SUPPORTING INFORMATION

GSK6853, a chemical probe for inhibition of the BRPF1 bromodomain

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Contents

General Methods

General: All solvents were purchased from Sigma Aldrich (Hy-Dry) anyhydrous solvents) and commercial chemicals were used without further purification unless otherwise specified.

All reactions, except those in aqueous media, were carried out with the use of standard techniques for the exclusion of moisture.
Reactions were monitored by thin-layer chromatography on 0.2 mm silica gel plates, or LCMS (liquid chromatography mass spectrometry) using a Waters ZQ instrument.
Column chromatography was performed on pre-packed silica gel columns using a Biotage SP4, or by mass directed autopreparative chromatography (MDAP).

NMR spectra were recorded at ambient temperature, unless otherwise stated, using standard pulse methods on the following spectrometers, at the frequencies stated: Brucker DPX 400 MHz (^{1}H = 400 MHz, ^{13}C = 100.6 MHz), Brucker AV 600 MHz (^{1}H = 600 MHz, ^{13}C = 151 MHz). Chemical shifts are reported in parts per million (ppm, δ units) and are referenced to tetramethylsilane (TMS) (^{1}H = 0.00), $CDCl_3$ (^{1}H = 7.27, ^{13}C = 77.0), DMSO-d_6 (^{1}H = 2.50, ^{13}C = 39.5), CD_3OD (^{1}H = 3.31, ^{13}C = 49.0). Coupling constants are quoted to the nearest 0.1 Hz, and multiplicities are given by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quadruplet), p (pentuplet), spt (septuplet), m (multiplet), br (broad).

IR spectra were obtained on a Perkin Elmer Spectrum 1 FT-IR spectrometer, and key well-defined peaks were recorded in cm^{-1}.

High resolution mass spectra (HRMS) were recorded on a Micromass Q-Tof Ultima hybrid quadrupole time-of-flight mass spectrometer, with analytes separated on an Agilent 1100 Liquid Chromatograph equipped with a Phenomenex Luna C18(2) reversed phase column (100 mm x 2.1 mm, 3 μm packing diameter).

Optical rotation measurements were recorded using a Jasco P-1030 polarimeter. The concentration was recorded in g/ml, path length in mm, and temperature in ºC.

The purity of all compounds tested was greater than 95%, as determined by LCMS and ^{1}H NMR.

LCMS methodology:

1) **Formic method** (standard method)

LC conditions

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50 mm x 2.1 mm, i.d. 1.7 µm packing diameter) at 40°C. The solvents employed were:

A = 0.1% v/v solution of formic acid in water

B = 0.1% v/v solution of formic acid in acetonitrile

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1	99	1
1.5	1	3	97
1.9	1	3	97
2.0	1	0	100

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS	:	Waters ZQ
Ionisation mode	:	Alternate-scan positive and negative electrospray
Scan range	:	100 to 1000 AMU
Scan time	:	0.27 sec
Inter scan delay	:	0.10 sec

2) **Formic 4.5 min method** (only used when explicitly stated)

LC conditions

The UPLC analysis was conducted on an Acquity BEH C18 column (50 mm x 2.1 mm, i.d. 1.7 µm packing diameter) at 35°C. The solvents employed were:

A = 0.1% v/v solution of formic acid in water

B = 0.1% v/v solution of formic acid in acetonitrile

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	0.6	97	3
0.4	0.6	97	3
3.2	0.6	2	98
3.8	0.6	2	98
4.2	0.6	97	3
4.5	0.6	97	3

The UV detection was a summed signal from wavelength of 200 nm to 400 nm.

MS conditions

MS	:	Waters SQD - 3100 Mass Detector
Ionisation mode	:	Electrospray Ionisation (ESI)
Polarity Switching	:	Positive/Negative
Scan range	:	100-1000
Scan time	:	0.5 (sec)
Inter scan delay	:	0.1 (sec)

3) High pH Method (standard method)

LC conditions

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50 mm x 2.1 mm, i.d. 1.7 µm packing diameter) at 40°C. The solvents employed were:

A = 10 mM ammonium hydrogen carbonate in water adjusted to pH10 with ammonia solution
B = acetonitrile

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1	99	1
1.5	1	3	97
1.9	1	3	97
2.0	1	0	100

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS	:	Waters ZQ
Ionisation mode	:	Alternate-scan positive and negative electrospray
Scan range	:	100 to 1000 AMU
Scan time	:	0.27 sec
Inter scan delay	:	0.10 sec

4) High pH 6 min method (only used when explicitly stated)

LC conditions

The UPLC analysis was conducted on an XBridge C18 column (50 mm x 4.6 mm, i.d. 2.5 µm packing diameter) at 35°C. The solvents employed were:

A = 5 mM ammonium acetate in water
B = acetonitrile

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1.3	95	5
0.5	1.3	95	5
1	1.3	85	15
3.3	1.3	2	98
5.2	1.3	2	98
5.5	1.3	95	5
6.0	1.3	95	5

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS	:	Waters TQD - Micromass
Ionisation mode	:	Electrospray Ionisation (ESI)
Polarity Switching	:	Positive/Negative
Scan range	:	100-1000
Scan time	:	0.5 (sec)
Inter scan delay	:	0.1 (sec)

MDAP methodology:

1) Method Formic

LC conditions
The HPLC analysis was conducted on either a Sunfire C18 column (100 mm x 19 mm, i.d. 5 μm packing diameter) or a Sunfire C18 column (150 mm x 30 mm, i.d. 5 μm packing diameter) at ambient temperature.
The solvents employed were:

A = 0.1% v/v solution of formic acid in water
B = 0.1% v/v solution of formic acid in acetonitrile

Run as a gradient over either 15 or 25 min (extended run) with a flow rate of 20 mL/min (100 mm x 19 mm, i.d. 5 μm packing diameter) or 40 mL/min (150 mm x 30 mm, i.d. 5 μm packing diameter).

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS	:	Waters ZQ
Ionisation mode	:	Alternate-scan positive and negative electrospray
Scan range	:	100 to 1000 AMU
Scan time	:	0.50 sec
Inter scan delay	:	0.20 sec

2) Method high pH

LC conditions
The HPLC analysis was conducted on either an Xbridge C18 column (100 mm x 19 mm, i.d. 5 μm packing diameter) or a Xbridge C18 column (100 mm x 30 mm, i.d. 5 μm packing diameter) at ambient temperature.
The solvents employed were:

A = 10 mM ammonium bicarbonate in water, adjusted to pH10 with ammonia solution
B = acetonitrile

Run as a gradient over either 15 or 25 min (extended run) with a flow rate of 20 mL/min (100 mm x 19 mm, i.d. 5 μm packing diameter) or 40 mL/min (100 mm x 30 mm, i.d. 5 μm packing diameter).

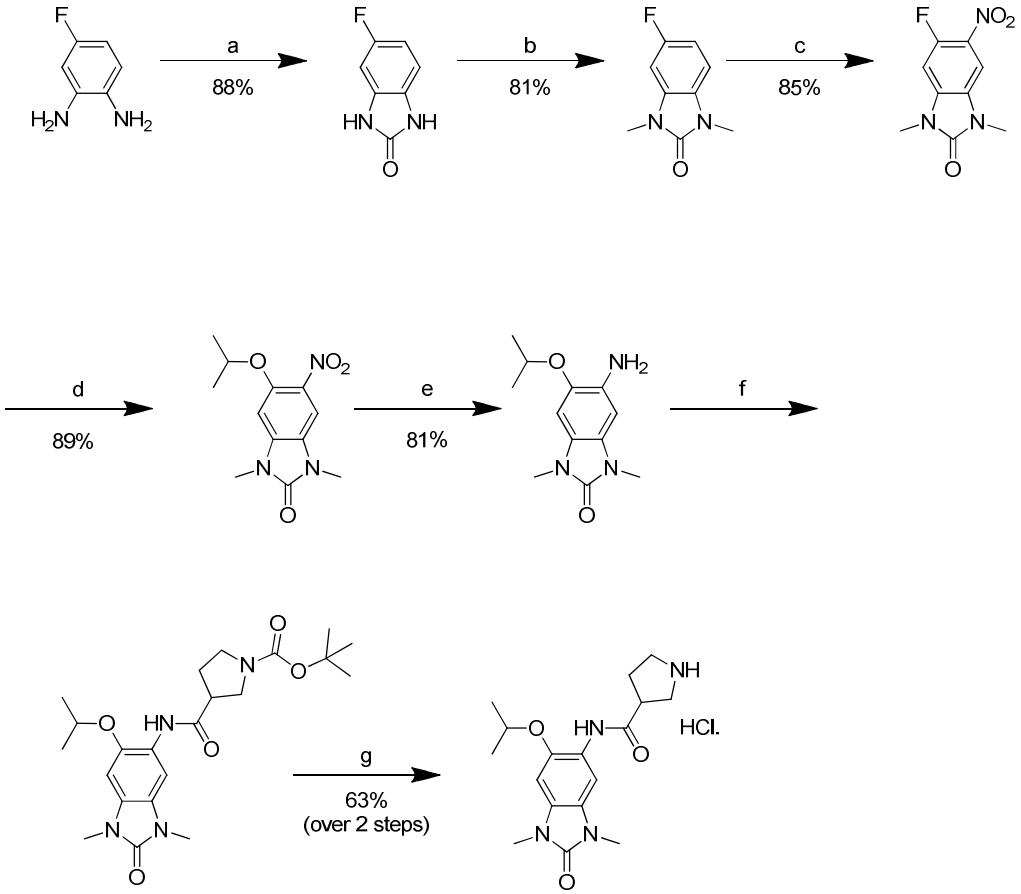
The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS	:	Waters ZQ
Ionisation mode	:	Alternate-scan positive and negative electrospray
Scan range	:	100 to 1000 AMU
Scan time	:	0.50 sec
Inter scan delay	:	0.20 sec

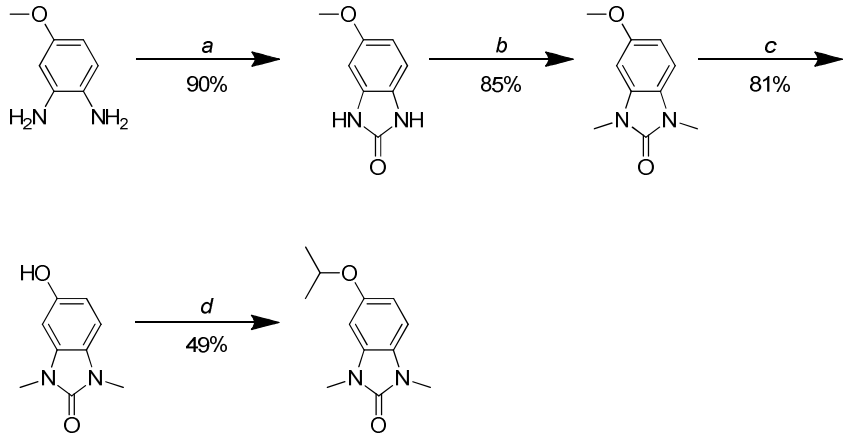
Reaction Schemes:

