Design of a biased potent small molecule inhibitor of the bromodomain and PHD finger-containing (BRPF) proteins suitable for cellular and *in vivo* studies

SUPPORTING INFORMATION

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MATERIALS and METHODS

General synthetic methods:

All anhydrous solvents and reagents were obtained from commercial suppliers and used without further purification. Flash chromatography refers to medium pressure silica gel (C60 (40-60 μm)) column chromatography, unless otherwise stated. The progress of reactions was monitored by thin layer chromatography (TLC) performed on Keiselgel 60 F_{254} (Merck) silica plates and visualised by exposure to UV light at 254 nm.

Melting points (mp) were determined in open capillary tubes on a Stuart SMP10 apparatus and are uncorrected.

Infrared (IR) analysis was performed on a Perkin Elmer Spectrum 1000 FT-IR in the 4000-400 cm^{-1} range.

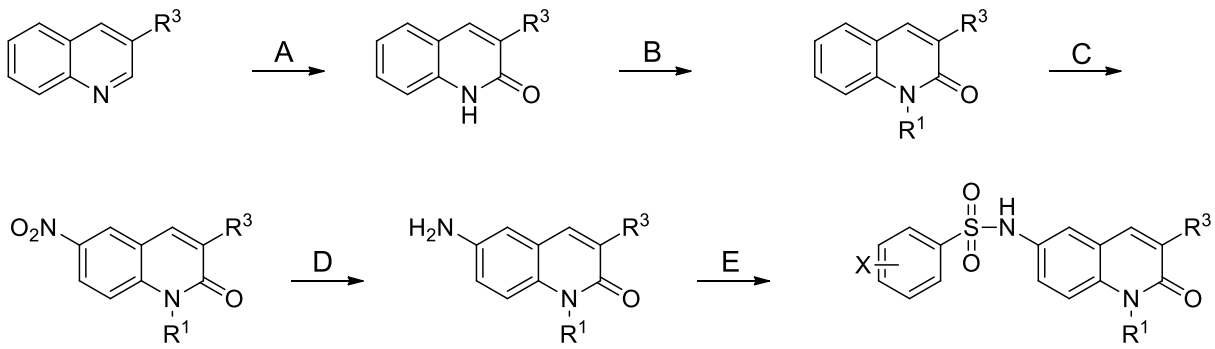
^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectra were recorded on a Bruker Advance 400 Spectrophotometer at 400 MHz or Bruker Advance 500 Spectrophotometer at 500 MHz. Chemical shifts were measured in parts per million (ppm) relative to tetramethylsilane (δ = 0) using the following internal references: $CDCl_3$ (δ 7.26), CD_3OD (δ 3.32), DMSO-d_6 (δ 2.50). Multiplicities in ^{1}H NMR spectra are quoted as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, ddd = double double doublet. ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectra were recorded on a Bruker Advance 500 Spectrophotometer at 125 MHz. Chemical shifts were measured in parts per million (ppm) relative to tetramethylsilane (δ = 0) using the following internal references: $CDCl_3$ (δ 77.16), CD_3OD (δ 49.00), DMSO-d_6 (δ 39.52). 2D NMR techniques HSQC, HMQC and HMBC were also utilised for the assignment of ^{1}H and ^{13}C NMR signals.

High-resolution mass spectra (HRMS) were recorded on a Thermo Navigator mass spectrometer coupled to an HPLC instrument using electrospray (ES) ionisation and time-of-flight (TOF) mass spectrometry. Alternatively, HRMS were recorded at the EPSRC UK National Mass Spectrometry Facility (NMSF) at Swansea University.

Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out on a XSELECT™ CSH™ C-18 column (2.5 μm; 6 x 50 mm). HPLC experiments (system A) were performed with gradient conditions: initial fixed composition 5% B to 50% B over 20 min, then increased to 95% B over 2 min, held for 2 min at 95% B, then returned to 5% B in 1 min. Total duration of gradient run was 25 min. Eluents used were solvent A (H_2O with 0.02% TFA) and solvent B (MeCN with 0.02% TFA). Flow rate: 1.00 mL/min.

Purity of screening compounds **12**-**15**, **21** and **22** was evaluated by NMR spectroscopy and HPLC analysis. All compounds had purity ≥ 95 % except **21** which had a purity of 91 % by HPLC.

Synthetic procedures for compounds 6-16:



General Procedure A: Oxidation of quinolines to quinolin-2(1*H*)-ones

To a stirred solution of the quinoline (1 eq.) in DCM (1 mL/mmol) at 0 °C was added 3-chloroperbenzoic acid (77 % w/w, 1 eq.) portionwise over 10 minutes. The resulting solution was allowed to warm to room temperature and then stirred overnight. After completion of the reaction, the solution was washed with sodium hydroxide (1.0 M, 3 × volume of DCM) and the aqueous phase extracted with DCM (3 x vol of DCM). The organic layers were combined and dried over *anhydrous* $MgSO_4$, filtered and the solvent removed *in vacuo* to yield the appropriate quinoline-*N*-oxide which was used in the next step without further purification.

To a stirred solution of quinoline-*N*-oxide in DCM (2 mL/mmol) was added sodium hydroxide (1.0 M, 1.5 mL/mmol) and the resulting biphasic mixture was cooled to 0 °C. To this was added, under rapid agitation, benzoyl chloride (1.2 eq) dropwise. The suspension was stirred for 2 hours and the resulting precipitate was collected by filtration, washed with water (50 mL) and dried under vacuum to give the desired quinolin-2(1*H*)-one.

General Procedure B: *N*-Alkylation of quinolin-2(1*H*)-ones

To a solution of quinolin-2(1*H*)-one (1 eq.) in dry DMF (2mL/mmol) under an argon atmosphere was added NaH (60 % wt, 1.2 eq.) in one portion. Upon the completion of gas evolution, the appropriate iodoalkane (1.2 eq) was added in 1 portion and the resulting solution was stirred overnight. Excess sodium hydride was quenched by the addition of water (3 × vol. of DMF). If precipitation was observed the precipitate was isolated by filtration, washed with water and dried to afford the desired *N*-alkylquinolin-2(1*H*)-one.

Otherwise, the solution was extracted with ethyl acetate (3 × vol. of DMF), washed with water and then brine. The organic phase was dried over *anhydrous* $MgSO_4$, filtered and then concentrated *in vacuo*. The crude solid was purified by column chromatography to afford the desired *N*-alkylquinolin-2(1*H*)-one.

General Procedure C: Nitration of quinolin-2(1*H*)-ones

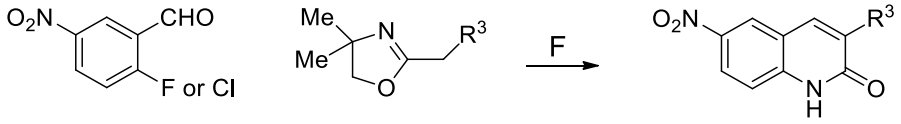
To a suspension of the appropriate quinolin-2(1*H*)-one (1 eq.) in conc. H_2SO_4 (2 mL/mmol) at -5 °C was added conc. HNO_3 (70% w/w, 0.5 mL/mmol) dropwise. The resulting yellow solution was stirred at -5 °C for 2.5 hours, before being allowed to warm to room temperature. The solution was poured over crushed ice and the resultant suspension stirred for 5 minutes. The precipitate was collected by filtration and dried under vacuum to give the appropriate 6-nitroquinolin-2(1*H*)-one.

General Procedure D: Reduction of 6-nitroquinolin-2(1*H*)-ones

To a suspension of the appropriate 6-nitroquinolin-2(1*H*)-one (1 eq.) in conc. HCl (5 mL/mmol) was added $SnCl_2 \cdot 2H_2O$ (5 eq.) and the resulting suspension was stirred overnight. Sodium hydroxide was added with cooling until the pH had reached ~pH 10. The aqueous solution was then extracted with DCM (3 × 100 mL) and the organic layers were combined and the solvent removed *in vacuo* to give the appropriate 6-aminoquinolin-2(1*H*)-one

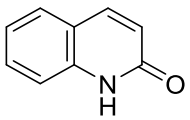
General procedure E: Preparation of sulphonamides.

To a solution of amine (1 eq.) in DCM or DMF (0.2 M, minimum 1 mL) was added pyridine (2 eq.) and the resulting solution was stirred for 5 minutes before the sulfonyl chloride (1.5 eq.) was added. The resulting solution was stirred overnight and then diluted with acetone (~20 mL). Celite® was added and the suspension was concentrated *in vacuo.* Purification of the crude product by column chromatography afforded the quinolin-2(1*H*)-one sulfonamides.



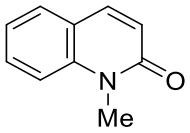
General Procedure F:

2-Halo-5-nitrobenzaldehyde (1 eq.) and 4,4-dimethyl-2-alkyl-4,5-dihydrooxazole (1 eq.) were dissolved in NMP (0.75 mL/mmol) and then xylenes (0.4 mL/mmol) and sodium bisulfate (0.1 eq.) were added. A Dean-Stark apparatus was attached and the mixture slowly heated to 200 °C and maintained at this temperature for 90 min, and then raised to 225 °C for 45 min. After this time, the reaction was allowed to cool to room temperature, added to water (10 × vol of NMP), filtered and dried to give the desired 3-alkyl-6-nitroquinolin-2(1*H*)-one.

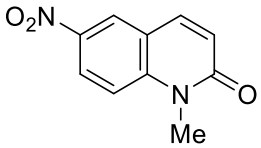


Quinolin-2(1*H*)-one: Prepared according to general procedure A from quinoline (1.0 g, 7.75 mmol) to give the title compound (0.88 g, 6.13 mmol, 78% over 2 steps) as a colourless solid. ^{1}H NMR (400

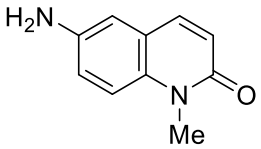
MHz, DMSO-d_6): δ ppm 11.75 (1H, br s), 7.90 (1H, d, *J* = 9.5 Hz), 7.65 (1H, dd, *J* = 7.8, 1.6 Hz), 7.49 (1H, t, *J* = 8.1 Hz), 7.35–7.26 (1H, m), 7.22–7.09 (1H, m), 6.50 (1H, d, *J* = 9.5 Hz); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 161.7, 140.2, 138.8, 130.3, 127.8, 121.9, 121.7, 119.1, 115.1. Data are in agreement with literature values.



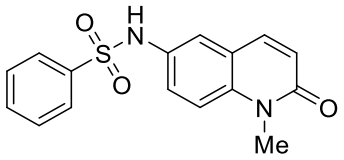
1-Methylquinolin-2(1*H*)-one was prepared by general procedure B from quinolin-2(1*H)*-one (530 mg, 3.63 mmol). Purification by column chromatography (ethyl acetate:hexanes, 30:20) gave the title compound (382 mg, 2.51 mmol, 69%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.91 (1H, d, *J* = 9.5 Hz), 7.72 (1H, dd, *J* = 7.7, 1.5 Hz), 7.67–7.61 (1H, m), 7.53 (1H, d, *J* = 8.5 Hz), 7.30–7.25 (1H, m), 6.62 (1H, d, *J* = 9.5 Hz), 3.62 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 162.3, 140.0, 139.0, 130.6, 128.8, 122.1, 121.8, 120.7, 114.1, 29.4. Data are in agreement with literature values.



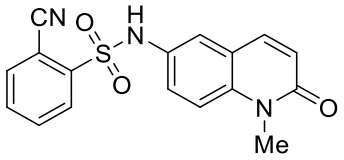
1-Methyl-6-nitroquinolin-2(1*H*)-one was prepared by general procedure C from 1-methylquinolin-2(1*H*)-one (1.20 g, 7.55 mmol) to give the title compound (1.34 g, 6.57 mmol, 87%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.75 (1H, d, *J* = 2.7 Hz), 8.41 (1H, dd, *J* = 9.4, 2.7 Hz), 8.16 (1H, d, *J* = 9.5 Hz), 7.73 (1H, d, *J* = 9.4 Hz), 6.81 (1H, d, *J* = 9.5 Hz), 3.68 (3H, s); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 161.7, 149.3, 140.9, 128.1, 124.6, 123.8, 123.3, 115.3, 62.1, 14.3. Data are in agreement with literature values.



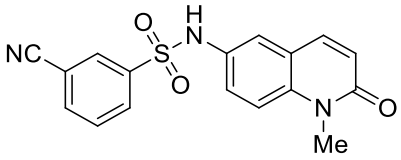
6-Amino-1-methylquinolin-2(1*H*)-one (6) was prepared according to general procedure D from 1-methyl-6-nitroquinolin-2(1*H*)-one (180 mg, 0.88 mmol) to give the title compound (0.85 mmol, 97%) as a bright yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.45 (1H, d, *J* = 9.5 Hz), 7.13 (1H, d, *J* = 8.9 Hz), 6.90 (1H, dd, *J* = 8.9, 2.7 Hz), 6.76 (1H, d, *J* = 2.7 Hz), 6.60 (1H, d, *J* = 9.5 Hz), 3.66–3.58 (5H, m); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm 160.9, 141.2, 138.1, 132.6, 122.3, 121.7, 119.4, 115.2, 112.8, 29.4.



***N*-(1-Methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (12-a):** Prepared by general procedure E from **6** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexanes, 30:70) to give the title compound (16 mg, 0.06 mmol, 47%). mp 201-204 ^{o}C (acetone-hexanes); IR (neat): v_{max} 3080, 1641, 1581, 1485, 1310, 1157, 1091, 979, 989, 819, 754, 687, 592, 567, 506, 474 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.32 (1H, br s), 7.83 (1H, d, *J* = 9.6 Hz), 7.76 (1H, s), 7.64–7.50 (3H, m), 7.46–7.39 (2H, m), 7.31 (1H, dd, *J* = 10.4, 2.0 Hz), 6.58 (1H, d, *J* = 9.4 Hz), 3.54 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.7, 139.2, 138.7, 136.8, 132.9, 131.7, 129.3, 126.6, 124.3, 121.8, 120.3, 120.1, 115.6, 29.0; HRMS: *m/z* (ESI$^-$) Found (M-H)$^-$ 313.0633, $C_{11}H_{12}N_2O_3S$ requires (M-H)$^-$ 313.0725; HPLC: Retention time (system A): t_R= 9.92 min. Purity: >95%.

