Supplementary Material for Organic & Biomolecular Chemistry

Synthetic chemistry-led creation of a difluorinated biaryl ether non-nucleoside reverse transcriptase inhibitor

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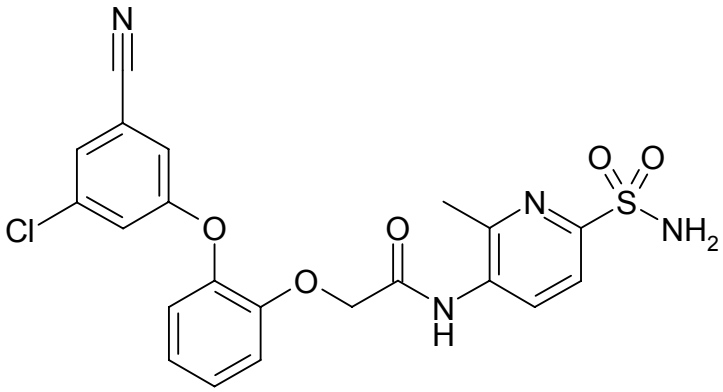
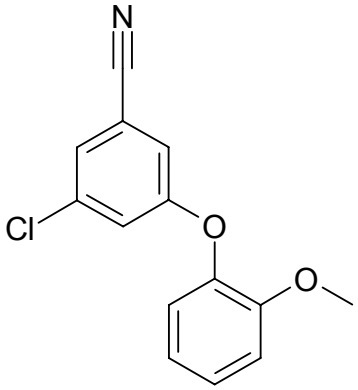
General. Melting points were determined on a Gallenkamp melting point apparatus using glass capillary tubes and are uncorrected. Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere, using commercially available anhydrous solvents. Thin-layer chromatography was performed on glass-backed pre-coated Merck silica gel (60 F254) plates, and FCC (flash column chromatography) was carried out using 40-63 μm silica gel. NMR spectra were carried out on a Varian Mercury 400, or Varian Inova 500 spectrometer in the solvents specified. Mass spectra were recorded on a Waters Micromass ZQ using atmospheric pressure chemical ionization (APCI). Combustion analyses were conducted by Exeter Analytical UK, Ltd, Uxbridge, Middlesex. Other abbreviations are used in conjunction with standard chemical practice.

2-[2-(3-Chloro-5-cyano-phenoxy)-phenoxy]-N-(2-methyl-6-sulfamoyl-pyridin-3-yl)-acetamide (1)

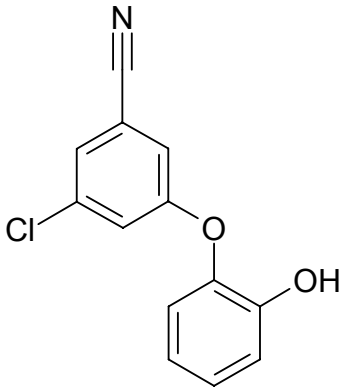


Step A
3-Chloro-5-(2-methoxy-phenoxy)-benzonitrile **(6)**



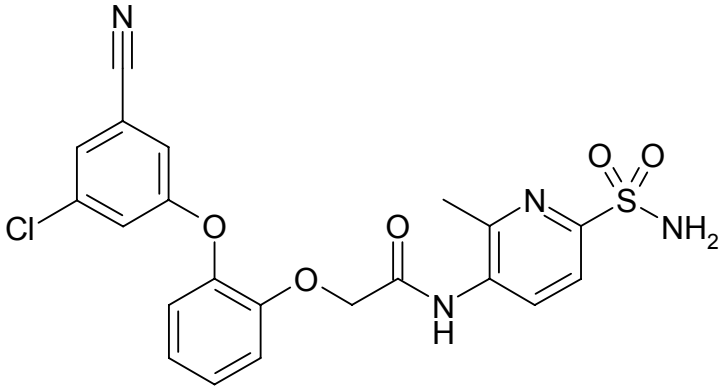
2-methoxy-phenol **(4)** (0.37mL, 3.4mmol) and 3-Chloro-5-fluoro-benzonitrile (500mg, 3.2mmol) were taken up in DMF (5mL) and Cs_2CO_3 (1.3g, 4.0mmol) added. Resultant solution was then heated to 80°C for 6 hours. After which reaction mixture was partitioned between water (50mL) and Ethyl acetate (2x50mL), combined organic phases were then washed with additional water (50mL) and brine (50mL) then dried ($MgSO_4$) and concentrated prior to purification by column chromatography (1:0 —>2:1, Pentane:EtOAc) to give title compound as a white solid (230mg, 28% yield): mp 90-92°C; (Found C, 64.4; H, 3.8. calc. for $C_{14}H_8ClF_2NO_2$: C, 64.8; H, 3.9) δ_H(400MHz, $CDCl_3$) 3.80 (3H, s), 6.99 (1H, m), 7.01 - 7.08 (3H, m), 7.12 (1H, m), 7.28 (2H, m); *m/z* (APCI) 259 $(M+H)^+$.

