Design of a chemical probe for the Bromodomain and Plant Homeodomain Finger-containing (BRPF) family of proteins

SUPPORTING INFORMATION

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MATERIALS and METHODS

General 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 **1**-**42** was evaluated by NMR spectroscopy and HPLC analysis. All compounds had purity ≥ 95 % by HPLC.

General synthetic procedures:

General Procedure A: Preparation of quinolin-2(1*H*)-ones from quinolines

To a stirred solution of 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 quinolone-*N*-oxide which was used in the next step without further purification.

To a stirred solution of quinolone-*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-one.

General Procedure B: *N1* 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% w/w, 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 × volume of DMF). If precipitation was observed, the solid was collected by filtration, washed with water and dried to afford the desired *N1*-alkylquinolin-2-one.

Otherwise, the solution was extracted with ethyl acetate (3 × volume 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 *N1*-alkylquinolin-2-one.

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

To a suspension of the appropriate quinolin-2-one (1 eq.) in concentrated H_2SO_4 (2 mL/mmol) at -5 °C was added HNO_3 (70 % w/w, 0.5 mL/mmol) dropwise [***CAUTION!***]. The resulting yellow solution was stirred 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-2one.

General Procedure D: Reduction of 6-nitroquinolin-2(1*H*)-ones with $SnCl_2$

To a suspension of the appropriate 6-nitroquinolin-2-one (1 eq.) in concentrated 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-one.

General procedure E: Coupling of 6-aminoquinolin-2-ones to sulfonyl chlorides

To a solution of amine (1 eq.) in DMF (0.2 M, minimum 1 mL) was added pyridine (2 eq.) and the resulting solution was stirred for 5 minutes and then 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 solid by column chromatography afforded pure sulfonamide.

General procedure F: Coupling of amines to 1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonyl chloride (59).

To a solution of 1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonyl chloride (**59**) (100 mg, 0.39 mmol, 1.1 eq.) and pyridine (31 mg, 32 µL, 1.1 eq.) in DCM (5 mL) was added the appropriate amine (0.35 mmol, 1 eq.) and the resulting solution was stirred overnight. After this time, the organic solvents were removed *in vacuo* and the residue purified by column chromatography (ethyl acetate:hexane) to give the product.

General Procedure H: Sulfonamide *N* alkylation

To a solution of sulfonamide (1.0 eq.) in dry DMF (3 mL) under an argon atmosphere was added NaH (60 % in mineral oil, 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 (30 mL) and the aqueous solution was extracted with EtOAc (3 × 50 mL). The combined organic fractions were dried over anhydrous $MgSO_4$, filtered and concentrated onto Celite®. Purification of the residue by column chromatography provided the desired *N*-alkylated sulfonamide.

General Procedure I: Oxidation of tetrahydroquinolin-2(1*H*)-ones with DDQ

To a solution of the appropriate of tetrahydroquinolin-2-one (1 eq.) in dry 1,2-DCE (5 mL/mmol) under an argon atmosphere was added freshly crystallised DDQ (3 eq.) and the resulting dark suspension was stirred at reflux for 3 hours before being allowed to cool to RT. NaOH (1.0 M, 25 eq.) was added with stirring and the mixture was then extracted with EtOAc (3 × 100 mL). The organic fractions were pooled, washed with NaOH (3 × 100 mL), saturated brine, dried over anhydrous Mg SO_4, filtered and concentrated to provide the quinolin-2-one which was either used without further purification or purified by column chromatography.

General Procedure J: Microwave conversion of aryl bromides to aryl amines

The appropriate aryl bromide (1 eq.) was dissolved in NMP (1.5 mL) in a Biotage 10 mL microwave vial. Cu_2O (0.1 eq) and 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 an 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 to give a solution of the aryl amine in NMP.

General Procedure K: Synthesis of cinnamides

A solution of the appropriate aniline (1 eq.) and potassium carbonate (1.5 eq.) in acetone (1 mL/mmol of amine) and water (2 mL/mmol of amine) was cooled to 0 °C and cinnamoyl chloride (1.25 eq.) was added portionwise over 10 minutes. Stirring was continued for 1 hour at 0 °C, then warmed to RT and stirred for an additional 1 hour. The product was isolated by filtration and either purified by crystallisation or used without further purification

General Procedure L: Cyclisation of cinnamides

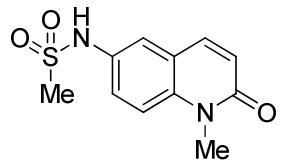
A solution of the appropriate cinnamide (1 eq.) in chlorobenzene (7.5 mL/mmol) was cooled to 0 °C and aluminium chloride (3 eq,) was added portionwise. The resulting suspension was slowly heated to 120 °C and maintained at that temperature for 3 hours. After this time, the solution was cooled to RT and poured over ice water. The precipitate was removed by filtration and dried to give the crude quinolin-2(1*H)*-ones which were either crystallised or used without further purification.

General Procedure M: Preparation of quinolin-2(1*H*)-ones from 2-*N*-protected benzaldehydes

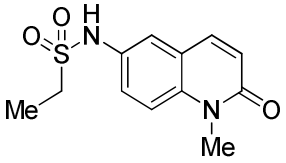
Step 1: To a solution of *N,N*-diisopropylamine (2.1 eq.) in dry Ether (1.25 mL/mmol) under an argon atmosphere at –78 °C was added *n*-butyllithium solution (1.6 M in Hexane, 2.1 eq.) and the solution was stirred for 30 minutes. *tert*-Butyl acetate (2.1 eq.) was added dropwise and the solution was allowed to stir for 30 minutes. The appropriate benzaldehyde (1.0 eq.) in dry Ether (1 mL/mmol) was added dropwise and the bright yellow solution was allowed to warm to RT over 2 hours. Ammonium chloride solution (1.0 M, 20 mL) was added and the reaction mixture stirred for a further 10 minutes. The aqueous layer was separated and extracted twice with ether. The combined organic layers were washed with water and brine, dried over *anhydrous* $MgSO_4$, filtered and the solvent removed *in vacuo* to provide the β-hydroxyester, which was used without further purification in the next step.

Step 2: Crude β-hydroxyester was dissolved in 1,4 dioxane (1 mL/mmol of benzaldehyde) and HCl (3 M, 1 mL/mmol of benzaldehyde) was added. The solution was heated at reflux for 4 hours and then allowed to cool to RT. The precipitated product was collected by filtration and dried under vacuum to yield the quinolin-2(1*H*)-one.

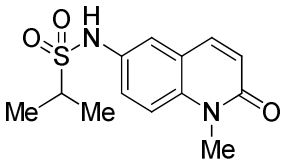
Synthetic procedures and characterization data for screening compounds 1-42:



***N*-(1-Methyl-2-oxo-1,2-dihydroquinolin-6-yl)methanesulfonamide (1):** Prepared by general procedure E from **43** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane, 3:7) to give the title compound (16 mg, 0.06 mmol, 59 %) as a pale yellow solid: mp 239-242 °C (acetone-hexane); IR (neat) ν_{max}: 3129, 2925, 1635, 1580, 1565, 1422, 1319, 1148, 1119, 971, 815, 776, 735, 655, 542, 513, 467, 410; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.79 (1H, s), 7.91 (1H, d, J = 9.3 Hz), 7.58 - 7.52 (2H, m), 7.46 (1H, dd, J = 9.6, 2.5 Hz), 6.63 (1H, d, J = 9.3 Hz), 3.60 (3H, s), 2.99 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.4, 138.6, 136.8, 132.4, 124.4, 121.8, 120.5, 119.7, 115.7, 39.5, 28.3; HRMS: *m/z* ESI$^+$ Found (M+H)$^+$ 253.0648. $C_{11}H_{12}N_2O_3S$ requires (M+H)$^+$ 253.0569; HPLC: Retention time (system A): t_R= 4.27 min. Purity: >95%.

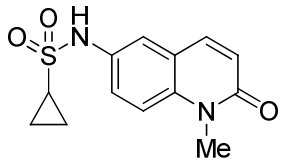


***N*-(1-Methyl-2-oxo-1,2-dihydroquinolin-6-yl)ethanesulfonamide (2):** Prepared by general procedure E from **43** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane, 3:7) to give the title compound (21 mg, 0.07 mmol, 64 %) as a pale yellow solid: mp 195-199 °C (acetone-hexane); IR (neat) ν_{max}: 3130, 2931, 1640, 1566, 1420, 1313, 1233, 1141, 977, 881, 818, 768, 527, 470 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.86 (1H, s), 7.90 (1H, d, J = 9.3 Hz), 7.56 - 7.51 (2H, m), 7.47 (1H, dd, J = 10.4, 2.0 Hz), 6.62 (1H, d, J = 9.3 Hz), 3.60 (3H, s), 3.08 (2H, q, J = 7.3 Hz), 1.21 (3H, t, J = 7.3 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.3, 138.9, 136.6, 132.5, 123.9, 121.8, 120.5, 119.2, 115.8, 44.9, 29.1, 8.0; HRMS *m/z* ESI$^+$ Found (M+H)$^+$ 267.0802. $C_{11}H_{12}N_2O_3S$ requires (M+H)$^+$ 267.0725; HPLC: Retention time (system A): t_R= 3.94 min. Purity: >95%.

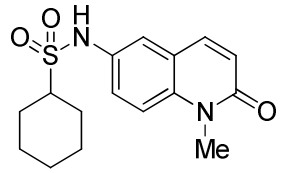


***N*-(1-Methyl-2-oxo-1,2-dihydroquinolin-6-yl)propane-2-sulfonamide (3):** Prepared by general procedure E from **43** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane, 3:7) to give the title compound (13 mg, 0.05 mmol, 43 %) as a brown solid: mp 191-193 °C (acetone-hexane); IR (neat) ν_{max}: 3084, 2933, 1642, 1622, 1572, 1486, 1334, 1306, 1259, 1168, 1139, 1121, 984, 886, 808, 754, 695, 672, 604, 586, 529, 492, 473, 446, 411 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6):

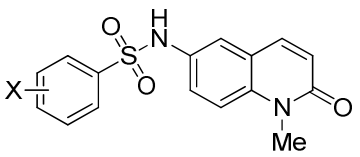
δ ppm 9.85 (1H, s), 7.90 (1H, d, *J*=9.6 Hz), 7.57 - 7.45 (3H, m), 6.61 (1H, d, *J* = 9.3 Hz), 3.59 (3H, s), 3.22 (1H, quin, *J* = 6.8 Hz), 1.25 (6H, d, *J* = 6.8 Hz); ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ ppm 160.7, 138.8, 136.5, 132.7, 123.7, 121.8, 120.5, 118.9, 115.8, 51.2, 29.0, 16.1; HRMS *m/z* ESI$^+$ Found (M+H)$^+$ 281.0954. $C_{11}H_{12}N_2O_3S$ requires (M+H)$^+$ 281.0882; HPLC: Retention time (system A): t_R= 5.53 min. Purity: >95%.



***N*-(1-Methyl-2-oxo-1,2-dihydroquinolin-6-yl)cyclopropanesulfonamide (4):** Prepared by general procedure E from **43** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane, 3:7) to give the title compound (31 mg, 0.10 mmol, 97 %) as a bright yellow solid: mp: 181-182 °C (acetone-hexane); IR (neat) ν_{max}: 3095, 2928, 1639, 1570, 1481, 1310, 1143, 978, 882, 809, 584, 525, 471 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ ppm 9.79 (1H, s), 7.91 (1H, d, *J* = 9.5 Hz), 7.57 (1H, s), 7.54 (1H, d, *J* = 9.5 Hz), 7.49 (1H, d, *J* = 8.5 Hz), 6.63 (1H, d, *J* = 9.5 Hz), 3.61 (3H, s), 0.91 (4H, br s); ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ ppm 160.1, 138.8, 136.8, 132.4, 124.7, 121.8, 120.4, 120.2, 115.6, 29.3, 29.1, 4.9; HRMS *m/z* ESI$^+$ Found (M+H)$^+$ 279.0800. $C_{13}H_{14}N_2O_3S$ requires (M+H)$^+$ 279.0825; HPLC: Retention time (system A): t_R= 4.99 min. Purity: >95%.



***N*-(1-Methyl-2-oxo-1,2-dihydroquinolin-6-yl)cyclohexanesulfonamide (5):** Prepared by general procedure E from **43** (20 mg, 0.11 mmol), purified by column chromatography (acetone:hexane, 3:7) to give the title compound (20 mg, 0.06 mmol, 59 %) as a pale yellow solid: mp: 169-172 °C (acetone-hexane); IR (neat) ν_{max}: 3098, 2933, 1640, 1620, 1572, 1483, 1332, 1308, 1258, 1142, 1121, 980, 882, 807, 758, 706, 604, 585, 526, 492, 472, 412 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ ppm 9.85 (1H, s), 7.90 (1H, d, *J* = 9.6 Hz), 7.56 - 7.45 (3H, m), 6.61 (1H, d, *J* = 9.6 Hz), 3.59 (3H, s), 3.04 – 2.82 (1H, m), 2.03 (2H, d, *J* = 11.1 Hz), 1.75 (2H, d, *J* = 12.6 Hz), 1.57 (1H, d, *J* = 11.6 Hz), 1.51 - 1.32 (2H, m), 1.26 - 1.02 (3H, m); ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ ppm 160.7, 138.9, 136.4, 132.7, 123.6, 121.8, 120.5, 118.8, 115.8, 58.8, 29.0, 26.0, 24.7, 24.3; HRMS *m/z* ESI$^+$ Found (M+H)$^+$ 321.1267. $C_{16}H_{20}N_2O_3S$ requires (M+H)$^+$ 321.1195; HPLC: Retention time (system A): t_R= 8.06 min. Purity: >95%.



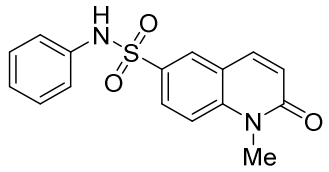
6: X = H

7: X = 2-CN

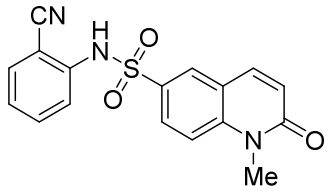
8: X = 3-CN

9: X = 4-CN

Detailed synthetic procedures and characterisation data for ***N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide** (**6**), **2-cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzene sulfonamide** (**7**), **3-cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide** (**8**) and **4-cyano-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide** (**9**) have been reported previously by this group.[1]

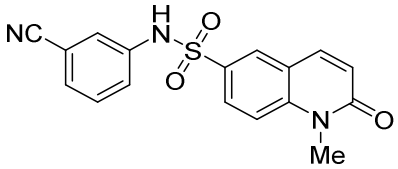


1-Methyl-2-oxo-*N*-phenyl-1,2-dihydroquinoline-6-sulfonamide (10): Prepared from **59** (100 mg, 0.39 mmol, 1.1 eq.) and aniline (33 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (ethyl acetate:hexane, 2:8) to give the title compound (82 mg, 0.27 mmol, 79 %) as a colourless solid: mp: 246-247 °C (ethyl acetate-hexane); IR (neat) ν_{max}: 3120, 3081, 3050, 2965, 2844, 1641, 1575, 1498, 1418, 1306, 1222, 1149, 1117, 1095, 1095, 936, 911, 762, 698, 690, 635, 594, 505, 476 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ ppm 10.31 (1H, s), 8.17 (1H, d, *J* = 2.0 Hz), 8.03 (1H, d, *J* = 9.6 Hz), 7.90 (1H, dd, *J* = 9.0, 2.1 Hz), 7.67 (1H, d, *J* = 9.1 Hz), 7.25 - 7.19 (2H, m), 7.14 - 7.09 (2H, m), 7.01 (1H, tt, *J* = 7.3, 1.1 Hz), 6.70 (1H, d, *J* = 9.6 Hz), 3.60 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.0, 142.2, 138.9, 137.6, 132.5, 129.2, 128.1, 127.9, 124.1, 122.6, 120.0, 119.5, 115.9, 29.0; HRMS *m/z* ESI^- Found $(M-H)^-$ 313.0635. $C_{16}H_{14}N_2O_3S$ requires $(M-H)^-$ 313.0647; HPLC: Retention time (system A): t_R= 8.29 min. Purity: >95%.

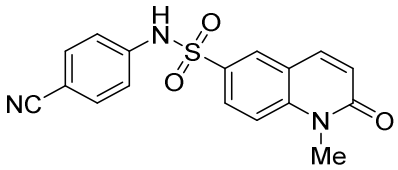


***N*-(2-Cyanophenyl)-1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonamide (11):** 1-Methyl-2-oxo-1,2-dihydroquinoline-6-sulfonyl chloride (**59**) (100 mg, 0.39 mmol, 1.1 eq.), 2-aminobenzonitrile (41 mg, 0.35 mmol, 1 eq.), 4-(dimethylamino)pyridine (4 mg, 0.4 mmol, 0.1 eq.) and pyridine (31 mg, 32 µL, 1.1 eq.) were dissolved in DCM and heated at reflux overnight. The solvent was removed *in vacuo* and the residue purified by column chromatography (ethyl acetate:hexane, 1:9 to 1:1) to give the title compound (18 mg, 0.05 mmol, 15 %) as a brown solid: IR (neat) ν_{max}: 1639, 1570, 1496, 1464, 1440, 1418, 1352, 1314, 1287, 1237, 1157, 1117, 1097, 1048, 935, 909, 832, 816, 787, 690, 648, 601, 599, 512, 475 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ ppm 10.62 (1H, br. s.), 8.14 (1H, d, *J* = 2.0 Hz), 8.05 (1H, d, *J* = 9.6 Hz), 7.90 (1H, dd, *J* = 9.1, 2.3 Hz), 7.84 - 7.79 (1H, m), 7.73 (1H, d, *J* = 9.1 Hz), 7.59

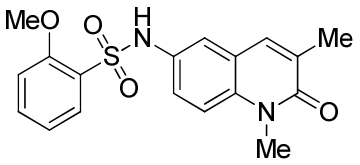
(1H, td, *J* = 7.8, 1.5 Hz), 7.40 (1H, td, *J* = 8.1, 0.8 Hz), 7.07 (1H, d, *J* = 8.1 Hz), 6.74 (1H, d, *J* = 9.3 Hz), 3.64 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.1, 142.4, 139.1, 138.6, 134.1, 134.0, 132.5, 128.3, 128.0, 127.2, 126.3, 122.6, 119.6, 116.6, 115.9, 109.9, 29.4; HRMS *m/z* ESI$^-$ Found (M-H)$^-$ 338.0591. $C_{17}H_{13}N_3O_3S$ requires (M-H)$^-$ 338.0599; HPLC: Retention time (system A): t_R= 7.90 min. Purity: >95%.