Scheme S1: Example scheme for Compounds **1**, **2**, **5-12**, **14-25**



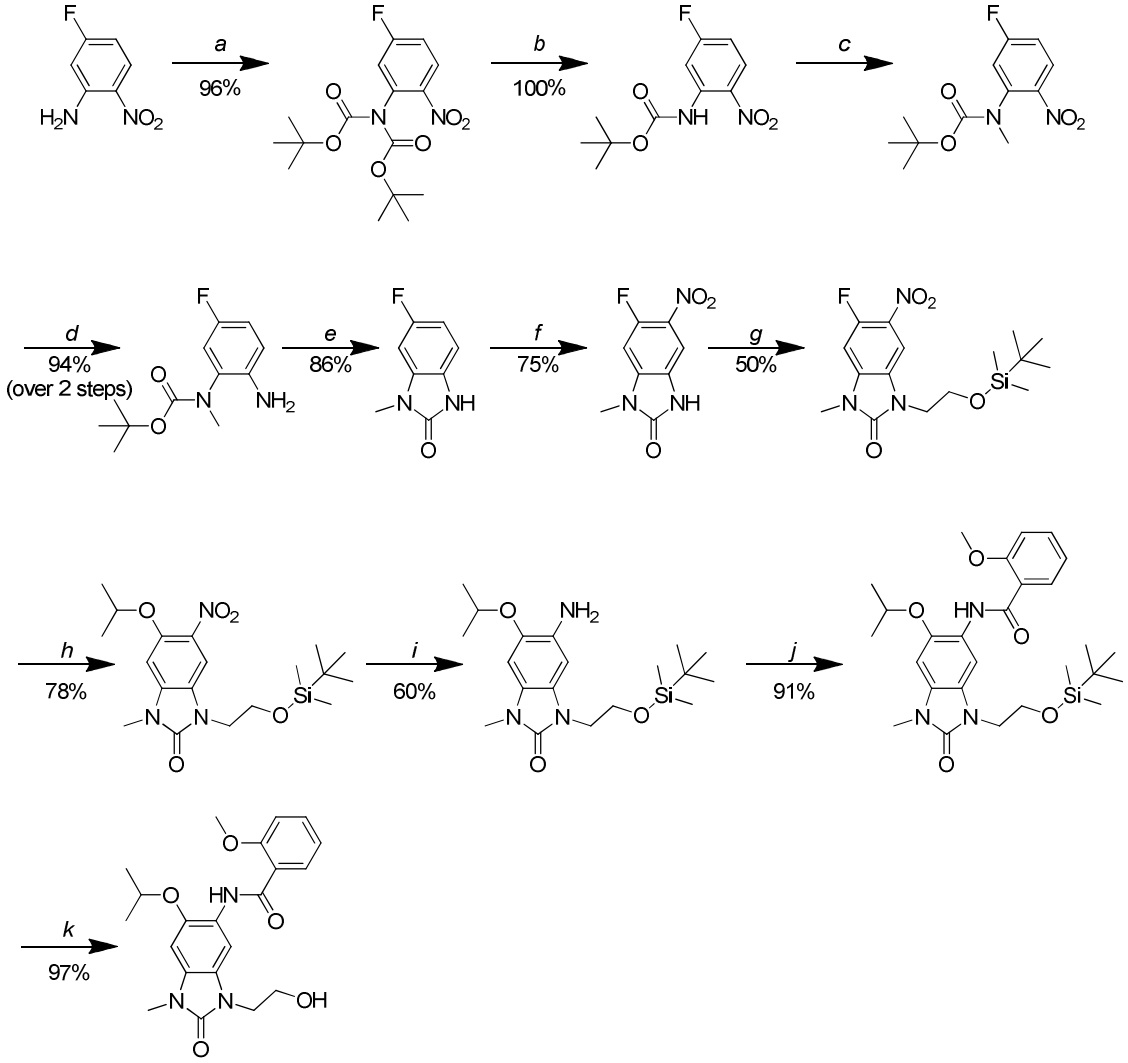
Conditions: (a) di(1*H*-imidazol-1-yl)methanone, THF, r.t.; (b) NaH, MeI, DMF, r.t.; (c) nitric acid, acetic anhydride, -30 °C to 0 °C; (d) NaH, propan-2-ol, 2-MeTHF, rt; (e) 10% Pd/C, H_2, EtOH, r.t.; (f) 1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid, HATU , DIPEA, DMF, r.t.; (g) HCl (4 M in dioxane), DCM, r.t.

Scheme S2: Synthesis of Compound **13**



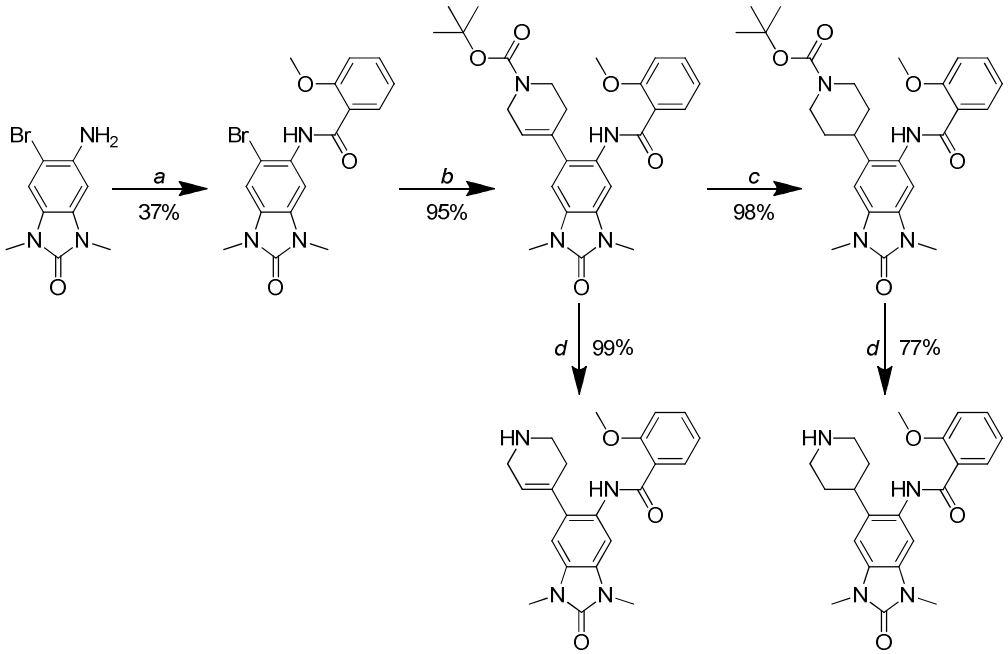
Conditions: (a) triphosgene, TEA, THF, r.t.; (b) NaH, MeI, DMF, r.t.; (c) BBr_3, DCM, r.t.; (d) DIAD, triphenylphosphine, propan-2-ol, THF, r.t.

Scheme S3: Synthesis of Compound **26**



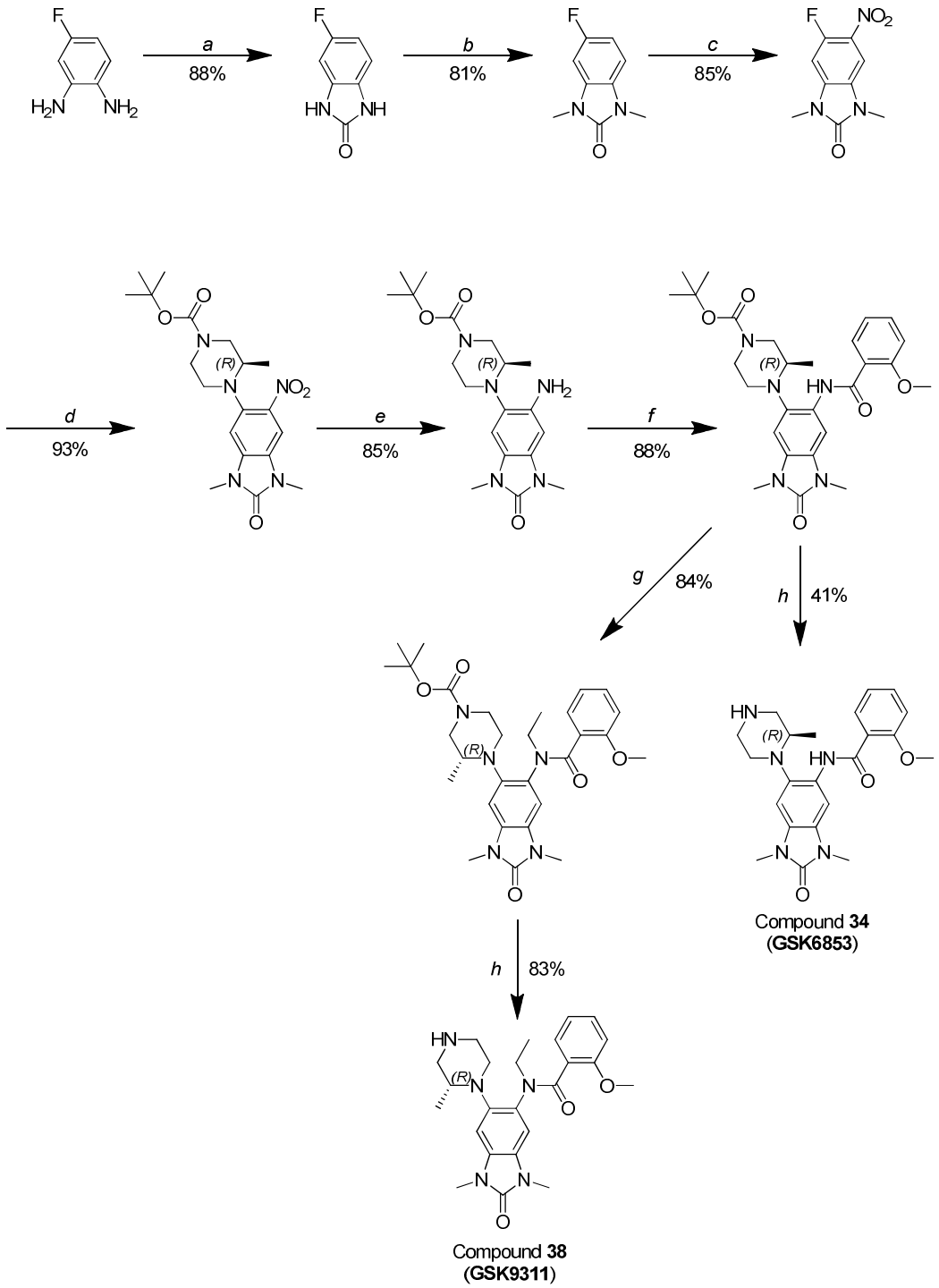
Conditions: (a) di-*tert*-butyl dicarbonate, DMAP, 2-Me THF, r.t.; (b) TFA, DCM, r.t.; (c) NaH, MeI, DMF/ THF (2:1), 0 ^{o}C; (d) 10% Pd/C, H_2, EtOH, r.t., 1 bar; (e) NaH, EtOH, DMF/ THF (1.5:1), 60 ^{o}C; (f) nitric acid, acetic anhydride, -30 ^{o}C to 0 ^{o}C (g) NaH, (2-bromoethoxy)(*tert*-butyl)dimethylsilane, DMF, r.t. then 40 ^{o}C; (h) NaH, propan-2-ol, THF, r.t.; (i) 10% Pd/C, H_2, propan-2-ol, r.t.; (j) 2-methoxybenzoyl chloride, pyridine, DCM; (k) TFA, DCM, r.t.

Scheme S4: Synthesis of Compounds **30** and **31**



Conditions: (a) 2-Methoxybenzoyl chloride, DIPEA, DCM; (b) *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate, potassium carbonate, $PdCl_2$(dppf)-CH_2Cl_2 adduct, dioxane/water (4:1), microwave, 100 ^oC; (c) 10% Pd/C, H_2, EtOH, r.t.; (d) TFA, r.t.

Scheme S5: Example Scheme for Compounds **28**-**29**, **32**-**38**



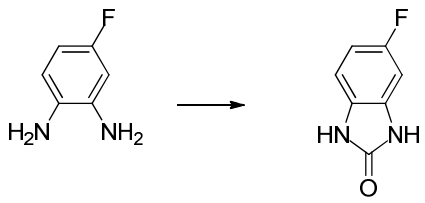
Conditions: (a) di(1*H*-imidazol-1-yl)methanone, THF, r.t.; (b) NaH, MeI, DMF, r.t.; (c) nitric acid, acetic anhydride, -30 ^{o}C to 0 ^{o}C; (d) (*R*)-*tert*-butyl 3-methylpiperazine-1-carboxylate, DIPEA, DMSO, microwave, 120 ^{o}C; (e) 10% Pd/C, H_2, propan-2-ol, r.t.; (f) 2-methoxybenzoyl chloride, pyridine, DCM; (g) NaH, EtI, DMF, r.t.; (h) TFA, DCM, r.t.