Step B
3-Chloro-5-(2-hydroxy-phenoxy)-benzonitrile **(8)**



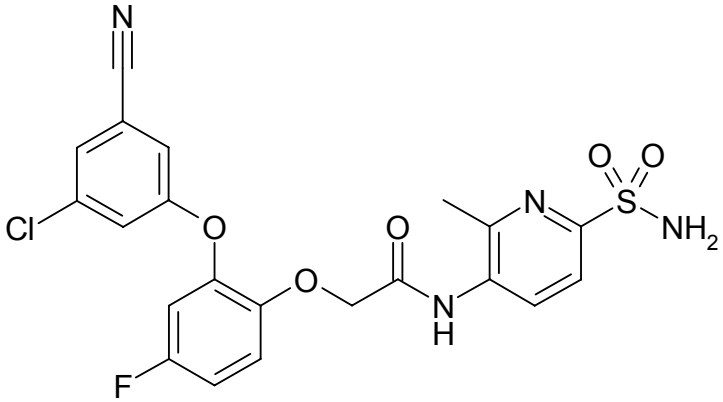
6 (245mg, 0.99mmol) was taken up in DCM (5mL) and cooled on ice prior to the dropwise addition of boron tribromide (1M in DCM, 4.7mL, 4.7mmol). Solution was then allowed to warm to room temperature and stirred for 4 hours. Reaction mixture was poured onto ice (~50mL) and extracted into DCM (2 x 100mL), combined organics were washed with brine (~50mL) then dried ($MgSO_4$) and concentrated to give title compound as a off white solid which was taken on crude to the next step (220mg, 95% yield): δ_H(400MHz, $CDCl_3$), 5.37 (1H, bs), 6.95 (2H, m), 7.08 (1H, d), 7.13 (1H, m), 7.17 (1H, m), 7.22 (1H, t), 7.35 (1H, t); *m/z* (APCI) 244 $(M-H)^-$.

Step C
2-[2-(3-Chloro-5-cyano-phenoxy)-phenoxy]-N-(2-methyl-6-sulfamoyl-pyridin-3-yl)-acetamide **(1)**



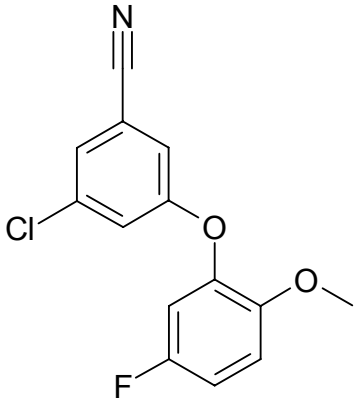
Phenol **(8)**, (220mg, 0.9mmol) was dissolved in DMF (2mL) and potassium carbonate (148mg, 1.1mmol), sodium iodide (165mg, 1.1mmol) and chloride **(10)** (354mg, 1.1mmol) added. Resulting solution was then stirred at 40°C for 18hours. Reaction mixture was then concentrated under vacuum. Crude material was suspended in water and extracted into 5% methanol in DCM (3x50mL), combined organics were then washed with brine (50mL), dried ($MgSO_4$), concentrated and crude material purified by column chromatography (90:10:1, DCM:MeOH:NH_3), this material was then recrystallised from H_2O/MeOH to give title compound as a white solid (90mg, 21% yield): mp 220-222°C; (Found C, 52.8; H, 3.6. calc. for $C_{21}H_{15}ClF_2N4O_5S.0.25H_2O$: C, 52.8; H, 3.7) δ_H(400MHz, DMSO-d6) 2.40 (3H, s), 4.87 (2H, s), 7.07 (1H, td), 7.21 (2H, m), 7.30 (1H, td), 7.33 (1H, t), 7.37 (3H, m), 7.69 (1H, t), 7.74 (1H, d), 8.13 (1H, d), 9.71 (1H, bs); *m/z* (APCI) 473 $(M+H)^+$.