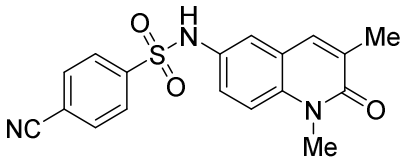
***N*-(3-Cyanophenyl)-1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonamide (12):** Prepared from **59** (100 mg, 0.39 mmol, 1.1 eq.) and 3-aminobenzonitrile (41 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (ethyl acetate:hexane, 3:7 to 1:1) to give the title compound (59 mg, 0.17 mmol, 50 %) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.63 (1H, d, *J*=2.0 Hz), 8.47 (1H, dd, *J*=9.3, 2.8 Hz), 8.34 (1H, dd, *J*=8.1, 2.0 Hz), 8.28 (1H, d, *J*=2.5 Hz), 7.96-7.88 (m, 4H), 6.73 (1H, s), 3.66 (3H, s); HRMS *m/z* ESI$^-$ Found (M-H)$^-$ 338.0593. $C_{17}H_{13}N_3O_3S$ requires (M-H)$^-$ 338.0599; HPLC: Retention time (system A): t_R= 7.48 min. Purity: >95%.



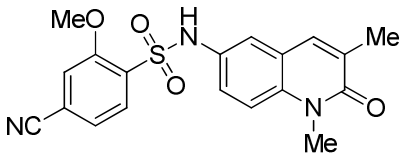
***N*-(4-Cyanophenyl)-1-methyl-2-oxo-1,2-dihydroquinoline-6-sulfonamide (13):** Prepared from **59** (100 mg, 0.39 mmol, 1.1 eq.) and 4-aminobenzonitrile (41 mg, 0.35 mmol, 1 eq.) using general procedure F. Purified by column chromatography (ethyl acetate:hexane, 3:7 to 1:1) to give the title compound (47 mg, 0.14 mmol, 39 %) as a colourless solid; mp: 290-292 °C (dec); IR (neat) v_{max}: 3128, 3050, 2937, 2876, 2221, 1646, 1575, 1503, 1349, 1303, 1159, 1098, 918, 836, 788, 690, 648, 01, 599, 512, 475; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 11.11 (1H, br. s.), 8.31 (1H, d, *J* = 2.0 Hz), 8.08 (1H, d, *J* = 9.6 Hz), 7.96 (1H, dd, *J* = 9.0, 2.1 Hz), 7.70 (3H, m), 7.27 (2H, d, *J* = 8.6 Hz), 6.74 (1H, d, *J* = 9.3 Hz), 3.61 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.0, 142.6, 142.1, 138.9, 133.7, 131.9, 128.0, 127.9, 122.8, 119.6, 118.7, 118.5, 116.2, 105.4, 29.4; HRMS *m/z* ESI$^-$ Found (M-H)$^-$ 338.0591. $C_{17}H_{13}N_3O_3S$ requires (M-H)$^-$ 338.0599; HPLC: Retention time (system A): t_R= 8.10 min. Purity: >95%.



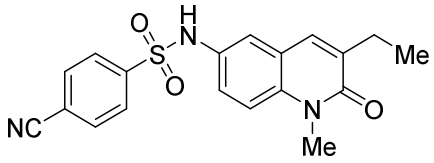
Detailed synthetic procedures and characterisation data for ***N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide** (**14**) have been reported previously by this group.[1]



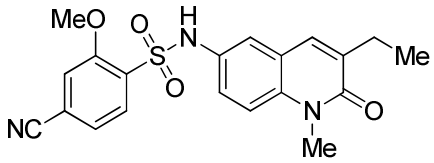
Detailed synthetic procedures and characterisation data for **4-cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide** (**15; NI-42**) have been reported previously by this group.[1]



Detailed synthetic procedures and characterisation data for **4-cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide** (**16; NI-57**) have been reported in the main Article.

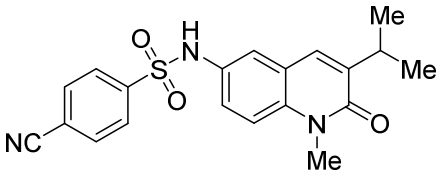


Detailed synthetic procedures and characterisation data for **4-cyano-*N*-(3-ethyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide** (**17**) have been reported previously by this group.[1]

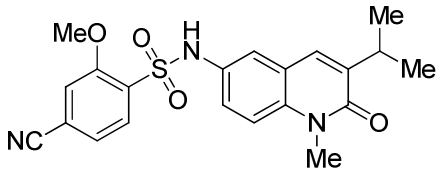


4-Cyano-*N*-(3-ethyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (18): Prepared according to general procedure E from **45** (30 mg, 0.15 mmol). Purified by column chromatography (acetone:hexane, 1:9 to 2:8) to give the title compound (23 mg, 0.06 mmol, 39 %) as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.37 (1H, s), 7.92 (1H, d, *J* = 8.1 Hz), 7.80 (1H, d, *J* = 1.3 Hz), 7.71 (1H, s), 7.54 (1H, dd, *J* = 8.1, 1.5 Hz), 7.47 - 7.40 (2H, m), 7.31 (1H, dd, *J* = 9.0, 2.4 Hz), 4.04 (3H, s), 3.61 (3H, s), 1.20 (3H, t, *J* = 7.3 Hz) *NB CH_2 overlaps with* DMSO; ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.7, 156.3, 156.3, 135.7, 135.2, 133.5, 131.1, 130.7, 124.1, 123.0,

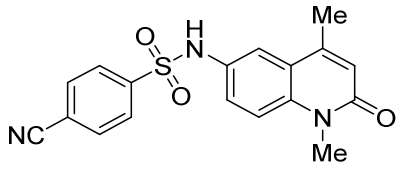
120.3, 119.4, 117.5, 116.8, 116.7, 115.3, 57.0, 29.3, 23.7, 12.6.; HRMS: *m/z* ESI^- Found $(M-H)^-$ 396.1032. $C_{20}H_{19}N_3O_4S$ requires $(M-H)^-$ 396.1013; HPLC: Retention time (system A): t_R= 9.85 min. Purity: >95%.



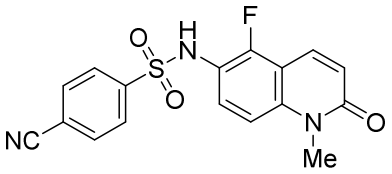
4-Cyano-*N*-(3-isopropyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (19): Prepared according to general procedure E from **46** (33 mg, 0.15 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 4:6) to give the title compound (20 mg, 0.05 mmol, 34 % over 2 steps) as a colourless solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.54 (1H, s), 8.04 (2H, d, *J* = 8.6 Hz), 7.88 (2H, d, *J* = 8.3 Hz), 7.67 (1H, s), 7.44 (1H, d, *J* = 2.5 Hz), 7.40 (1H, d, *J* = 9.1 Hz), 7.22 (1H, dd, *J* = 9.0, 2.4 Hz), 3.62 - 3.53 (3H, m), 3.11 (1H, spt, *J* = 6.9 Hz), 1.17 (6H, d, *J* = 7.1 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.6, 143.4, 139.6, 135.9, 133.5, 131.8, 130.7, 127.4, 123.6, 120.5, 120.4, 117.5, 115.4, 115.3, 29.4, 27.8, 21.5; HRMS: *m/z* ESI^- Found $(M-H)^-$ 380.1063. $C_{20}H_{19}N_3O_3S$ requires $(M-H)^-$ 380.1069; HPLC: Retention time (system A): t_R= 10.78 min. Purity: >95%.



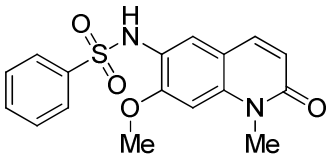
4-Cyano-*N*-(3-isopropyl-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (20): Prepared according to general procedure E from **46** (20 mg, 0.11 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 4:6) to give the title compound (18 mg, 0.04 mmol, 29 % over 2 steps) as a colourless solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.27 (1H, s), 7.86 (1H, d, *J* = 8.1 Hz), 7.74 (1H, d, *J* = 1.3 Hz), 7.63 (1H, s), 7.47 (1H, dd, *J* = 8.1, 1.5 Hz), 7.40 (1H, d, *J* = 2.3 Hz), 7.37 (1H, d, *J* = 9.1 Hz), 7.25 (1H, dd, *J* = 9.0, 2.4 Hz), 3.99 (3H, s), 3.56 (3H, s), 3.10 (1H, spt, *J* = 6.8 Hz), 1.16 (6H, d, *J* = 7.1 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.5, 156.3, 139.5, 135.6, 131.8, 131.0, 130.8, 124.1, 123.1, 120.2, 119.7, 117.5, 116.8, 116.7, 115.2, 57.0, 29.4, 27.9, 21.5; HRMS: *m/z* ESI^- Found $(M-H)^-$ 410.117. $C_{21}H_{21}N_3O_4S$ requires $(M-H)^-$ 410.1175; HPLC: Retention time (system A): t_R= 10.60 min. Purity: >95%.



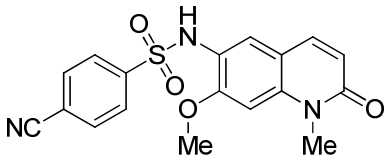
4-Cyano-*N*-(1,4-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (21): Prepared by general procedure E from **47** (100 mg, 0.53 mmol). Purified by column chromatography (ethyl acetate) to give the title compound (73 mg, 0.21 mmol, 43 %) as a white solid: mp: 281-282 °C (ethyl acetate); IR (neat) ν_{max}: 2915, 2849, 2776, 1637, 1616, 1564, 1482, 1339, 1309, 1283, 1159, 1091, 979, 869, 835, 815, 732, 702, 660, 628, 578, 546, 519, 499, 441cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.04 (2H, d, *J* = 8.3 Hz), 7.90 (2H, d, *J* = 8.3 Hz), 7.48–7.39 (2H, m), 7.31 (1H, dd, *J* = 9.0, 2.4 Hz), 6.54 (1H, s), 3.53 (3H, s), 2.31 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6) δ ppm 160.4, 145.5, 143.3, 136.9, 133.5, 127.5, 124.4, 121.2, 120.9, 117.5, 117.4, 116.0, 115.3, 28.8, 18.1; HRMS *m/z* ESI$^-$ Found (M-H)$^-$ 352.0749. $C_{18}H_{15}N_3O_3S$ requires (M-H)$^-$ 352.0756; HPLC: Retention time (system A): t_R= 7.78 min. Purity: >95%.



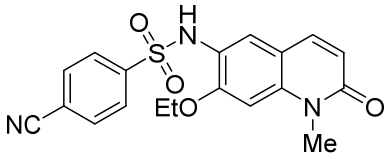
4-Cyano-*N*-(5-fluoro-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (22): Prepared by general procedure E from 5-fluoro-1-methyl-6-aminoquinolin-2(1*H*)-one (30 mg, 0.16 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 3:7) to give the title compound (44 mg, 0.13 mmol, 80 %): ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.54 (1H, br. s), 8.06 (2H, d, *J* = 8.6 Hz), 7.92 - 7.82 (3H, m), 7.45 - 7.37 (1H, m), 7.33 (1H, d, *J* = 9.6 Hz), 6.66 (1H, d, *J* = 9.6 Hz), 3.58 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.2, 154.1 (d, *J* = 244.6 Hz), 150.9, 144.7 (d, *J* = 13.4 Hz), 135.5, 135.4, 128.9 (d, *J* = 18 Hz), 126.2, 122.7, 119.2 , 117.0, 116.0, 102.6, 102.2, 29.2; HRMS: *m/z* ESI$^-$ Found (M-H)$^-$ 356.0496. $C_{17}H_{12}N_3O_3SF_3$ requires (M-H)$^-$ 356.0505; HPLC: Retention time (system A): t_R= 7.12 min. Purity: >95%.



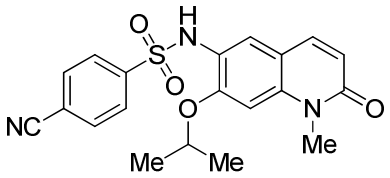
***N*-(7-Methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (23):** Prepared by general procedure E from a solution of **49** in NMP (ca. 39 mg, 0.19 mmol). Purified by column chromatography (acetone:hexane, 3:7 to 1:1) to give the title compound (24 mg, 0.07 mmol, 37 % over 2 steps) as a colourless solid: mp: 236-237 °C (acetone-hexane); IR (neat) ν_{max}: 3200, 1697, 1591, 1430, 1397, 1350, 1324, 1250, 1165, 1119, 1054, 823, 777, 666, 634, 598, 558, 506 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.62 (1H, s), 7.83 (1H, d, *J* = 9.4 Hz), 7.69 (2H, d, *J* = 7.3 Hz), 7.64–7.48 (4H, m), 6.83 (1H, s), 6.47–6.42 (1H, m), 3.58 (6H, app. s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.2, 155.2, 140.4, 139.5, 138.7, 132.5, 128.8, 126.6, 126.3, 120.3, 118.2, 113.3, 97.5, 55.8, 29.2; HRMS: *m/z* ESI$^-$ Found (M-H)$^-$ 343.0739. $C_{17}H_{16}N_2O_4S$ requires (M-H)$^-$ 343.0753; HPLC: Retention time (system A): t_R= 7.79 min. Purity: >95%.



4-Cyano-*N*-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (24): Prepared by general procedure E from a solution of **49** in NMP (ca. 39 mg, 0.19 mmol). Purified by column chromatography (acetone:hexane, 3:7 to 6:4) to give the title compound (15 mg, 0.04 mmol, 21 % over 2 steps) as a colourless solid: mp: 291-292 °C (acetone-hexane); ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.99 (1H, br. s), 8.03 (2H, d, J = 8.3 Hz), 7.89–7.80 (3H, m), 7.60 (1H, s), 6.83 (1H, s), 6.46 (1H, d, J = 9.6 Hz), 3.58 (3H, s), 3.54 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.2, 155.6, 144.7, 140.0, 138.7, 133.0, 127.6, 127.4, 119.3, 118.3, 117.8, 114.8, 113.4, 97.6, 55.7, 29.2; HRMS: *m/z* ESI$^+$ Found (M+H)$^+$ 370.0855. $C_{17}H_{16}N_2O_4S$ requires (M+H)$^+$ 370.0854; HPLC: Retention time (system A): t_R= 6.17 min. Purity: >95%.

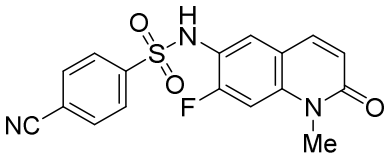


4-Cyano-*N*-(7-ethoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (25): Prepared by general procedure E from a solution of **50** in NMP (ca. 50 mg, 0.18 mmol). Purification by column chromatography (acetone:hexane, 4:6 to 6:4) gave the title compound (12 mg, 0.03 mmol, 17 % over 2 steps): ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 9.96 (1H, br. s), 8.03 (2H, d, J = 7.9 Hz), 7.87 (1H, d, J = 9.5 Hz), 7.81 (2H, d, J = 7.9 Hz), 7.63 (1H, s), 6.81 (1H, s), 6.45 (1H, d, J = 9.1 Hz), 3.87 (2H, q, J = 6.3 Hz), 3.56 (3H, s), 1.06 (3H, t, J = 6.8 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.2, 154.8, 144.7, 140.0, 138.7, 133.0, 127.7, 127.4, 119.3, 118.2, 117.7, 114.8, 113.3, 97.9, 64.0, 29.2, 13.8; HRMS: *m/z* ESI$^-$ Found (M-H)$^-$ 382.0862. $C_{19}H_{17}N_3O_4S$ requires (M-H)$^-$ 382.0862; HPLC: Retention time (system A): t_R= 8.08 min. Purity: >95%.

