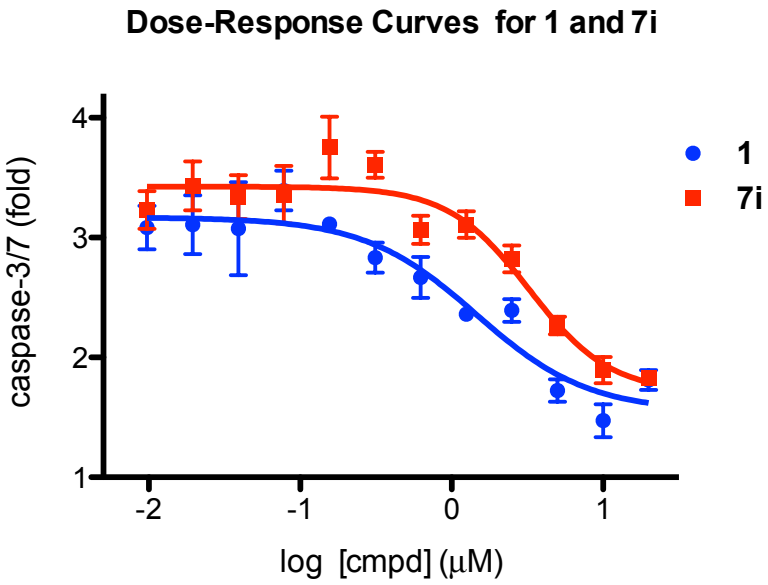
IV. Cell Culture and Reagents

INS-1E cells (generously provided by C. Wollheim and P. Maechler, University of Geneva) were maintained in RPMI 1640 containing 11 mM glucose, 10% fetal bovine serum (FBS), 10 mM HEPES, 50 μM 2-mercaptoethanol, and 1 mM sodium pyruvate, cultivated at 37 °C with 5% CO_2 in a humidified atmosphere, and split every week. Recombinant mouse IL-β, recombinant rat TNF-α, and recombinant rat IFN-γ were purchased from R&D Systems. Caspase-Glo® 3/7 reagent was purchased from Promega. STAT1 and pSTAT1 primary antibodies were purchased from Cell Signaling. The β-actin primary antibody was purchased from Sigma-Aldrich. Secondary horseradish peroxidase-conjugated goat anti-mouse and anti-rabbit antibodies were purchased from Cell Signaling. The secondary Alexafluor-conjugated goat anti-rabbit antibody was purchased from Invitrogen.

V. Caspase-3/7 Assay

INS-1E cells were seeded at 5,000 cells per well using a Multidrop Combi (Thermo Labsystems) in white optical 384-well plates. After 24 h incubation, medium was removed using a plate washer (ELx 405, BioTek Instruments) and 50 μL of RPMI containing 1% FBS and a combination of cytokines (0.5 ng mL^{-1} IL-β, 2.5 ng mL^{-1} TNF-α, 500 ng mL^{-1} IFN-γ) were added to every well. Using compounds dissolved in DMSO and titrated in 384-well plates, 0.1 μL of each dose of compound was added using a Cybi-Well pin-transfer robot (CyBio Corp.). After treatment with cytokines and compounds for 48 h, medium was removed and 20 μL Caspase-Glo® 3/7 reagent was added. Luminescence was measured after 2 h of incubation using an EnVision plate reader (PerkinElmer).

Data are expressed as mean values with standard deviation of triplicate wells and were analyzed by nonlinear regression analysis with Prism Graphpad 5 software. IC_{50} values were determined using a sigmoidal dose response (variable slope) equation and maximum activity was calculated with the predicted bottom of the sigmoidal curve for compounds as a function of DMSO and cytokines controls using the equation: Y = (bottom – cytokines) / (DMSO – cytokines) * 100%. Representative dose-response curves for active compounds are provided below:



VI. Western Blot Analysis for STAT1 phosphorylation

INS-1E cells (1 million cells/well in a 6-well plate) were incubated for 48 h, treated, and lysed in passive lysis buffer with proteasome inhibitors. Total protein was separated by 4-12% SDS-PAGE and transferred to a PVDF membrane. The membranes were incubated with primary antibodies overnight at 4 °C and with secondary antibodies for 1 h at rt. Blots were developed using the SuperSignal® West Femto chemiluminescence detection system (Thermo Scientific) and light emission was captured using an Imaging Station 4000MM (Carestream).

VII. Immunofluorescence STAT1 Nuclear Translocation Assay

INS-1E cells (20,000 cells/well in a 96-well black plate) were incubated for 48 h, treated for 30 minutes, and fixed with 4% paraformaldehyde (PFA) for 15 minutes at rt. Fixation was stopped by aspirating the PFA and wells were washed with PBS twice. Fixed cells were permeabilized with 0.2% Triton X-100 in PBS for 20 min and blocked with 2% BSA for 2 h at rt. The primary STAT1 antibody was added to the cells and incubated at 1:500 dilution overnight at 4 °C, washed three times with 2% BSA, and incubated with the Alexafluor-conjugated secondary antibody at 1:2000 dilution for 1 h at rt. Nuclei were stained with the Hoechst 33342 reagent (Invitrogen). Wells were washed with PBS, and then imaged with an ImageXpress Micro automated microscope (Molecular Devices, Sunnyvale, CA).

References:

(1) Chou et al. *ACS Med. Chem. Lett.* **2011**, *2*, 698-702.

(2) Kung, A. C.; Falvey, D. E. *J. Org. Chem.* **2005**, *70*, 3127-3132.