Synthetic Methods:

Intermediate 1

Step 1

5-Fluoro-1*H*-benzo[d]imidazol-2(3*H*)-one



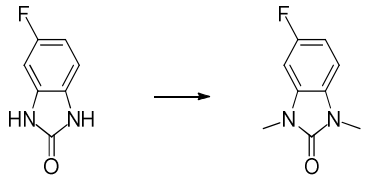
A stirred solution of 4-fluorobenzene-1,2-diamine (15.1 g, 120 mmol) in tetrahydrofuran (120 mL) under nitrogen was cooled using an ice-bath and then was treated with di(1*H*-imidazol-1-yl)methanone (23.4 g, 144 mmol) portion-wise over 15 minutes. The resulting mixture was slowly warmed to room temperature then was concentrated *in vacuo* after 2.5 h. The residue was suspended in a mixture of water and dichloromethane (250 mL each) and filtered off. This residue was then washed with water (50 mL) and dichloromethane (50 mL), before being dried at 40 ^{o}C *in vacuo* for 16 h to give the **title compound** (16.0 g, 105 mmol, 88%) as a brown solid.

LCMS (High pH): Rt 0.57 min; $[M\text{-}H^{+}]^{-}$ = 151.1

δ_H NMR (400 MHz, DMSO-d_6) 10.73 (br s, 1H), 10.61 (br s, 1H), 6.91 - 6.84 (m, 1H), 6.78 - 6.70 (m, 2H).

Step 2

5-Fluoro-1,3-dimethyl-1*H*-benzo[d]imidazol-2(3*H*)-one



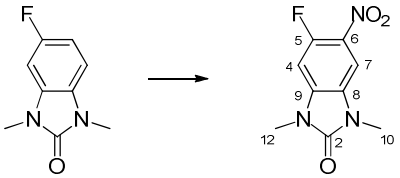
A solution of 5-fluoro-1*H*-benzo[d]imidazol-2(3*H*)-one (16.0 g, 105 mmol) in *N*,*N*-dimethylformamide (400 mL) under nitrogen was cooled with an ice-bath, using a mechanical stirrer for agitation. It was then treated over 10 minutes with sodium hydride (60% w/w in mineral oil, 13.1 g, 327 mmol) and the resulting mixture was stirred at this temperature for 30 min before being treated with iodomethane (26.3 mL, 422 mmol) over 30 min. The resulting mixture was then allowed to warm to room temperature and after 1 h was carefully treated with water (500 mL). The aqueous phase was extracted with ethyl acetate (3 x 800 mL) and the combined organics were washed with brine (1 L), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the brown residue by flash chromatography on silica gel (SP4, 1.5 kg column, gradient: 0-25% (3:1 EtOAc:EtOH) in cyclohexane) gave the **title compound** (15.4 g, 86 mmol, 81%) as pink solid.

LCMS (High pH): Rt 0.76 min; $[M+H^+]^+$ = 181.1

δ_H NMR (400 MHz, $CDCl_3$) 6.86 - 6.76 (m, 2H), 6.71 (dd, *J* = 8.3, 2.3 Hz, 1H), 3.39 (s, 3H), 3.38 (s, 3H).

Step 3

5-Fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[d]imidazol-2(3*H*)-one



A stirred solution of 5-fluoro-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (4.55 g, 25.3 mmol) in acetic anhydride (75 mL) under nitrogen was cooled to -30 ^{o}C and then was slowly treated with fuming nitric acid (1.13 mL, 25.3 mmol) making sure that the temperature was kept below -25 ^{o}C. The solution turned brown once the first drop of acid was added and a thick brown precipitate formed after the addition was complete. The mixture was allowed to slowly warm up to 0 ^{o}C then was carefully treated after 1 h with ice-water (100 mL). Ethyl acetate (15 mL) was then added and the resulting mixture was stirred for 20 min. The precipitate formed was filtered off, washed with water (10 mL) and ethyl acetate (10 mL), and then was dried *in vacuo* at 40^{o}C for 16 h to give the **title compound** (4.82 g, 21.4 mmol, 85%) as a yellow solid.

LCMS (High pH): Rt 0.76 min; $[M+H^{+}]^{+}$ not detected

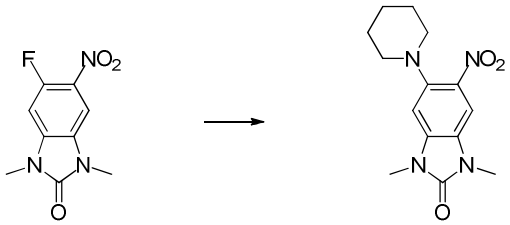
δ_H NMR (600 MHz, DMSO-d_6) 7.95 (d, J = 6.4 Hz, 1H, (H-7)), 7.48 (d, J = 11.7 Hz, 1H, (H-4)), 3.38 (s, 3H, (H-10)), 3.37 (s, 3H, (H-12)).

δ_C NMR (151 MHz, DMSO-d_6) 154.3 (s, 1C, (C-2)), 152.3 (d, J = 254.9 Hz, 1C, (C-5)), 135.5 (d, J = 13.0 Hz, 1C, (C-9)), 130.1 (d, J = 8.0 Hz, 1C, (C-6)), 125.7 (s, 1C, (C-8)), 104.4 (s, 1C, (C-7)), 97.5 (d, J = 28.5 Hz, 1C, (C-4)), 27.7 (s, 1C, (C-12)), 27.4 (s, 1C, (C-10)).

Compound 1

Step 1

1,3-Dimethyl-5-nitro-6-(piperidin-1-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one



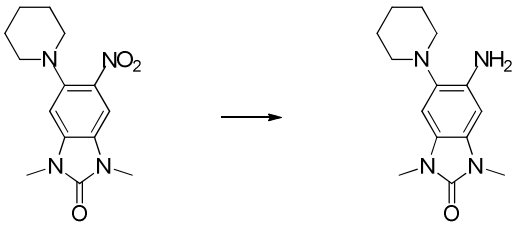
A mixture of 5-fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.45 g, 2.0 mmol), piperidine (0.59 mL, 6.0 mmol) and *N*,*N*-diisopropylethylamine (1.75 mL, 10.0 mmol) in dimethylsulfoxide (2 mL) was stirred for 1 h under nitrogen at 120 °C under microwave irradiation then was cooled to room temperature and diluted with water (20 mL). The aqueous phase was extracted three times with ethyl acetate and the combined organics were washed with water then brine, dried over $MgSO_4$ and concentrated *in vacuo* to give, after trituration with diethyl ether, the **title compound** (0.460 g, 1.58 mmol, 79%) as an orange solid which was used in the next step without further purification.

LCMS (High pH): Rt 1.11 min; $[M+H^+]^+$ =291.3

δ_H NMR (400 MHz, DMSO-d_6) 7.73 (s, 1H), 7.12 (s, 1H), 3.36 (s, 3H), 3.33 (s, 3H), 2.93 (m, 4H), 1.65 (m, 4H), 1.54 (m, 2H).

Step 2

5-Amino-1,3-dimethyl-6-(piperidin-1-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one



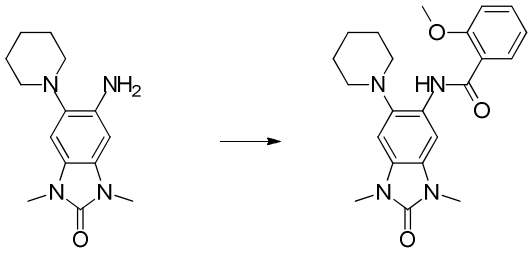
A solution of 1,3-dimethyl-5-nitro-6-(piperidin-1-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.440 g, 1.52 mmol) in ethanol (30 mL) at room temperature was treated with palladium on carbon (10% w/w, 50% paste, 0.150 g) and the resulting mixture was stirred at this temperature under hydrogen (1 atm) for 16 h. The catalyst was filtered off using a pad of celite (2.5 g) and rinsed with ethyl acetate. The combined organics were concentrated *in vacuo* to give the **title compound** (0.390 g, 1.50 mmol, 99%) as a white solid.

LCMS (Formic): Rt 0.47 min; $[M+H^+]^+$ =261.3

δ_H NMR (400 MHz, DMSO-d_6) 6.79 (s, 1H), 6.47 (s, 1H), 4.55 (br s, 2H), 3.23 (s, 3H), 3.20 (s, 3H), 2.74 (br s, 4H), 1.66 (m, 4H), 1.52 (br s, 2H).

Step 3

_N_-(1,3-Dimethyl-2-oxo-6-(piperidin-1-yl)-2,3-dihydro-1_H_-benzo[_d_]imidazol-5-yl)-2-methoxybenzamide



A solution of 5-amino-1,3-dimethyl-6-(piperidin-1-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.200 g, 0.768 mmol) in dichloromethane (8 mL) at room temperature was treated with pyridine (0.081 mL, 1.0 mmol) then 2-methoxybenzoyl chloride (0.11 mL, 0.84 mmol) and the resulting mixture was stirred at this temperature for 45 min before being diluted with dichloromethane. The organic phase was washed with a saturated aqueous solution of sodium bicarbonate and the aqueous phase was extracted with dichloromethane. The combined organics were washed with aqueous hydrochloric acid (2 N) and the aqueous phase was extracted with dichloromethane. The combined organics were washed with water, dried using a separator frit and concentrated *in vacuo*.

Purification of the residue by flash chromatography on silica gel (25 g column, 8-38% (3:1 EtOAc: EtOH) in cyclohexane) gave **1** (0.068 g, 0.17 mmol, 22%) as a white solid. The remaining fractions containing the product were concentrated *in vacuo* and purified again by flash chromatography on silica gel (25 g column, 5-20% (3:1 EtOAc: EtOH) in cyclohexane) to give **1** (0.100 g, 0.254 mmol, 33%) as a white solid.

LCMS (High pH): Rt 1.23 min; $[M+H^+]^+$ =395.4

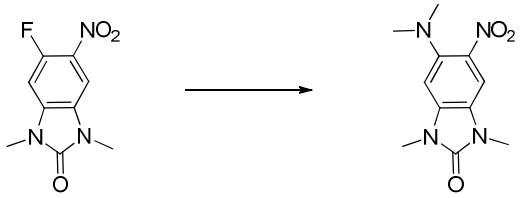
δ_H NMR (600 MHz, $CDCl_3$) 10.61 (s, 1H), 8.43 (s, 1H), 8.29 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.52 - 7.50 (m, 1H), 7.17 - 7.14 (m, 1H), 7.07 (d, *J* = 8.1 Hz, 1H), 6.83 (s, 1H), 4.10 (s, 3H), 3.44 (s, 3H), 3.42 (s, 3H), 2.93 - 2.80 (m, 4H), 1.78 - 1.75 (m, 4H), 1.63 (br s, 2H).

δ_C NMR (151 MHz, $CDCl_3$) 163.24 (s, 1C), 157.36 (s, 1C), 155.05 (s, 1C), 138.43 (s, 1C), 132.89 (s, 1C), 132.36 (s, 1C), 128.75 (s, 1C), 126.45 (s, 1C), 125.96 (s, 1C), 122.84 (s, 1C), 121.49 (s, 1C), 111.68 (s, 1C), 101.11 (s, 1C), 99.93 (s, 1C), 56.32 (s, 1C), 54.49 (s, 2C), 27.28 (s, 1C), 27.13 (s, 1C), 26.71 (s, 2C), 24.00 (s, 1C).