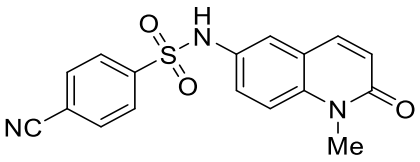


2-Cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (12-b): Prepared by general procedure E from **6** (100 mg, 0.55 mmol). Purified by column chromatography (ethyl acetate) to give the title compound (114 mg, 0.34 mmol, 61%) as an off-white solid. mp 231 – 234 °C (ethyl acetate-hexanes); IR (neat): v_{max} 3185, 2225, 1646, 1578, 1500, 1408, 1348, 1309, 1162, 1098, 956, 933, 908, 820, 797, 712, 681, 638, 590, 531, 472, 417 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.19 (1H, t, *J*= 1.5 Hz), 8.15–8.07 (1H, m), 8.04–7.96 (1 H, m), 7.87 (1H, d, *J* = 9.5 Hz), 7.81–7.71 (1H, m), 7.50–7.40 (2H, m), 7.31 (1 H, s), 6.60 (1H, d, *J* = 9.5 Hz), 3.54 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.0, 142.5, 138.9, 138.7, 131.9, 130.8, 128.0, 127.9, 127.6, 124.1, 122.7, 122.1, 119.6, 118.2, 116.1, 112.1, 29.4; HRMS *m/z* (ESI$^+$) Found (M+H)$^+$ 340.0678, $C_{17}H_{13}N_3O_3S$ requires (M+H)$^+$ 340.0751; HPLC: Retention time (system A): t_R= 9.28 min. Purity: >95%.

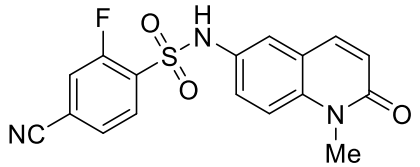


3-Cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (12-c): Prepared using general procedure E from **6** (100 mg, 0.55 mmol). Purified by column chromatography (ethyl acetate) to give the title compound (88 mg, 0.26 mmol, 46%) as an off-white solid. mp 256-257 °C (ethyl acetate); IR (neat) v_{max}: 1637, 1565, 1503, 1462, 1436, 1416, 1336, 1239, 1204, 1156, 1124, 1087, 1062, 976, 934, 904, 873, 802, 715, 82, 625, 583, 560, 495, 472 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.53 (1H, br. s), 8.19 (1H, t, *J* = 1.5 Hz), 8.11 (1H, dt, *J* =7.8, 1.3 Hz), 8.03–7.97 (1H, m), 7.87 (1H, d, *J* = 9.5 Hz), 7.80 –7.72 (1H, m), 7.48–7.41 (2H, m), 7.33–7.26 (1H, m), 6.63–6.56 (1H, m),

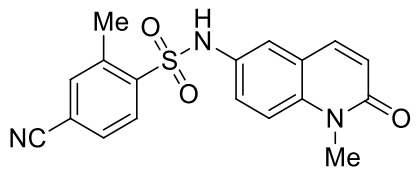
3.55 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 160.6, 140.3, 138.7, 137.2, 136.6, 131.1, 130.9, 130.8, 130.2, 124.7, 121.9, 120.8, 120.4, 117.4, 115.8, 112.5, 29.0; HRMS *m/z* (ESI$^+$) Found $(M+H)^+$ 340.0678, $C_{17}H_{13}N_3O_3S$ requires $(M+H)^+$ 340.0751; HPLC: Retention time (system A): t_R= 9.21 min. Purity: >95%.



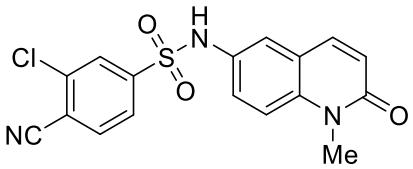
4-Cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (12-d): Prepared using general procedure E from **6** (100 mg, 0.55 mmol). Purified by column chromatography (ethyl acetate) to give the title compound (77 mg, 0.23 mmol, 41%) as an off-white solid, mp >300 °C; IR (neat) ν_{max}: 3091, 2846, 1644, 1574, 1523, 1435, 1338, 1274, 1159, 1091, 952, 905, 871, 844, 663, 649, 584, 556, 496 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.59 (1H, br. s), 8.06–8.01 (2H, m), 7.91–7.84 (3H, m), 7.47–7.42 (2H, m), 7.30 (1H, d, *J* = 2.6 Hz), 6.60 (1H, d, *J* = 9.5 Hz), 3.55 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.8, 146.1, 143.2, 136.6, 133.3, 127.9, 124.5, 122.0, 120.1, 118.0, 117.2, 116.3, 116.0, 29.0; HRMS *m/z* (ESI$^+$) Found $(M+H)^+$ 340.0755, $C_{17}H_{13}N_3O_3S$ requires $(M+H)^+$ 340.0751; HPLC: Retention time (system A): t_R= 9.33 min. Purity: >95%.



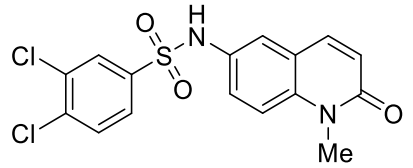
4-Cyano-2-fluoro-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (12-e): Prepared according to general procedure E from **6** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexanes gradient elution 20:80 to 30:70) to give the title compound (31 mg, 0.09 mmol, 79%) as a pink solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 8.10 (1H, d, *J* = 10.1 Hz), 7.95 (1H, t, *J* = 7.7 Hz), 7.84 (2H, t, *J* = 9.5 Hz), 7.47 - 7.39 (2H, m), 7.33 (1H, dd, *J* = 9.1, 2.5 Hz), 6.58 (1H, d, *J* = 9.5 Hz), 3.55 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.7, 157.5 (d, *J* = 263.0 Hz), 138.7, 137.2, 131.6 (d, *J* = 14.7 Hz), 131.3, 130.5, 129.4 (d, *J* = 4.6 Hz), 124.6, 121.9, 121.6 (d, *J* = 25.7 Hz), 120.6, 120.4, 117.4 (d, *J* = 10.1 Hz), 116.4, 115.8, 29.0; HRMS *m/z* (ESI$^-$) Found $(M-H)^-$ 356.0508, $C_{17}H_{12}FN_2O_3S$ requires $(M-H)^-$ 356.0505; HPLC: Retention time (system A): t_R= 7.92 min. Purity: 94%.



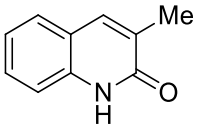
4-Cyano-2-methyl-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (12-f): Prepared according to general procedure E from **6** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexanes, gradient elution 20:80 to 30:70) to give the title compound (5 mg, 0.01 mmol, 12%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.96 (1H, d, *J* = 8.3 Hz), 7.90 (1H, s), 7.80 (2H, t, *J* = 9.2 Hz), 7.47 - 7.19 (3H, m), 6.57 (1H, d, *J* = 9.1 Hz), 3.53 (3H, s), 2.64 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.7, 138.7, 138.3, 136.1, 130.2, 129.9, 124.0, 121.9, 120.4, 119.8, 117.5, 115.8, 115.2, 29.0, 19.5; HRMS *m/z* (ESI$^-$) Found (M-H)$^-$ 352.0764, $C_{18}H_{15}N_3O_3S$ requires (M-H)$^-$ 352.0756; HPLC: Retention time (system A): t_R= 8.84 min. Purity: >95%.



3-Chloro-4-cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (12-g): Prepared according to general procedure E from **6** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexanes, gradient elution 20:80 to 30:70) to give the title compound (30 mg, 0.08 mmol, 73%) as a colourless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.16 (1H, d, *J* = 8.1 Hz), 8.03 (1H, d, *J* = 1.5 Hz), 7.88 (1H, d, *J* = 9.6 Hz), 7.79 (1H, dd, *J* = 8.2, 1.6 Hz), 7.49 - 7.42 (2H, m), 7.31 (1H, dd, *J* = 9.1, 2.5 Hz), 6.61 (1H, d, *J* = 9.3 Hz), 3.56 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.7, 144.6, 138.7, 137.4, 136.5, 135.9, 130.5, 127.5, 125.8, 125.0, 121.9, 121.2, 120.5, 116.1, 115.9, 115.0, 29.0; HRMS *m/z* (ESI$^-$) Found (M-H)$^-$ 372.0219, $C_{17}H_{12}ClN_3O_3S$ requires (M-H)$^-$ 372.0221; HPLC: Retention time (system A): t_R= 9.19 min. Purity: >95%.

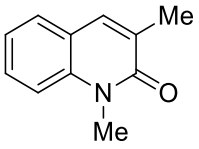


3,4-Dichloro-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (12-h): Prepared according to general procedure E from **6** (20 mg, 0.11 mmol). The reaction solution was added to H_2O (10 mL) and the precipitate collected by filtration. Purification of the crude solid by crystallisation (MeOH) gave the title compound (38 mg, 0.10 mmol, 91%) as a colourless solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.62–10.32 (1H, br. s), 7.95 (1H, d, *J* = 2.2 Hz), 7.88 (1H, d, *J* = 9.5 Hz), 7.83 (1H, d, *J* = 8.5 Hz), 7.65 (1H, dd, *J* = 8.5, 2.2 Hz), 7.45 (2H, m, *J* = 2.0 Hz), 7.31 (1H, dd, *J* = 9.0, 2.6 Hz), 6.60 (1H, d, *J* = 9.5 Hz), 3.55 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.7, 139.5, 138.7, 137.3, 136.1, 132.2, 131.7, 130.9, 128.4, 126.8, 124.8, 121.9, 120.9, 120.4, 115.8, 29.0; HRMS *m/z* (ESI$^-$) Found (M-H)$^-$ 380.9871, $C_{17}H_{12}Cl_2N_2O_3S$ requires (M-H)$^-$ 380.9868; HPLC: Retention time (system A): t_R= 10.34 min. Purity: >95%.



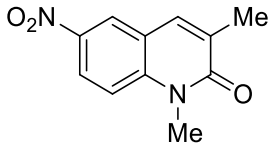
3-Methylquinolin-2-(1*H*)-one (**18**). To a stirred solution of 3-methylquinoline (**17**) (25.0 g, 175 mmol, 1.0 eq.) in dichloromethane (875 mL) in a 2 litre round bottom flask at 0 °C was added 3-chloroperbenzoic acid (70 %, 47.3 g, 192 mmol, 1.1 eq.) portionwise over 10 minutes. The resulting solution was allowed to warm to room temperature and then stirred overnight. After completion of the reaction, the solution was washed with sodium hydroxide (1.0 M, 300 mL) and the aqueous phase extracted with dichloromethane (2 x 300 mL). The organic layers were combined, dried over *anhydrous* $MgSO_4$, filtered and the solvent removed under reduced pressure to yield crude 3-methylquinolone-*N*-oxide as a white solid, which was used without further purification.

To a 2 L three-necked round bottom flask fitted with an overhead mechanical stirrer was charged crude 3-methylquinolone-*N*-oxide followed by dichloromethane (430 mL) and aqueous sodium hydroxide solution (1.0 M, 400 mL). The resulting biphasic mixture was cooled to 0 °C and to this was added, under rapid agitation, benzoyl chloride (24.4 mL, 210 mmol, 1.2 eq.) dropwise *via* a side-arm pressure equalised dropping funnel. The suspension was stirred overnight and the resulting heterogeneous solution was diluted with diethyl ether and stirred rapidly for 15 minutes. The resultant precipitate was collected by filtration, washed with diethyl ether (200 mL) and then water (200 mL) and dried under vacuum to give 3-methylquinolin-2(1*H*)-one (**18**) (16.3 g 59 % over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.72 (br. s, 1H), 7.75 (s, 1H), 7.56 (d, J = 7.8 Hz, 1H), 7.41 (t, J = 8.3 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 7.14 (t, J = 7.5 Hz, 1H), 2.09 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.4, 137.9, 136.3, 129.8, 129.0, 126.9, 121.6, 119.4, 114.7, 16.5.

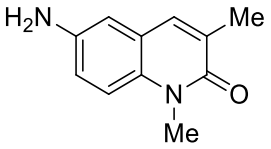


1,3-Dimethylquinolin-2-(1*H*)-one (19). To a solution of 3-methylquinolin-2(1*H*)-one (**18**) (16.3 g, 103 mmol, 1.0 eq.) in *anhydrous* DMF (500 mL) in a 1 litre round bottom flask cooled to 0 °C and under an argon atmosphere was added NaH (60 % wt., 4.50 g , 113 mmol, 1.1 eq.) and the reaction mixture stirred for 0.5 hours. Iodomethane (8.3 mL, 133 mmol, 1.3 eq.) was added dropwise *via* syringe and the reaction mixture stirred for 5 hours. Excess sodium hydride was quenched by the addition of water (30 mL) and the solvents removed under reduced pressure. The resultant slurry was taken up in the minimum volume of ethyl acetate and poured onto crushed ice (approximately 200 g). The resultant heterogeneous mixture was stirred rapidly for 15 minutes and then the precipitate collected by filtration, washed with cold diethyl ether (100 mL) and dried under vacuum to give 1,3-dimethylquinolin-2-(1*H*)-one (**19**) (16.9 g, 95 %) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.78 (s, 1H), 7.63 (dd, J = 7.8, 1.3 Hz, 1H), 7.58–7.53 (m, 1H), 7.50–7.48 (m, 1H), 7.26–7.22 (m, 1H),

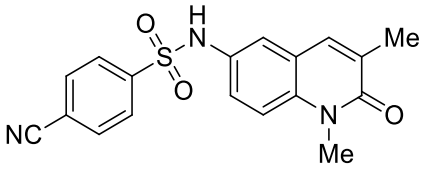
3.64 (s, 3H) 2.13 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.7, 138.7, 135.5, 129.5, 128.9, 127.7, 121.8, 120.0, 114.3, 29.3, 16.5.