2-[2-(3-Chloro-5-cyano-phenoxy)-4-fluoro-phenoxy]-N-(2-methyl-6-sulfamoyl-pyridin-3-yl)-acetamide (2)



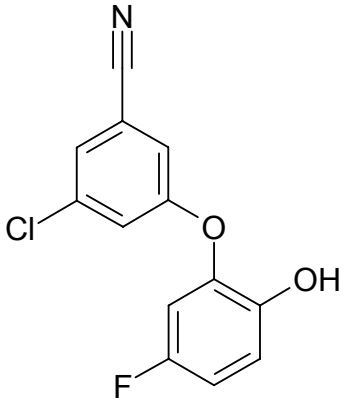
Step A

3-Chloro-5-(5-fluoro-2-methoxy-phenoxy)-benzonitrile **(7)**



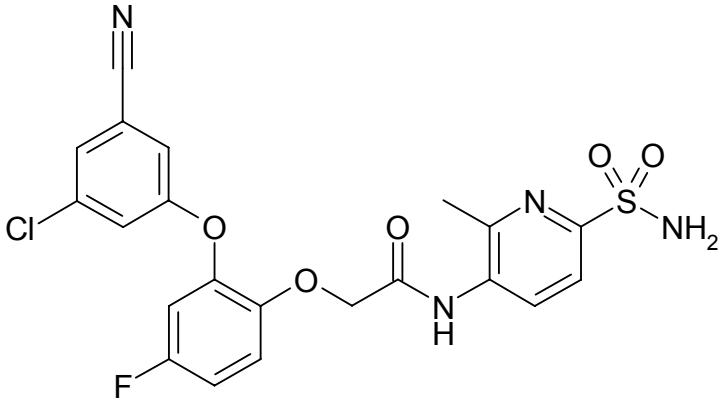
5-fluoro-2-methoxy-phenol **(6)** (4.5g, 31.66mmol) was taken up in DMF (50mL) and Cs_2CO_3 (13.4g, 41.16mmol) added, solution was stirred for 10mins and then 3-Chloro-5-fluoro-benzonitrile (6.4g, 41.16mmol) added. Resultant solution was heated to 80^{o}C for 18 hours, after which reaction mixture was diluted with ethyl acetate (200mL) and washed with brine (3x 200mL), organic phase was then dried ($MgSO_4$) and concentrated prior to purification by column chromatography (70:30, Pentane:EtOAc) to give title compound as a white solid (9g, 100% yield) δ_H(400MHz, CDOD) 3.78 (3H, s), 6.85 (1H, m), 6.98 (1H, m), 7.25-7.30 (2H, m), 7.36 (1H, m), 7.46 (1H, s); *m/z* (APCI) 278 $(M+H)^+$.

Step B
3-Chloro-5-(5-fluoro-2-hydroxy-phenoxy)-benzonitrile **(9)**



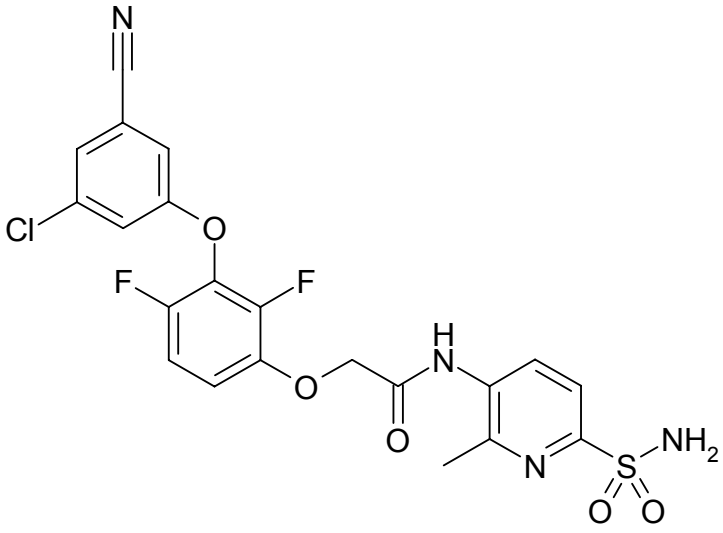
7 (9g, 31.66mmol) was taken up in DCM (50mL) and cooled to -78^{o}C prior to the dropwise addition of Boron tribromide (5mL, 53.82mmol) over 10 mins. Solution was then allowed to warm to room temperature and stirred for 18 hours. Reaction mixture was poured onto ice (~150mL) and extracted into DCM (3 x 150mL), combined organics were then dried ($MgSO_4$) and concentrated prior to purification by column chromatography (90:10, Pentane:EtOAc) to give title compound as a white solid (6.6g, 78% yield): δ_H(400MHz, $CDCl_3$), 6.70 (1H, m), 6.89 (1H, m), 7.03 (1H, m), 7.15 (1H, m), 7.23 (1H, m) 7.37 (1H,m), 7.46 (1H, m); *m/z* (APCI) 264 $(M+H)^+$.