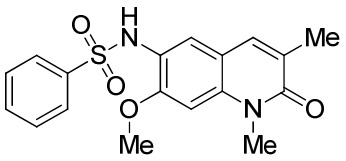


4-Cyano-*N*-(7-isopropoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (26): Prepared by general procedure E from a solution of **51** in NMP (ca. 50 mg, 0.17 mmol). Purification by column chromatography (acetone:hexane, 4:6 to 6:4) provided the title compound (16 mg, 0.04 mmol, 24 % over 2 steps): ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.87 (1H, br. s.), 8.03 (2H, d, J = 8.6 Hz), 7.84 - 7.78 (3H, m), 7.64 (1H, s), 6.80 (1H, s), 6.44 (1H, d, J = 9.6 Hz), 4.67 (1H, spt, J = 6.0 Hz), 3.55 (3H, s), 1.01 (6H, d, J = 6.1 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.2, 153.5, 144.8, 139.9, 138.7, 133.1, 127.7, 127.5, 119.8, 118.1, 117.7, 114.8, 113.1, 98.5, 70.0, 29.2, 20.9; HRMS: *m/z* ESI$^-$

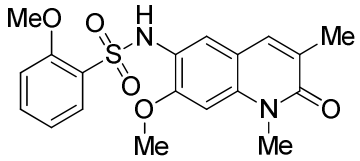
Found $(M-H)^-$ 396.1015. $C_{20}H_{19}N_3O_4S$ requires $(M-H)^-$ 396.1018; HPLC: Retention time (system A): t_R= 9.51 min. Purity: >95%.



4-Cyano-*N*-(7-fluoro-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (27): Prepared according to general procedure E from **52** (20 mg, 0.10 mmol). Purified by column chromatography (acetone:hexane, 3:7 to 6:4) to give the title compound (31 mg, 0.08 mmol, 83 %): ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.52 (1H, br. s), 8.06 (2H, d, *J* = 8.6 Hz), 7.92 (1H, d, *J* = 9.6 Hz), 7.87 (2H, d, *J* = 8.6 Hz), 7.65 (1H, d, *J* = 8.6 Hz), 7.41 (1H, d, *J* = 12.6 Hz), 6.59 (1H, d, *J* = 9.6 Hz), 3.53 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.9, 157.8 (d, *J* = 250.2 Hz), 143.9, 139.7 (d, *J* = 11.0 Hz), 138.5, 133.4, 128.3, 127.4, 120.8, 117.6 , 116.9, 115.3, 102.8, 102.6, 29.5; HRMS: *m/z* ESI$^-$ Found $(M-H)^-$ 356.508. $C_{17}H_{12}N_3O_3SF$ requires $(M-H)^-$ 356.505; HPLC: Retention time (system A): t_R= 7.79 min. Purity: >95%.

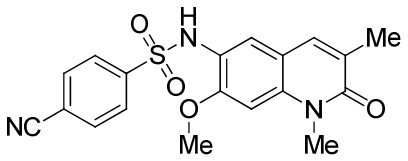


***N*-(7-Methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (28):** Prepared by general procedure E from a solution of **53** in NMP (ca. 38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 3:7) to give the title compound (36 mg, 0.10 mmol, 57 % over 2 steps) as a colourless solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 9.60 (1H, br. s), 7.72–7.66 (3H, m), 7.63–7.59 (1H, m), 7.55–7.47 (3H, m), 6.81 (1H, s), 3.60 (3H, s), 3.57 (3H, s), 2.08 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.8, 154.4, 140.4, 138.4, 135.2, 132.5, 128.7, 126.6, 126.1, 125.2, 120.2, 113.2, 97.4, 55.7, 29.6, 17.1; HRMS: *m/z* ESI$^-$ Found $(M-H)^-$ 357.0920. $C_{18}H_{18}N_2O_4S$ requires $(M-H)^-$ 357.0909; HPLC: Retention time (system A): t_R= 9.02 min. Purity: >95%.

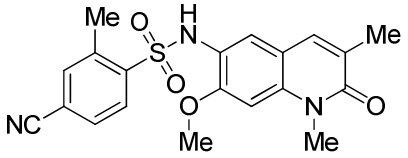


2-Methoxy-*N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (29): Prepared by general procedure E from a solution of **53** in NMP (ca. 38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 3:7) to give the title compound (35 mg, 0.09 mmol, 51 % over 2 steps) as a colourless solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.82 (1H, s), 7.67 (1H, s), 7.61 - 7.53 (2H, m), 7.48 (1H, s), 7.21 (1H, d, *J* = 7.8 Hz), 6.95 (1H, td, *J* = 7.6, 1.0 Hz), 6.83 (1H, s), 3.92 (3H, s), 3.73 (3H, s), 3.59 (3H, s), 2.06 (3H, d, *J* = 1.0 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ

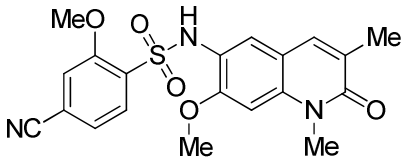
ppm 161.7, 156.6, 153.5, 137.8, 135.2, 134.8, 129.5, 127.1, 126.1, 123.5, 120.8, 119.6, 113.1, 112.5, 97.2, 56.1, 56.1, 29.5, 17.1; HRMS: *m/z* ESI$^-$ Found (M-H)$^-$ 387.1024. $C_{19}H_2N_2O_5S$ requires (M-H)$^-$ 387.1014; HPLC: Retention time (system A): t_R= 9.33 min. Purity: >95%.



4-Cyano-*N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (30): Prepared by general procedure E from a solution of **53** in NMP (ca. 38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 6:4) to give the title compound (35 mg, 0.09 mmol, 49 % over 2 steps) as a pale pink solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 9.98 (1H, br. s), 8.03 (2H, d, *J* = 8.5 Hz), 7.82 (2H, d, *J* = 8.2 Hz), 7.74 (1H, s), 7.51 (1H, s), 6.81 (1H, s), 3.61 (3H, s), 3.53 (3H, s), 2.09 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.8, 154.7, 144.8, 138.8, 135.2, 133.0, 127.4, 126.5, 126.2, 119.4, 117.8, 114.8, 113.3, 97.5, 55.6, 29.6, 17.1; HRMS: *m/z* ESI$^-$ Found (M-H)$^-$ 382.0862. $C_{19}H_{17}N_3O_4S$ requires (M-H)$^-$ 382.0862; HPLC: Retention time (system A): t_R= 9.19 min. Purity: >95%.

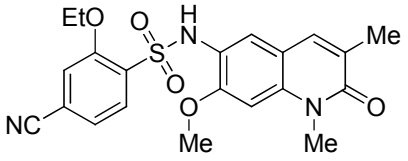


4-Cyano-*N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methylbenzenesulfonamide (31): Prepared by general procedure E from a solution of **53** in NMP (ca. 38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 4:6) to give the title compound (26 mg, 0.07 mmol, 37 % over 2 steps) as a pale yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 9.97 (1H, s), 7.95 (1H, s), 7.74 - 7.66 (3H, m), 7.50 (1H, s), 6.78 (1H, s), 3.59 (3H, s), 3.52 (3H, s), 2.70 (3H, s), 2.08 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.8, 155.1, 142.9, 139.0, 138.8, 135.6, 135.1, 129.6, 129.4, 127.2, 126.2, 118.9, 117.7, 114.7, 113.3, 97.4, 55.5, 29.6, 19.5, 17.1; HRMS: *m/z* ESI$^-$ Found (M+CO_2^-)$^-$ 441.0743, $C_{20}H_{19}N_3O_4S$ requires (M+CO_2^-)$^-$ 441.0995; HPLC: Retention time (system A): t_R= 11.04 min. Purity: >95%.

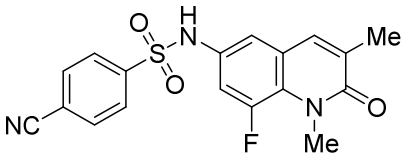


4-Cyano-2-methoxy-*N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (32): Prepared by general procedure E from a solution of **53** in NMP (ca.

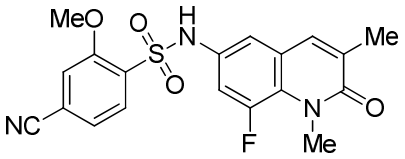
114 mg, 0.54 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 1:1) to give the title compound (81 mg, 0.20 mmol, 37 % over 2 steps) as an off-white solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.35 (1H, br. s), 7.76 (1H, s), 7.70 (2H, d, *J* = 7.8 Hz), 7.46 (1H, s), 7.43 (1H, dd, *J* = 8.0, 1.4 Hz), 6.81 (1H, s), 3.97 (3H, s), 3.65 (3H, s), 3.60 (3H, s), 2.07 (3H, d, *J* = 0.8 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.8, 156.7, 154.6, 138.5, 135.1, 132.4, 130.0, 126.1, 126.0, 123.6, 119.8, 117.7, 116.3, 116.2, 113.2, 97.3, 56.9, 55.9, 29.5, 16.8; HRMS: *m/z* ESI^{+} Found (M+H)$^{+}$ 414.1113. $C_{20}H_{19}N_3O_5S$ requires (M+H)$^{+}$ 414.1112; HPLC: Retention time (system A): t_R= min. Purity: >95%.



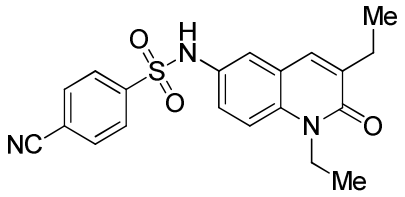
4-Cyano-2-ethoxy-*N*-(7-methoxy-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (33): Prepared by general procedure E from a solution of **53** in NMP (ca. 38 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 1:1) to give the title compound (16 mg, 0.04 mmol, 22 % over 2 steps) as an orange solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 9.06 (1H, s), 7.75 (1H, d, *J* = 3.8 Hz), 7.76 - 7.70 (2H, m), 7.50 (1H, s), 7.43 (1H, dd, *J* = 8.0, 1.1 Hz), 6.84 (1H, s), 4.26 (2H, q, *J* = 6.9 Hz), 3.67 (3H, s), 3.61 (3H, s), 2.07 (3H, s), 1.35 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.8, 156.0, 154.2, 138.3, 135.1, 132.3, 130.3, 126.2, 125.0, 123.5, 120.0, 117.7, 116.9, 116.4, 113.2, 97.3, 65.3, 55.9, 29.6, 17.1, 14.0; HRMS: *m/z* ESI^{-} Found (M-H)$^{-}$ 426.1133. $C_{21}H_{21}N_3O_5S$ requires (M-H)$^{-}$ 426.1124; HPLC: Retention time (system A): t_R= 10.44 min. Purity: >95%.



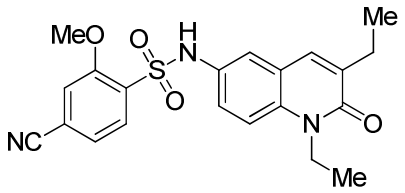
4-Cyano-*N*-(8-fluoro-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (34): Prepared by general procedure E from **54** (29 mg, 0.14 mg). Purified by column chromatography (acetone:hexane, 3:7 to 6:4) to give the title compound (11 mg, 0.03 mmol, 21 % over 2 steps) as a colourless solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.04 (2H, d, *J* = 8.1 Hz), 7.92 (2H, d, *J* = 8.3 Hz), 7.73 (1H, s), 7.13 (1H, d, *J* = 0.8 Hz), 7.08 (1H, dd, *J* = 15.7, 2.0 Hz), 3.72 (3H, d, *J* = 8.3 Hz), 2.09 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.6, 149.1 (d, *J* = 246.5 Hz), 143.1 (d, *J* = 1.8 Hz), 135.1, 133.6, 131.0, 127.7, 124.9 (d, *J* = 7.3 Hz), 123.0 (d, *J* = 3.7 Hz), 117.5, 115.5, 115.1 (d, *J* = 2.7 Hz), 110.1 (d, *J* = 26.6 Hz), 32.7 (d, *J* = 14.7 Hz), 17.2; HRMS: *m/z* ESI^{-} Found (M-H)$^{-}$ 370.0659. $C_{18}H_{14}N_3O_3SF$ requires (M-H)$^{-}$ 370.0662; HPLC: Retention time (system A): t_R= 8.68 min. Purity: >95%.



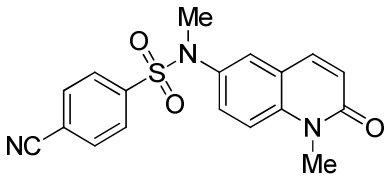
4-Cyano-*N*-(8-fluoro-1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (35): Prepared by general procedure E from **54** (29 mg, 0.14 mg). Purified by column chromatography (acetone:hexane, 2:8) to give the title compound (7 mg, 0.02 mmol, 13 % over 2 steps) as a colourless solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 10.55 (1H, s), 7.94 (1H, d, *J* = 8.2 Hz), 7.73 (2H, d, *J* = 12.9 Hz), 7.52 (1H, s), 7.14 - 7.04 (2H, m), 3.96 (3H, s), 3.71 (3H, d, *J* = 8.2 Hz), 2.08 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.6, 156.3, 149.0 (d, *J* = 246.5 Hz), 135.1, 131.7, 131.6, 131.1, 130.7 (d, *J* = 58.7 Hz), 124.5 (d, *J* = 6.4 Hz), 124.2, 122.9 (d, *J* = 3.7 Hz), 117.4, 117.1, 116.9, 114.2 (d, *J* = 2.7 Hz), 109.4 (d, *J* = 27.5 Hz), 57.0, 32.7 (d, *J* = 14.7 Hz), 17.2; HRMS: *m/z* ESI$^-$ Found (M-H)$^-$ 400.0765. $C_{19}H_{16}N_3O_4SF$ requires (M-H)$^-$ 400.0767; HPLC: Retention time (system A): t_R= 9.95 min. Purity: >95%.



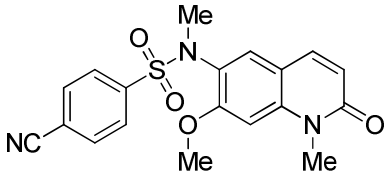
Detailed synthetic procedures and characterisation data for **4-cyano-*N*-(1,3-diethyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide** (**36; NI-198**) have been reported previously by this group.[1]



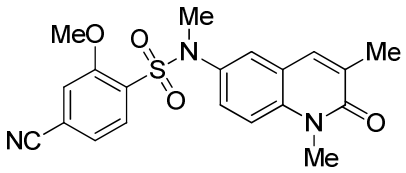
4-Cyano-*N*-(1,3-diethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide (37): Prepared according to general procedure E from **55** (40 mg, 0.18 mmol). Purified by column chromatography (acetone:hexane, 1:9 to 2:8) to give the title compound (51 mg, 0.12 mmol, 20 % over 3 steps) as a colorless solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 10.30 (1H, s), 7.88 (1H, d, *J* = 8.2 Hz), 7.75 (1H, d, *J* = 0.9 Hz), 7.64 (1H, s), 7.48 (1H, dd, *J* = 8.2, 1.3 Hz), 7.43 (1H, d, *J* = 8.8 Hz), 7.36 (1H, d, *J* = 2.2 Hz), 7.26 (1H, dd, *J* = 9.1, 2.5 Hz), 4.20 (2H, q, *J* = 7.1 Hz), 3.98 (3H, s), 1.15 (6H, app. t, *J* = 7.4 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 160.4, 156.3, 135.2, 134.6, 133.6, 131.0, 131.0, 130.9, 124.1, 123.1, 120.6, 119.5, 117.5, 116.8, 116.7, 115.0, 56.9, 36.9, 23.5, 12.7, 12.5; HRMS: *m/z* ESI$^-$ Found (M-H)$^-$ 410.1185. $C_{21}H_{21}N_3O_4S$ requires (M-H)$^-$ 410.1253; HPLC: Retention time (system A): t_R= 10.54 min. Purity: >95%.



4-Cyano-*N*-methyl-*N*-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)benzenesulfonamide (38): Prepared by general procedure H from **9** (50 mg, 0.15 mmol). Purified by column chromatography (acetone:hexane, 3:7) to give the title compound (33 mg, 0.09 mmol, 63%) as a colourless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.08 (2H, d, *J* = 8.3 Hz), 7.85 (1H, d, *J* = 9.6 Hz), 7.70 (2H, d, *J* = 8.6 Hz), 7.54 - 7.49 (2H, m), 7.37 (1H, dd, *J* = 9.1, 2.5 Hz), 6.65 (1H, d, *J* = 9.6 Hz), 3.61 (3H, s), 3.22 (3H, s); ^{13}C NMR (151 MHz, DMSO-d_6) □ 161.0, 139.7, 138.9, 138.8, 134.4, 133.5, 129.1, 128.3, 126.3, 121.9, 120.3, 117.7, 115.8, 115.5, , 38.2, 29.2; LRMS: *m/z* ESI^{+} Found (M+H)$^{+}$ 354.1. $C_{18}H_{16}N_3O_3S$ requires (M+H)$^{+}$ 354.1; HPLC: Retention time (system A): t_R= 7.59 min. Purity: >95%.

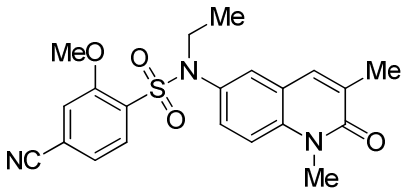


4-Cyano-*N*-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-N-methylbenzenesulfonamide (39): Prepared by general procedure H from **24** (20 mg, 0.05 mmol). Purified by column chromatography (acetone:hexane, 3:7) to give the title compound (13 mg, 0.03 mmol, 63 %) as a colourless solid: ^{1}H NMR (400 MHz, DMSO-d_6): δppm 8.09 (1H, d, *J* = 8.6 Hz), 7.86 (1H, d, *J* = 9.6 Hz), 7.82 (2H, d, *J* = 8.6 Hz), 7.66 (1H, s), 6.89 (1H, s), 6.49 (1H, d, *J* = 9.3 Hz), 3.62 (3H, s), 3.50 (3H, s), 3.19 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.3, 157.7, 142.7, 141.3, 138.6, 133.2, 131.3, 127.9, 123.0, 118.5, 117.8, 115.1, 113.5, 98.1, 55.6, 37.9, 29.3; HRMS: *m/z* ESI^{-} Found (M+Cl)$^{-}$ 418.0862. $C_{19}H_{17}N_3O_4S$ requires (M+Cl)$^{-}$ 418.0628; HPLC: Retention time (system A): t_R= 9.39 min. Purity: >95%.