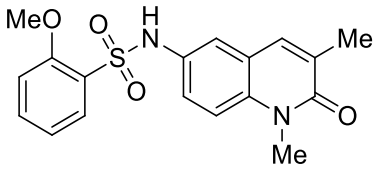
1,3-Dimethyl-6-nitroquinolin-2-(1*H*)-one (**20**). A 500 mL round bottom flask was charged with 1,3-dimethylquinolin-2-(1*H*)-one (**19**) (16.9 g, 97.5 mmol, 1.0 eq.) and cooled to 0 °C. Concentrated sulfuric acid (120 mL) was added and the reaction mixture stirred for 0.5 hours during which point most of the solid dissolved. To the resultant pale red solution, potassium nitrate (10.4 g, 102.4 mmol, 1.05 eq.) was added in two portions. The reaction mixture rapidly turned yellow and was stirred for a further 4 hours, after which it was poured onto crushed ice (approximately 400 g) and stirred rapidly for 0.5 hours. The yellow precipitate was collected by filtration onto a sintered-glass funnel and washed with water (2 x 250 mL). Still on the sintered disk, the resultant filter cake was slurried with hot ethanol (200 mL) using a glass rod and allowed to stand for 10 minutes. The solid was collected by filtration to give 1,3-dimethyl-6-nitroquinolin-2-(1*H*)-one (**20**) (18.5 g, 87 %) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.63 (s, 1H), 8.35 (d, *J* = 8.8 Hz, 1H), 8.03 (s, 1H), 7.7 (d, *J* = 8.8 Hz, 1H), 3.71 (s, 3H) 2.16 (s, 3H); ^{13}C NMR (125 MHz, , DMSO-d_6): δ 161.8, 142.9, 141.4, 135.3, 131.2, 123.9, 123.5, 119.7, 115.7, 30.1, 17.4.



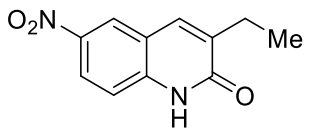
6-Amino-1,3-dimethyl-6-quinolin-2-(1*H*)-one (**7**). A 500 mL round bottom flask was charged with 1,3-dimethyl-6-nitroquinolin-2-(1*H*)-one (**20**) (5.00 g, 22.9 mmol, 1.0 eq.) and palladium on carbon (5 % wt., 2.44 g, 1.45 mmol, 0.05 eq.). The flask was sealed then evacuated and back-filled with nitrogen three times before adding ethanol (300 mL). The stirred slurry was then re-evacuated until the solvent began to bubble and back-filled with nitrogen once more. A balloon of hydrogen was attached to the flask *via* a syringe fitted with a stop-cock. The flask was evacuated until the solvent began to boil and then back-filled with hydrogen gas five times. The reaction was then stirred for seven hours after which point the complete consumption of starting material was confirmed by TLC. At the end of the reaction, the reaction flask was evacuated and back-filled with nitrogen three times, filtered through a pad of Celite® onto a glass-sintered funnel. The Celite® pad was further washed with ethanol (300 mL) and then the filtrate concentrated under reduced pressure to give 6-amino-1,3-dimethyl-6-quinolin-2-(1*H*)-one (**7**) (4.10 g, 95 %) as a pale yellow solid that was not purified further. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.55 (s, 1H), 7.21 (d, *J* = 8.8 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 1H), 6.71 (s, 1H), 5.04 (br. s, 2H), 3.56 (s, 3H) 2.08 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.9, 143.6, 135.0, 130.5, 128.7, 120.9, 117.7, 115.0, 110.0, 29.2, 17.5.



4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (**13-d, NI-42**): To a solution of 6-amino-1,3-dimethylquinolin-2(1*H*)-one (**7**) (188 mg, 1.00 mmol) and pyridine (158 mg, 2.00 mmol, 2.0 eq.) in DMF (5 mL) was added 4-cyanobenzenesulfonyl chloride (201 mg, 1.00 mmol, 1.0 eq.) at room temperature and the reaction mixture stirred overnight. The resulting suspension was homogenised by addition of DMF (3 mL), concentrated onto Celite® and purified by column chromatography (acetone:hexane, gradient elution 30:70 to 60:40) to give **NI-42** (129 mg, 0.37 mmol, 37%) as a white solid. m.pt. 279-282 ^{o}C (acetone-hexane)**;** ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 10.52 (1H, s), 8.06 - 8.00 (2H, m), 7.90 - 7.84 (2H, m), 7.73 (1H, s), 7.41 (1H, d, *J* = 9.1 Hz), 7.33 (1H, d, *J* = 2.5 Hz), 7.23 (1H, dd, *J* = 9.1, 2.5 Hz), 3.57 (3H, s), 2.09 (3H, d, *J* = 1.0 Hz); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm: 161.4, 143.3, 136.3, 135.0, 133.5, 130.8, 130.0, 127.4, 123.5, 120.3, 119.9, 117.5, 115.5, 115.3, 29.4, 17.3; HRMS *m/z* (ESI$^+$) found $(M+H)^+$ 354.0908, $C_{18}H_{15}N_3O_3S$ requires $(M+H)^+$ 354.0907; HPLC: Retention time (system A): t_R= 8.97 min. Purity: >95%.

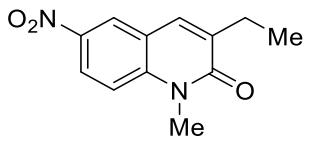


***N*-(1,3-Dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (13-i):** Prepared according to general procedure E from **7** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexanes, 10:90) to give the title compound (25 mg, 0.07 mmol, 65%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.97 (1H, s), 7.72 (1H, dd, *J* = 7.8, 1.8 Hz), 7.67 (1H, s), 7.57 - 7.50 (1H, m), 7.35 (1H, d, *J* = 9.1 Hz), 7.31 - 7.25 (2H, m), 7.16 (1H, d, *J* = 7.8 Hz), 6.99 (1 H, td, *J* = 7.6, 1.0 Hz), 3.91 (3H, s), 3.54 (3H, s), 2.07 (3H, d, *J* = 1.0 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.4, 156.3, 135.6, 135.0, 135.0, 131.9, 130.2, 129.8, 126.1, 122.5, 120.1, 120.0, 118.4, 115.2, 112.7, 56.0, 29.3, 17.2; HRMS: *m/z* (ESI$^-$) Found $(M-H)^-$ 357.0921, $C_{18}H_{18}N_2O_4S$ requires $(M-H)^-$ 357.0909; HPLC: Retention time (system A): t_R= 8.56 min. Purity: >95%.

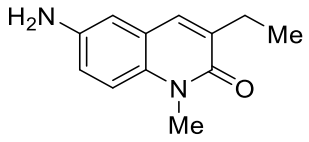


3-Ethyl-6-nitroquinolin-2(1*H*)-one was prepared by general procedure F from 2-chloro-5-nitrobenzaldehyde (2.50 g, 13.5 mmol, 1 eq.) and 4,4-dimethyl-2-propyl-4,5-dihydrooxazole (1.90 g, 13.5 mmol, 1 eq.) to give the title compound (2.62 g,12.0 mmol, 89%) as a brown solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 12.29 (1H, s), 8.64 (1H, d, *J* = 2.5 Hz), 8.28 (1H, dd, *J* = 9.1, 2.5 Hz), 7.97

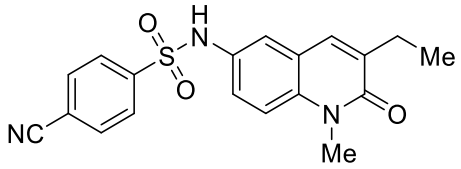
(1H, s), 7.42 (1H, d, *J* = 9.1 Hz), 1.19 (3H, t, *J*=7.4 Hz), [CH_2 overlaps with DMSO]; ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 162.1, 142.1, 141.4, 137.4, 134.5, 124.0, 123.5, 118.9, 115.6, 22.8, 12.3.



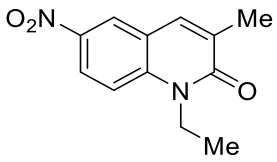
3-Ethyl-1-methyl-6-nitroquinolin-2(1*H*)-one was prepared according to general procedure B from 3-ethyl-6-nitroquinolin-2(1*H*)-one (1.20 g, 5.50 mmol) to give the title compound (1.0 g, 4.34 mmol, 79%) as a brown solid, which was used without further purification. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 8.58 (1H, d, *J* = 2.5 Hz), 8.19 (1H, dd, *J* = 9.0, 2.5 Hz), 7.94 (1H, s), 7.41 (1H, d, *J* = 9.0 Hz), 1.18 (3H, t, *J*=7.4 Hz), [CH_2 overlaps with DMSO].



6-Amino-3-ethyl-1-methylquinolin-2(1*H*)-one (8) was prepared according to the general procedure D from 3-ethyl-1-methyl-6-nitroquinolin-2(1*H*)-one (500 mg, 1.03 mmol) to give the title compound (129 mg, 0.63 mmol 31%) as a black solid that was used without further purification.

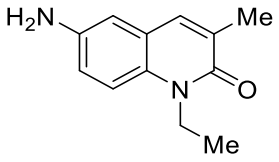


4-Cyano-*N*-(3-ethyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (14-d): Prepared according to general procedure E from **8** (30 mg, 0.15 mmol). Purified by column chromatography to give the title compound (30 mg, 0.08 mmol, 17% over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.57 (1H, s), 8.04 (2H, d, *J* = 8.6 Hz), 7.88 (1H, d, *J* = 8.6 Hz), 7.69 (1H, s), 7.46 - 7.35 (2H, m), 7.22 (1H, dd, *J* = 8.8, 2.5 Hz), 3.57 (3H, s), 1.15 (3H, t, *J* = 7.5 Hz), [CH_2 overlaps with DMSO]*;* ^{13}C NMR (126 MHz, *DMSO*-d_6): δ ppm 160.9, 143.3, 136.1, 135.3, 133.5, 133.5, 130.8, 127.4, 123.6, 120.4, 120.2, 117.5, 115.5, 115.3, 29.4, 23.7, 12.5; HRMS: *m/z* (ESI$^-$) Found (M-H)$^-$ 366.0915, $C_{19}H_{17}N_3O_3S$ requires (M-H)$^-$ 366.0912; HPLC: Retention time (system A): t_R= 9.83 min. Purity: >95%.

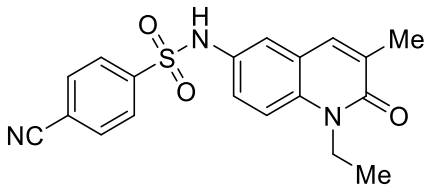


1-ethyl-3-methyl-6-nitroquinolin-2(1*H*)-one was prepared by general procedure B from 3-methyl-6-nitroquinolin-2(1*H*)-one (500 mg, 2.45 mmol) to give the title compound (470 mg, 2.0 mmol, 83%) as a

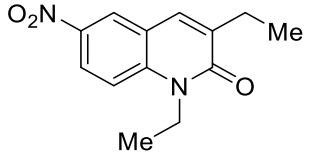
pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.45 (1H, d, *J* = 2.5 Hz), 8.37 (1H, dd, *J* = 9.5, 2.5 Hz), 7.66 (1H, s), 7.45 (1H, d, *J* = 9.5 Hz), 4.43 (2H, q, *J* = 7.1 Hz), 2.32 (3H, d, *J* = 0.9 Hz), 1.41 (3H, t, *J* = 7.1 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 162.2, 142.1, 141.9, 135.1, 132.8, 124.0, 123.9, 120.5, 114.3, 38.3, 17.7, 12.7.



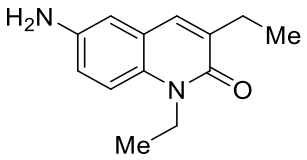
6-Amino-1-ethyl-3-methylquinolin-2(1*H*)-one (9) was prepared by general procedure D from 1-ethyl-3-methyl-6-nitroquinolin-2(1*H*)-one (250 mg, 1.08 mmol) to give the title compound (135 mg, 0.67 mmol, 62%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.42 (1 H, s), 7.21 (1 H, d, *J* = 8.8 Hz), 6.94 (1 H, dd, *J* = 8.8, 2.5 Hz), 6.82 (1 H, d, *J* = 2.5 Hz), 4.36 (2 H, q, *J* = 7.0 Hz), 3.79 (1 H, br. s.), 2.26 (3 H, s), 1.36 (3 H, t, *J* = 7.3 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 161.8, 140.7, 134.9, 131.6, 130.6, 122.1, 118.3, 114.8, 112.3, 37.5, 17.8, 12.9.



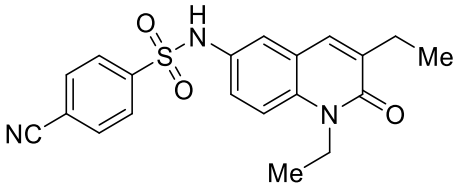
4-Cyano-*N*-(1-ethyl-3-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (15-d): Prepared according to general procedure E from **9** (25 mg, 0.13 mmol). Purified by column chromagraphy (acetone:hexanes, gradient elution 10:90 to 30:70) to give the title compound (22mg, 0.06 mmol, 48%) as a pale pink solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 10.55 (1H, s), 8.04 (2H, d, *J* = 8.2 Hz), 7.89 (2H, d, *J* = 8.5 Hz), 7.73 (1H, s), 7.46 (1H, d, *J* = 9.1 Hz), 7.35 (1H, d, *J* = 2.2 Hz), 7.23 (1H, dd, *J* = 9.1, 2.5 Hz), 4.22 (2H, q, *J* = 6.9 Hz), 2.09 (3H, s), 1.16 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.9, 143.4, 135.1, 135.1, 133.5, 130.7, 130.0, 127.4, 123.6, 120.6, 120.0, 117.5, 115.3, 115.3, 36.9, 17.2, 12.6; HRMS *m/z* (ESI-) Found $(M-H)^-$ 366.0923, $C_{19}H_{17}N_3O_3S$ requires $(M-H)^-$ 366.0912; HPLC: Retention time (system A): t_R= 9.40 min. Purity: >95%.