Step C
2-[2-(3-Chloro-5-cyano-phenoxy)-4-fluoro-phenoxy]-N-(2-methyl-6-sulfamoyl-pyridin-3-yl)-acetamide **(2)**

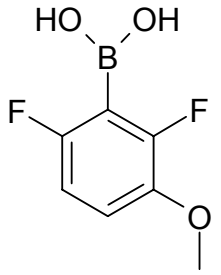


Phenol **(9)**, (650mg, 2.41mmol), chloride **(10)** (900mg, 3.61mmol), sodium iodide (443mg, 2.9mmol) and potassium carbonate (400mg, 2.9mmol) were combined in DMF (2mL) and stirred at 40°C for 18hours. Reaction mixture was then concentrated under vacuum and crude material purified by column chromatography (90:10:1, DCM:MeOH:NH_3) to give title compound as a white solid (600mg, 50% yield): (Found C, 51.01; H, 4.0. calc. for $C_{21}H_{15}ClF_2N4O_5S$: C, 50.8; H, 4.0); δ_H(400MHz, DMSO-d6) 2.40 (3H, s), 4.83 (2H, s), 7.15 (1H, m), 7.25 (2H, m), 7.37 (2H, m), 7.40 (1H, m), 7.42 (1H, s), 7.60 (1H, s), 7.75 (1H, s), 8.09 (1H, d), 9.70(1H, bs); *m/z* (APCI) 491 $(M+H)^+$.

2-[3-(3-Chloro-5-cyano-phenoxy)-2,4-difluoro-phenoxy]-N-(2-methyl-6-sulfamoyl-pyridin-3-yl)-acetamide (20)

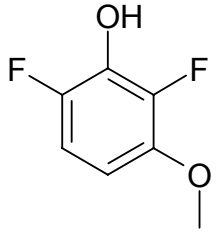


Step A
2,6-Difluoro-3-methoxy-phenylboronic acid **(18)**



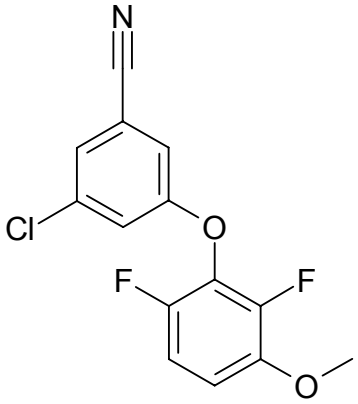
1-Bromo-3,5-difluoro-2-methoxy-benzene **(17)** (5g, 22.4mmol) was dissolved in THF (50mL) and cooled to -78°C, prior to the dropwise addition of n-BuLi (2.5M in THF, 9mL, 22.4mmol). Solution was stirred for 20mins then triisopropyl borate (6g, 31.6mmol) added and resultant solution maintained at -78°C for 1 hour. HCl (2M, 30mL) was then added and solution allowed to warm to room temperature. Product was extracted with EtOAc (200mL), washed with brine (200mL), dried ($MgSO_4$) and concentrated. Crude material was by purified by column chromatography (1:1, Pentane:EtOAc) to give title compound as an off white solid which was taken on directly to the next step(1.8g, 43% yield).