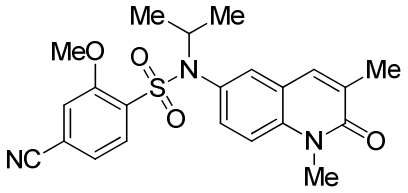


4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxy-N-methylbenzenesulfonamide (40): Prepared according to general procedure H from **16** (50 mg, 0.13 mmol). Purified by column chromatography (acetone:hexane, 1:9) to give the title compound (32 mg, 0.08 mmol, 62 %) as a yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 7.82 (1H, d, *J* = 0.9 Hz),

7.75 - 7.72 (2H, m), 7.50-7.47 (2H, m), 7.45 - 7.41 (3H, m), 7.36 (3H, d, *J* = 2.2 Hz), 3.88 (3H, s), 3.61 (3H, s), 3.34 (3H, s), 2.11 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.5, 156.6, 137.5, 135.0, 134.5, 131.7, 130.4, 129.8, 127.6, 125.1, 124.1, 120.1, 117.5, 117.0, 116.9, 115.0, 56.8, 29.5, 17.3; HPLC: Retention time (system A): t_R= 9.98 min. Purity: >95%.

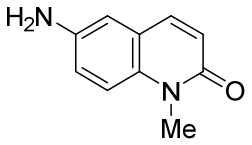


4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-*N*-ethyl-2-methoxybenzenesulfonamide (41): Prepared according to general procedure H from **16** (50 mg, 0.13 mmol). Purified by column chromatography (acetone:hexane, 1:9) to give the title compound (44 mg, 0.11 mmol 82 %) as a pale yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 7.85 (1H, s), 7.75 (1 H, s), 7.66 (1H, d, *J* = 7.9 Hz), 7.46 - 7.42 (3H, m), 7.26 (1H, dd, *J* = 9.0, 2.4 Hz), 4.00 (3H, s), 3.83 (2H, q, *J* = 6.9 Hz), 3.61 (3H, s), 2.11 (3H, s), 1.03 (3H, t, *J* = 7.1 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 162.3, 161.6, 156.5, 138.0, 135.0, 131.5, 131.2, 129.8, 129.8, 127.9, 124.0, 120.2, 117.6, 116.9, 116.7, 115.3, 56.9, 46.6, 29.5, 17.3, 14.6; HRMS: *m/z* ESI$^-$ Found (M+Cl)$^-$ 446.0942. $C_{21}H_{21}N_3O_4S$ requires (M+Cl)$^-$ 446.0943; HPLC: Retention time (system A): t_R= 10.69 min. Purity: >95%.

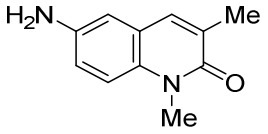


4-Cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-*N*-isopropyl-2-methoxybenzenesulfonamide (42): Prepared according to general procedure H from **16** (50 mg, 0.13 mmol). Purified by column chromatography (acetone:hexane, 1:9) to give the title compound (27 mg, 0.06 mmol, 49 %) as a pale yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 7.88 (1H, d, *J* = 0.9 Hz), 7.77 (1H, s), 7.66 (1H, d, *J* = 8.2 Hz), 7.50 - 7.40 (2H, m), 7.30 (1H, d, *J* = 2.2 Hz), 7.08 (1H, dd, *J* = 8.8, 2.2 Hz), 4.65 (1H, spt, *J* = 6.6 Hz), 4.08 (3H, s), 3.62 (3H, s), 2.11 (3H, s), 1.07 (6H, d, *J* = 6.6 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 161.6, 156.3, 138.6, 135.1, 133.0, 132.3, 131.2, 131.2, 129.7, 127.4, 124.0, 120.0, 117.6, 116.9, 116.6, 115.0, 56.8, 51.4, 29.5, 22.2, 17.3; HRMS: *m/z* ESI$^-$ Found (M+Cl)$^-$ 460.1093. $C_{22}H_{23}N_3O_4S$ requires (M+Cl)$^-$ 460.1094; HPLC: Retention time (system A): t_R= 11.14 min. Purity: >95%.

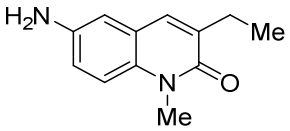
Synthetic procedures and characterization data for amines 43-55:



Detailed synthetic procedures and characterisation data for **6-amino-1-methylquinolin-2(1*H*)-one** (**43**) have been reported previously by this group.[1]

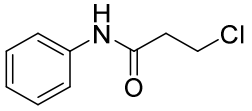


Detailed synthetic procedures and characterisation data for **6-amino-1,3-dimethylquinolin-2(1*H*)-one** (**44**) have been reported previously by this group.[1]

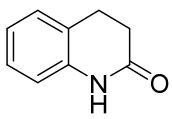


Detailed synthetic procedures and characterisation data for **6-amino-3-ethyl-1-methylquinolin-2(1*H*)-one** (**45**) have been reported previously by this group.[1]

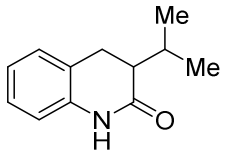
6-Amino-3-isopropyl-1-methylquinolin-2(1*H*)-one (46):



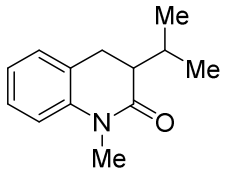
Step 1: 3-Chloro-*N*-phenylpropanamide: To a solution of aniline (9.3 g, 100 mmol, 1 eq.) in acetone (100 mL) and water (200 mL) was added K_2CO_3 (20.8 g, 150 mmol, 1.5 eq.). The suspension was cooled to 0 °C and 3-chloropropanoyl chloride (15.9 g, 125 mmol, 1.25 eq.) was added dropwise. The solution was maintained at 0 °C and stirred for 1 hour before being poured onto ice (~200 g). The precipitate was collected by filtration and dried under reduced pressure to afford the title compound (18.1 g 97 mmol, 97 %) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.54 (2H, d, *J* = 7.8 Hz), 7.46 - 7.30 (3H, m), 7.15 (1H, t, *J* = 7.6 Hz), 3.91 (2H, t, *J* = 6.3 Hz), 2.84 (2H, t, *J* = 6.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 167.8, 137.4, 129.1, 124.7, 120.1, 40.5, 39.9. The data is in agreement with that previously reported in the literature.[2]



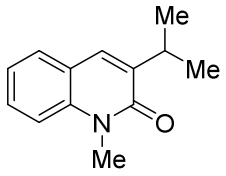
Step 2: 3,4-Dihydroquinolin-2(1*H*)-one: $AlCl_3$ (43 g, 328 mmol, 4 eq.) was added to 3-chloro-*N*-phenylpropanamide (15 g, 82 mmol, 1 eq.) under an argon atmosphere and the mixture was heated slowly to 120 °C and then maintained at this temperature for 3 hours. The solution was allowed to cool to 60 °C and then poured onto ice (~500 g) and stirred for 30 mins. The precipitate was filtered, washed with water and dried to provide the crude title product. Crystallisation of the crude material from MeOH gave the title compound (5.8 g, 39 mmol, 48 %) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.66 (1H, br. s.), 7.23 - 7.13 (2 H, m), 7.01 (1H, td, *J* = 7.5, 1.0 Hz), 6.83 (1H, d, *J* = 7.8 Hz), 3.00 (2H, t, *J* = 7.6 Hz), 2.67 (2H, t, *J* = 7.3 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 171.6, 137.3, 128.0, 127.5, 123.7, 123.1, 115.7, 30.7, 25.3. The data is in agreement with that previously reported in the literature.[3]



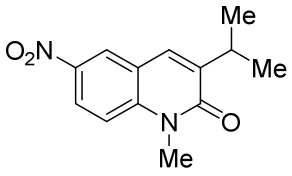
Step 3: 3-Isopropyl-3,4-dihydroquinolin-2(1*H*)-one: To a solution of 3,4-dihydroquinolin-2(1*H*)-one (1.5 g, 10 mmol, 1 eq.) in dry THF (50 mL) under an argon atmosphere at -78 °C was added freshly prepared LDA (2.0M in THF, 25 mmol, 2.5 eq.) and the solution was stirred at -78 °C for 1 hour. 2-Bromopropane (1.5 g, 12 mmol, 1.2 eq.) was then added dropwise and the mixture was stirred at -78 °C for a further 30 minutes before warming to RT. The mixture was quenched with sat. NH_4Cl and extracted with EtOAc (3 × 50 mL). The combined organic fractions were dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo.* Purification of the residue by column chromatography (hexane:ethyl acatate, 1:9 to 3:7) gave the title compound (912 mg, 4.8 mmol, 48 %) as a colourless solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.63 (1H, br. s.), 7.23 - 7.13 (2H, m), 6.99 (1H, td, *J* = 7.5, 1.0 Hz), 6.80 (1H, dd, *J* = 8.1, 1.0 Hz), 3.04 – 2.84 (2H, m), 2.40 (1H, dt, *J* = 8.3, 6.2 Hz), 2.27 - 2.16 (1H, m), 1.03 (6H, dd, *J* = 30.8, 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 172.1, 140.3, 127.9, 127.3, 125.8, 122.6, 114.3, 47.0, 29.7, 27.2, 26.6, 20.9, 19.1.



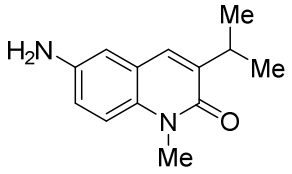
Step 4: 3-Isopropyl-1-methyl-3,4-dihydroquinolin-2(1*H*)-one: Prepared by general procedure B from 3-isopropyl-3,4-dihydroquinolin-2(1*H*)-one (280 mg, 1.48 mmol). Purification by column chromatography (hexane:ethyl acetate, 1:9 to 2:8) gave the title compound (237 mg, 1.17 mmol, 79 %) as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.17 (1H, td, *J* = 7.8, 1.0 Hz), 7.09 (1H, d, *J* = 7.1 Hz), 6.93 (1H, t, *J* = 7.1 Hz), 6.88 (1H, d, *J* = 8.1 Hz), 3.29 (3H, s), 2.85 (1H, dd, *J*=15.7, 5.6 Hz), 2.73 (1H, dd, *J* = 16.1, 8.3 Hz), 2.27 (1H, dt, *J* = 8.2, 6.0 Hz), 2.08 - 2.02 (1 H, m), 0.89 (6H, dd, *J* = 27.3, 7.1 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 172.1, 140.3, 127.9, 127.3, 125.8, 122.6, 114.3, 47.0, 29.6, 27.2, 26.6, 20.8, 19.0.



Step 5: 3-Isopropyl-1-methylquinolin-2(1*H*)-one: Prepared by general procedure I from 3-isopropyl-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (520 mg, 2.56 mmol). A second portion of DDQ (3 eq.) was added after 24 hours. Purification by column chromatography (hexane:ethyl acatate, 1:9 to 2:8) gave the title compound (117 mg, 0.58 mmol, 23 %) as a colourless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.59 - 7.48 (3H, m), 7.35 (1H, d, *J* = 8.3 Hz), 7.24 (1H, td, *J* = 7.5, 1.0 Hz), 3.77 (3H, s), 3.33 (1H, spt, *J* = 7.1 Hz), 1.29 (6H, d, *J* = 6.8 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 162.2, 139.9, 138.8, 132.2, 129.3, 128.2, 121.9, 120.8, 113.7, 29.7, 28.2, 21.8.

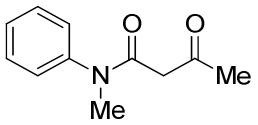


Step 6: 3-isopropyl-1-methyl-6-nitroquinolin-2(1*H*)-one: To a solution of 3-isopropyl-1-methylquinolin-2(1*H*)-one (104 mg, 0.52 mmol) in AcOH (5 mL) at 0 °C was added KNO_3 (52 mg, 0.52 mmol, 1 eq.) in 1 portion and the resulting solution was stirred at 5 °C for 2 hours and then poured onto ice. The resulting suspension was filtered, washed with H_2O and dried to give the crude compound. Crystallisation of the crude material from EtOH provided the title compound (81 mg, 0.33 mmol, 63 %) as pale yellow needles: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.49 (1H, d, *J* = 2.5 Hz), 8.37 (1H, dd, *J* = 9.3, 2.5 Hz), 7.60 (1H, s), 7.43 (1H, d, *J* = 9.3 Hz), 3.81 (3H, s), 3.32 (1H, spt, *J* = 7.1 Hz), 1.30 (6H, d, *J* = 7.1 Hz); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 161.9, 142.7, 142.5, 131.8, 124.0, 124.0, 120.2, 114.3, 30.3, 28.4, 21.6.

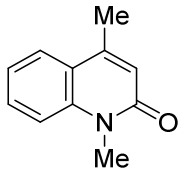


Step 7: 6-Amino-3-isopropyl-1-methylquinolin-2(1*H*)-one (46): Prepared according to general procedure D from 3-isopropyl-1-methyl-6-nitroquinolin-2(1*H*)-one (75 mg, 0.30 mmol) to give the title compound as a yellow oil which was used without further purification.

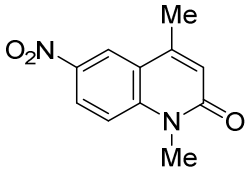
6-Amino-1,4-dimethylquinolin-2(1*H*)-one (47):



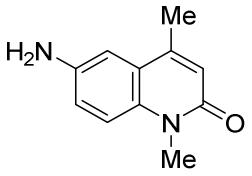
Step 1: *N*-Methyl-3-oxo-*N*-phenylbutanamide: To a solution of *N*-methylaniline (3 g, 26.5 mmol, 1 eq.) in xylenes as added 2,2,6-Trimethyl-4*H*-1,3-dioxin-4-one (3.77 g, 26.5 mmol, 1 eq.) and the resulting solution was heated at reflux for 3 hours. After this time, the solution was cooled to RT and the solvent removed *in vacuo*. The crude oil was purified by column chromatography (ethyl acetates:hexane, 4:1) to give the title compound (3.02 g, 15.6 mmol, 59 %) as a dark red oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 14.26 (0.2 H, br. s), 7.45 - 7.39 (2H, m), 7.38 - 7.32 (1H, m), 7.22 - 7.16 (2H, m), 4.68 (0.2H, br. s), 3.35 - 3.24 (5H, m), 2.09 (2H, s), 1.79 (0.7H, s); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 202.4, 166.4, 143.5, 129.9, 128.3, 127.2, 49.90 , 37.31, 30.33. The data is in agreement with that previously reported in the literature.[4]



Step 2: 1,4-Dimethylquinolin-2(1*H*)-one: Conc. sulfuric acid (10 mL) was cooled to 0 °C and *N*-methyl-3-oxo-*N*-phenylbutanamide (3 g, 15.6 mmol 1 eq.) was added dropwise, the flask was fitted with a calcium chloride guard tube and then heated to 95 °C for 2 hours. After this time, the solution was cooled to RT, poured over crushed ice and stirred for 30 minutes. The resultant precipitate was collected by filtration, washed with water and dried under vacuum to give the title compound (1.83 g, 10.5 mmol, 58 %) as a purple solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.73 (1H, dd, J = 8.1, 1.5 Hz), 7.60 (1H, ddd, *J* = 8.5, 7.1, 1.5 Hz), 7.40 (1H, d, *J* = 8.6 Hz), 7.31 - 7.25 (1H, m $CHCl_3$ overlap), 6.62 (1H, d, *J* = 1.0 Hz), 3.73 (3H, s), 2.49 (3H, d, *J* = 1.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 162.1, 146.4, 139.8, 130.4, 125.2, 121.9, 121.4, 121.2, 114.2, 29.2, 18.9. The data is in agreement with that previously reported in the literature.[5]

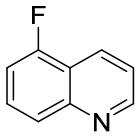


Step 3: 1,4-Dimethyl-6-nitroquinolin-2(1*H*)-one: Prepared according to general procedure C from 1,4-dimethylquinolin-2(1*H*)-one (1 g, 5.78 mmol, 1 eq.) to give the title compound (1.15 g, 5.27 mmol, 91 %) as a pale yellow solid: ^{1}H NMR: (400 MHz, DMSO-d_6): δ ppm 8.42 (1H, d, *J* = 8.3 Hz), 8.05 (1H, s), 7.96 (1H, s), 7.09 (1H, d, *J* = 8.6 Hz), 3.60 (s, 3H), 2.19 (s, 3H).



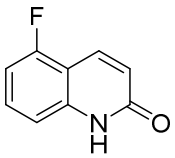
Step 4: 6-Amino-1,4-dimethylquinolin-2(1*H*)-one (47): Prepared according to general procedure D from 1,4-dimethyl-6-nitroquinolin-2(1*H*)-**one** (900 mg, 4.12 mmol) to give the title compound (603 mg, 3.2 mmol, 78 %) as a golden solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.45 (1H, d, *J* = 8.1 Hz), 6.77 (1H, s), 6.65 (1H, s), 6.60 (1H, d, *J* = 8.2 Hz), 3.69–3.60 (5H, m), 2.10 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ ppm 161.6, 145.4, 141.1, 133.2, 122.4, 121.6, 119.1, 115.5, 109.7, 29.2, 19.0.