Compound 2

Step 1

5-(Dimethylamino)-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one



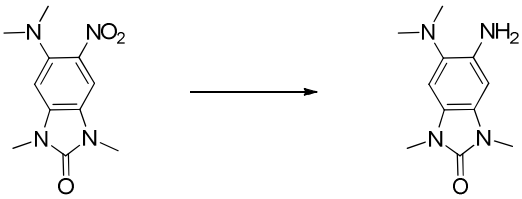
5-Fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (1.49 g, 6.62 mmol) was suspended in dimethylsulfoxide (13 mL) in a microwave vial. Dimethylamine hydrochloride (1.62 g, 19.8 mmol) and *N*,*N*-diisopropylethylamine (5.78 mL, 33.1 mmol) were added and the vial was sealed and placed under an atmosphere of nitrogen. The reaction heated to 120 ˚C in a Biotage Initiator+ microwave reactor for 2 h. The reaction mixture was poured into 50% aq. NaCl (50 mL), and the resulting orange precipitate was collected by filtration, washed with 50% aq. NaCl (50 mL), water (40 mL) and diethyl ether (40 mL), then dried *in vacuo* to afford the **title compound** (1.32 g, 2.52 mmol, 79%) as an orange solid.

LCMS (High pH): Rt 0.79 min, $[M+H^+]^+$ 251.2.

δ_H NMR (400 MHz, $CDCl_3$) 7.61 (d, *J* = 2.4 Hz, 1H), 6.68 (br. s., 1H), 3.47 - 3.39 (m, 6H), 2.98 - 2.91 (m, 6H).

Step 2:

5-Amino-6-(dimethylamino)-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one



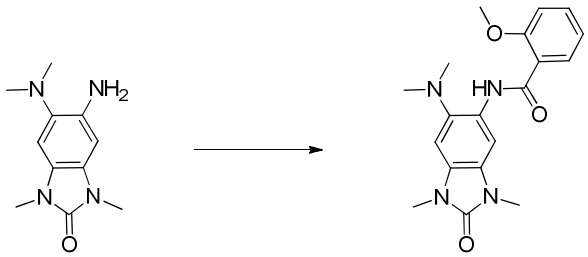
5-(Dimethylamino)-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (1.32 g, 5.25 mmol) was suspended in ethanol (45 mL) and water (15 mL). Iron powder 325 mesh (1.62 g, 29.0 mmol) and ammonium chloride (1.09 g, 20.4 mmol) were added and the reaction mixture was heated to 90 ˚C under nitrogen for 4 h. The reaction mixture was allowed to cool to room temperature, filtered through celite and washed with methanol (100 mL). The filtrate was concentrated *in vacuo* to give the crude product, which was purified by ion exchange chromatography (Biotage 70 g SCX-2 cartridge, eluting with methanol, followed by methanolic ammonia [2 M]). The appropriate fractions were concentrated *in vacuo* to afford the **title compound** (1.05 g, 4.79 mmol, 91%) as a grey solid.

LCMS (High pH): Rt 0.65 min, $[M+H^+]^+$ 221.3.

δ_H NMR (400 MHz, DMSO-d_6) 6.82 (s, 1 H), 6.46 (s, 1 H), 4.58 (br. s., 2 H), 3.24 (s, 3 H), 3.20 (s, 3 H), 2.58 (s, 6 H).

Step 3:

***N*-(6-(Dimethylamino)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide**



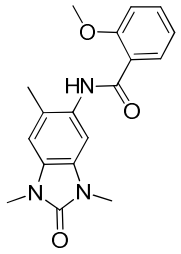
5-Amino-6-(dimethylamino)-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.049 g, 0.22 mmol) was dissolved in *N*,*N*-dimethylformamide (1 mL). Pyridine (0.025 mL, 0.31 mmol) and 2-methoxybenzoyl chloride (0.070 mL, 0.50 mmol) were added and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with 1:1 DMSO:MeCN and purified by MDAP (High pH). The appropriate fractions were concentrated *in vacuo* to afford **2** (0.072 g, 0.20 mmol, 91%) as a light brown solid.

LCMS (High pH): Rt 1.03 min, $[M+H^+]^+$ 355.3.

δ_H NMR (400 MHz, $CDCl_3$) 11.15 (s, 1H), 8.50 (s, 1H), 8.33 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.50 (ddd, *J* = 8.3, 7.6, 1.8 Hz, 1H), 7.18 - 7.12 (m, 1H), 7.05 (d, *J* = 8.3 Hz, 1H), 6.86 (s, 1H), 4.07 (s, 3H), 3.44 (s, 3H), 3.42 (s, 3H), 2.75 (s, 6H).

Compound 3

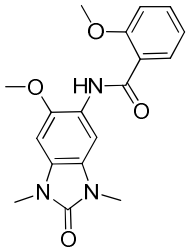
2-methoxy-*N*-(1,3,6-trimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)benzamide



Available from ChemDiv. Catalogue number C301-7345.

Compound 4

2-Methoxy-*N*-(6-methoxy-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)benzamide

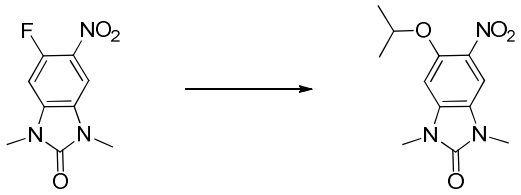


Available from ChemDiv. Catalogue number C301-8069.

Compound 5

Step 1:

5-Isopropoxy-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one



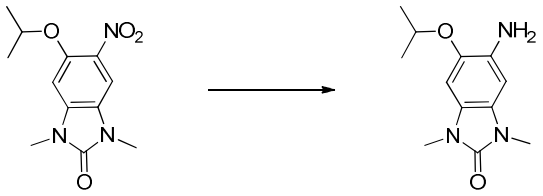
Propan-2-ol (0.80 g, 13 mmol) was taken up in 2-MeTHF (75 mL) under nitrogen and cooled in an ice-bath. Sodium hydride (60% in mineral oil, 0.53 g, 13 mmol) was added and the reaction was stirred at 0 ˚C for 20 min. 5-Fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (2.0 g, 8.9 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. Additional propan-2-ol (0.27 g, 4.4 mmol) was added and the reaction stirred at room temperature for 18 h. The reaction was quenched with water (50 mL) and extracted with 20% methanol in dichloromethane (2 x 100 mL). The combined organics were eluted through a hydrophobic frit then concentrated *in vacuo* to afford the **title compound** (2.1 g, 7.9 mmol, 89%) as an orange solid.

LCMS (High pH): Rt 0.90 min, no mass ion detected.

δ_H NMR (400 MHz, $CDCl_3$) 7.57 (s, 1H), 6.64 (s, 1H), 4.60 (spt, *J* = 6.1 Hz, 1H), 3.43 (s, 3H), 3.43 (s, 3H), 1.42 (d, *J* = 6.1 Hz, 6H).

Step 2:

5-Amino-6-isopropoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one



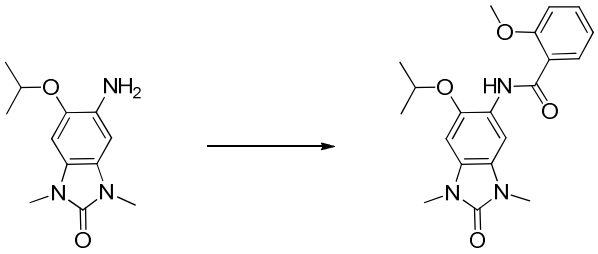
5-Isopropoxy-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (2.1 g, 7.9 mmol) was suspended in ethanol (150 mL). Palladium on carbon (10% w/w, 50% paste, 0.5 g) was added and the mixture was stirred under an atmosphere of hydrogen for 5 h. The reaction mixture was filtered through Celite and the filter cake was washed with ethanol (50 mL). The filtrate was concentrated *in vacuo* and purified by silica chromatography (5-40% (3:1 EtOAc:EtOH) in cyclohexane). The appropriate fractions were concentrated *in vacuo* to give the **title compound** (1.51 g, 6.42 mmol, 81%) as a cream solid.

LCMS (High pH): Rt 0.74 min, $[M+H^+]^+$ 236.2.

δ_H NMR (400 MHz, $CDCl_3$) 6.54 (s, 1H), 6.42 (s, 1H), 4.43 (spt, *J* = 6.1 Hz, 1H), 3.69 (br. s., 2H), 3.35 (s, 3H), 3.33 (s, 3H), 1.36 (d, *J* = 6.1 Hz, 6H).

Step 3:

***N*-(6-Isopropoxy-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide**

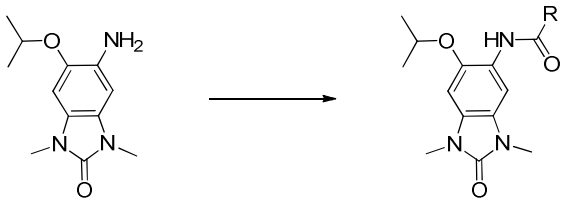


5-Amino-6-isopropoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.044 g, 0.19 mmol) was dissolved in *N*,*N*-dimethylformamide (1 mL). Pyridine (0.031 mL, 0.38 mmol) and 2-methoxybenzoyl chloride (0.031 mL, 0.23 mmol) were added and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with 1:1 DMSO:MeCN and purified by MDAP (High pH). The appropriate fractions were evaporated *in vacuo* to give **5** (0.052 g, 0.14 mmol, 74%) as a cream solid.

LCMS (High pH): Rt 1.06 min, $[M+H^+]^+$ 370.3.

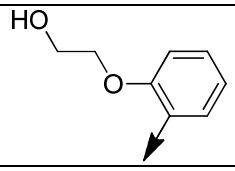
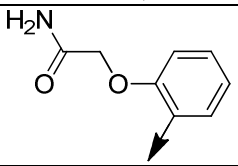
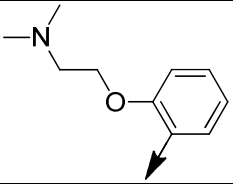
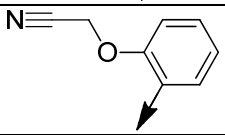
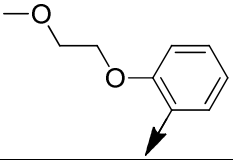
δ_H NMR (400 MHz, $CDCl_3$) 10.46 (s, 1H), 8.50 (s, 1H), 8.32 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.50 (ddd, *J* = 8.3, 7.6, 1.8 Hz, 1H), 7.19 - 7.12 (m, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.64 (s, 1H), 4.59 (spt, *J* = 6.1 Hz, 1H), 4.10 (s, 3H), 3.43 (s, 3H), 3.40 (s, 3H), 1.42 (d, *J* = 6.1 Hz, 6H).

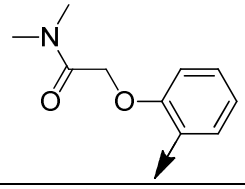
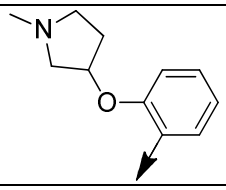
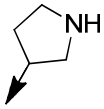
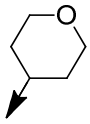
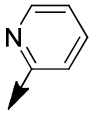
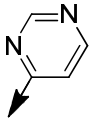
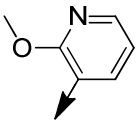
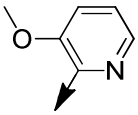
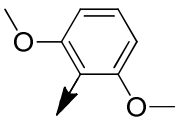
Compounds 6-12, 14-17, 19, 22-25



To a solution of acid (0.171 mmol), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (0.065 g, 0.17 mmol) and *N*,*N*-diisopropylethylamine (0.078 mL, 0.45 mmol) in *N*,*N*-dimethylformamide (1 mL) was added 5-amino-6-isopropoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.035 g, 0.15 mmol), and the reaction was stirred at room temperature for 18 h. The reaction mixtures were purified by MDAP (High pH). The solvent was dried under a stream of nitrogen to afford the products.