Step B
2,6-Difluoro-3-methoxy-phenol **(19)**



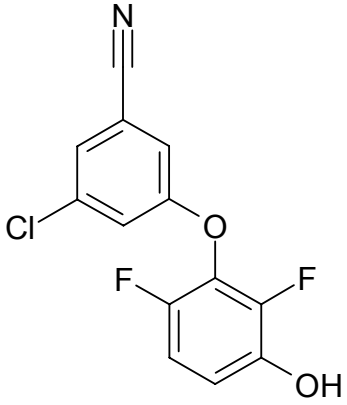
Boronic acid **(18)** (1.8g, 9.6mmol) was dissolved in THF (30mL) and acetic acid (1mL) and hydrogen peroxide (30%, 1.3mL) added, solution was then stirred for 1 week. Sodium metabisulfite. aq (100mL) was added and product extracted into EtOAc (100mL), organics were then washed with brine (50mL), water (50mL), dried ($MgSO_4$) and concentrated. Crude material was purified by column chromatography (2:1, Pentane:EtOAc) to give title compound as a white solid (900mg, 59% yield): δ_H(400MHz, $CDCl_3$) 3.78 (3H, s), 6.56 (1H, dd), 6.92 (1H, dd); *m/z* (APCI) 159 $(M)^-$. COSY attached as Supplementary Information. The COSY data show two mutually coupled C-Hs which indicates that the protons are in an ortho configuration. The fluorine coupling also suggests the presence of a proton with two meta couplings, 6.56ppm (9Hz and 4.8Hz) and a proton with an ortho and para coupling, 6.92ppm (11Hz and 2.4Hz).

Step C
3-Chloro-5-(2,6-difluoro-3-methoxy-phenoxy)-benzonitrile



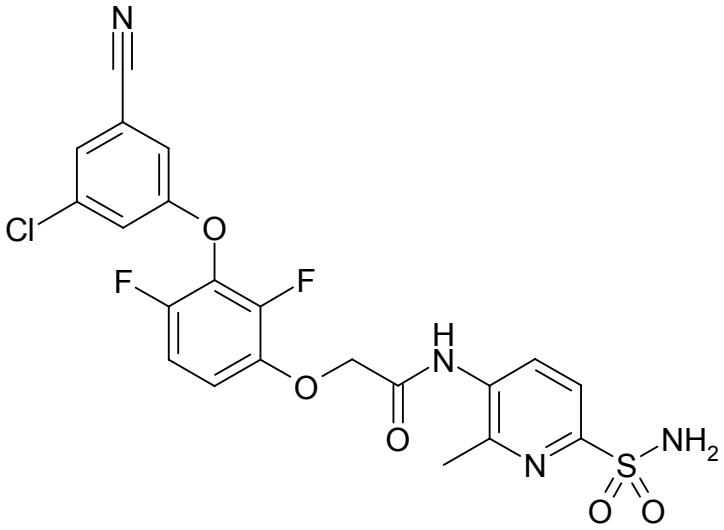
2,6-Difluoro-3-methoxy-phenol **(19)** (200mg, 1.24mmol) was taken up in DMF (2mL) and Cs_2CO_3 (406mg, 1.24mmol) added, solution was stirred for 10mins and then 3-Chloro-5-fluoro-benzonitrile (252mg, 1.6mmol) added. Resultant solution was then heated to reflux for 18 hours, after which reaction mixture was partitioned between citric acid (25mL) and Ethyl acetate (25mL), organic phase was then dried ($MgSO_4$) and concentrated prior to purification by column chromatography (4:1, Pentane:EtOAc) to give title compound as a white solid (125.9mg, 34% yield): mp 118-120°C; (Found C, 56.7; H, 2.7. calc. for $C_{14}H_8ClF_2NO_2$: C, 56.9; H, 2.7) δ_H(400MHz, $CDCl_3$) 3.92 (3H, s), 6.68 (1H, m), 6.98 (1H, m), 7.08 (1H, s), 7.20 (1H, s), 7.36 (1H, s); *m/z* (APCI) 295 $(M+H)^+$.