5-Fluoro-1-methyl-6-aminoquinolin-2(1*H*)-one (48):



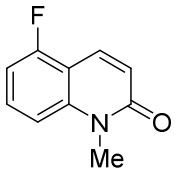
Step 1: 5-Fluoroquinoline: To a suspension of 5-aminoquinoline (2.50 g, 17.36 mmol, 1 eq.) in 48 % aqueous HBF_4 (20 mL) at 0°C was added sodium nitrite (1.32 g, 19.09 mmol, 1.1 eq.) in several portions. The resulting solution was stirred for 1 hour at 0 °C and then poured into EtOAc:Et_2O (1:1, 50 mL). The diazonium tetrafluoroborate salt was collected by filtration, washed with Et_2O (100 mL) and dried under vacuum.

The tetrafluoroborate salt was added portionwise to xylene (80 mL) heated to reflux and then stirred at reflux for an additional 2 hours before cooling to RT. The xylene was decanted off and the residue was dissolved in HCl (1 M, 50 mL). The solution was neutralized with $NaHCO_3$ and extracted with EtOAc (3 × 50 mL). The extracts were combined, dried over *anhydrous* Na_2SO_4, filtered and the solvent removed *in vacuo*. The residue was purified by column chromatography (ethyl acetate:hexane, 1:99 to 2:98) to give the title compound (970 mg, 6.50 mmol, 38 %) as a pink oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.99 (1H, dd, *J* = 4.2, 1.6 Hz), 8.47 (1H, d, *J* = 8.3 Hz), 7.95 (1H, d, *J* = 8.6 Hz), 7.67 (1H, td, *J* = 8.2, 6.1 Hz), 7.49 (1H, dd, *J* = 8.3, 4.3 Hz), 7.28 - 7.21 (1H, m); ^{13}C NMR (126 MHz, $CDCl_3$): δ 158.1 (d, *J* = 252.4 Hz), 151.2, 149.0, 129.7, 129.0 (d, *J* = 9.0 Hz), 124.8 (d, *J* = 5.1 Hz), 121.3 (d, *J* = 3 Hz), 119.0, 110.0 (d, *J* = 19.2 Hz).

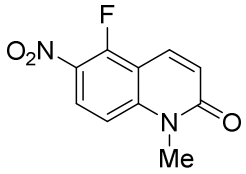


Step 2: 5-Fluoroquinolin-2(1*H*)-one: Prepared by general procedure A from 5-fluoroquinoline (950 mg, 6.46 mmol) to give the title compound (864 mg, 5.30 mmol, 82 %) as a pale pink solid: ^{1}H NMR

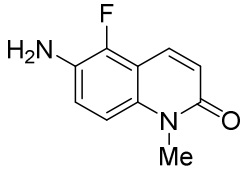
(400 MHz, DMSO-d_6) δ ppm 12.04 – 11.95 (1H, br. s.), 7.99 (1H, d, *J* = 9.6 Hz), 7.51 (1H, d, *J* = 6.3 Hz), 7.14 (1H, d, *J* = 8.3 Hz), 7.04 - 6.95 (1H, m), 6.57 (1H, d, *J* = 9.9 Hz).



Step 3: 5-Fluoro-1-methylquinolin-2(1*H*)-one: Prepared by general procedure B from 5-fluoroquinolin-2(1*H*)-one (700 mg, 4.30 mg) to give the title compound (534 mg, 3.00 mmol, 70 %) as a pale pink solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.01 (1H, d, *J* = 9.6 Hz), 7.65 (1H, td, *J* = 8.4, 6.4 Hz), 7.39 (1H, d, *J*=8.6 Hz), 7.13 (1 H, ddd, *J*=9.7, 8.2, 0.8 Hz), 6.69 (1 H, d, *J*=9.9 Hz), 3.63 (3 H, s).

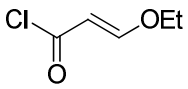


Step 4: 5-Fluoro-1-methyl-6-nitroquinolin-2(1*H*)-one: Prepared by general procedure C from 5-fluoro-1-methylquinolin-2(1*H*)-one (400 mg, 2.26 mmol). The crude solid was crystallised (acetone) to give the title compound (217 mg, 0.97 mmol, 43 %) as a yellow solid that was used without further purification.

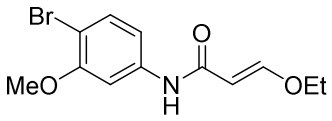


Step 5: 5-Fluoro-1-methyl-6-aminoquinolin-2(1*H*)-one (48): Prepared by general procedure D from 5-fluoro-1-methyl-6-nitroquinolin-2(1*H*)-one (100 mg, 0.45 mmol) to give the title compound (45 mg, 0.24 mmol, 54 %) as a bright yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 8.21 (1H, dd, *J* = 9.0, 5.8 Hz), 8.12 (1H, d, *J* = 9.8 Hz), 7.34 (1H, t, *J* = 8.7 Hz), 6.85 (1H, d, *J*=9.5 Hz), 3.50 (2H, br. s.), 3.29 (3H, s).

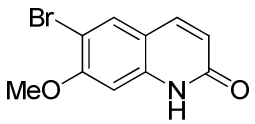
6-Amino-7-methoxy-1-methylquinolin-2(1*H*)-one (49):



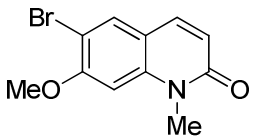
Step 1: (*E*)-3-Ethoxyacryloyl chloride: Oxalyl chloride (12.9 mL, 150 mmol, 1.5 eq.) was cooled to 0 °C and ethyl vinyl ether (9.56 mL, 100 mmol, 1 eq.) was added dropwise. The solution was allowed to warm to RT and then stirred overnight. The volatiles were removed *in vacuo* and the residue was heated under a positive pressure of argon to 110 °C to give the title compound (9.73 g, 73 mmol, 73%) as a black oil that was used without further purification.



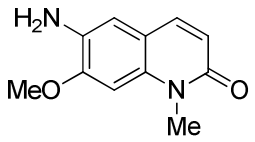
Step 2: (*E*)-*N*-(4-Bromo-3-methoxyphenyl)-3-ethoxyacrylamide: To a solution of 3-methoxy-4-bromoaniline (1.01 g, 5.00 mmol, 1 eq.) and pyridine (474 mg, 484 µL, 6 mmol, 1.2 eq.) in DCM (20 mL) was added (*E*)-3-ethoxyacryloyl chloride (432 mg, 6 mmol, 1.2 eq.). The solution was stirred for 3 hours, the solvent removed *in vacuo* and the residue purified by column chromatography (ethyl acetate:hexane, 1:9 to 6:4) to give the title compound (885 mg, 2.95 mmol, 59%) as sticky brown solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 9.86 (1H, s), 7.55–7.47 (2H, m), 7.44 (1H, d, J = 8.6 Hz), 7.11 (1H, dd, J = 8.6, 2.3 Hz), 5.51 (1H, d, J = 12.1 Hz), 3.96 (2H, q, J = 6.9 Hz), 3.83 (3H, s), 1.27 (3 H, q, J = 7.6 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 165.2, 161.7, 156.2, 138.8, 132.9, 112.6, 105.6, 104.5, 98.7, 67.6, 56.2, 14.7.



Step 3: 6-Bromo-7-methoxyquinolin-2(1*H*)-one: Conc. sulphuric acid (8 mL) was cooled to 0 °C and (*E*)-*N*-(4-bromo-3-methoxyphenyl)-3-ethoxyacrylamide (880 mg, 2.93 mmol) was added portionwise. The dark solution was allowed to stir for 20 minutes and was then poured onto ice. The resulting precipitate was collected by filteration, washed with water and dried under vacuum to afford the title compound (745 mg, 2.93 mmol, quant.) as a brown solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 7.94 (1H, s), 7.80 (1H, d, J = 9.6 Hz), 6.94 (1H, s), 6.37 (1H, d, J = 9.6 Hz), 3.89 (3H, s).

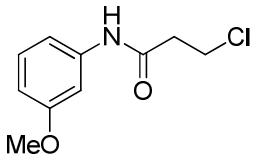


Step 4: 6-Bromo-7-methoxy-1-methylquinolin-2(1*H*)-one: Prepared by general procedure B from 6-bromo-7-methoxyquinolin-2(1*H*)-one (200 mg, 0.81 mmol, 1 eq.) Purification by column chromatography (ethyl acetate:hexane, 1:4) gave the title compound (132 mg, 0.50 mmol, 56 %) as a pale yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.00 (1H, s), 7.81 (1H, d, J = 9.6 Hz), 7.04 (1H, s), 6.49 (1H, d, J = 9.6 Hz), 4.02 (3H, s), 3.64 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 162.3, 157.6, 141.0, 137.7, 132.3, 119.5, 115.6, 105.9, 97.1, 56.5, 29.6.

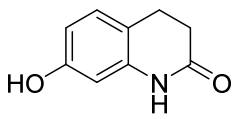


Step 5: 6-Amino-7-methoxy-1-methylquinolin-2(1*H*)-one (49): Prepared by general procedure J from 6-bromo-7-methoxy-1-methylquinolin-2(1*H*)-one (200 mg, 0.76 mmol, 1.0 eq.). The resulting residue was purified by column chromatography (hexane:acetone, 7:3 to 3:7) to give the title compound as a solution in NMP. Relevant ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.63 (1H, d, *J* = 9.3 Hz) 6.86 (2H, d, *J* = 11.4 Hz) 6.36 (1H, d, *J* = 9.3 Hz) 4.77 (2H, s) 3.95 (3H, s) 3.60 (3H, s).

6-Amino-7-ethoxy-1-methylquinolin-2(1*H*)-one (50):

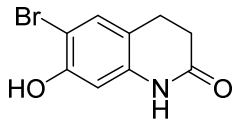


Step 1: 3-Chloro-*N*-(3-methoxyphenyl)propanamide: 3-Methoxyaniline (10.00 g, 81.3 mmol, 1 eq.) was dissolved in toluene (20 mL) and $NaHCO_3$ (10.29 g, 122 mmol, 1.5 eq.) was added. The resulting suspension was cooled to 0 °C and 3-chloropropionyl chloride (10.33 g, 81.3 mmol, 1 eq.) was added dropwise with rapid stirring. On completion, a further 10 mL of toluene was added and the suspension was heated to 50 °C for 2 hours and then poured with stirring onto HCl (3 M, 50 mL). The precipitate was filtered, washed with H_2O and toluene and then dried to give the title compound (13.70 g, 64.2mmol, 79 %) as a brown solid that was used without further purification. Analytical sample purified by column chromatography (ethyl acetate:hexane, 1:4). 200 mg of crude solid gave 187 mg of the title compound as white needles: ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.33 (1H, br. s.), 7.23 (1H, t, *J* = 2.5 Hz), 7.14 (1H, t, *J* = 8.2 Hz), 6.91 (1H, d, *J* = 7.6 Hz), 6.61 (1H, dd, *J* = 8.5, 1.9 Hz), 3.81 (2H, t, *J* = 6.4 Hz), 3.73 (3H, s), 2.74 (2H, t, *J*=6.4 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 167.7, 160.2, 139.0, 130.4, 112.1, 110.6, 106.0, 55.3, 40.6, 39.9. The data is in agreement with that previously reported in the literature.[6]

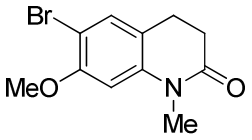


Step 2: 7-Hydroxy-3,4-dihydroquinolin-2(*1H*)-one (104): 3-Chloro-*N*-(3-methoxyphenyl)propan-amide (15.0 g, 70.2 mmol. 1.0 eq.) was dissolved in *N,N*-dimethylacetamide (12 mL) and cooled to 0 °C. $AlCl_3$ (37.4 g, 281 mmol, 4 eq.) was added portionwise and the resulting solution was fitted with a calcium chloride guard tube and heated to 150 °C for 2 hours. On completion, the reaction was allowed to cool to ~60 °C and then poured over ice (~300 g) and stirred for 20 mins. The precipitate was filtered, washed with H_2O, dried and then crystallized (EtOH) to give the title compound (6.16 g,

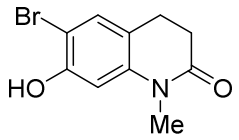
37.80 mmol, 54 %) as a pale pink solid**:** ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 9.93 (1H, br. s), 9.25 (1H, s), 6.92 (1H, d, *J* = 7.9 Hz), 6.33 (1H, d, *J* = 2.5 Hz), 6.31 (1H, dd, *J* = 8.2, 2.5 Hz), 2.73 (2H, t, *J* = 7.4 Hz), 2.39 (2H, t, *J* = 7.3 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 170.3, 156.4, 139.0, 128.1, 113.7, 108.6, 102.3, 30.8, 23.9. The above data is in agreement with that previously reported in the literature.[7]



Step 3: 6-Bromo-7-hydroxy-3,4-dihydroquinolin-2(*1H*)-one was synthesised according to the method of Hu *et al* from 7-hydroxy-3,4-dihydroquinolin-2(*1H*)-one (2.0 g, 12.3 mmol) and NBS (2.4 g, 13.5 mmol, 1.1 eq.) to give the title compound (1.71 g, 7.12 mmol, 58 %) as a colourless powder: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 10.08 (2H,app. s), 7.25 (1H, s), 6.54 (1H, s), 2.76 (2H, t, *J* = 7.6 Hz), 2.39 (2H, t, *J*= 7.6 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 170.2, 152.8, 138.5, 131.2, 115.9, 103.1, 101.0, 30.5, 23.6. The data is in agreement with that previously reported in the literature.[8]

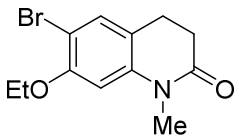


Step 4: 6-Bromo-7-methoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one: 6-Bromo-7-hydroxy-3,4-dihydroquinolin-2(*1H*)-one (1.1 g, 4.6 mmol, 1 eq.) was dissolved in dry DMF (9 mL) under argon and NaH (60% in mineral oil, 405 mg, 10.1 mmol, 2.2 eq.) was added in 1 portion with rapid stirring. After gas evolution had ceased, the solution was cooled to 0 °C and iodomethane (1.5 mL, large excess) was added dropwise. The resulting solution was stirred overnight and then poured onto ice and stirred for 20 mins. The precipitate was filtered, washed with water and crystallized (MeOH) to give the title compound (905 mg, 3.36 mmol, 73 %) as white needles: ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.34 (1H, s), 6.55 (1H, s), 3.94 (3H, s), 3.39 (3H, s), 2.85 (2H, t, *J* = 7.9 Hz), 2.66 (2H, t, *J* = 7.9 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.1, 155.2, 141.0, 131.8, 119.6, 104.4, 100.0, 56.5, 31.7, 29.7, 24.2. The above data is in agreement with that previously reported in the literature.[8]

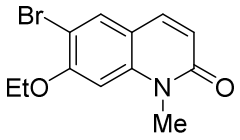


Step 5: 6-Bromo-7-hydroxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one: 6-Bromo-7-methoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (500 mg, 1.85 mmol, 1 eq.) was dissolved in dry THF (9 mL) and the resulting solution was cooled to -78 °C. BBr_3 (1.0 M in heptane, 5.56 mmol, 3 eq.) was added dropwise and the solution was then allowed to warm to RT and stirred for 3 hours. The solution was poured over ice and the precipitate collected by filtration, washed with H_2O and dried to give the title

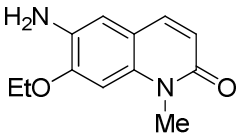
compound (287 mg, 1.12 mmol, 61 %) as yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 10.16 (1H, s), 7.31 (1H, s), 6.66 (1H, s), 3.18 (3H, s), 2.76 (2H, t, *J* = 7.4 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 169.3, 152.9, 140.6, 130.9, 118.3, 103.5, 101.5, 31.3, 28.9, 23.2.



Step 6: 6-Bromo-7-ethoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one: 6-Bromo-7-hydroxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (100 mg, 0.39 mmol, 1 eq.) was dissolved in dry DMF (2 mL) under an argon atmosphere and NaH (60 % in mineral oil, 17 mg, 0.43 mmol, 1.1 eq.) was added in one portion. Upon completion of gas evolution, iodoethane (61 mg, 0.43 mmol, 1.1 eq.) was added in one portion and the resulting solution was stirred overnight. Water (10 mL) was added and the resulting solution was extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with saturated brine (3 × 20 mL), dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to give the title compound (112 mg, 0.39 mmol, quant.) as a pale yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.31 (1H, s), 6.53 (1H, s), 4.12 (2H, q, *J* = 6.9 Hz), 2.83 (2H, t, *J* = 7.9 Hz), 2.63 (2H, t, *J* = 7.9 Hz), 1.49 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.3, 154.7, 140.9, 131.7, 119.7, 105.1, 101.6, 65.5, 31.8, 29.6, 24.2, 14.8.

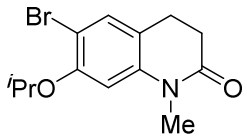


Step 7: 6-Bromo-7-ethoxy-1-methylquinolin-2(1*H*)-one: Prepared by general procedure I from 6-bromo-7-ethoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (80 mg, 0.28 mmol) to give the title compound (79 mg, 0.28 mmol, quant) as a pale yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.06 (1H, s), 7.45 (1H, d, *J* = 8.8 Hz), 6.86 (1H, dd, *J* = 8.5, 2.2 Hz), 6.80 (1H, d, *J* = 2.2 Hz), 4.17 (2H, q, *J* = 6.9 Hz), 3.78 (3H, s), 1.51 (3H, t, *J* = 6.9 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 161.4, 158.8, 141.1, 140.3, 129.5, 114.7, 113.7, 110.7, 99.4, 64.0, 31.1, 14.3.