LCMS (Formic): See table.

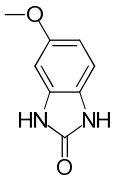
Compound #	R	Yield (%)	Retention time (min)	$[M+H^+]^+$
6		34	0.89	400.3
7		8	0.79	413.3
8		70	0.57	427.3
9		39	1.01	395
10		67	1.03	414.3

11		61	0.88	441.3
12		59	0.59	439.3
14		63	0.69	278.2
15		68	0.88	306.3
16	 .HCl	63 (over 2 steps)	0.40	333.3
17		67	0.77	348.3
19		52	1.03	341.3
22		18	0.91	342.2
23		24	1.05	371.3
24		63	0.84	371.3
25		50	0.95	400.3

Compound 13

Step 1

5-Methoxy-1*H*-benzo[*d*]imidazol-2(3*H*)-one

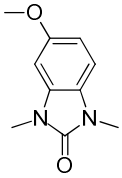


Triethylamine (15.1 mL, 109 mmol) was added to a solution of 4-methoxybenzene-1,2-diamine (15.0 g, 109 mmol) in tetrahydrofuran (150 mL) at room temperature. After stirring for 20 min, triphosgene (32.2 g, 109 mmol) was added portionwise over 30 min at 0 °C, and reaction mixture was allowed to stir at room temperature 1 h. The reaction mixture was poured into ice water (500 mL), and the mixture was extracted with ethyl acetate (3 x 500 mL). The combined organic phases were washed with water (250 mL), brine (250 mL), dried over sodium sulphate and evaporated *in vacuo* to give the **title compound** (16 g, 97 mmol, 90% yield) as a light brown solid.

LCMS (Formic, 4.5 min method): Rt 1.29 min, $[M+H^+]^+$ 165.1.

Step 2

5-Methoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one



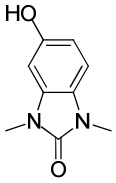
5-Methoxy-1*H*-benzo[*d*]imidazol-2(3*H*)-one (10 g, 61 mmol) was added to a suspension of sodium hydride (60% w/w in mineral oil, 8.77 g, 219 mmol) in *N*,*N*-dimethylformamide (150 mL) at 0 °C. After stirring for 20 min, methyl iodide (34.3 mL, 548 mmol) was added dropwise over 20 min. After stirring at room temperature for 3 h, the reaction was quenched with addition of ice cold water (300 mL). The mixture was partitioned between ethyl acetate (2 x 200 mL) and water. The combined organic phases were washed with water (200 mL), brine (200 mL), dried over sodium sulphate and evaporated *in vacuo* to give the **title compound** (10 g, 52 mmol, 85% yield) as a light yellow solid.

LCMS (High pH, 6 min method): Rt 2.75 min, $[M+H^+]^+$ 193.2.

δ_H NMR (400 MHz, DMSO-d_6) 7.0 (d, *J* = 12.8 Hz, 1H), 6.8 (s, 1H), 6.62 (d, *J* = 12.8 Hz, 1H), 3.78 (s, 3H), 3.3 (s, 3H), 3.25 (s, 3H).

Step 3

5-Hydroxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one



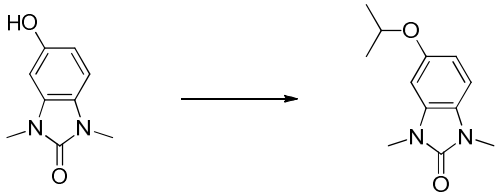
A solution of BBr_3 (1 M in dichloromethane) (41.6 mL, 41.6 mmol) was added dropwise to a solution of 5-methoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (4.00 g, 20.8 mmol) in dichloromethane (50 mL) at -78°C under nitrogen. The reaction mixture was allowed to warm to room temperature and was stirred for 6 h. The reaction was then cooled to 0 °C, quenched with addition of a saturated $NaHCO_3$ aqueous solution (100 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic layers were dried over sodium sulphate and concentrated *in vacuo*. The crude product was triturated with *n*-pentane (30 mL), filtered under vacuum to give the **title compound** (3.00 g, 16.8 mmol, 81%) as a pale yellow solid.

LCMS (Formic, 4.5 min method): Rt 1.26 min, $[M+H^+]^+$ 179.0

δ_H NMR (400 MHz, DMSO-d_6) 9.05 (s, 1H), 6.87 (d, *J* = 12.8 Hz, 1H), 6.57 (s, 1H), 6.46 (d, *J* = 12.8 Hz, 1H), 3.06 (s, 6H).

Step 4

5-Isopropoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one



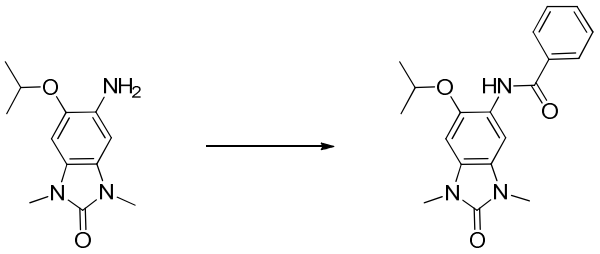
To a solution of 5-hydroxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.050 g, 0.28 mmol), triphenylphosphine (0.110 g, 0.421 mmol) and 2-propanol (0.032 mL, 0.42 mmol) in tetrahydrofuran (3 mL) at 0 °C was added diisopropyl azodicarboxylate (0.082 mL, 0.42 mmol). The reaction mixture was gradually allowed to warm to room temperature and stirred for 2 h. The reaction mixture was evaporated to dryness and the residue redissolved in ethyl acetate (20 mL) and water (20 mL). The aqueous phase was extracted with ethyl acetate (2 x 20 mL) and the combined organics were dried through a hydrophobic frit and evaporated to dryness. The residue was purified by MDAP (high pH) and then silica chromatography (0-100% ethyl acetate/cyclohexane) to afford **13** (0.030 g, 0.14 mmol, 49%) as a white solid.

LCMS (High pH): Rt 0.93 min, $[M+H^+]^+$ 221.1.

δ_H NMR (400 MHz, $CDCl_3$) 6.83 (d, *J* = 8.5 Hz, 1H), 6.66 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.58 (d, *J* = 2.2 Hz, 1H), 4.43 - 4.55 (m, 1H), 3.39 (s, 3H), 3.38 (s, 3H), 1.34 (d, *J* = 6.0 Hz, 6H).

Compound 18

N-(6-Isopropoxy-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)benzamide



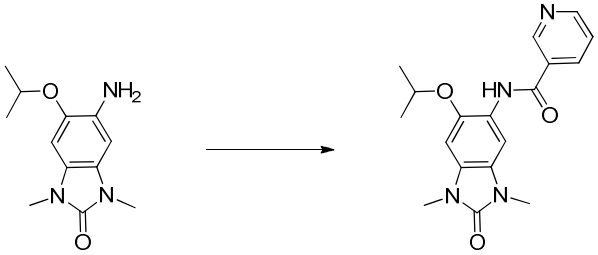
To a solution of 5-amino-6-isopropoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.045 g, 0.19 mmol) in *N*,*N*-dimethylformamide (1 mL) was added pyridine (0.031 mL, 0.38 mmol) then benzoyl chloride (0.027 mL, 0.23 mmol). The reaction was stirred at room temperature overnight. The reaction mixture was then purified by MDAP (formic). The appropriate fractions were concentrated *in vacuo* to give **18** (0.036 g, 0.11 mmol, 56 %) as a cream solid.

LCMS (High pH): Rt 1.02 min, $[M+H^+]^+$ 340.2.

δ_H NMR (400 MHz, $CDCl_3$) 8.73 (s, 1 H) 8.39 (s, 1 H) 7.95 - 7.88 (m, 2 H) 7.63 - 7.50 (m, 3 H) 6.66 (s, 1 H) 4.61 (dt, *J*=12.2, 6.1 Hz, 1 H) 3.41 (s, 3H), 3.42 (s, 3H), 1.44 (d, *J*=6.1 Hz, 6 H)

Compound 20

_N_-(6-Isopropoxy-1,3-dimethyl-2-oxo-2,3-dihydro-1_H_-benzo[_d_]imidazol-5-yl)nicotinamide



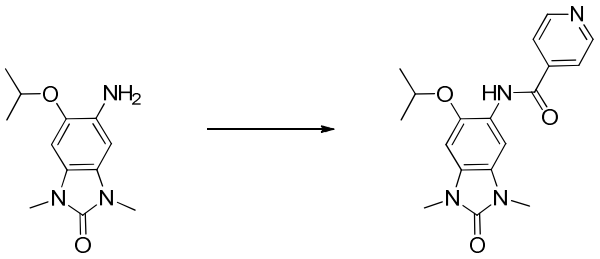
To a solution of nicotinic acid (0.021 g, 0.17 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (0.065 mg, 0.17 mmol) and *N*,*N*-diisopropylethylamine (0.078 mL, 0.45 mmol) in *N*,*N*-dimethylformamide (1 mL) was added 5-amino-6-isopropoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.035 g, 0.15 mmol), and the reaction was stirred at room temperature for 2 h. The reaction mixture was filtered and purified by MDAP (High pH). The solvent was evaporated *in vacuo* to afford **20** (0.042 g, 0.12 mmol, 83%).

LCMS (High pH): Rt 0.83 min, $[M+H^+]^+$ 341.2.

δ_H NMR (400 MHz, $CDCl_3$) 9.12 (d, *J* = 1.6 Hz, 1H), 8.80 (dd, *J* = 4.9, 1.6 Hz, 1H), 8.71 (br. s., 1H), 8.32 (s, 1H), 8.25 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.48 (ddd, *J* = 8.0, 4.9, 0.7 Hz, 1H), 6.65 (s, 1H), 4.67 - 4.54 (m, 1H), 3.44 (s, 3H), 3.41 (s, 3H), 1.43 (d, *J* = 6.1 Hz, 6H).

Compound 21

***N*-(6-Isopropoxy-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)isonicotinamide**



To a solution of isonicotinic acid (0.021 g, 0.17 mmol), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (0.065 g, 0.17 mmol) and *N*,*N*-diisopropylethylamine (0.078 mL, 0.45 mmol) in *N*,*N*-dimethylformamide (1 mL) was added 5-amino-6-isopropoxy-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.035 g, 0.149 mmol), and the reaction was stirred at room temperature for 2 h. The reaction mixture was filtered and purified by MDAP (High pH). The solvent was evaporated *in vacuo* to afford **21** (0.044 g, 0.13 mmol, 87%)

LCMS (High pH): Rt 0.83 min, $[M+H^+]^+$ 341.3.

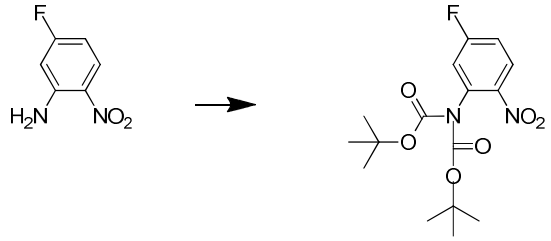
δ_H NMR (400 MHz, $CDCl_3$) 8.86 - 8.81 (m, 2H), 8.75 (br. s., 1H), 8.32 (s, 1H), 7.74 - 7.70 (m, 2H), 6.65 (s, 1H), 4.67 - 4.55 (m, 1H), 3.44 (s, 3H), 3.41 (s, 3H), 1.43 (d, *J* = 6.1 Hz, 6H).