Step D
3-Chloro-5-(2,6-difluoro-3-hydroxy-phenoxy)-benzonitrile



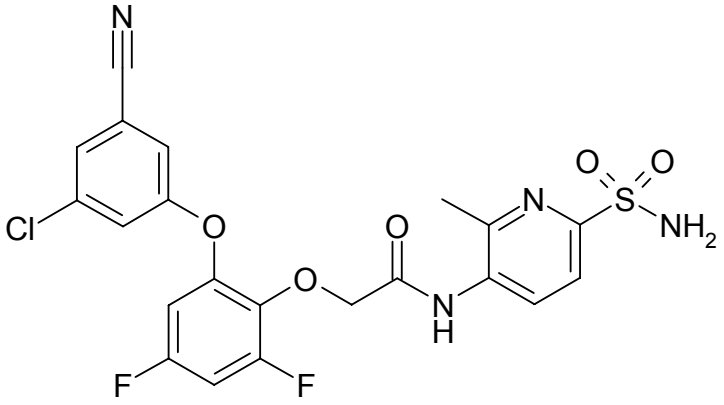
3-Chloro-5-(2,6-difluoro-3-methoxy-phenoxy)-benzonitrile (120mg, 0.4mmol from step C) was taken up in DCM (2.7mL) and cooled on ice prior to the dropwise addition of boron tribromide (1M in DCM, 2mL, 2mmol). Solution was then allowed to warm to room temperature and stirred for 18 hours. Reaction mixture was poured onto ice (~15mL) and extracted into DCM (3 x 15mL), combined organics were then dried ($MgSO_4$) and concentrated prior to purification by column chromatography (10:1, Pentane:EtOAc) to give title compound as a white solid (57mg, 50% yield): δ_H(400MHz, $CDCl_3$), 5.20 (1H, s), 6.90 (2H, m), 7.10 (1H, s), 7.20 (1H, s), 7.38 (1H, s); *m/z* (APCI) 280 $(M-H)^-$.

Step E
2-[3-(3-Chloro-5-cyano-phenoxy)-2,4-difluoro-phenoxy]-N-(2-methyl-6-sulfamoyl-pyridin-3-yl)-acetamide **(20)**

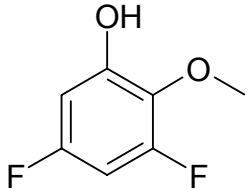


3-Chloro-5-(2,6-difluoro-3-hydroxy-phenoxy)-benzonitrile (57mg, 0.2mmol from step D), chloride **(10)** (80mg, 0.3mmol), sodium iodide (36mg, 0.24mmol) and potassium carbonate (34mg, 0.24mmol) were combined in DMF (2mL) and stirred at 40°C for 18hours. Reaction mixture was then concentrated under vacuum and crude material purified by column chromatography (90:10:1, DCM:MeOH:NH_3) to give title compound as a white solid (87mg, 84% yield): mp 198-201°C; (Found C, 49.4; H, 3.0. calc. for $C_{21}H_{15}ClF_2N4O_5S$: C, 49.6; H, 3.0); δ_H(400MHz, DMSO) 2.48 (3H, s), 4.97 (2H, s), 7.20 (1H, m), 7.25-7.40 (3H, bm), 7.53 (1H, s), 7.61 (1H, s), 7.77 (1H, d), 7.82 (1H, s), 8.14 (1H, d); *m/z* (APCI) 509 $(M+H)^+$.

2-[2-(3-Chloro-5-cyano-phenoxy)-4,6-difluoro-phenoxy]-N-(2-methyl-6-sulfamoyl-pyridin-3-yl)-acetamide (3)