1,3-Diethyl-6-nitroquinolin-2(1*H*)-one was prepared according to general procedure B from 3-ethyl-6-nitroquinolin-2(1*H*)-one (300 mg 1.38 mmol) to give the title compound (222 mg, 0.90 mmol, 65%) as a brown solid which was used without further purification.

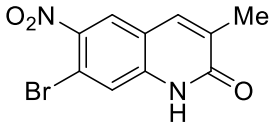


1,3-Diethyl-6-aminoquinolin-2(1*H*)-one (10) was prepared according to general procedure D from 1,3-diethyl-6-nitroquinolin-2(1*H*)-one (210 mg, 0.85 mmol). Purification by filtration through a silica gel plug (acetone:hexanes, 70:30) gave the title compound as a yellow oil (81 mg, 38 mmol, 44%) which was used without further purification.

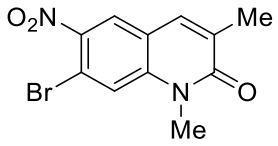


4-Cyano-*N*-(1,3-diethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (16-d): Prepared according to general procedure E from **10** (40 mg, 0.18 mmol) with 4-cyanobenzenesulfonyl chloride to give **NI-198** (37 mg, 0.09 mmol) as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 10.56 (1H, s), 8.04 (2H, d, *J* = 8.5 Hz), 7.89 (2H, d, *J* = 8.5 Hz), 7.68 (1H, s), 7.46 (1H, d, *J* = 8.8 Hz), 7.41 (1H, d, *J* = 2.5 Hz), 7.23 (1H, dd, *J* = 9.1, 2.5 Hz), 4.22 (2H, q, *J* = 6.9 Hz), 1.21 - 1.10 (6H, m), [1xCH_2 overlaps with DMSO]; ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.5, 143.4, 135.3, 134.9, 133.6, 133.5, 130.7, 127.4, 123.6, 120.7, 120.3, 117.5, 115.3, 115.2, 36.9, 23.5, 12.7, 12.5; HRMS *m/z* (ESI^-) Found $(M-H)^-$ 380.1057, $C_{20}H_{19}N_3O_3S$ requires $(M-H)^-$ 380.1069; HPLC: Retention time (system A): t_R= 10.36 min. Purity: >95%.

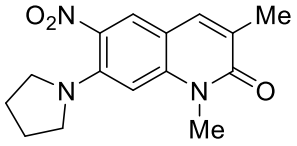
Synthetic procedures for compound 21:



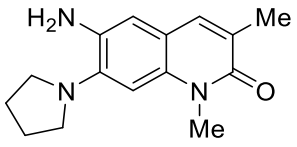
7-Bromo-3-methyl-6-nitroquinolin-2(1*H*)-one was prepared according to general procedure F from 4-bromo-2-fluoro-5-nitro-benzaldehyde (4.20 g, 16.8 mmol, 1 eq.) and 4,4-dimethyl-2-ethyl-4,5-dihydrooxazole (2.13 g, 16.8 mmol, 1 eq.) to give the title compound (3.92 g, 13.8 mmol, 82%) as a black solid that was used without further purification. Relevant ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 12.22 (1H, br. s.), 8.46 (1H, s), 7.90 (1H, s), 7.63 (1H, s), 2.10 (3H, d, *J*=0.9 Hz).



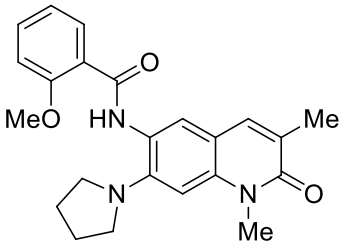
7-Bromo-1,3-dimethyl-6-nitroquinolin-2(1*H*)-one was prepared by general procedure B from 7-bromo-3-methyl-6-nitroquinolin-2(1*H*)-one (1.2 g, 4.2 mmol). Product precipitated on addition of H_2O and was filtered, washed with H_2O and dried to give the title compound (983 mg, 3.30 mmol, 78%) as a brown solid that was used without further purification. Relevant ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 8.57 (1H, s), 8.04 (1H, s), 8.00 (1H, s), 3.77 (3H, s), 2.23 (3H, s).



1,3-Dimethyl-6-nitro-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one. 7-Bromo-1,3-dimethyl-6-nitroquinolin-2(1*H*)-one (100 mg, 0.35 mmol, 1 eq.), CuI (7 mg, 0.04 mmol, 0.1 eq.) and pyrrolidine (125 mg, 1.75 mmol, 5 eq.) were dissolved in dry DMF and heated at 80 °C for 3 hours. After cooling to RT, the solution was poured onto ice (~50 g) stirred for 10 mins and filtered to give the title compound (62 mg, 0.22 mmol, 65%) as a brown solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.96 (1H, s), 7.43 (1H, s), 6.51 (1H, s), 3.70 (3H, s), 3.41 - 3.21 (4H, m), 2.23 (3H, s), 2.11 – 1.98 (4H, m); ^{13}C NMR (126 MHz, *$CDCl_3$*): δ ppm 163.5, 143.1, 142.7, 135.0, 133.6, 126.8, 126.7, 110.7, 98.3, 50.9, 29.8, 25.8, 17.5.

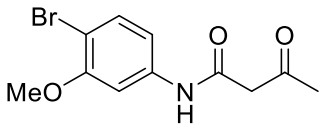


6-Amino-1,3-dimethyl-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one was prepared by general procedure D from 1,3-dimethyl-6-nitro-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (40 mg, 0.11 mmol). Compound precipitated on basification to pH 11 and was filtered, washed with H_2O and dried to give the title compound (24 mg, 0.1 mmol, 91%) as a dark red solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 (1H, s), 6.84 (1H, s), 6.81 (1H, s), 3.85 - 3.70 (5H, m), 3.31 – 3.14 (4H, m), 2.24 (3H, s), 2.07 – 1.98 (4H, m); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 162.7, 135.7, 134.7, 133.4, 131.2, 127.2, 116.3, 112.7, 102.8, 50.8, 29.6, 24.3, 17.8.

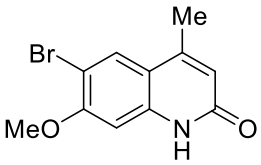


2-Methoxy-*N*-(1,3-dimethyl-2-oxo-7-(pyrrolidin-1-yl)-1,2-dihydroquinolin-6-yl)benzamide (21): Prepared by a method similar to general procedure E from 6-amino-1,3-dimethyl-7-(pyrrolidin-1-yl)quinolin-2(1*H*)-one (20 mg, 0.08 mmol) and 2-methoxybenzoyl chloride. Purified by column chromatography (acetone:hexanes 20:80) to give the title compound (12 mg, 0.03 mmol, 36%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 10.12 (1H, s), 8.36 (1H, s), 8.30 (1H, dd, *J* = 7.7, 1.7 Hz), 7.48 - 7.42 (2H, m), 7.10 (1H, t, *J* = 7.1 Hz), 6.99 (1H, d, *J* = 8.5 Hz), 6.82 (1H, s), 3.98 (3H, s), 3.66 (3H, s), 3.22 - 3.15 (4H, m), 2.16 (3H, d, *J* = 0.9 Hz), 1.98 - 1.92 (4H, m); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 163.3, 163.1, 157.4, 144.1, 136.8, 135.8, 133.2, 132.7, 127.2, 126.2, 121.8, 121.7, 121.7, 115.7, 111.5, 102.4, 56.0, 51.6, 29.6, 24.8, 17.7; HRMS *m/z* (ESI$^+$) Found $(M+H)^+$ 392.1958, $C_{23}H_{25}N_3O_3$ requires $(M+H)^+$ 392.1896; HPLC: Retention time (system A): t_R= 10.40 min. Purity: 91%.

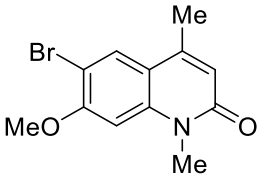
Synthetic procedures for compound 22:



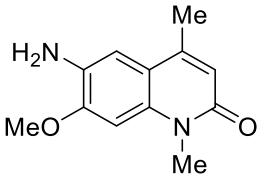
***N*-(4-Bromo-3-methoxyphenyl)-3-oxobutanamide** To a solution of 4-bromo-3-methoxyaniline (2.5 g, 12.32 mmol, 1 eq.) in xylenes (25 mL) at 110 °C was added 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (1.93 g, 13.55 mmol, 1.1 eq.). The solution was stirred for 2 hours and allowed to cool to RT. The solvents were removed *in vacuo* and the residue purified by column chromatography (hexanes:ethyl acetate, gradient elution 80:20 to 60:40) to yield the title compound (2.32 g, 8.14 mmol, 66%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 9.07 (1H, br. s), 7.30 (1H, t, *J* = 2.2 Hz), 7.24 (1H, t, *J* = 8.1 Hz), 7.06 (1H, dd, *J* = 8.0, 1.1 Hz), 6.70 (1H, dd, *J* = 8.2, 1.9 Hz), 3.83 (3H, s), 3.61 (2H, s), 2.35 (3H, s).



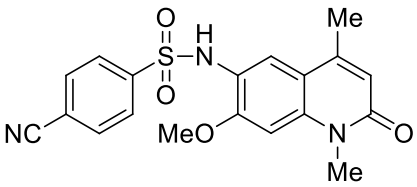
6-Bromo-7-methoxy-4-methylquinolin-2(1*H*)-one. A mixture of *N*-(4-bromo-3-methoxyphenyl)-3-oxobutanamide (1.21 g, 4.05 mmol) and polyphosphoric acid (10 g) was heated at 90 °C for 2 hours. The reaction mixture was allowed to cool to approximately 60 °C and then ice was added until a freely stirring mixture was achieved. The precipitate was isolated by filtration and dried under vacuum to yield the title compound (1.05 g, 3.90 mmol 96%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.87 (1H, s), 6.94 (1H, s), 6.27 (1H, s), 3.89 (3H, s), 2.38 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.7, 156.5, 147.2, 139.7, 128.7, 118.8, 114.7, 104.5, 98.1, 56.3, 18.4.



6-Bromo-7-methoxy-1,4-dimethylquinolin-2(1*H*)-one was prepared according to general procedure B from 6-bromo-7-methoxy-4-methylquinolin-2(1*H*)-one (500 mg, 1.88 mmol). Product was precipitated by addition of H_2O, filtered and dried to give the title compound (275 mg, 0.98 mmol, 52%) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.92 (1H, s), 7.02 (1H, s), 6.41 (1H, s), 4.02 (3H, s), 3.62 (3H, s), 2.39 (3H, d, J = 0.5 Hz); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 162.2, 157.3, 145.3, 140.7, 129.4, 119.1, 116.3, 105.7, 97.2, 56.4, 28.7, 18.4.



6-Amino-7-methoxy-1,4-dimethylquinolin-2(1*H*)-one. 6-Bromo-7-methoxy-1,4-dimethylquinolin-2(1*H*)-one (100 mg, 0.35 mmol, 1 eq.) was dissolved in NMP (1.5 mL) in a Biotage 10 mL microwave vial. Cu_2O (5 mg, 0.035 mmol, 0.1 eq) and aq. NH_4OH (28–30% NH_3, 2 mL) were added and the vial was sealed and heated at 110 °C under microwave irradiation for 3 hours. After cooling to RT the solution was filtered through a pad of celite® and washed with DCM (20 mL). The filtrate was washed with aqueous lithium chloride solution (0.5 M, 10 mL) and the organic fractions were combined and concentrated *in vacuo*. The resulting residue was purified by column chromatography (hexanes:acetone, gradient elution 70:30 to 30:70) to give the title compound (75 mg, ca. 0.35 mmol) as a mixture with NMP. Relevant ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 6.96 (1H, s), 6.87 (1H, s), 6.29 (1H, s), 4.82–4.76 (2H, br. s), 3.95 (3H, s), 3.59 (3H, s), 2.31 (3H, s).



4-Cyano-*N*-(7-methoxy-1,4-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (22, NI-48): Prepared by general procedure E from a solution of 6-amino-7-methoxy-1,4-dimethylquinolin-2(1*H*)-one in NMP (75 mg, ca. 0.35 mmol). Purified by column chromatography (acetone:hexanes, gradient elution 20:80 to 50:50) to give **NI-48** (28 mg, 0.07 mmol, 20% over 2 steps) as a pale yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 10.05 (1H, br. s), 8.04 (2H, d, J = 8.2 Hz), 7.83 (2H, d, J = 8.5 Hz), 7.52 (1H, s), 6.84 (1H, s), 6.40 (1H, s), 3.56 (6H, m), 2.34 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.9, 155.5, 145.8, 144.7, 139.7, 133.0, 127.5, 124.1, 119.3, 118.0, 117.7, 114.8,

113.8, 97.8, 55.7, 29.0, 18.3; HRMS *m/z* (NSI) Found $(M+H)^+$ 384.1012, $C_{19}H_{17}N_3O_4S$ requires $(M+H)^+$ 384.0940; HPLC: Retention time (system A): t_R= 7.21 min. Purity: >95%.