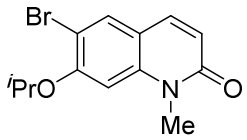


Step 8: 6-Amino-7-ethoxy-1-methylquinolin-2(1*H*)-one (50): The required amine was prepared by general procedure from **6-bromo-7-ethoxy-1-methylquinolin-2(1*H*)-one** (50 mg, 0.18 mmol). Purification of the residue by column chromatography (acetone:hexane, 3:7 to 4:6) provided the amine as a solution in NMP.

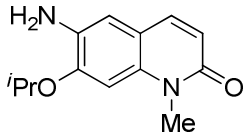
6-Amino-7-isopropoxy-1-methylquinolin-2(1*H*)-one (51):



Step 1: 6-Bromo-7-isopropoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one: Prepared as described above from 6-bromo-7-hydroxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (100 mg, 0.39 mmol) and 2-iodopropane (74 mg, 0.43 mmol, 1.1 eq.) to give the title compound (73 mg, 0.25 mmol, 63 %): ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.23 (1H, s), 6.49 (1H, s), 4.45 (1H, spt, *J* = 6.0 Hz), 3.25 (3H, s), 2.75 (2H, t, *J* = 7.9 Hz), 2.56 (2H, t, *J* = 7.9 Hz), 1.32 (6H, d, *J* = 6.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 170.2 , 153.9, 140.8, 131.8, 120.4, 107.0, 104.6, 73.3, 31.8, 29.7, 24.3, 22.1.

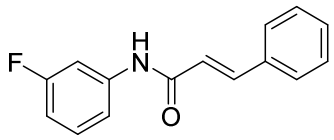


Step 2: 6-Bromo-7-isopropoxy-1-methylquinolin-2(1*H*)-one: Prepared by general procedure I from 6-bromo-7-isopropoxy-1-methyl-3,4-dihydroquinolin-2(1*H*)-one (60 mg, 0.20 mmol) to give the title compound (60 mg, 0.20 mmol, quant) as a pale yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.02 (1H, s), 7.51 (1H, d, *J* = 8.9 Hz), 6.84 (1H, dd, *J* = 8.6, 2.1 Hz), 6.80 (1H, d, *J* = 2.1 Hz), 4.45 (1H, spt, *J* = 6.0 Hz), 1.32 (6H, d, *J* = 6.0 Hz); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 161.0, 158.8, 139.6, 139.5, 130.0, 114.9, 114.6, 110.9, 99.0, 65.2, 29.8, 22.2.

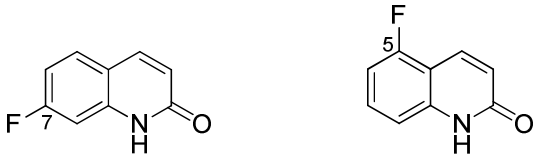


Step 3: 6-Amino-7-isopropoxy-1-methylquinolin-2(1*H*)-one (51): Prepared by general procedure J from 6-bromo-7-isopropoxy-1-methylquinolin-2(1*H*)-one (50 mg, 0.17 mmol). Purification of the residue by column chromatography (acetone:hexane, 3:7 to 4:6) provided the amine as a solution in NMP.

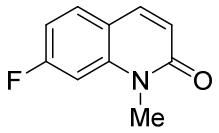
7-Fluoro-1-methyl-6-aminoquinolin-2(1*H*)-one (52):



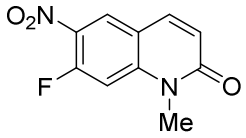
Step 1: *N*-(3-Fluorophenyl)cinnamamide: Prepared by general procedure K from 2-fluoroaniline (4.0 g, 36.03 mmol) to give the title compound (8.07 g, 33.51 mmol, 93%) as a colourless solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 10.43 (1H, s), 7.82 - 7.58 (4H, m), 7.50 - 7.32 (5H, m), 6.95 - 6.87 (1H, m), 6.83 (1H, d, *J* = 15.7 Hz).



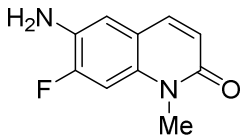
Step 2: 7-Fluoroquinolin-2(1*H*)-one: Prepared by general method L from *N*-(3-fluorophenyl)cinnamamide (5.00 g, 20.58 mmol). Product was crystallised (EtOH) to give the title compound (2.05 g, 12.6 mmol, 61 %) as a pink solid which contains 24 % 5-F isomer by ^{1}H NMR: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 11.82 (1 H, br s), 7.91 (1 H, d, *J* = 9.6 Hz), 7.73 (1 H, dd, *J* = 8.5, 6.2 Hz), 7.10 – 6.99 (2H, m), 6.46 (1H, d, *J* = 9.6 Hz). Used without further purification.



Step 3: 7-Fluoro-1-methylquinolin-2(1*H*)-one: Prepared by general procedure B from 7-fluoroquinolin-2(1*H*)-one (1.00 g, 6.13 mmol). Purified by column chromatography (acetone:hexane, 1:19 to 1:9) to give the title compound (836 mg, 4.72 mmol, 77 %) as a pale pink solid (contains 11% 5-F isomer by ^{1}H NMR): ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.91 (1H, d, *J* = 9.3 Hz), 7.79 (1H, dd, *J* = 8.6, 6.6 Hz), 7.40 (1H, dd, *J* = 11.6, 2.3 Hz), 7.14 (1H, td, *J* = 8.6, 2.5 Hz), 6.57 (1H, d, *J* = 9.6 Hz), 3.58 (3H, s). Used without further purification.

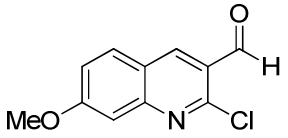


Step 4: 7-Fluoro-1-methyl-6-nitroquinolin-2(1*H*)-one: Prepared by general procedure C from 7-fluoro-1-methylquinolin-2(1*H*)-one (700 mg, 3.95 mmol). Product was crystallised (EtOH) to give the title compound (300 mg, 1.34 mmol, 34%) as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.51 (1H, d, *J* = 8.3 Hz), 7.88 (1H, d, *J* = 9.6 Hz), 7.51 (1H, d, *J* = 14.1 Hz), 6.53 (1H, d, *J* = 9.6 Hz), 3.40 (3H, s).

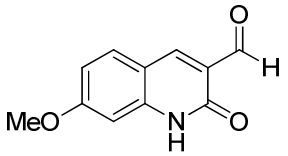


Step 5: 7-Fluoro-1-methyl-6-aminoquinolin-2(1*H*)-one (52): Prepared by general procedure D from 7-fluoro-1-methyl-6-nitroquinolin-2(1*H*)-one (100 mg, 0.45 mmol) to give the title compound (33 mg, 0.17 mmol, 39 %) as a bright yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.52 (1H, d, *J* = 9.8 Hz), 7.06 (1H, d, *J* = 12.6 Hz), 6.95 (1H, d, *J* = 9.1 Hz), 6.64 (1H, d, *J* = 9.5 Hz), 3.78 (2H, br. s.), 3.66 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 161.9, 153.8 (d, *J* = 244.7 Hz), 137.7, 133.7 (d, *J* = 10.1 Hz), 130.2 (d, *J* = 13.7 Hz), 121.1 (d, *J* = 3.7 Hz), 117.7 (d, *J* = 2.7 Hz), 114.8 (d, *J* = 5.5 Hz), 101.7 (d, *J* = 24.7 Hz), 29.6.

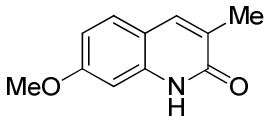
6-amino-7-methoxy-1,3-dimethylquinolin-2(1*H*)-one (53):



Step 1: 7-Methoxyquinoline-3-carbaldehyde: Prepared according to the procedure of Meth-Cohn *et al* starting from *N*-(3-methoxyphenyl)acetamide (6 g, 36.3 mmol) to give the title compound (4.82 g, 25.8 mmol, 71 %) as a pale yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 10.53 (1H, s), 8.68 (1H, s), 7.87 (1H, d, *J* = 9.1 Hz), 7.40 (1H, d, *J* = 2.5 Hz), 7.29 (2H, dd, *J* = 9.1, 2.5 Hz), 4.01 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 189.1, 164.2, 152.0, 151.1, 139.5, 130.8, 124.4, 121.8, 121.6, 106.3, 55.9. The data is in agreement with that previously reported in the literature.[9]

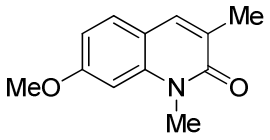


Step 2: 7-Methoxy-2-oxo-1,2-dihydroquinoline-3-carbaldehyde: 7-Methoxyquinoline-3-carbaldehyde (3.0 g, 13.5 mmol, 1 eq.) was suspended in HCl (6.0 M, 45 mL) and heated at reflux for 6 hours. The suspension was allowed to cool to RT and filtered. The precipitate was washed with H_2O and dried to give the title compound (1.76 g, 8.68 mmol, 64 %) as a brown solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 12.01 (1H, s), 8.43 (1H, s), 7.78 – 7.71 (1H, m), 7.66 (1H, d, *J* = 8.8 Hz), 6.90 - 6.82 (2H, m), 3.85 (3H, s); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 162.3, 161.1, 141.5, 141.0, 130.4, 126.8, 122.6, 113.5, 111.8, 97.5, 55.5.

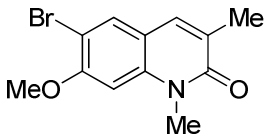


Step 3: 7-Methoxy-3-methylquinolin-2(1*H*)-one: To a solution of 7-methoxy-2-oxo-1,2-dihydroquinoline-3-carbaldehyde (1.50 g, 7.39 mmol, 1 eq.) in TFA (15 mL) at 0 °C was added triethylsilane (2.57 g, 22.2 mmol, 3 eq.) dropwise. The solution was allowed to warm to RT, stirred

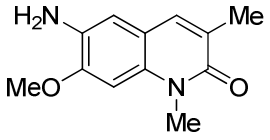
overnight and then poured onto ice (~250 g). The resulting precipitate was collected by filtration, washed with H_2O, triturated with Et_2O and dried to provide the title compound (796 mg, 4.21 mmol, 57 %) as a pale yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 11.60 (1H, br. s.), 7.68 (1H, s), 7.49 (1H, d, *J* = 8.5 Hz), 6.81 - 6.75 (2H, m), 3.79 (3H, s), 2.05 (3H, d, *J* = 0.9 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 162.7, 160.1, 139.5, 136.2, 128.3, 126.3, 113.6, 110.2, 97.9, 55.2, 16.3.



Step 4: 7-Methoxy-1,3-dimethylquinolin-2(1*H*)-one: Prepared according to general procedure B from 7-methoxy-3-methylquinolin-2(1*H*)-one (750 mg, 3.97 mmol). Purified by column chromatography (acetone:hexane, 1:9) to provide the title compound (652 mg, 3.21 mmol, 81 %) as a colourless solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.40 (1H, s), 7.33 (1H, d, *J* = 8.6 Hz), 6.77–6.67 (2H, m), 3.84 (3H, s), 3.64 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 163.3, 160.7, 140.6, 135.5, 129.1, 126.7, 115.0, 109.4, 98.5, 55.6, 29.7, 17.2.

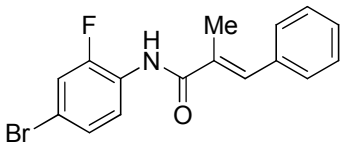


Step 5: 6-Bromo-7-methoxy-1,3-dimethylquinolin-2(1*H*)-one: 7-Methoxy-1,3-dimethylquinolin-2(1*H*)-one (812 mg, 4 mmol 1 eq.) was dissolved in DMF (4 mL) and *N*-bromosuccinimide (855 mg, 4.8 mmol, 1.2 eq.) was added in one portion. The solution was stirred overnight at RT. Cold water (30 mL) was added and the resulting precipitate was filtered off to give the title compound (826 mg, 2.93 mmol, 73 %) as a colourless solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.68 (1H, s), 7.40 (1H, s), 6.75 (1H, s), 4.03 (3H, s), 3.75 (3H, s), 2.25 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 162.9, 156.5, 139.8, 134.4, 131.7, 128.0, 115.6, 105.7, 97.0, 56.5 , 29.9, 17.5.

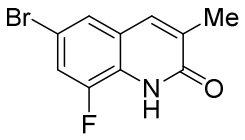


Step 6: 6-Amino-7-methoxy-1,3-dimethylquinolin-2(1*H*)-one (53): Prepared by general procedure J from 6-bromo-7-methoxy-1,3-dimethylquinolin-2(1*H*)-one (500 mg, 1.77 mmol). Purified by column chromatography (acetone:hexane, 2:8 to 6:4) to give the title compound as a solution in NMP that was used without further purification.

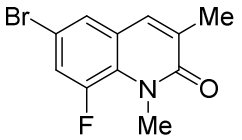
6-Amino-8-fluoro-1,3-dimethylquinolin-2(1*H*)-one (54):



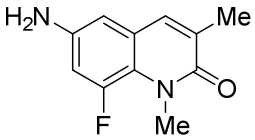
Step 1: (*E*)-*N*-(4-Bromo-2-fluorophenyl)-2-methyl-3-phenylacrylamide: Prepared by general procedure K from 2-fluoro-4-bromoaniline (2.50 g, 13.2 mmol) to give the title compound (4.32 g, 12.94 mmol, 98 %) as a colourless solid: ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 9.81 (1H, s), 7.65 - 7.55 (2H, m), 7.50 - 7.33 (7H, m), 2.12 (3H, d, *J* = 1.3 Hz); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 167.9, 155.3 (d, *J* = 253.9 Hz), 135.7, 134.1, 131.8, 129.4, 128.5, 128.0, 127.9 (d, *J* = 1.8 Hz), 127.3 (d, *J* = 3.7 Hz), 125.7 (d, *J* =11.9 Hz), 119.1 (d, *J* = 23.8 Hz), 117.1 (d, *J* = 9.2 Hz), 14.2.



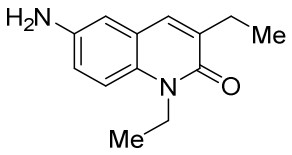
Step 2: 6-Bromo-8-fluoro-3-methylquinolin-2(1*H*)-one: Prepared by general procedure L from (*E*)-*N*-(4-bromo-2-fluorophenyl)-2-methyl-3-phenylacrylamide (3.5 g, 10.5 mmol). The title compound (443 mg, 1.73 mmol, 17 % yield) was isolated as a waxy red solid which was used without further purification in the next step: Relevant ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 11.91 (1H, br s), 7.78 (1H, t, *J* = 1.3 Hz), 7.68 (1H, t, *J* = 2.0 Hz), 7.62 (1H, dd, *J* = 10.4, 2.0 Hz), 2.11 (3H, d, *J* = 1.0 Hz).



Step 3: 6-Bromo-8-fluoro-1,3-dimethylquinolin-2(1*H*)-one: Prepared by general procedure B from 6-bromo-8-fluoro-3-methylquinolin-2(1*H*)-one (430 mg, 1.68 mmol) to give the title compound (112 mg, 0.41 mmol, 25 %) as a dark red solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 7.43 (1H, t, *J*=1.8 Hz), 7.42 - 7.40 (1H, m), 7.36 (1H, dd, *J* = 13.6, 2.3 Hz), 3.92 (3H, d, *J* = 8.1 Hz), 2.27 (3H, d, *J* = 1.0 Hz).

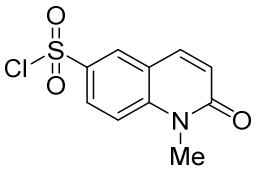


Step 4: 6-Amino-8-fluoro-1,3-dimethylquinolin-2(1*H*)-one (54): Prepared by general procedure J from 6-bromo-8-fluoro-1,3-dimethylquinolin-2(1*H*)-one (75 mg, 0.28 mmol). Purified by column chromatography (acetone:hexane, 3:7 to 4:6) to give a solution of the title compound in NMP which was used without further purification in the next step.

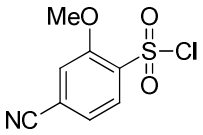


Detailed synthetic procedures and characterisation data for **1,3-diethyl-6-aminoquinolin-2(1*H*)-one (55)** have been reported previously by this group.[1]

Synthetic procedures and characterization data for sulfonyl chlorides 59 and 65:



1-Methyl-2-oxo-1,2-dihydroquinoline-6-sulfonyl chloride (59): A solution of 1-methyl-2-oxo-1,2-dihydroquinoline (**58**) (400 mg, 2.55 mmol, 1 eq.) in chlorosulfonic acid (4 mL) was heated at 95 °C for 3 hours. After completion of the reaction the solution was cooled to 0 °C and added dropwise to ice water (~100 g). [***Caution****. Very violent reaction!*]. The resulting precipitate was filtered, washed with water and dried *in vacuo* to give the title compound (503 mg, 1.96 mmol, 77 %) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.28 (1H, d, *J* = 2.0 Hz), 8.19 (1H, dd, *J* = 9.1, 2.3 Hz), 7.79 (1H, d, *J* = 9.6 Hz), 7.56 (1H, d, *J* = 9.1 Hz), 6.89 (1H, d, *J* = 9.6 Hz), 3.80 (3H, s); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 161.8, 144.2, 138.3, 137.4, 128.4, 128.3, 124.3, 120.3, 115.4, 30.0.



Synthetic procedures and characterisation data for **4-cyano-2-methoxybenzenesulfonyl chloride** (**65**) have been reported have been reported in the main Article.[10]

Biological Screening Methods:

Cloning, protein expression and purification

Cloning, protein expression and purification were performed as described previously by this group.[11-15]

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

DSF were performed as described previously by this group.[11-15]

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. 16.