Compound 26

Compound 26 was obtained according in part to: Chen, H.; McDaniel, K. F.; Green, B. E.; Shanley, J. P.; Kruger, A. W.; Gandarilla, J.; Welch, D. S.; Cink, R. D.; Gai, Y.; Wang, G. Or, Y. S. Preparation of macrocyclic proline-containing peptides as hepatitis C serine protease inhibitors.PCT Int. Appl.(2011), WO2011156337A2.

Step 1

Di-*tert*-butyl (5-fluoro-2-nitrophenyl)carbamate



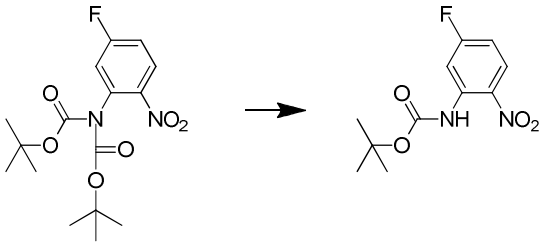
A solution of 5-fluoro-2-nitroaniline (10 g, 64 mmol) in 2-methyltetrahydrofuran (200 mL) at room temperature was treated with di-*tert*-butyl dicarbonate (29.7 mL, 128 mmol) and 4-dimethylaminopyridine (0.160 g, 1.31 mmol) and the resulting mixture was stirred at this temperature for 3 days. The mixture was washed with water and the layers were separated (an aliquot of brine was added to facilitate the separation of the phases). The aqueous phase was extracted with ethyl acetate and the combined organic phases were washed with water (again an aliquot of brine was added to facilitate the separation of the phases), dried over $MgSO_4$ and concentrated *in vacuo* to give the **title compound** (21.8 g, 61.2 mmol, 96%) as a yellow solid which was used in the next step without further purification.

LCMS (High pH): Rt 1.27 min; $[M+H^+]^+$ = 357.3

δ_H NMR (400 MHz, $CDCl_3$) 8.21 - 8.12 (m, 1H), 7.26 - 7.17 (m, 1H), 7.11 - 7.04 (m, 1H), 1.43 (s, 18H).

Step 2

***Tert*-butyl (5-fluoro-2-nitrophenyl)carbamate**



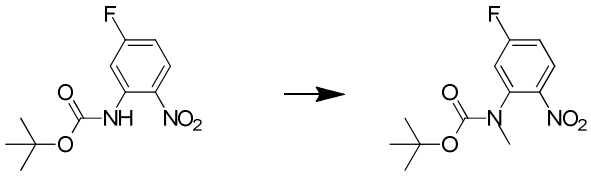
A solution of di-*tert*-butyl (5-fluoro-2-nitrophenyl)carbamate (11.3 g, 31.7 mmol) in dichloromethane (100 mL) at room temperature was treated with trifluoroacetic acid (3.66 mL, 47.5 mmol) drop wise and the resulting mixture was stirred at this temperature for 80 min before being treated with a saturated $NaHCO_3$ aqueous solution. The layers were separated and the organic phase was washed with a saturated $NaHCO_3$ aqueous solution, dried using a phase separator and concentrated *in vacuo* to give the **title compound** (8.10 g, 31.6 mmol, 100%) as a yellow solid which was used in the next step without further purification.

LCMS (High pH): Rt 1.31 min; $[M\text{-}H^+]^-$ = 255.4

δ_H NMR (400 MHz, $CDCl_3$) 9.90 (br s, 1H), 8.42 (dd, *J* = 11.7, 2.7 Hz, 1H), 8.28 (dd, *J* = 9.4, 5.7 Hz, 1H), 6.79 (m, 1H), 1.57 (s, 9H).

Step 3

***Tert*-butyl (5-fluoro-2-nitrophenyl)(methyl)carbamate**



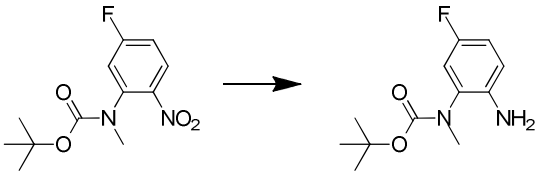
A solution of *tert*-butyl (5-fluoro-2-nitrophenyl)carbamate (13 g, 51 mmol) in tetrahydrofuran (80 mL) and *N*,*N*-dimethylformamide (160 mL) at 0 °C under nitrogen was treated with sodium hydride (60% w/w in mineral oil, 2.43 g, 60.9 mmol) and the resulting mixture was stirred at this temperature for 15 minutes before being treated with methyl iodide (6.34 mL, 101 mmol). The resulting mixture was stirred at this temperature for 1 h. Ethanol (2 mL) was added followed by a few drops of acetic acid. The bright yellow solution was allowed to warm to room temperature and was concentrated *in vacuo*. The solid residue was partitioned between water and ethyl acetate and the layers were separated. The aqueous phase was extracted with ethyl acetate. The combined organics were washed twice with water then with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give a mixture containing the **title compound** (14.9 g) as a yellow oil which was used the next step without further purification.

LCMS (High pH): Rt 1.15 min; $[M+H^+]^+ = 271.2$

δ_H NMR (400 MHz, $CDCl_3$) 8.03 - 7.94 (m, 1H), 7.13 - 7.02 (m, 2H), 3.32 (s, 3H), 1.33 (br s, 9H).

Step 4

***Tert*-butyl (2-amino-5-fluorophenyl)(methyl)carbamate**



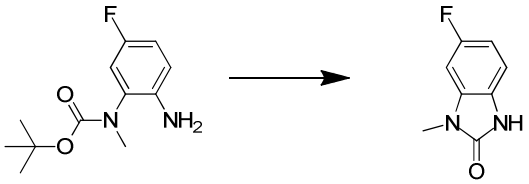
A solution of crude *tert*-butyl (5-fluoro-2-nitrophenyl)(methyl)carbamate (14.9 g) in ethanol (300 mL) was treated with palladium on carbon (10% w/w, 50% paste, 1.5 g) and the resulting mixture was stirred under hydrogen (1 bar) for 16 h. The catalyst was removed using a pad of celite (10 g) and rinsed with ethanol. The combined organics were concentrated *in vacuo* to give the **title compound** (11.4 g, 94% over 2 steps) as a white solid which was used in the next step without further purification.

LCMS (High pH): Rt 1.01 min; $[M+H^+]^+$ = 241.2

δ_H NMR (400 MHz, DMSO-d_6, T = 393K) 6.86 - 6.70 (m, 3H), 4.46 - 4.28 (br s, 2H), 3.05 (s, 3H), 1.40 (br s, 9H).

Step 5

6-Fluoro-1-methyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one



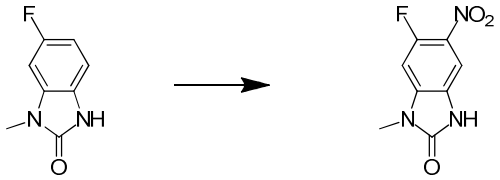
A solution of *tert*-butyl (2-amino-5-fluorophenyl)(methyl)carbamate (12.7 g, 53.0 mmol) in *N*,*N*-dimethylformamide (120 mL) and tetrahydrofuran (80 mL) under nitrogen at room temperature was treated with sodium hydride (60% w/w in mineral oil, 2.76 g, 68.9 mmol) and the resulting mixture was stirred at 60 °C for 2 h then was cooled to room temperature, treated with ethanol (2 mL, excess) and concentrated *in vacuo*. The residue was dissolved in water and treated with a few drops of acetic acid until a precipitate appeared. This precipitate was filtered off and rinsed with water. It was then suspended in diethyl ether, filtered off and dried under vacuum at 40 ^oC for 16 h to give the **title compound** (7.6 g, 46 mmol, 86%) as a grey solid which was used in the next step without further purification.

LCMS (High pH): Rt 0.66 min; $[M+H^+]^+$ = 167.0

δ_H NMR (400 MHz, DMSO-d_6) 11.09 - 10.74 (m, 1H), 7.05 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.93 (dd, *J* = 8.4, 4.8 Hz, 1H), 6.78 (ddd, *J* = 10.4, 8.4, 2.4 Hz, 1H), 3.26 (s, 3H).

Step 6

6-Fluoro-1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one



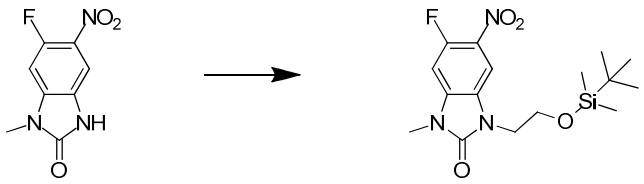
A suspension of 6-fluoro-1-methyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (5 g, 30 mmol) in acetic anhydride (75 mL) under nitrogen was cooled to -30 ^{o}C and slowly treated with fuming nitric acid (1.34 mL, 30.1 mmol), keeping the temperature below -20 ^{o}C. The resulting mixture was then allowed to warm slowly to 0 ^{o}C. After 1 h, the mixture was poured into ice-water (100 mL) and ethyl acetate (10 mL) was added. The resulting mixture was stirred for 30 min. The precipitate formed was collected by filtration and washed with water (20 mL) before being dried *in vacuo* at 40 ^{o}C for 16 h to give the **title compound** (4.75 g, 22.5 mmol, 75%) as a light yellow solid which was used in the next step without further purification.

LCMS (High pH): Rt 0.66 min; $[M\text{-}H^{+}]^{-}$ = 210.3

δ_H NMR (400 MHz, DMSO-d_6) 11.39 (s, 1H), 7.63 (d, *J* = 6.6 Hz, 1H), 7.44 (d, *J* = 11.7 Hz, 1H), 3.33 (s, 3H).

Step 7

1-(2-((*Tert*-butyldimethylsilyl)oxy)ethyl)-5-fluoro-3-methyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one



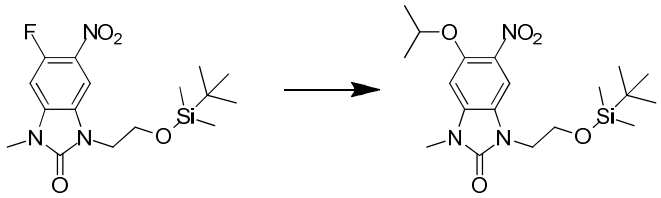
To a stirred solution of 6-fluoro-1-methyl-5-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (1.15 g, 5.43 mmol) in *N*,*N*-dimethylformamide (10 mL) was added portionwise sodium hydride (60% w/w in mineral oil, 0.326 g, 8.14 mmol). The mixture was stirred for 30 min, then treated with (2-bromoethoxy)(*tert*-butyl)dimethylsilane (1.40 mL, 6.52 mmol), and additional *N*,*N*-dimethylformamide (15 mL). After 15 minutes at room temperature the mixture was heated to 40 ^{o}C. After a further 18 hours additional sodium hydride (60% w/w in mineral oil, 0.109 g, 2.71 mmol) and (2-bromoethoxy)(*tert*-butyl)dimethylsilane (0.349 mL, 1.63 mmol) were added. The mixture was stirred for 4 h, then concentrated *in vacuo*. The residue was diluted with a saturated sodium bicarbonate aqueous solution, and extracted with EtOAc. The organic extracts were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give a residue which was purified by silica chromatography (0-4% methanolic ammonia [2 M] in dichloromethane) to afford the **title compound** (1.008 g, 2.727 mmol, 50%) as an orange/yellow solid.

LCMS (Formic): Rt 1.37 min, $[M+H^+]^+$ 370.4.

δ_H NMR (400 MHz, DMSO-d_6) 8.00 (d, *J* = 6.4 Hz, 1H), 7.52 (d, *J* = 12.0 Hz, 1H), 4.05 (t, *J* = 5.0 Hz, 2H), 3.84 (t, *J* = 5.0 Hz, 2H), 3.39 (s, 3H), 0.71 (s, 9H), -0.15 (s, 6H).