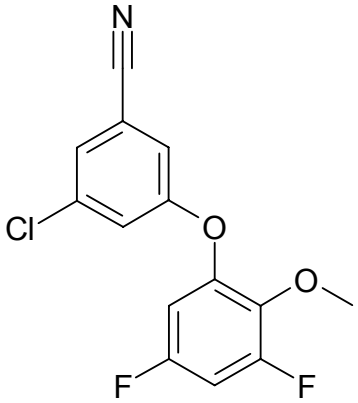


Step A
3,5-Difluoro-2-methoxy-phenol **(12)**



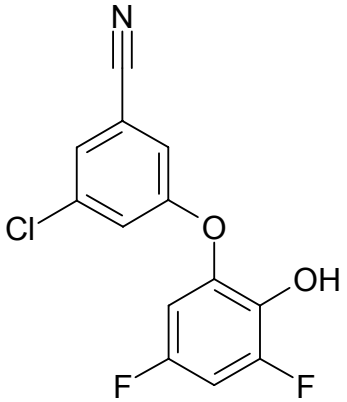
1-Bromo-3,5-difluoro-2-methoxy-benzene **(17)** (500mg, 2.24mmol) was dissolved in diethyl ether (5mL) and cooled to -78°C, prior to the dropwise addition of n-BuLi (2.5M in THF, 0.9mL, 2.24mmol). Solution was stirred for 2mins then triisopropyl borate (600mg, 3.16mmol) added and resultant solution allowed to warm slowly to room temperature overnight. HCl (2M, 3mL) was then added and stirred for 30 minutes. Product was extracted with EtOAc (20mL), washed with brine (20mL), dried ($MgSO_4$) and concentrated to give 338mg of a yellow foam.
The crude boronic acid (90mg, 0.48mmol) was dissolved in THF (6.2mL) and acetic acid (0.2mL) and hydrogen peroxide (30%, 0.27mL) added, solution was then stirred for 3 days. Sodium metabisulfite. aq (10mL) was added and product extracted into EtOAc (10mL), organics were then washed with brine (5mL), water (5mL), dried ($MgSO_4$) and concentrated. Crude material was purified by column chromatography (2:1, Pentane:EtOAc) to give title compound as a colourless gum (60mg, 62% yield over two steps). Identical by ^1H NMR to J.Med.Chem .1993,36, 3947 (ref. 4).

Step B
3-Chloro-5-(3,5-difluoro-2-methoxy-phenoxy)-benzonitrile



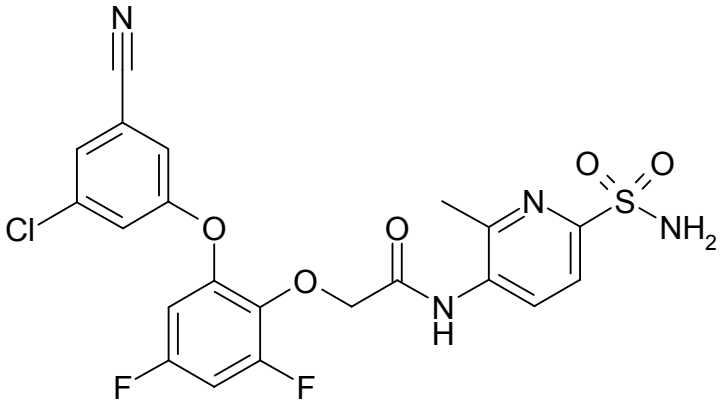
Prepared following step C from compound **20** - (415mg, 75% yield): δ_H(400MHz, $CDCl_3$) 3.80 (3H, s), 6.62 (1H, m), 6.82 (1H, m), 7.08 (1H, bs), 7.16 (1H, bs), 7.37 (1H, m); *m/z* (APCI) 295 $(M+H)^+$.

Step C
3-Chloro-5-(3,5-difluoro-2-hydroxy-phenoxy)-benzonitrile



Prepared following step D from compound **20** - (275mg, 70% yield): δ_H(400MHz, $CDCl_3$) 5.28 (1H, bs), 6.60 (1H, m), 6.83 (1H, m), 7.14 (1H, m), 7.22 (1H, m), 7.40 (1H, m); *m/z* (APCI) 280 $(M-H)^-$.

Step D
2-[2-(3-Chloro-5-cyano-phenoxy)-4,6-difluoro-phenoxy]-N-(2-methyl-6-sulfamoyl-pyridin-3-yl)-acetamide **(3)**



Prepared following step E from compound **20** - (200mg, 40% yield): δ_H(400MHz, DMSO) 2.40 (3H, s), 4.74 (2H, s), 7.08 (1H, d), 7.32-7.41 (3H, bm), 7.56 (1H, s), 7.61 (1H, s), 7.75 (1H, d), 7.80 (1H, s), 8.03 (1H, d), 9.70 (1H, bs); *m/z* (APCI) 509 $(M+H)^+$.

Summary of method to determine potencies

The assay uses a DNA/RNA primer/template where the 5' biotinylated primer DNA is a 16mer oligo d(T) and is annealed to a poly(rA) template, approximately 300 bases in length. Incorporation of [^{3}H]TTP by reverse transcription, results in extension of the primer. During the reaction the primer/template is bound to a streptavidin-coated 'flashplate'. The incorporated tritiated nucleotides can then stimulate the scintillant to produce a signal that is measured using a scintillation counter. Compounds that inhibit reverse transcription will give rise to a reduced signal and a dose-response curve for each compound can be used to calculate the IC_{50} values.