Biological Screening Methods:

Cloning, protein expression and purification

Cloning, protein expression and purification were performed as described previously by this group.[1-5]

Differential scanning fluorimetry (DSF), Thermal Shift (ΔT_m) assay

DSF were performed as described previously by this group.[1-5]

BROMO*scan*™ assay (DiscoverX Corp., San Diego CA, USA)

https://www.discoverx.com/technologies-platforms/competitive-binding-technology/bromoscan-technology-platform

Bromodomain assays: T7 phage strains displaying bromodomains were grown in parallel in 24-well blocks in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (5,000 x g) and filtered (0.2µm) to remove cell debris. Streptavidin-coated magnetic beads were treated with biotinylated small molecule or acetylated peptide ligands for 30 minutes at room temperature to generate affinity resins for bromodomain assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining bromodomains, liganded affinity beads, and test compounds in 1x binding buffer (17% SeaBlock, 0.33x PBS, 0.04% Tween 20, 0.02% BSA, 0.004% Sodium azide, 7.4 mM DTT). Test compounds were prepared as 1000X stocks in 100% DMSO. Kds were determined using an 11-point 3-fold compound dilution series with one DMSO control point. All compounds for Kd measurements are distributed by acoustic transfer (non-contact dispensing) in 100% DMSO. The compounds were then diluted directly into the assays such that the final concentration of DMSO was 0.09%. All reactions performed in polypropylene 384- well plates. Each was a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 2 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The bromodomain concentration in the eluates was measured by qPCR.

For a more detailed description of this assay technology, see ref. 6.

AlphaScreen™ assay

AlphaScreen™ assays were performed with minor modifications from the manufacturers protocol (PerkinElmer, USA). All reagents were diluted in the recommended buffer (50 mMHEPES, 100 mM NaCl, 0.1% BSA; pH = 7.4) supplemented with 0.05% CHAPS and allowed to equilibrate to room temperature prior to addition to plates. 4 ml of HIS-tagged protein was added to low-volume 384-well plates (ProxiPlatet-384 Plus, PerkinElmer, USA), followed by 4 ml of either buffer, non-biotinylated peptide, solvent or compound. Plates were sealed and incubated at room temperature for 30 minutes, before the addition of 4 ml biotinylated peptide, resealing and incubation for a further 30 minutes. 4 ml of streptavidin-coated donor beads (25 µg/ml) and 4 µl of nickel chelate acceptor beads (25 µg/ml) were then added under low light conditions. Plates were foil sealed to protect from light, incubated at room temperature for 60 minutes and read on a PHERAstar FS plate reader (BMG Labtech,Germany) using an AlphaScreen™ 680 excitation/570 emission filter set. IC50s were calculated in GraphPad Prism 5 (GraphPad Software, USA). Results for compounds dissolved in DMSO were normalised against corresponding DMSO controls prior to IC_{50} determination, which are given as the final concentration of compound in the 20 µl reaction volume.

Isothermal titration calorimetry (ITC)

Experiments were carried out on a VP-ITC microcalorimeter (MicroCal™). All experiments were performed at 15 °C in 20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM TCEP. BRPF1B, BRPF2, BRPF3 and BRD9 protein solutions were buffer exchanged by dialysis into the ITC buffer. Protein concentrations were between 200-240 µM and the NI-42 inhibitor concentration used was 20 µM. The titrations were conducted using an initial injection of 2 µl followed by 34 identical injections of 8 µl. The dilution heats were measured on separate experiments and were subtracted from the titration data. Thermodynamic parameters were calculated using $\Delta G = \Delta H - T\Delta S = -RT\ln K_B$, where ΔG, ΔH and ΔS are the changes in free energy, enthalpy and entropy of binding respectively. In all cases a single binding site model was employed.

Caco2 cell permeability assay

Cell permeability was measured by transit performance in the Caco2 cell line at Cyptotex, UK. See: http://www.cyprotex.com/admepk/in-vitro-permeability/caco-2-permeability

Crystallization

Aliquots of purified BRPF1B were set up for crystallization using a mosquito® crystallization robot (TTP Labtech, Royston UK). Coarse screens were typically setup onto Greiner 3-well plates using three different drop ratios of precipitant to protein per condition (100+50 nl, 75+75 nl and 50+100 nl).

Initial hits were optimized further scaling up the drop sizes. All crystallizations were carried out using the sitting drop vapor diffusion method at 4 °C. BRPF1B crystals with **5b** compound were grown by mixing 50 nl of the protein (15 mg/ml and 10 mM final ligand concentration) with 100 nl of reservoir solution containing 20 % PEG3350, 0.2 M ammonium nitrate. BRPF1B crystals with **22** were grown by mixing 75 nl of the protein (15 mg/ml and 4 mM final ligand concentration) with 75 nl of reservoir solution containing 0.1 m bis-tris propane pH 8.5, 0.2 M sodium formate, 20% PEG 3350, 10% ethylene glycol. Diffraction quality crystals grew within a few days.

Data Collection and Structure solution

BRPF1B complex crystals were cryo-protected using the well solution supplemented with additional ethylene glycol and were flash frozen in liquid nitrogen. Data were collected at Diamond beamline I04 at a wavelength of 0.9686 Å (BRPF1B/**5b**) or Diamond beamline I02 at a wavelength of 0.9795 Å (BRPF1B/**22**). Indexing and integration was carried out using XDS[7] and scaling was performed with AIMLESS.[8] Initial phases were calculated by molecular replacement with PHASER[9] using the apo BRPF1B structure (PDB 4LC2). Initial models were built by ARP/wARP[10] followed by manual building in COOT.[11] Refinement was carried out in REFMAC5.[12] GRADE (global phasing)[13] was used to generate compound coordinates and cif files. All model validations were carried out using MolProbity.[14] Data collection and refinement statistics can be found in **Supplemental Table S4**. The model and structure factors have been deposited with PDB accession codes: 5T4U (BRPF1B/**5b**) and 5T4V (BRPF1B/**22**).

Fluorescence Recovery After Photobleaching (FRAP)

FRAP studies were performed essentially as described.[15] In brief, U2OS cells were transfected (Fugene HD; Roche) with mammalian over-expression constructs encoding N-terminal GFP and a NLS followed by three tandem repeats of the BRPF1B bromodomain. The imaging system consisted of a Zeiss LSM 710 laser-scanning and control system (Zeiss) coupled to an inverted Zeiss Axio Observer.Z1 microscope equipped with a high-numerical-aperture (N. A. 1.3) 40 x oil immersion objective (Zeiss). Samples were placed in an incubator chamber in order to maintaining temperature and humidity. FRAP and GFP fluorescence imaging were both carried out with an argon-ion laser (488 nm) and with a PMT detector set to detect fluorescence between 500-550 nm. Once an initial scan had been taken, a region of interest corresponding to approximately 50 % of the entire GFP positive nucleus was empirically selected for bleaching. A time lapse series was then taken to record GFP recovery using 1% of the power used for bleaching. The image datasets and fluorescence recovery data were exported from ZEN 2009, the microscope control software, into Origin to determine the average half-time for full recovery for 5-20 cells per treatment point. Data were analysed using one-way ANOVA with Tukey's multiple comparisons test.

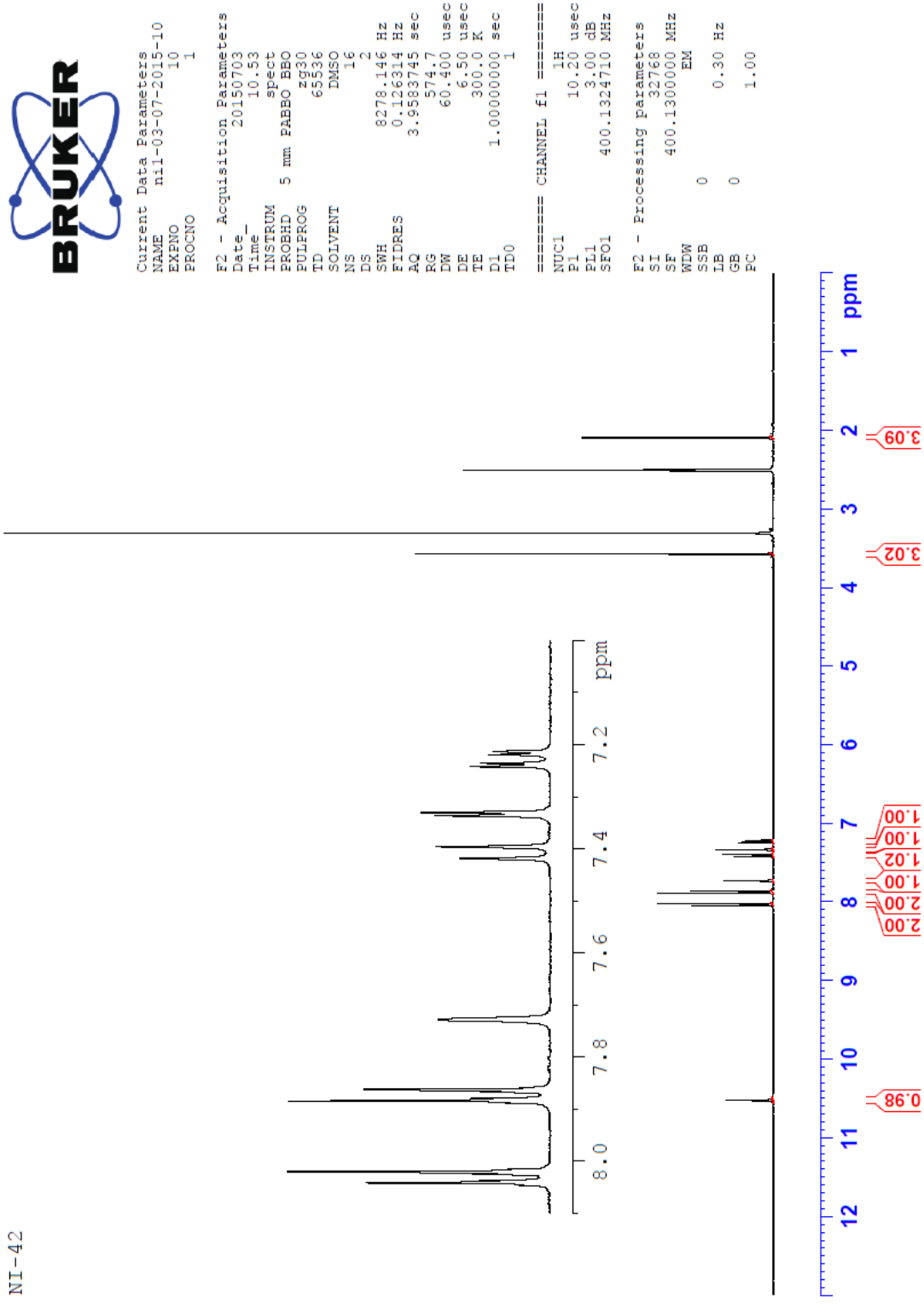
Triple Bromodomain Constructs for FRAP

Gateway entry clone containing triple bromodomain module was constructed by multiple LIC protocol[16, 17] Briefly, pDONR221 vector was linearized by PCR using primers encoding LIC sites and NLS. BRPF1B bromodomain sequence was amplified by either of three sets of primers. PCR fragments were purified using Qiagen PCR purification kit. Fragment encoding first and third repeats of BRPF1B bromodomain were treated with T4 DNA polymerase in the presence of dCTP and linearized pDONR221 vector and second bromodomain repeat were treated with T4 DNA polymerase in the presence of dGTP. Treated fragment were combined and directly transformed into E.coli MACH1 strain. Clones containing correct inserts were identified by colony PCR and confirmed by sequencing. GFP tagged triple BRPF1B bromodomain construct was constructed using Gateway LR reaction between pcDNA™6.2/N-EmGFP-DEST (Invitrogen, cat # V356-20) and entry clone encoding triple bromodomain construct.

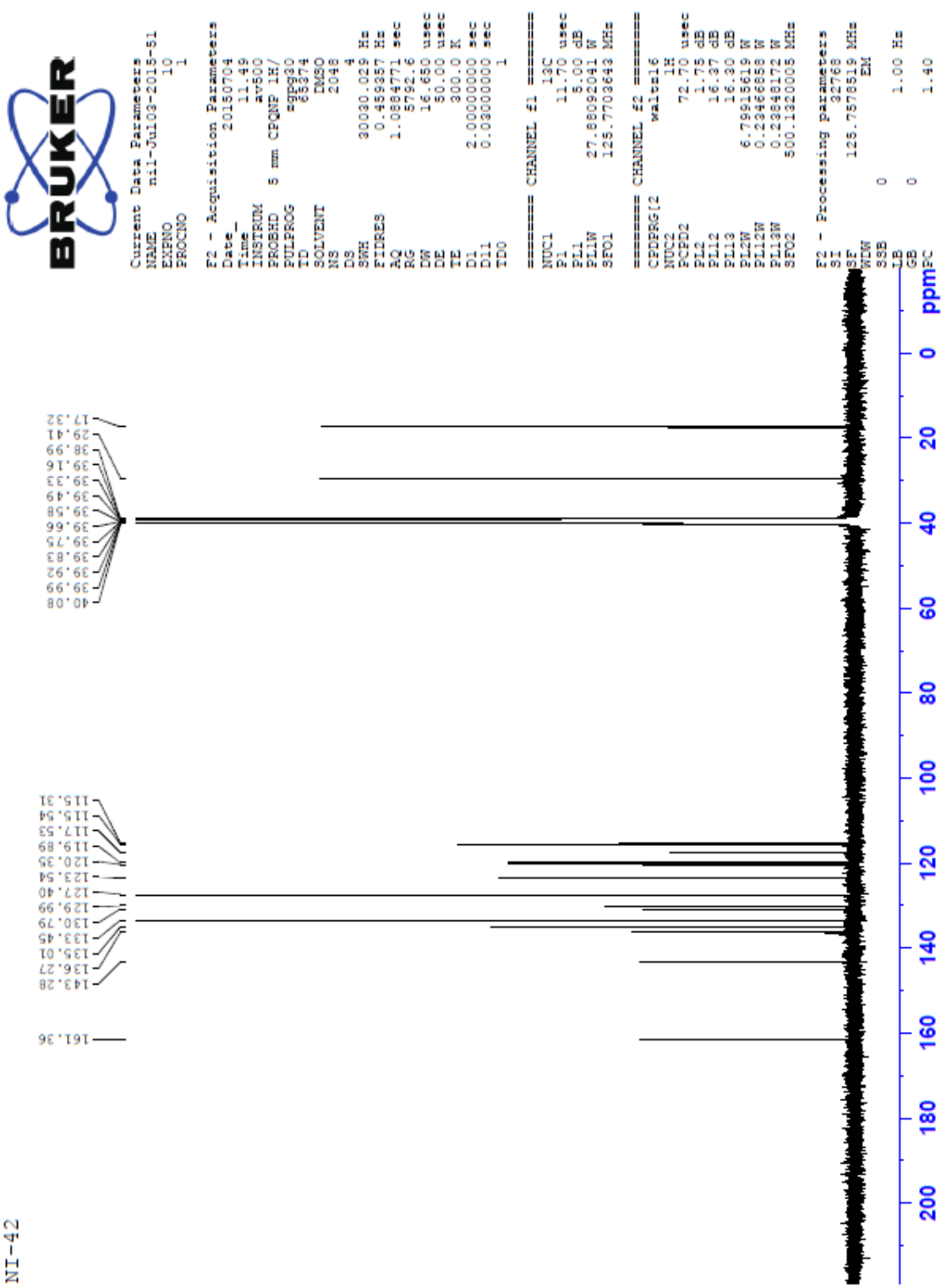
BRPF1-F1	CTACTTCCAATCCATgGAGATGCAGCTGACTCCTTTC
BRPF1-F2	GGAGAAGGTGGTGAGGGAGATGGTGGACCCGAGATGCAGCTGACTCCTTT
BRPF1-F3	TCCTCTCAATCACACCAATCTTCCTCCGGGGAGATGCAGCTGACTCCTTT
BRPF1-R1N	TCCACCATCTCCCTCACCACCTTCTCCGGGGCCCATTTTTTCTGCCTGG
BRPF1-R2N	GGAGGAAGATTGGTGTGATTGAGAGGACCCGCCCATTTTTTCTGCCTGG
BRPF1-R3N	ACCTTCCTCTTTTTCTTGGGGCCCATTTTTTCTGCCTGG