Step 8

1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-5-isopropoxy-3-methyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one



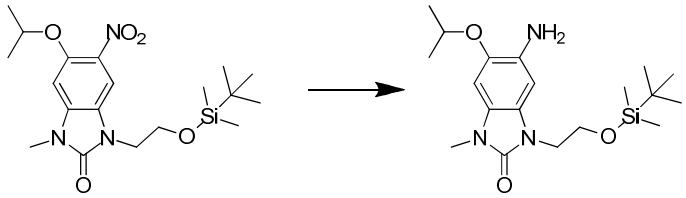
A stirred solution of propan-2-ol (0.047 mL, 0.609 mmol) in tetrahydrofuran (3 mL) was treated with sodium hydride (60% w/w in mineral oil, 0.024 g, 0.61 mmol). After 15 minutes 1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-5-fluoro-3-methyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.150 g, 0.406 mmol) was added, followed by sodium hydride (60% w/w in mineral oil, 0.010 g, 0.25 mmol) after a further 85 minutes. The mixture was stirred for 1.5 h then concentrated *in vacuo*. The residue was then partitioned between ethyl acetate and a saturated sodium bicarbonate aqueous solution and the layers were separated. The aqueous phase was further extracted with ethyl acetate. The organic extracts were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give a residue which was purified by silica chromatography (0-80% ethyl acetate in cyclohexane) to give the **title compound** (0.129 g, 0.315 mmol, 78%) as bright yellow solid.

LCMS (Formic): Rt 1.43 min, $[M+H^+]^+$ 410.4.

δ_H NMR (400 MHz, $CDCl_3$) 7.75 (s, 1H), 6.65 (s, 1H), 4.61 (spt, *J* = 6.1 Hz, 1H), 4.01 (t, *J* =4 .0 Hz, 2H), 3.91 (t, *J* = 4.0 Hz, 2H), 3.45 (s, 3H), 1.43 (d, *J* = 6.1 Hz, 6H), 0.79 (s, 9H), -0.05 (s, 6H).

Step 9

5-Amino-3-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-isopropoxy-1-methyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one



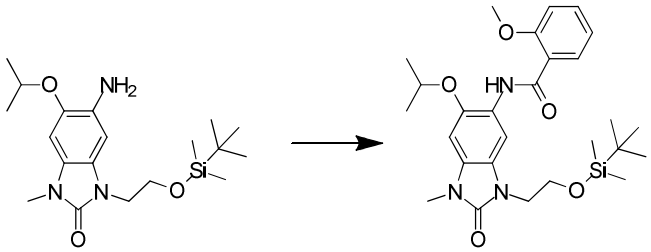
A mixture of 1-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-5-isopropoxy-3-methyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.129 g, 0.315 mmol) and palladium on carbon (10% w/w, 50% paste, 0.524 g) in isopropanol (30 mL) was hydrogenated at room temperature and atmospheric pressure for 24 h. The mixture was then filtered through Celite, and the residue washed with methanol and dichloromethane. The filtrate was concentrated *in vacuo* to give a residue which was taken up in DMSO:MeOH (1:1) and purified by MDAP (High pH) to give the **title compound** (0.072 g, 0.19 mmol, 60%) as a cream solid.

LCMS (High pH): Rt 1.27 min, $[M+H^+]^+$ 380.4.

δ_H NMR (400 MHz, $CDCl_3$) 6.57 (s, 1H), 6.54 (s, 1H), 4.44 (spt, *J* = 6.1 Hz, 1H), 3.95 - 3.90 (m, 2H), 3.89 - 3.84 (m, 2H), 3.35 (s, 3H), 1.36 (d, *J* = 6.1 Hz, 6H), 0.83 (s, 9H), -0.06 (s, 6H). Two NH's can't be seen.

Step 10

N-(3-(2-((*Tert*-butyldimethylsilyl)oxy)ethyl)-6-isopropoxy-1-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide



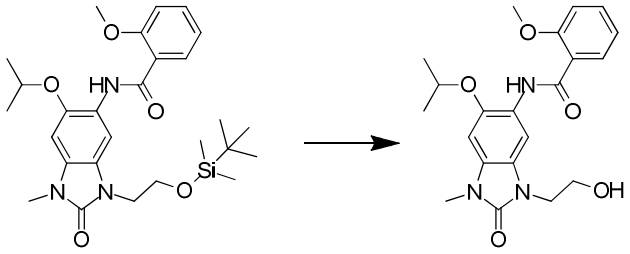
A stirred mixture of 5-amino-3-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-isopropoxy-1-methyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.072 mg, 0.19 mmol) and pyridine (0.046 mL, 0.57 mmol) in dichloromethane (2 mL) was treated with 2-methoxybenzoyl chloride (0.038 mL, 0.28 mmol). After 1 h the mixture was concentrated *in vacuo* to give a residue which was taken up in DMSO:MeOH (1:1) and purified by MDAP (High pH) to give the **title compound** (0.089 g, 0.17 mmol, 91%) as a cream solid.

LCMS (High pH): Rt 1.52 min, $[M+H^+]^+$ 514.5.

δ_H NMR (400 MHz, $CDCl_3$) 10.41 (s, 1H), 8.58 (s, 1H), 8.33 (dd, *J* = 7.6, 1.9 Hz, 1H), 7.51 (ddd, *J* = 8.3, 7.6, 1.9 Hz, 1H), 7.19 - 7.13 (m, 1H), 7.07 (d, *J* = 8.3 Hz, 1H), 6.64 (s, 1H), 4.60 (spt, *J* = 6.1 Hz, 1H), 4.11 (s, 3H), 4.04 (t, *J* = 6.0 Hz, 2H), 3.92 (t, *J* = 6.0 Hz, 2H), 3.40 (s, 3H), 1.43 (d, *J* = 6.1 Hz, 6H), 0.83 - 0.78 (m, 9H), -0.05 (s, 6H).

Step 11

_N_-(3-(2-Hydroxyethyl)-6-isopropoxy-1-methyl-2-oxo-2,3-dihydro-1_H_-benzo[_d_]imidazol-5-yl)-2-methoxybenzamide



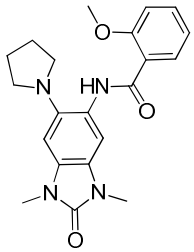
A stirred solution of *N*-(3-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-6-isopropoxy-1-methyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide (0.089 mg, 0.17 mmol) in dichloromethane (2 mL) was treated with trifluoroacetic acid (2 mL). After 20 minutes the mixture was concentrated *in vacuo* to give a residue which was taken up in DMSO:MeOH (1:1) and purified by MDAP (High pH) to give **26** (0.067 g, 0.17 mmol, 97%) as a cream solid.

LCMS (High pH): Rt 1.01 min, $[M+H^+]^+$ 400.4.

δ_H NMR (400 MHz, DMSO-d_6) 10.40 (s, 1H), 8.43 (s, 1H), 8.11 (dd, *J* = 1.8, 7.8 Hz, 1H), 7.63 - 7.55 (m, 1H), 7.33 - 7.27 (m, 1H), 7.20 - 7.13 (m, 1H), 7.10 (s, 1H), 4.90 (t, *J* = 5.4 Hz, 1H), 4.75 (spt, *J* = 6.1 Hz, 1H), 4.10 (s, 3H), 3.84 (t, *J* = 6.1 Hz, 2H), 3.68 - 3.61 (m, 2H), 3.33 (s, 3H), 1.37 (d, *J* = 6.1 Hz, 6H).

Compound 27

***N*-(1,3-Dimethyl-2-oxo-6-(pyrrolidin-1-yl)-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide**

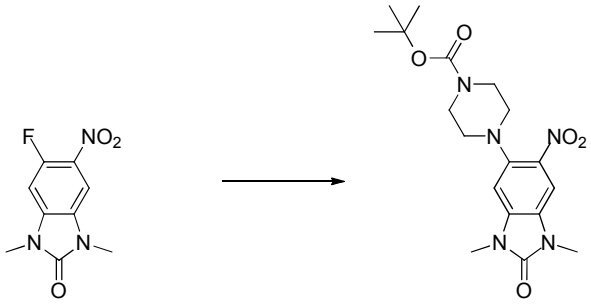


Available from ChemDiv. Catalogue number C301-6407

Compound 28

Step 1

***Tert*-butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)piperazine-1-carboxylate**



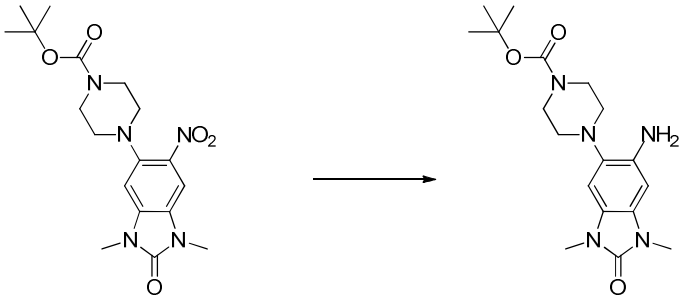
A mixture of 5-fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.498 g, 2.21 mmol), tert-butyl piperazine-1-carboxylate (0.453 g, 2.43 mmol), and *N*,*N*-diisopropylethylamine (0.773 mL, 4.42 mmol) in dimethyl sulfoxide (1 mL) was heated at 120 ºC in a microwave for 8 h. The mixture was then concentrated *in vacuo*, and the resulting residue partitioned between ethyl acetate and a saturated sodium bicarbonate aqueous solution. The layers were separated and the aqueous phase was further extracted with ethyl acetate. The organic extracts were combined, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give the impure **title compound** (0.939 g) as a deep orange/red solid. This material was taken on to the next step without further purification.

LCMS (Formic): Rt 1.11 min, $[M+H^+]^+$ 392.4 (~86% purity by UV).

δ_H NMR (400 MHz, DMSO-d_6) 7.77 (s, 1H), 7.25 (s, 1H), 3.50 - 3.43 (m, 4H), 3.36 (s, 3H), 3.35 (s, 3H), 2.97 - 2.93 (m, 4H), 1.43 (s, 9H).

Step 2

***Tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)piperazine-1-carboxylate**



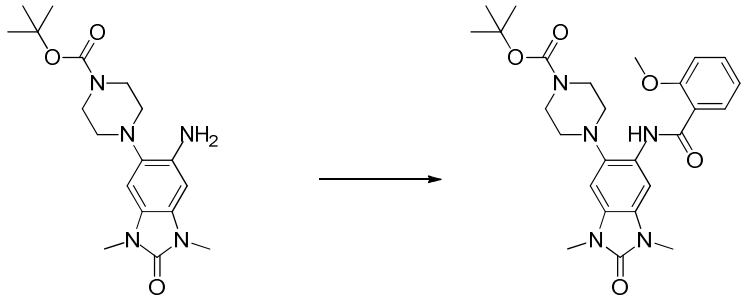
A stirred mixture of impure *tert*-butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)piperazine-1-carboxylate (0.939 g) and palladium on carbon (10% w/w, 50% paste, 1.7 g) in ethanol (120 mL) was hydrogenated at room temperature and atmospheric pressure. After 51 h an additional portion of palladium on carbon (10% w/w, 50% paste, 1.0 g) was added. The mixture was hydrogenated for a further 17 h then filtered through Celite. The residue was washed with ethanol and dichloromethane, and the filtrate concentrated *in vacuo* to give a residue which was purified by silica chromatography (0-3% methanolic ammonia [2 M] in dichloromethane) to give the **title compound** (0.449 g, 1.24 mmol, 56% over 2 steps) as cream solid.

LCMS (High pH): Rt 0.96 min, $[M+H^+]^+$ 362.3.