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.

Fluoresence polarization competition assay

The FP assay measures the displacement of a fluorescently labelled small molecule (Aleafluor 647-JQ1 probe) from the recombinant protein. The change in polarization value in the presence of a test compound is used to determine the relative affinity of the compound for BRD4(BD1). FP assay buffer was prepared on the day of the assay by addition of 0.1% Tween-20 (v/v) and 1mM DTT to PSA/A (2.7 mM KCl; 1.8 mM KH_2PO_4; 137 mM NaCl; 10 mM $Na_2HPO_4{\bullet}H_2O$). The assay was run in black 384 well, low volume, medium bind plates (Greiner Bio-One). 10 nM of fluorescent probe was added to assay ready plates containing 40 nL compound or DMSO (predispensed with Echo 555 acoustic dispenser (Labcyte)), followed by 100 nM BRD4(BD1). The positive control in the assay was JQ-1 at a concentration of 33 µM. Final concentration of DMSO in the 12 mL assay volume was 0.3% (v/v).

Isothermal titration calorimetry (ITC)[15]

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-57 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

Gene expression markers in human alveolar macrophage

Study population:
A total of 12 subjects were assessed, including non-smokers (n=3), ex-smokers (n=5) and smokers (n=4) with normal lung function.

Preparation and activation of alveolar macrophages:
Human alveolar macrophages (AM) were derived from lung resection tissue by flushing the tissue with PBS. Cells were plated and allowed to adhere for 1 hour at 37°C in a 5% CO_2 humidified incubator in serum-free/phenol red free RPMI 1640. Total number of cells was determined by Sysmex XT-2000i Cell counter and the cells were plated in 24- well flat bottom polystyrene dishes at a density of 2x 10^6 cells/ml. After 1 hour incubation non-adherent cells were removed by washing with RPMI 1640 and adherent cells, being the AM, were kept. Cell media was changed to X-Vivo 10 (Lonza), enriched with exogenous stimuli LPS/IFNy or IL-4 and treated with DMSO or **NI-57** (**16**) (10 µM for 24 hours.

RNA extraction and RNA sequencing:

Total RNA was extracted using RNeasy Plus Mini Kit (Qiagen) and reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturers' protocol. Gene expression was subsequently determined using the Taqman Fast protocol using the according to the manufacturers' protocol. Gene expression was normalized using human GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) as a housekeeping gene and relativized to the gene expression of untreated alveolar macrophages.

Statistics:

All comparisons were tested for statistical significance using the Wilcoxon test from GraphPad Prism 6.0 software

Protein crystallization

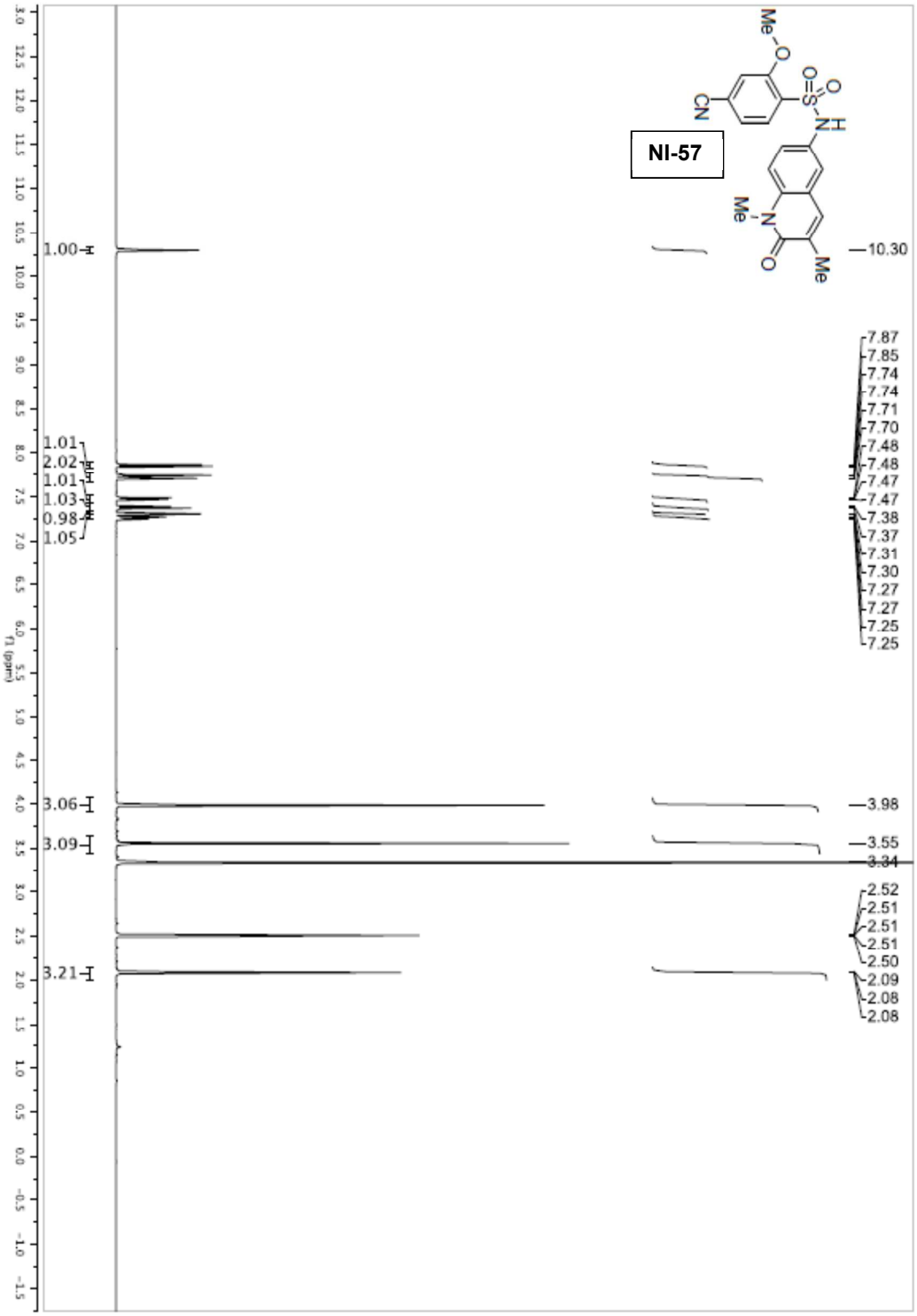
Aliquots of purified BRPF1B were set up for crystallization using a mosquito® crystallization robot (TTP Labtech, Royston UK). Coarse screens were typically setup onto Greiner 3-well plates using three different drop ratios of precipitant to protein per condition (100+50 nl, 75+75 nl and 50+100 nl). 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 **NI-57** chemical probe 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 0.1 M ammonium acetate, 17% PEG 10K, 0.1 M bis-tris pH 5.5. Diffraction quality crystals grew within a few days.

Data collection and structure solution

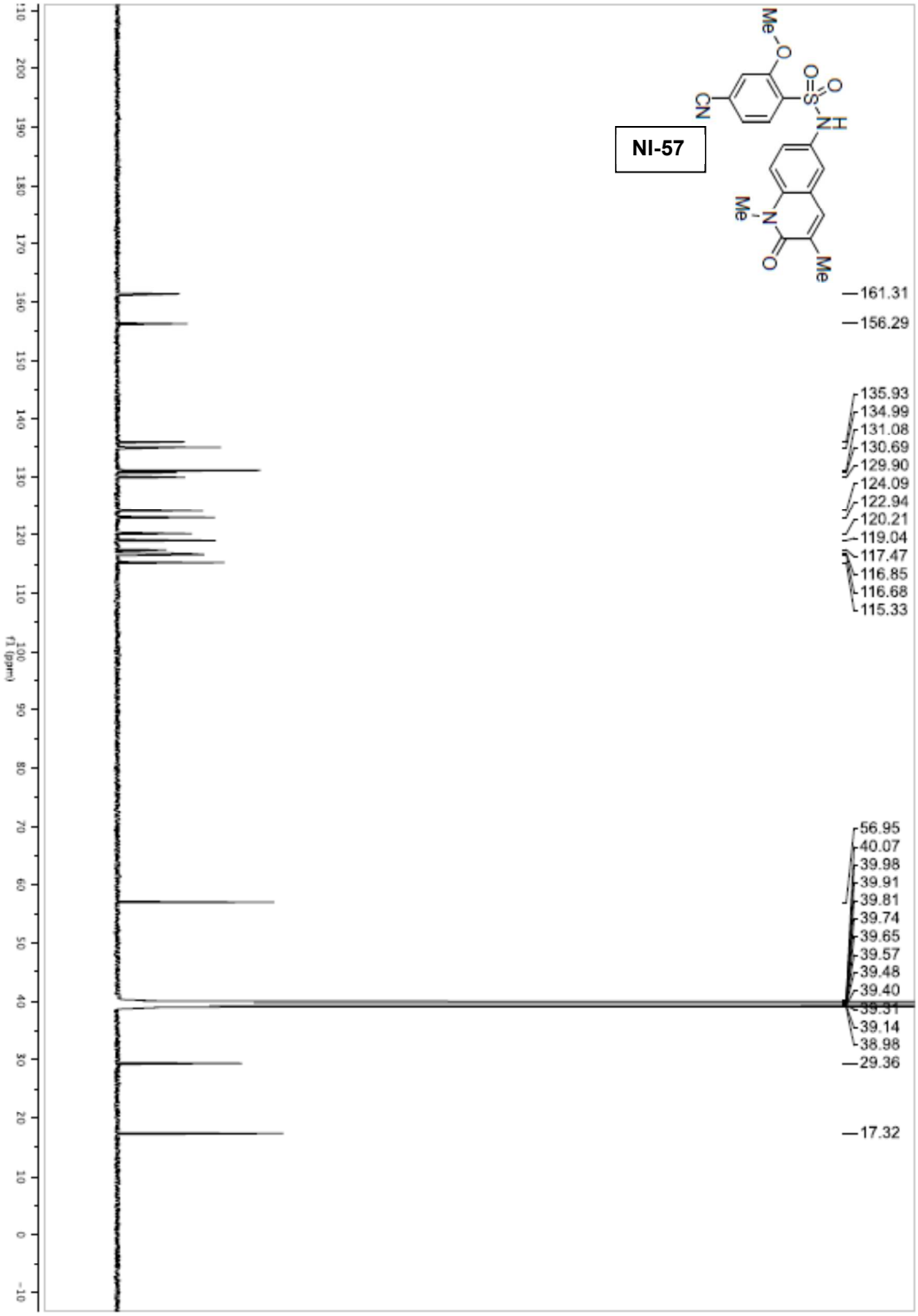
BRPF1B complex crystals were cryo-protected using the well solution supplemented with additional ethylene glycol and were flash frozen in liquid nitrogen. Data was collected at Diamond beamline I03 at a wavelength of 0.9763 Å. Indexing and integration was carried out using XDS[17] and scaling was performed with SCALA.[18] Initial phases were calculated by molecular replacement with PHASER[19] using the apo BRPF1B structure (PDB IDs 4LC2). Initial models were built by ARP/wARP[20] followed by manual building in COOT.[21] Refinement was carried out in combination of REFMAC5[22] and Phenix.[23] GRADE (global phasing)[24] was used to generate compound coordinates and .cif files. All model validations were carried out using MolProbity.[25] Data collection and refinement statistics can be found in **Supplemental Table S5**. The model and structure factors have been deposited with PDB accession code: 5MYG.pdb.

Figure S1: Spectroscopic and analytical data for 4-cyano-*N*-(1,3-dimethyl-2-oxo-1,2-dihydroquinolin-6-yl)-2-methoxybenzenesulfonamide NI-57 (16)

A: ^{1}H NMR (500MHz, DMSO-d_6) of NI-57:



B: ^{13}C NMR (125 MHz, DMSO-d_6) of NI-57:



C: HPLC of NI-57:

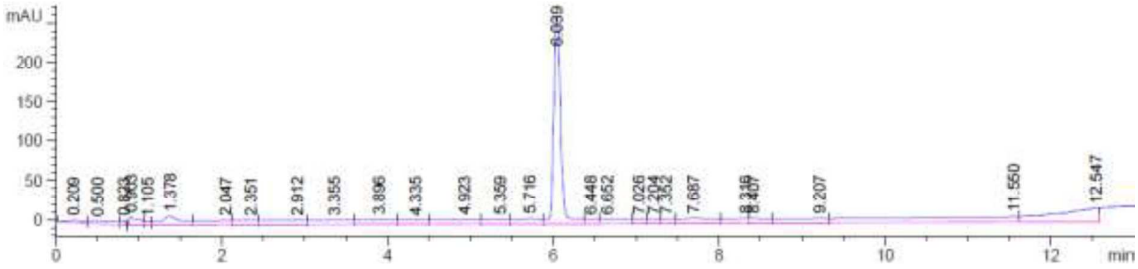
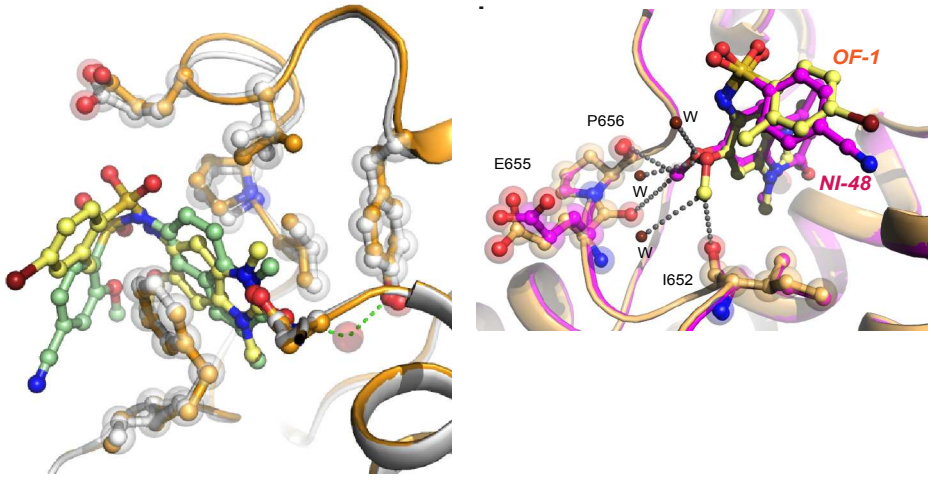


Figure S2: Comparison of NI-57 complex structure with OF-1 and NI-48 complexes.

Superposition of NI-57 and OF-1 chemical probes showed the quinolin-2-one and the 1,3-dimethyl benzimidazolone core scaffolds occupying similar chemical space for the acetyl-lysine substrate. However, as seen previously with NI-48, the benzene group of OF-1 was facing the solvent exposed area of the protein whereas for NI-57 this group was buried against the ZA-loop (Figure S2a).

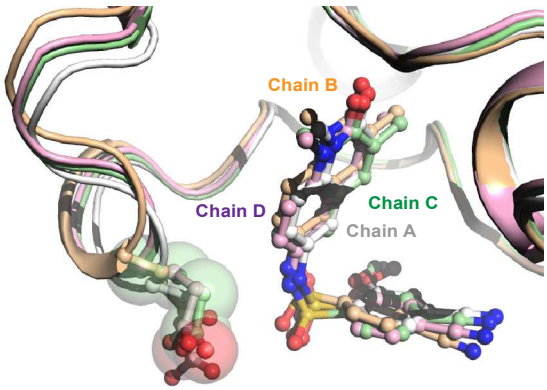


(a) Superposition of BRPF1B + NI-57 (white and green) structure with BRPF1B + OF-1 structure (orange and yellow). (b) Highlights of the 7-OMe group interaction and stability in the OF-1 and NI-48 compounds.

An interesting observation is the slightly more promiscuous poly-BRD activity of inhibitors that incorporate a 7-OMe group on the quinolin-2-one core, e.g. NI-48 and **32**. OF-1 incorporates a 6-OMe group which occupies a similar region in the protein as the 7-OMe of NI-48 (5FG4.pdb and 5T4V.pdb)(Figure S2b). These OMe groups contribute additional Van der Waals interactions and water-mediated hydrogen bonds that further stabilize the compound with residues that lie at the bottom of the binding site.

Figure S3. Asymmetric unit.

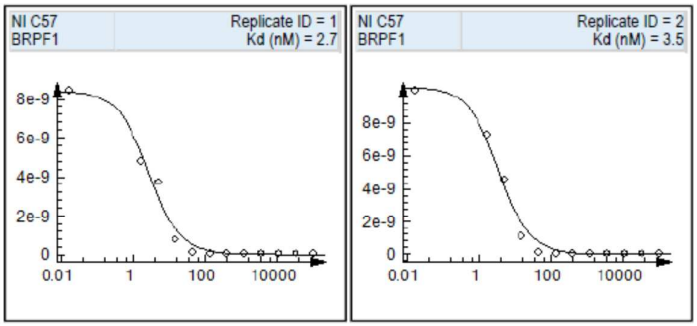
Structural insights of **NI-57** in complex to BRPF1B led us to understand the lower affinity for *N*-alkyl analogues for BRPF1. We examined all four molecules crystallized at the asymmetric unit of the orthorhombic crystal form. Overall, the NH of the sulfonamide group is facing towards the E661 residue of the ZA-loop. Distances from the NH group to the glutamic acid lipophilic side chain or the carboxylic group ranged between 3.6 – 4.3 Å (Figure S3) limiting the space to accommodate bulky substituents. Additionally, E661 adopts a closed conformation as a result of a water mediated H-bond interaction with a S(=O) of the sulfonamide group of NI-57.