Figure S1: Spectroscopic and analytical data for 4-cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide, NI-42 (13-d), and 4-cyano-*N*-(1,3-diethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide, (16-d):

A: ^{1}H NMR (400MHz) of NI-42:

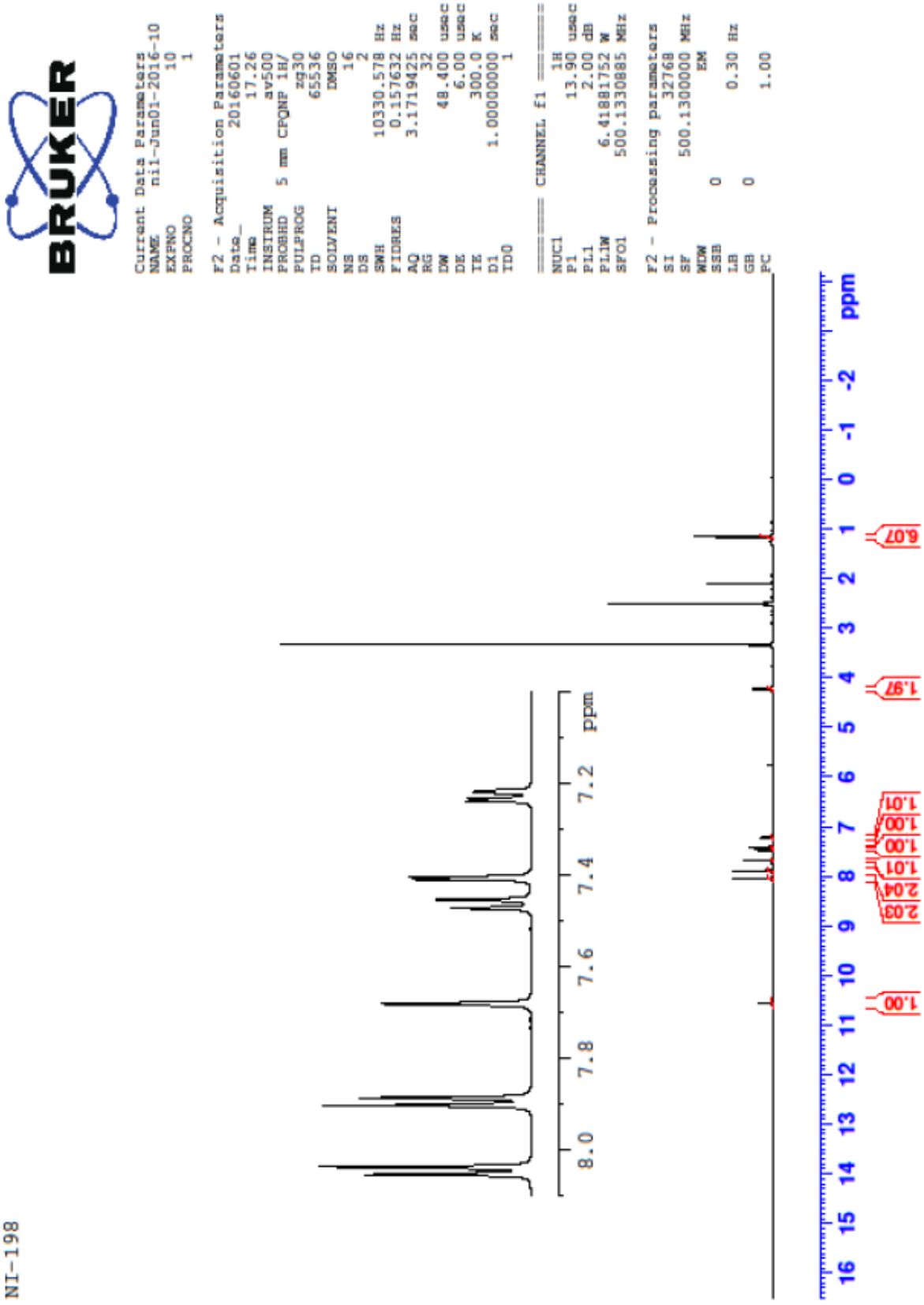


B: ^{13}C NMR (125 MHz) of NI-42:



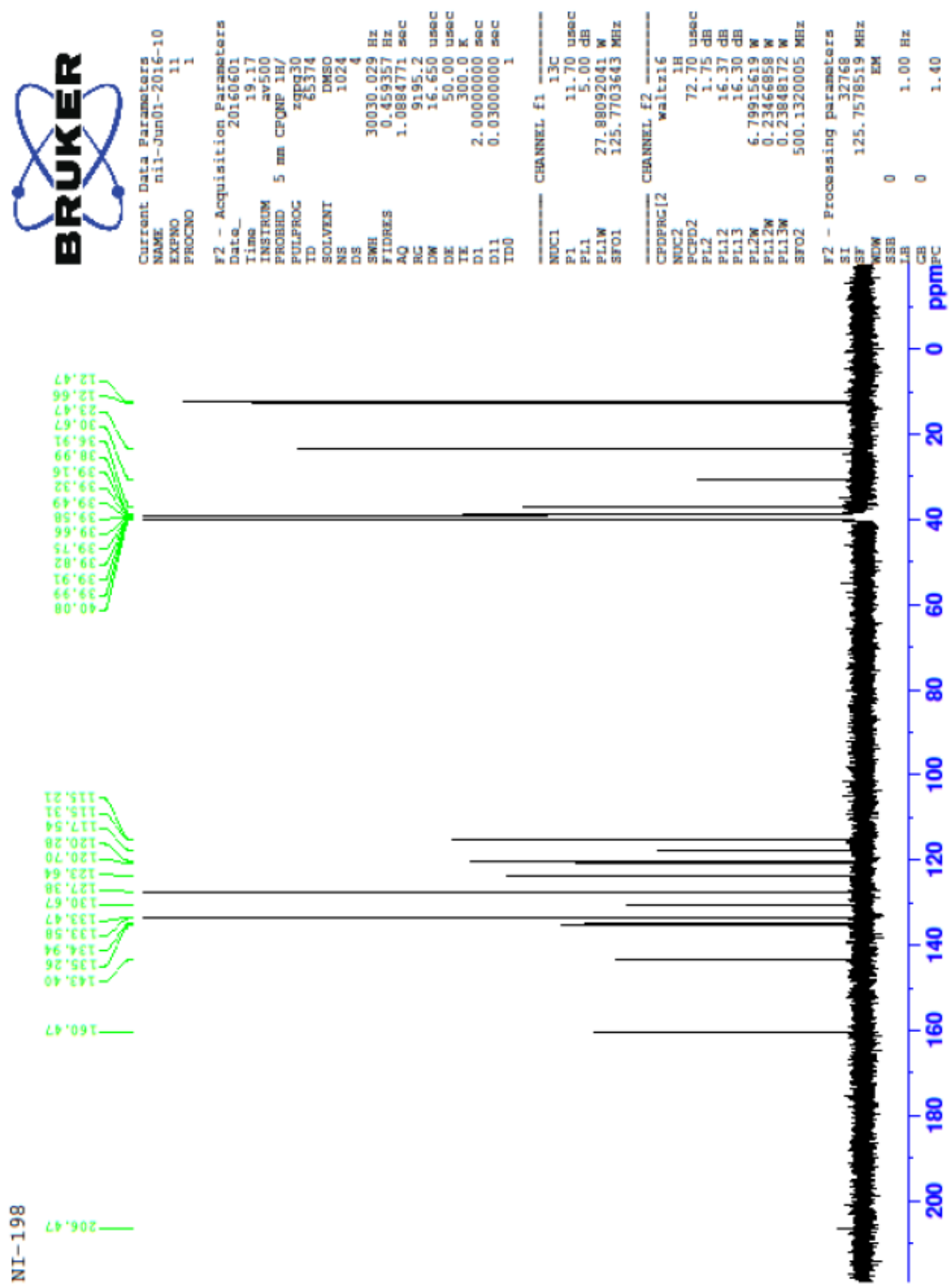
C: ^{1}H NMR (400MHz) of 16-d:

'NI-198' is the laboratory notebook and compound I.D. for **16-d**.

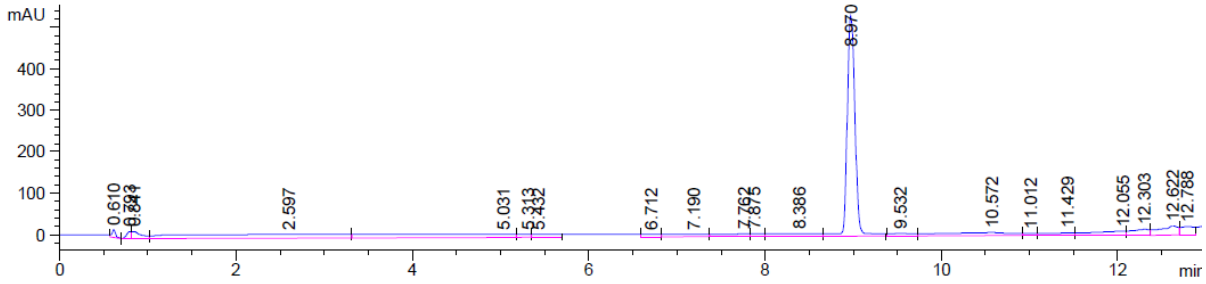


D: ^{13}C NMR (125 MHz) of 16-d:

'NI-198' is the laboratory notebook and compound I.D. for **16-d**.



E: HPLC of NI-42:



F: HPLC of 16-d:

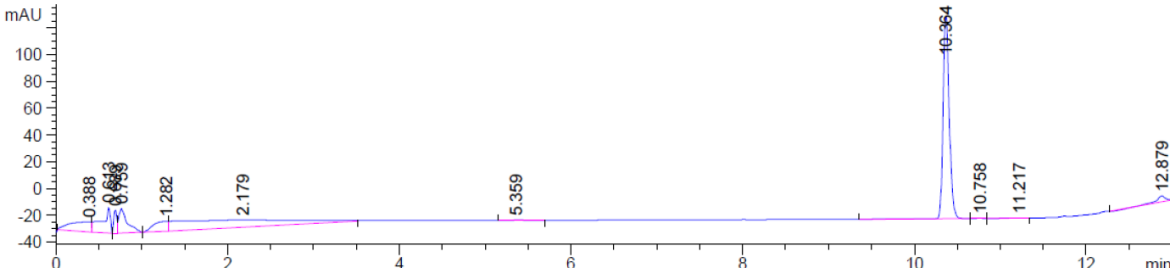
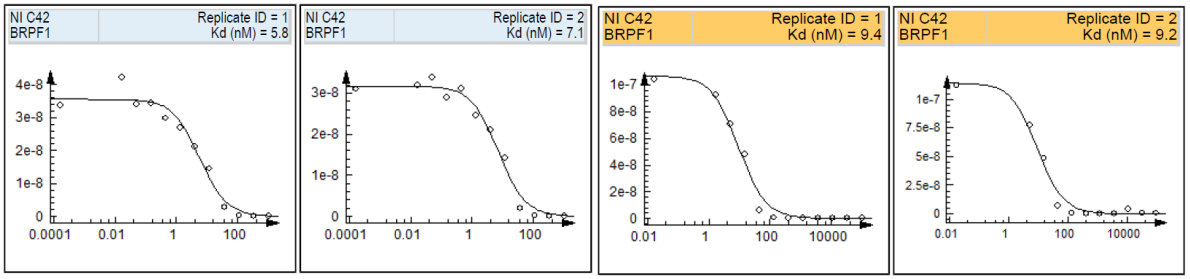
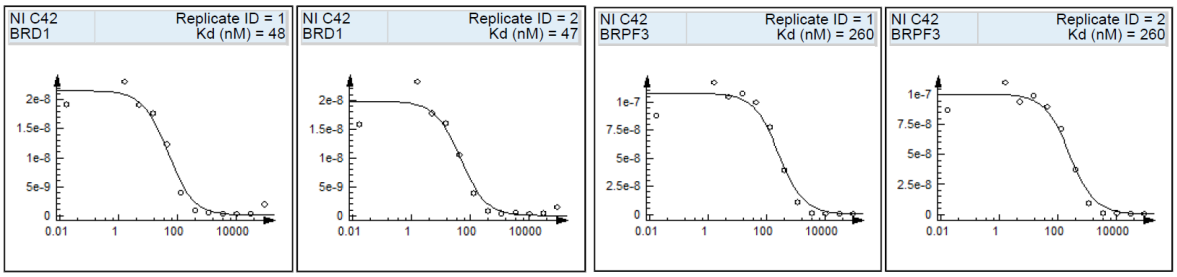


Figure S2: **NI-42: Dose-response-curves for inhibition of class IV and BRD4(BD1) BRD activity as measured by the BROMO*scan*™ assay.**

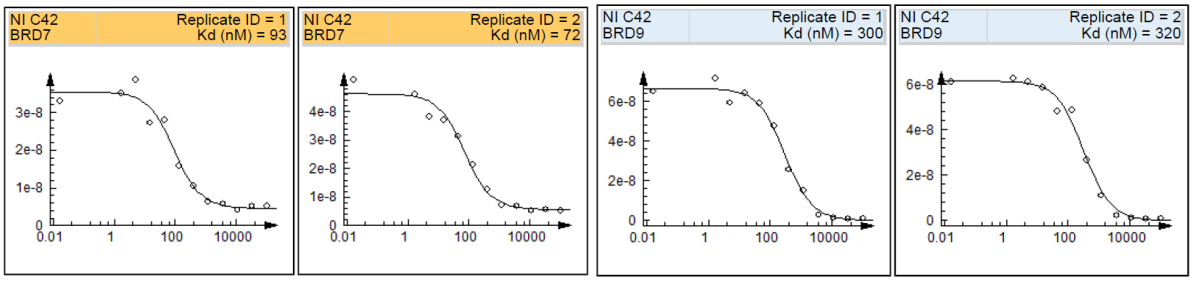
A BRPF1



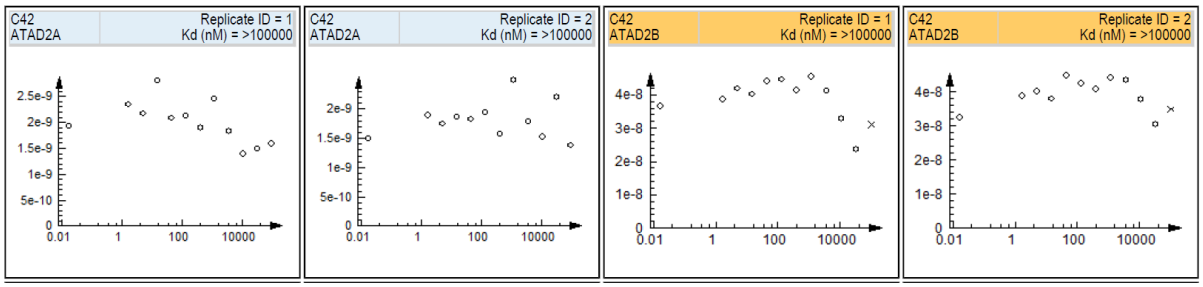
B BRD1 **C BRPF3**



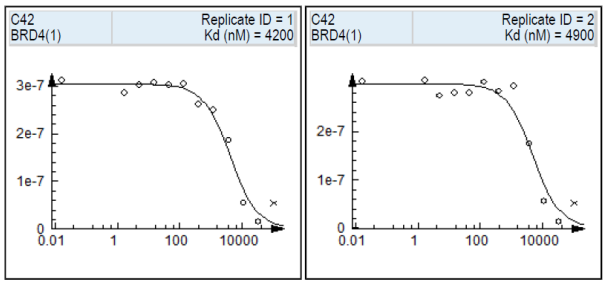
D BRD7 **E BRD9**



F ATAD2A **G ATAD2B**



H BRD4(BD1)

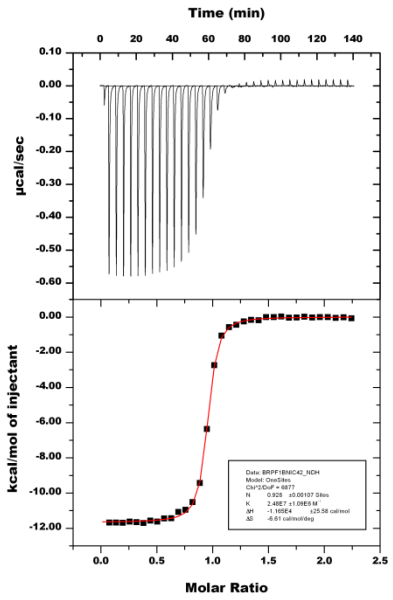


Dose-response curve images (n = 2 or 4). The amount of bromodomain measured by qPCR (Signal: y-axis) is plotted against the corresponding concentration in nM in log10 scale (x-axis). Data points marked with an "x" were not used for K_d determination.

'NI-C-42' (or C42) is the laboratory notebook reference and Compound I.D. for **NI-42**.

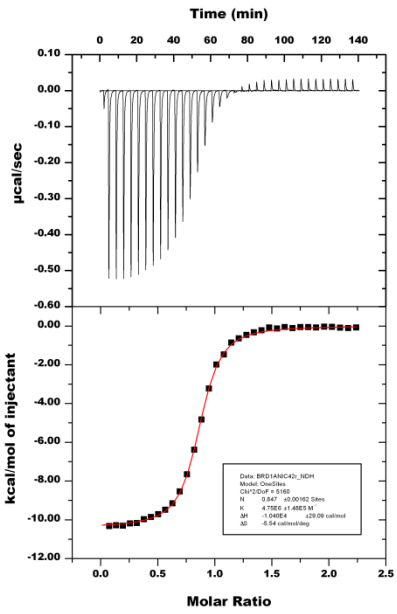
Figure S3: ITC analysis of NI-42 binding with BRPF1, BRD1, BRPF3 and BRD9.

A



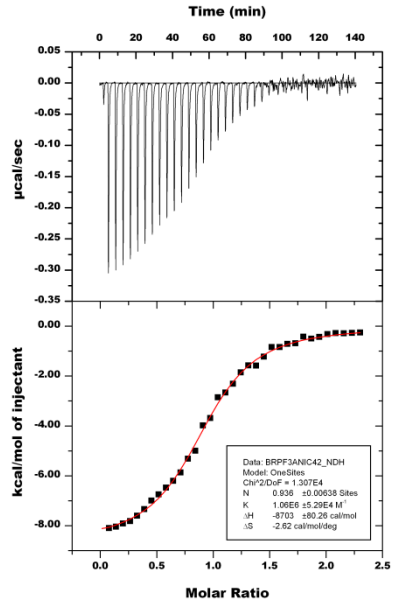
BRPF1
K_D = 40 nM
ΔH = -11.7 kcal/mol

B



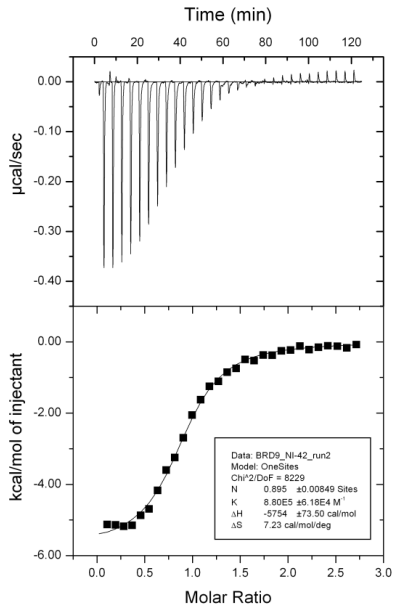
BRD1
K_D = 210 nM
ΔH = -10.4 kcal/mol

C



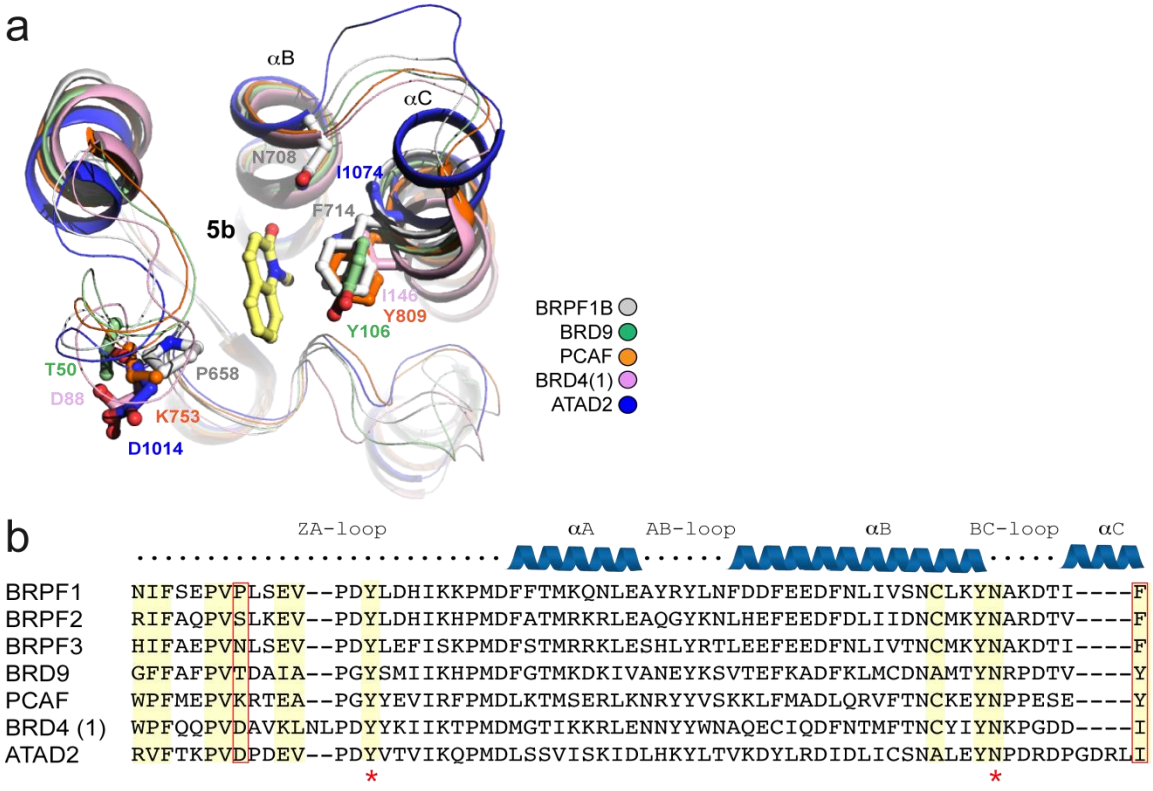
BRPF3
K_D = 940 nM
ΔH = -8.7 kcal/mol

D



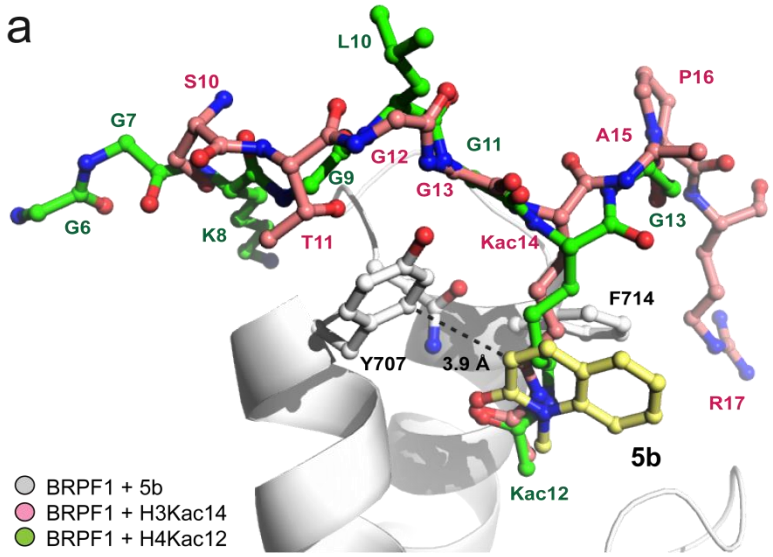
BRD9
K_D = 1130 nM
ΔH = -5.75 kcal/mol

Figure S4:



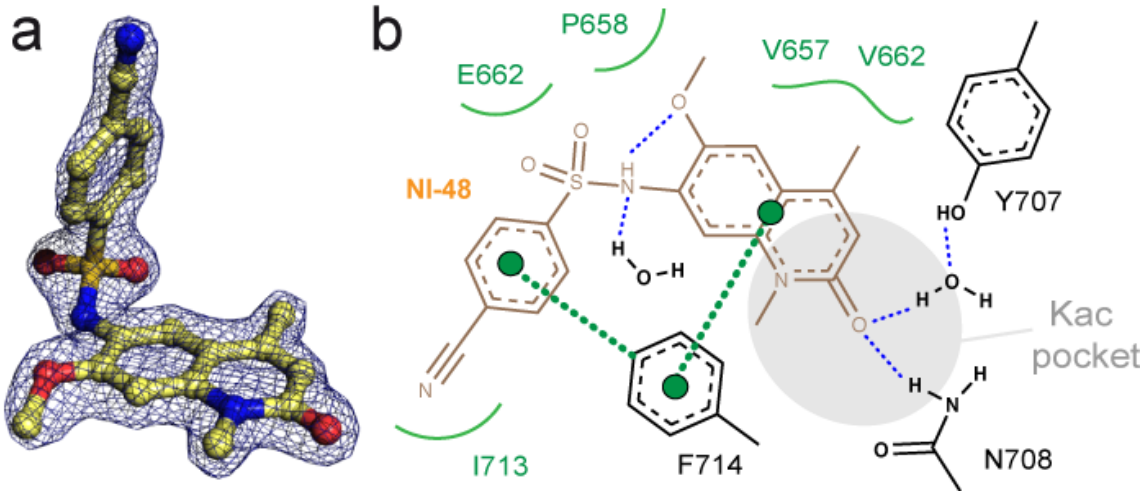
A) Structural superposition of **5b** + BRPF1B (5t4u.pdb) complex to free-ligand structures of BRD9 (3hme.pdb), PCAF (3gg3.pdb), BRD4(1) (2oss.pdb) and ATAD2 (3dai.pdb). B) Sequence alignment of bromodomain stretch including residues (highlighted in yellow) that point to the bromodomain-binding site.