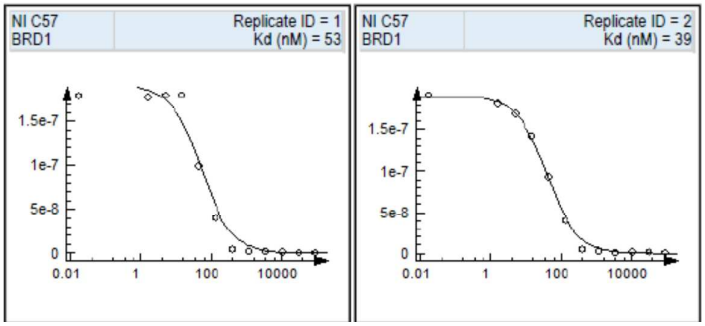
Superposition of the four molecules that form the asymmetric unit.

Figure S4: **NI-57: Concentration-activity-curves for inhibition of class IV BRDs and BRD4(BD1) activity as measured by the BROMO*scan*™ assay.**

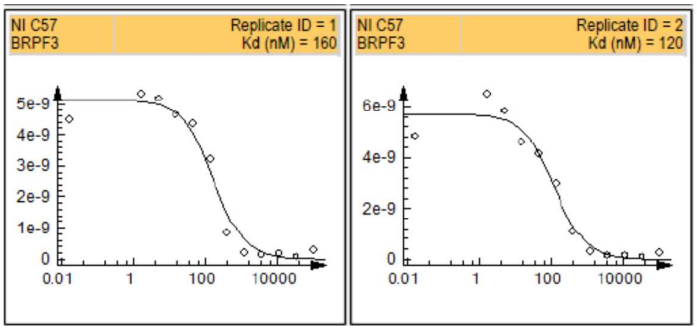
A **BRPF1B**



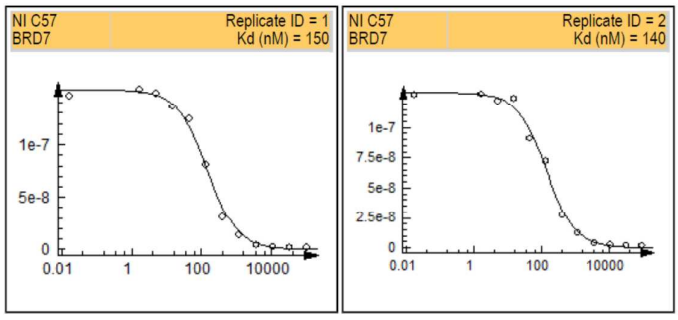
B **BRD1**



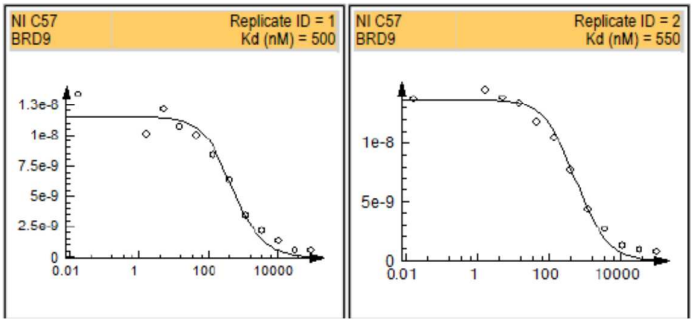
C **BRPF3**



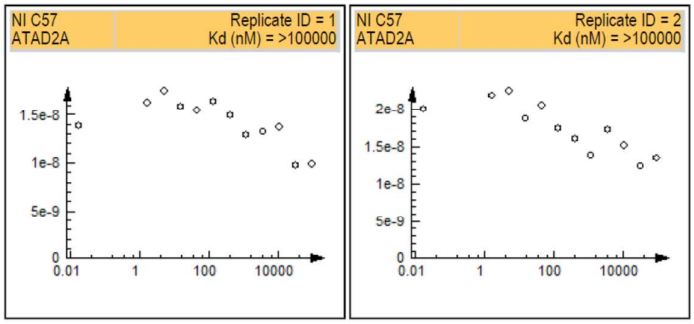
D **BRD7**



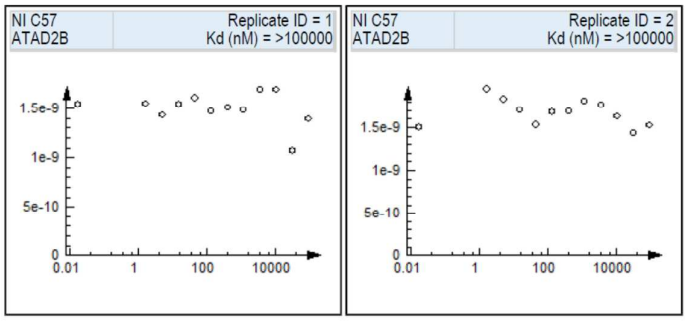
E **BRD9**



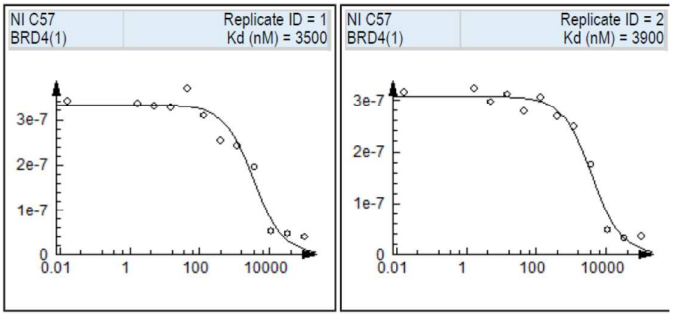
F **ATAD2A**



G **ATAD2B**



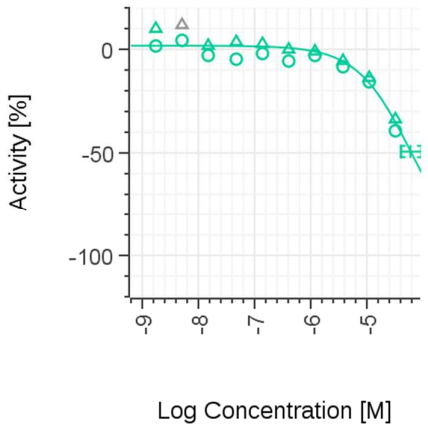
H **BRD4(BD1)**



Dose-response curve images (n = 2). 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 C57' is the laboratory notebook reference and Compound I.D. for **NI-57**.

Figure S5: **NI-57: Concentration-activity-curve for inhibition of BRD4(BD1) activity as measured by the FP competition assay.**



BRD4(BD1) IC_{50} = >33,000 nM

Table S1: **DSF Temperature shift data, ΔT_m (°C), for compounds 16 (NI-57) and 32 against a panel of 47 human bromodomain proteins at 10 μM (n = 2).**[a]

protein	Compound 16		Compound 32	
	ΔT_m Shift (°C)	standard deviation (°C)	ΔT_m Shift (°C)	standard deviation (°C)
ASH1L	-0.5	0.9	0.8	0.5
ATAD2	-0.5	0.1	0.2	0.4
BAZ1A	-0.3	0.9	1.1	1.3
BAZ1B	0.0	0.5	1.0	0.1
BAZ2A	-0.4	0.2	-0.3	0.0
BAZ2B	0.0	0.1	0.2	0.1
BRD1	5.6	0.1	6.0	0.3
BRD2(1)	0.4	0.3	2.2	0.4
BRD2(2)	0.0	0.1	0.5	0.2
BRD3(1)	0.8	0.1	2.3	0.1
BRD3(2)	0.2	0.0	0.9	0.2
BRD4(1)	0.8	0.6	2.6	0.1
BRD4(2)	-0.1	0.3	0.3	0.1
BRD9	3.1	0.7	6.5	0.8
BRDT(1)	0.2	0.1	1.1	0.5
BRDT(2)	0.2	0.2	1.0	0.0
BRPF1A	0.9	0.4	0.6	0.0
BRPF1B	10.7	0.3	10.9	0.4
BRPF3	5.3	0.3	5.6	0.8
BRWD3(2)	1.5	0.8	0.7	1.9
CECR2	0.3	0.2	0.6	0.0
CREBBP	1.1	0.2	2.2	0.1
EP300	1.1	0.1	2.2	0.1
FALZ	0.3	0.0	1.9	0.3
GCN5L2	0.3	0.2	1.0	0.5
ATAD2B	-0.1	0.1	0.4	0.1
SP140L	0.5	1.2	1.5	0.9
MLL	0.3	0.2	1.0	0.3
PB1(1)	0.5	0.4	0.9	1.0
PB1(2)	0.4	0.2	0.7	0.1
PB1(3)	0.1	0.3	0.3	0.4
PB1(4)	0.6	0.3	1.0	0.0
PB1(5)	0.3	0.3	0.8	0.2
PB1(6)	0.3	0.0	0.6	0.4
PCAF	0.5	0.0	0.9	0.2
PHIP(2)	-0.4	2.6	0.2	1.3
SMARCA2	0.5	0.2	0.3	0.1
SMARCA4	0.2	0.2	0.6	0.4

SP140	0.9	0.2	1.5	0.2
TAF1(2)	0.5	0.1	0.7	0.0
TAF1(1)	0.4	0.0	0.6	0.2
TAF1L(1)	0.7	0.1	1.1	0.3
TAF1L(2)	0.7	0.1	1.7	0.2
TIF1-bromo[b]	2.0	0.0	2.5	1.1
TIF1-phd-bromo	1.6	0.1	1.5	0.1
TRIM28	0.8	0.6	0.3	0.2
WDR9(2)	0.7	0.0	0.8	1.1

[a] The values in Table S1 are slightly lower than the values reported in Table 3 as a slightly modified experimental procedure was employed where compound was added to the protein.

[b] TIF1 is also known as TRIM24

Table S2: **BROMO*scan* data for NI-57 against a panel of 40 BRDs.**

Target	NIC57
Gene Symbol	Kd (nM)
ATAD2A	>50000
ATAD2B	>50000
BAZ2A	>50000
BAZ2B	9400
BRD1	46
BRD2(1)	5900
BRD2(1,2)	12000
BRD2(2)	>50000
BRD3(1)	7200
BRD3(1,2)	12000
BRD3(2)	>50000
BRD4(1)	3300
BRD4(1,2)	15000
BRD4(2)	>50000
BRD4(full-length,short-iso.)	4900
BRD7	100
BRD8(1)	>50000
BRD8(2)	>50000
BRD9	160
BRDT(1)	4800
BRDT(1,2)	>50000
BRDT(2)	>50000
BRPF1	4
BRPF3	110
CECR2	12000
CREBBP	13000
EP300	15000
FALZ	2300
GCN5L2	4100
PBRM1(2)	>50000
PBRM1(5)	11000
PCAF	4200
SMARCA2	>50000
SMARCA4	41000
TAF1(2)	4900
TAF1L(2)	5500
TRIM24(Bromo.)	1600
TRIM24(PHD,Bromo.)	5600
TRIM33(PHD,Bromo.)	>50000
WDR9(2)	>50000

Separate experiments (mean of n=2) to the data presented in Figure S2.

Table S3: IonChannelProfiler™ data for NI-57 (Eurofins Discovery Services, St. Charles MO, USA).

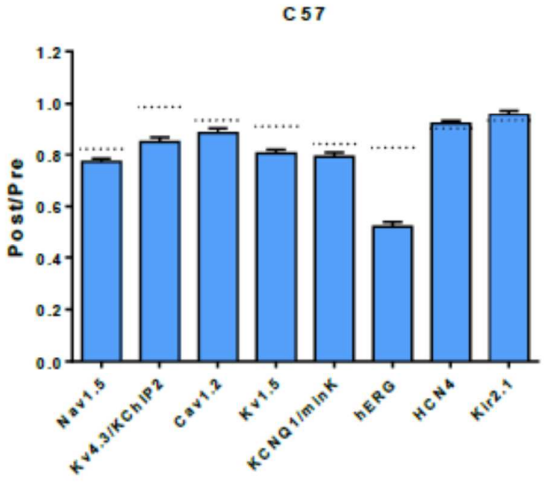
Eurofins Discovery services CardiacProfiler™ Panel:

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

Electrophysiological assays conducted on **NI-57** for activities at 30 μM on the ion channels specified below using the IonWorks Quattro electrophysiological platform.

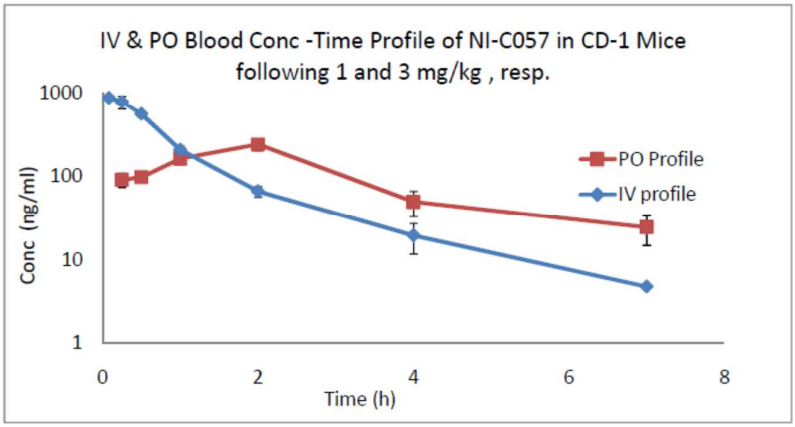
Channel	Normalized Percentage Inhibition at Test Concentration 30 μM	SE	N
Nav1.5	6.2	1.3	11
Kv4.3/KChIP2	14.9	1.8	12
Cav1.2	6.4	1.9	12
hKv1.5	11.9	1.4	12
KCNQ1/minK	6.7	1.8	12
hERG	37.0	1.9	12
HCN4	-2.2	1.1	12
Kir2.1	-2.9	1.6	12

The effects of **NI-57** 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).



'C57' is the laboratory notebook reference and Compound I.D. for **NI-57** in this experiment.

Table S4: NI-57: Methods and results for mouse i.v. and p.o. PK determination.



'NI-C057' is the laboratory notebook reference and Compound I.D. for **NI-57**

Methods: NI-57 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-57** 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-57 is a low clearance compound (21 mL/min/kg) with a moderate volume of distribution of 1.3 L/kg resulting in a moderate half-life of 1.2 hour in the female mouse following a 1 mg/kg solution IV dose. The intrinsic clearance of **NI-57** in mouse liver microsomes was 0.2 mL/min/g liver (4 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-57** was 29% with a C_{max} of 0.63 µM and an $AUC_{0\text{-}t}$ of 651 ng.h/mL. The oral half-life was 1.6 h. Based on blood clearance and oral bioavailability, the fraction absorbed was calculated as 35%.

Table S4A: Mouse pharmacokinetic data for **NI-57:**

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)	2.3
Cl_b (mL/min/kg)	21
V_{ss} (L/kg)	1.3
$T_{1/2}$ (h)	1.2
MRT (h)	1.0
Route	p.o. suspension
Target dose	3 mg/kg
Formulation	1% w/v methylcellulose in water
C_{max} (µM)	0.63
T_{max} (h)	2.0
$T_{1/2}$ (h)	1.6

$AUC_{0\text{-}t}$ (ng.h/mL)	651
$F_{po\ (0\text{-}\infty)}$ (%)	29

Table S4B: Summary of physicochemical properties and *in vitro* ADME profile of **NI-57.**

Physicochemical properties	
mw	383
ClogP	2.1
tPSA ($Å^2$)	99
ADME profile	
HLM, Cl_{int} (mL/min/g liver)	1.2
MLM, Cl_{int} (mL/min/g liver)	0.2
CYP1A2, IC_{50} (μM)	>25
CYP2C9, IC_{50} (μM)	4.9
CYP2C19, IC_{50} (μM)	>25
CYP2D6,IC_{50} (μM)	>25
CYP3A4, IC_{50} (μM)	3.6
Aqueous solubility	moderate (100 μM min)[a]
Caco2 transit AB/BA, P_{app} ($x10^{-6}$ cms^{-1})	11/52

[a] Maximum concentration used in screening assays created by serial dilution of a 100 mM stock solution of **NI-57** in DMSO.

NI-57 has good metabolic stability in HLM consistent with predicted low clearance, weak CYP450 enzyme inhibition and good cell permeability albeit with some evidence of efflux.

Table S5: Co-crystal structure determination of BRPF1B with NI-57. Data collection and refinement statistics

Data Collection	
PDB ID	5MYG.pdb
Protein	BRP1B
Ligand number	**16**
Ligand name	NI-57
Space group	$P2\,2_1\,2_1$
Cell dimensions: a, b, c (Å)	37.14 123.72 137.07
α, β, γ (deg)	90.00 90.00 90.00
Resolution* (Å)	28.88 (2.30)
Unique observations*	28990 (4116)
Completeness* (%)	99.6 (99.4)
Redundancy*	6.3 (6.3)
Rmerge*	0.116 (0.678)
I/ σI*	11.4 (3.1)
Refinement	
Resolution (Å)	2.3
R_{work} / R_{free} (%)	24.7 / 30.7
Number of atoms (protein/other/water)	3748 / 108 / 75
B-factors (Å^2) (protein/other/water)	41.68 / 50.34 / 35.91
r.m.s.d bonds (Å)	0.010
r.m.s.d angles (^{o})	1.344
Ramachadran Favoured (%)	97.98
Allowed (%)	2.02
Disallowed (%)	0.00

* Values in parentheses correspond to the highest resolution shell.

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