Figure S5:



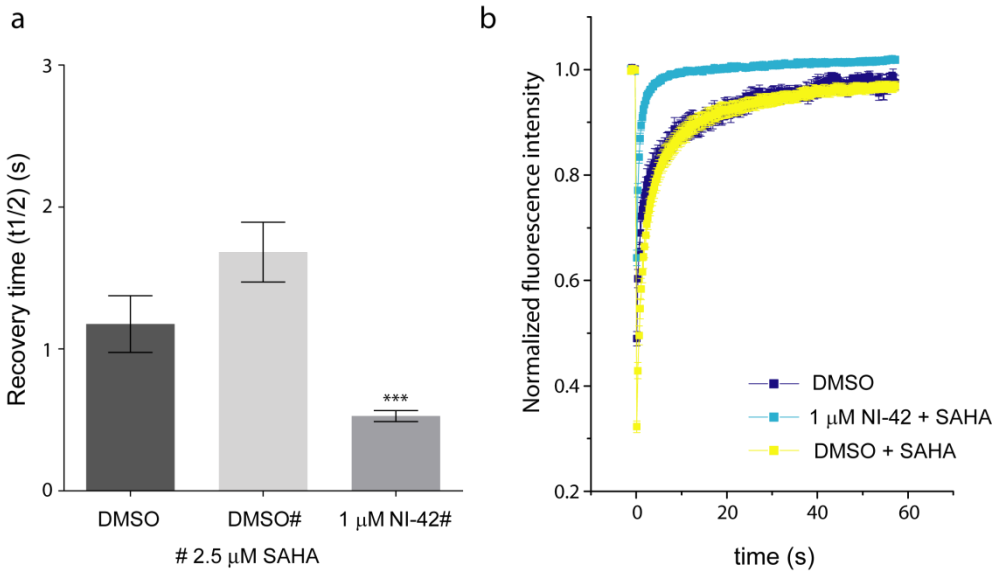
Superposition of histone complex structures (4QYD.pdb and 5FFY.pdb) with crystal structure of BRPF1B with compound **5b** (yellow) (5T4U.pdb).

Figure S6:



A) Experimental electron density map |2Fo-Fc| contoured at 1.6σ around **NI-48** (compound **22**). B) 2D diagram and ligand interactions map between **NI-48** and BRPF1B.

Figure S7: FRAP assay data for NI-42.



a) Fluorescence Recovery After Photobleaching (FRAP) assay data. Shown are recovery half times of cells overexpressing BRPF1B-BRDx3 and treated with SAHA as well as **NI-42** . ***P < 0.001, significant difference from wt treated with SAHA. b) Time dependence of fluorescence recovery in the bleached area of cells expressing GFP-BRPF1B with the corresponding treatment as in (a); Curves represent averaged data of at least 5 replicates.

Table S1: **DSF thermal shift data, ΔT_m (°C), for NI-42 against a panel of 48 human BRDs at 10 μM.**

BRD protein	ΔT_m Shift (°C)			comments
	Expt 1	Expt 2	Expt 3	
ASH1L		0.5		
ATAD2		-0.4		
BAZ1A		-0.9		
BAZ1B		0.2		
BAZ2A		-0.8		
BAZ2B		-0.5		
BRD1	5.1	4.2		
BRD2(1)		0.4		
BRD2(2)		-0.15		
BRD3(1)		0.6		
BRD3(2)		-0.2		
BRD4(1)		0.4		
BRD4(2)		0.3		
BRD7		4.8		Not reliable. Low mpt.
BRD9		1.8	3.5	
BRDT(1)		-0.05		
BRDT(2)		-0.3		
BRPF1A		0.15		
BRPF1B	9.7	7.8		
BRPF3	4.5	2.4		
BRWD3(2)		0.5		
CECR2		0.4		
CREBBP		0.5		
EP300		0.8		
FALZ		0.25		
GCN5L2		-0.25		
ATAD2B		-0.15		
SP140L		-0.2		
MLL		0.0		
PB1(1)		-0.1		
PB1(2)		0.0		
PB1(3)		0.2		
PB1(4)		0.0		
PB1(5)		0.2		
PB1(6)		-0.05		

PCAF		0.3
PHIP(2)		-0.8
SMARCA2		0.2
SMARCA4		-0.3
SP140		-0.45
TAF1(2)		-0.6
TAF1(1)		0.0
TAF1L(1)		0.7
TAF1L(2)		-1.9
TIF1-bromo	0.8	0.4
TIF1-phd-bromo		0.3
TRIM28		-0.9
WDR9(2)		-0.2

Table S2: IonChannelProfiler™ data for NI-42 (Eurofins Discovery Services, St. Charles MO, USA).

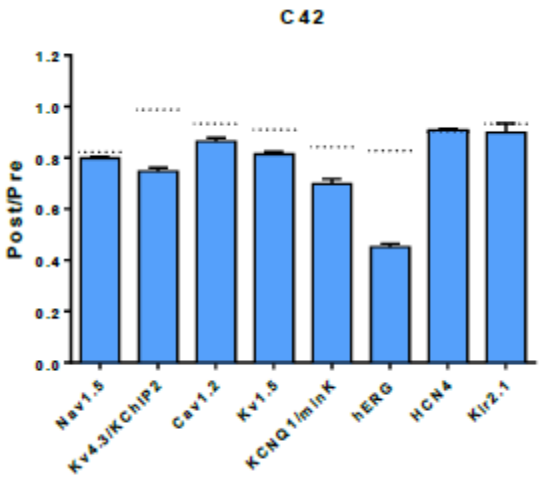
Eurofins Discovery services CardiacProfiler™ Panel:

http://www.eurofins.com/biopharma-services/discovery/services/in-vitro-pharmacology/ion-channels/cardiacprofiler/

Electrophysiological assays conducted on **NI-42** for activities at 30 μM on the ion channels specified below using the IonWorks Quattro electrophysiological platform.

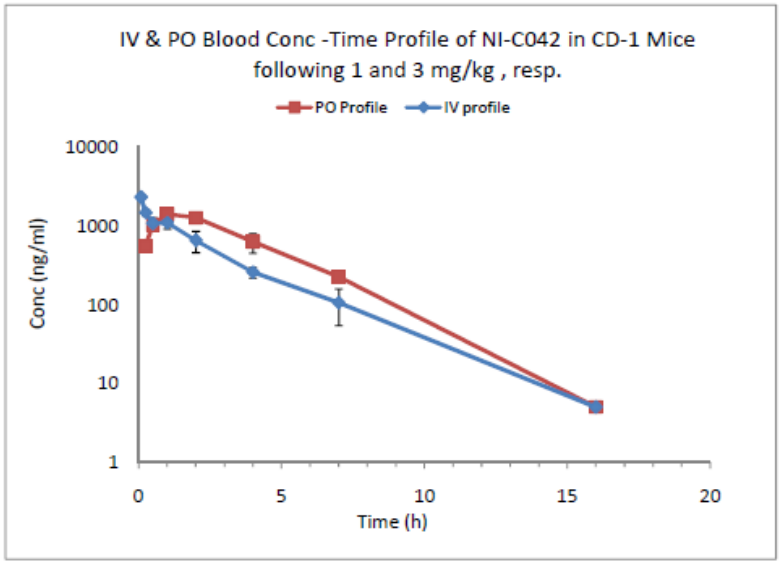
Channel	Normalized Percentage Inhibition at Test Concentration 30 μM	SE	N
Nav1.5	3.1	0.7	12
Kv4.3/KChIP2	25.9	1.5	12
Cav1.2	9.0	1.4	12
hKv1.5	10.9	0.9	12
KCNQ1/minK	18.7	2.0	12
hERG	45.6	1.3	12
HCN4	-0.7	0.5	12
Kir2.1	3.2	3.6	12

The effects of NI-42 on profiled ion channels are shown below. The dashed horizontal line associated with each bar indictes the mean effect of the vehicle in control wells (0.3% DMSO).



'C42' is the laboratory notebook reference and Compound I.D. for **NI-42** in this experiment.

Table S3A: Methods and results for mouse i.v. and p.o. PK determination.



'NI-C-042' is the laboratory notebook reference and Compound I.D. for **NI-42**

Methods: NI-42 was dosed to female CD-1 mice at 1 mg/kg, 5 mL/kg via an intravenous bolus dose to the tail vein formulated in 2% DMSO / 98% HP-β-CD (10% w/v in Saline 0.9%), and at 3 mg/kg,10mL/kg orally as a suspension in 1% methylcellulose in water. Three mice were dosed per time point per route. Blood was extracted by protein precipitation and **NI-42** analysed via UPLCMSMS using an internal standard. The assay meets the acceptance criteria with 9 calibration standards within ± 20% of nominal concentration. The lower limit of quantitation was 10 ng/mL.

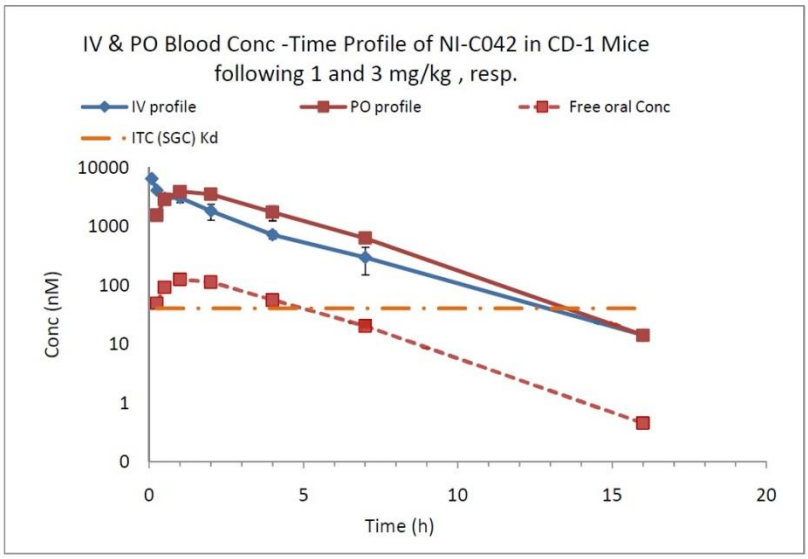
Results: NI-42 is a very low clearance compound (4 mL/min/kg) with a moderate volume of distribution of 0.7 L/kg resulting in a moderate half-life of 2 hour in the female mouse following a 1 mg/kg solution IV dose. The intrinsic clearance of **NI-42** in mouse liver microsomes was 0.8 mL/min/g liver (14 µL/min/mg protein) which predicts a low *in vivo* clearance in the mouse. Oral bioavailability ($F_{po(0\text{-}inf)}$) following a 3 mg/kg oral dose of **NI-42** was 49% with a C_{max} of 4 µM and an $AUC_{0\text{-}24h}$ of 5692 ng.h/mL. The oral half-life was 1.7 h. Based on blood clearance and oral bioavailability, the fraction absorbed was calculated as 51%.

Mouse pharmacokinetic data for **NI-42:**

Species	female CD-1 mouse
Route	i.v
Target dose	1 mg/kg
Formulation	2% DMSO / 98% HP-β-CD (10% w/v in 0.9% saline)
C_{max} (µM)	6.5
Cl_b (mL/min/kg)	4
V_{ss} (L/kg)	0.7
$T_{1/2}$ (h)	2.0
MRT (h)	2.6
Route	p.o. suspension
Target dose	3 mg/kg

Formulation	1% w/v methylcellulose in water
C_{max} (μM)	3.95
T_{max} (h)	1.0
$T_{1/2}$ (h)	1.7
AUC_{0-t} (ng.h/mL)	5692
$F_{po\ (0-\infty)}$ (%)	49

Table S3B: Results for mouse p.o. PK-PD determination.



'NI-C-042' is the laboratory notebook reference and Compound I.D. for **NI-42**

These results established that **NI-42** had pharmacokinetic properties compatible with oral dosing in mouse PK-PD models of disease with a 3 mg/kg p.o. dose achieving *free* drug levels above the BRPF1 K_D (ITC) for 4-5 h (Table S3B).

Table S4: Co-crystal structure determination of BRPF1B with 5b and NI-48. Data collection and refinement statistics

Data Collection		
PDB ID	**5T4U**	**5T4V**
Protein	BRP1B	BRPF1B
Ligand number	**5b**	**22**
Ligand name	*N*-Methylquinolin-2(1*H*)-one	NI-48
Space group	$P3_2\,2\,1$	I 1 2 1
Cell dimensions: a, b, c (Å)	60.32 60.32 63.39	47.09 60.15 51.95
α, β, γ (deg)	90.00 90.00 120.00	90.00 99.83 90.00
Resolution* (Å)	1.50 (1.58-1.50)	1.65 (1.XX-1.65)
Unique observations*	21838 (3172)	17186 (870)
Completeness* (%)	100.0 (100.0)	99.0 (97.6)
Redundancy*	9.5 (7.7)	5.1 (4.7)
Rmerge*	0.072 (0.350)	0.045 (0.500)
I/ σI*	17.8 (4.9)	16.5 (2.9)
Refinement		
Resolution (Å)	1.50	1.65
R_{work} / R_{free} (%)	20.6 / 23.1	21.0 / 25.0
Number of atoms (protein/other/water)	934 / 16 / 116	931 / 34 / 75
B-factors (Å^2) (protein/other/water)	23.02 / 25.65 / 31.08	35.30 / 36.68 / 38.66
r.m.s.d bonds (Å)	0.015	0.014
r.m.s.d angles (^{o})	1.656	1.586

Ramachadran Favoured (%)	99.09	99.10
Allowed (%)	0.91	0.90
Disallowed (%)	0.00	0.00

* Values in parentheses correspond to the highest resolution shell.

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