δ_H NMR (400 MHz, DMSO-d_6) 6.84 (s, 1H), 6.48 (s, 1H), 4.66 (br. s., 2H), 3.49 (br. s., 4H), 3.23 (s, 3H), 3.20 (s, 3H), 2.73 (br. s., 4H), 1.43 (s, 9H).

Step 3

***Tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)piperazine-1-carboxylate**



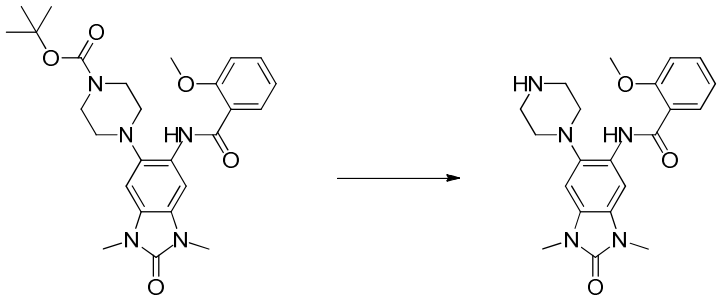
A stirred mixture of *tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)piperazine-1-carboxylate (0.051 mg, 0.14 mmol and pyridine (0.034 mL, 0.42 mmol) in *N*,*N*-dimethylformamide (0.5 mL) was treated with 2-methoxybenzoyl chloride (0.038 mL, 0.28 mmol). After 25 minutes the mixture was concentrated *in vacuo* and the residue taken up in DMSO:MeOH (1:1) and purified by MDAP (High pH) to give the **title compound** (0.054 g, 0.11 mmol, 77%) as an off-white solid.

LCMS (High pH): Rt 1.22 min, $[M+H^+]^+$ 496.4.

δ_H NMR (400 MHz, DMSO-d_6) 10.55 (s, 1H), 8.29 (s, 1H), 8.08 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.62 - 7.55 (m, 1H), 7.29 (d, *J* = 8.3 Hz, 1H), 7.25 (s, 1H), 7.18 - 7.13 (m, 1H), 4.07 (s, 3H), 3.54 (br. s., 4H), 3.33 (s, 3H), 3.31 (s, 3H), 2.82 (br. s., 4H), 1.44 (s, 9H).

Step 4

_N_-(1,3-Dimethyl-2-oxo-6-(piperazin-1-yl)-2,3-dihydro-1_H_-benzo[_d_]imidazol-5-yl)-2-methoxybenzamide



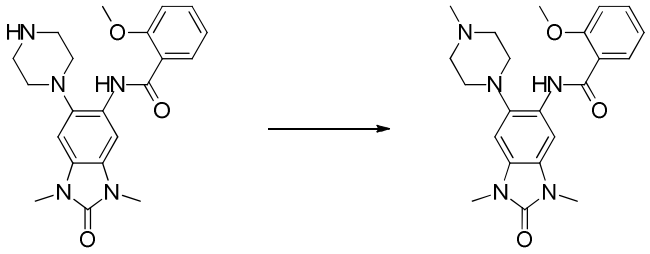
To *tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)piperazine-1-carboxylate (0.054 g, 0.11 mmol) was added trifluoroacetic acid (1 mL). After 3 h of stirring at room temperature the mixture was concentrated *in vacuo*. The residue was loaded on an SCX column (1 g), washed with MeOH, then eluted with methanolic ammonia (2M). The appropriate fractions were combined and concentrated *in vacuo* to give **28** (0.033 g, 0.083 mmol, 77%) as a yellow oil.

LCMS (High pH): Rt 0.84 min, $[M+H^+]^+$ 396.4.

δ_H NMR (400 MHz, DMSO-d_6) 10.55 (s, 1H), 8.28 (s, 1H), 8.08 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.58 (ddd, *J* = 8.4, 7.5, 1.7 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.19 - 7.11 (m, 2H), 4.10 (s, 3H), 3.34 (s, 3H), 3.31 (s, 3H), 2.94 - 2.85 (m, 4H), 2.80 - 2.72 (m, 4H). One of the NH's can't be seen.

Compound 29

_N_-(1,3-Dimethyl-6-(4-methylpiperazin-1-yl)-2-oxo-2,3-dihydro-1_H_-benzo[_d_]imidazol-5-yl)-2-methoxybenzamide



To a solution of *N*-(1,3-dimethyl-2-oxo-6-(piperazin-1-yl)-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide (0.015 g, 0.038 mmol) in formic acid (0.5 mL) at room temperature was added formaldehyde (37% wt. in H_2O, 0.141 mL, 1.90 mmol). The reaction mixture was stirred at 80 °C for 2 h then was cooled to room temperature and neutralised with a saturated $NaHCO_3$ aqueous solution. The aqueous phase was extracted with dichloromethane (3 x 10 mL) and the combined organics were dried through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (High pH). The solvent was evaporated to give **29** (0.009 g, 0.02 mmol, 58%) as a white solid.

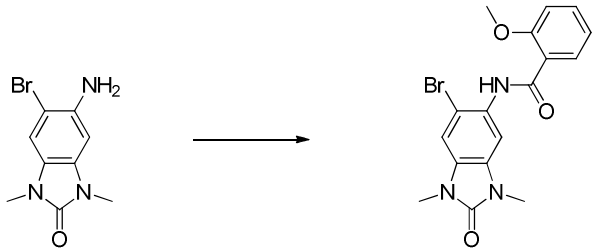
LCMS (High pH): Rt 0.95 min, $[M+H]^+$ 410.4.

δ_H NMR (400 MHz, $CDCl_3$) 10.51 (s, 1H), 8.38 (s, 1H), 8.28 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.55 - 7.48 (m, 1H), 7.19 - 7.13 (m, 1H), 7.08 (d, *J* = 8.3 Hz, 1H), 6.84 (s, 1H), 4.10 (s, 3H), 3.43 (s, 3H), 3.41 (s, 3H), 2.99 (t, *J* = 4.6 Hz, 4H), 2.76 - 2.60 (m, 4H), 2.42 (s, 3H).

Compound 30

Step 1

***N*-(6-Bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide**



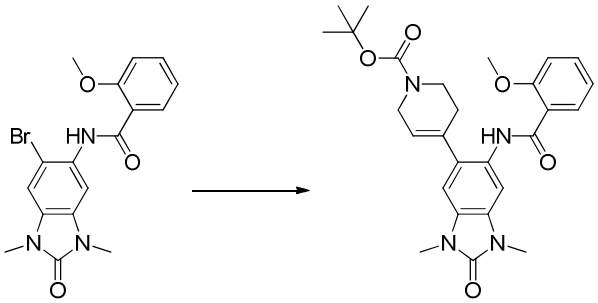
N,*N*-Diisopropylethylamine (0.689 mL, 3.94 mmol) and 2-methoxybenzoyl chloride (0.531 mL, 3.94 mmol) were added to a solution of 5-amino-6-bromo-1,3-dimethyl-1*H*-benzo[*d*]imidazol-2(3*H*)-one (1.01 g, 3.94 mmol) in dichloromethane (30 mL). After stirring at room temperature for 24 h under nitrogen, the reaction mixture was diluted with dichloromethane (20 mL) and washed with water (50 mL). The aqueous phase was further extracted with dichloromethane (50 mL), the combined organic layers were dried through a hydrophobic frit and concentrated *in vacuo* to give a residue which was purified by silica chromatography, (0-4% methanolic ammonia (2M) in dichloromethane). The appropriate fractions were combined and concentrated *in vacuo* to give the **title compound** (0.567 g, 1.45 mmol, 37%) as a white solid.

LCMS (Formic): Rt 1.08 min, $[M+H^+]^+$ 392.2.

δ_H NMR (400 MHz, DMSO-d_6) 10.42 (s, 1H), 8.21 (s, 1H), 8.09 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.67 - 7.58 (m, 1H), 7.56 (s, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.22 - 7.11 (m, 1H), 4.10 (s, 3H), 3.35 (s, 6H).

Step 2

***Tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate**



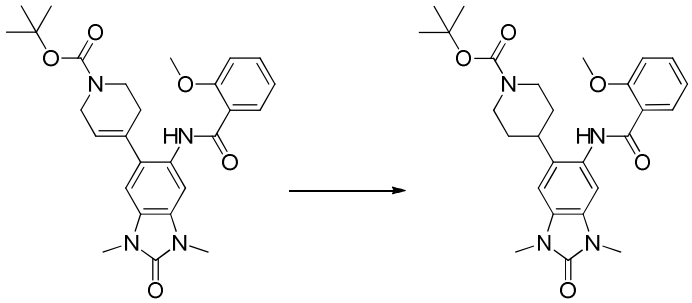
To a mixture of *N*-(6-bromo-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide (0.200 g, 0.513 mmol), *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (0.174 g, 0.564 mmol), potassium carbonate (0.142 g, 1.02 mmol), and $PdCl_2$(dppf)-CH_2Cl_2 adduct (0.042 g, 0.051 mmol) under nitrogen in a microwave vial was added 1,4-dioxane (2 mL) and water (0.400 mL). The mixture was then heated in the microwave at 100 ^oC for 2 h. The mixture was concentrated *in vacuo* to give a residue which was purified by silica chromatography (0-75% ethyl acetate in dichloromethane) to give the **title compound** (0.239 g, 0.485 mmol, 95%) as white foam.

LCMS (Formic): Rt 1.19 min, $[M+H^+]^+$ 493.5.

δ_H NMR (400 MHz, DMSO-d_6) 9.86 (s, 1H), 7.99 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.90 (s, 1H), 7.59 - 7.53 (m, 1H), 7.26 (d, *J* = 8.3 Hz, 1H), 7.16 - 7.10 (m, 1H), 7.03 (s, 1H), 5.79 (br. s., 1H), 4.03 - 3.99 (m, 2H), 3.94 (s, 3H), 3.59 - 3.54 (m, 2H), 3.33 (s, 6H), 2.38 (br. s., 2H), 1.42 (s, 9H).

Step 3

***Tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)piperidine-1-carboxylate**



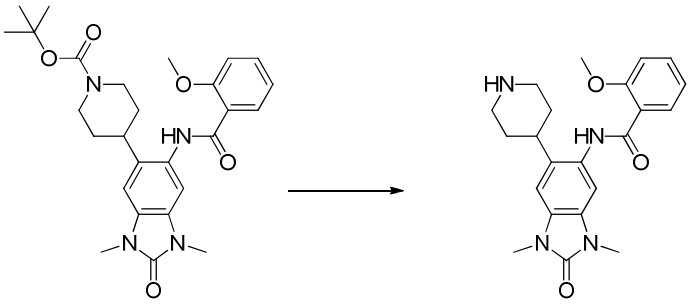
A stirred mixture of *tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (0.171 g, 0.347 mmol) and palladium on carbon (10% w/w, 50% paste, 0.406 g) in ethanol (30 mL) was hydrogenated at room temperature and atmospheric pressure. After 27 h the mixture was filtered through Celite, the residue washed with ethanol and dichloromethane, then the filtrate concentrated *in vacuo* to give the **title compound** (0.168 g, 0.339 mmol, 98%) as cream solid.

LCMS (High pH): Rt 1.16 min, $[M+H^+]^+$ 495.5.

δ_H NMR (400 MHz, DMSO-d_6) 9.72 (s, 1H), 7.80 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.56 - 7.50 (m, 1H), 7.31 - 7.20 (m, 2H), 7.16 - 7.06 (m, 2H), 4.19 - 4.10 (m, 2H), 3.98 (s, 3H), 3.31 (s, 6H), 3.07 - 2.97 (m, 1H), 2.78 (br. s., 2H), 1.81 - 1.71 (m, 2H), 1.66 - 1.52 (m, 2H), 1.43 (s, 9H).

Step 4

_N_-(1,3-Dimethyl-2-oxo-6-(piperidin-4-yl)-2,3-dihydro-1_H_-benzo[_d_]imidazol-5-yl)-2-methoxybenzamide



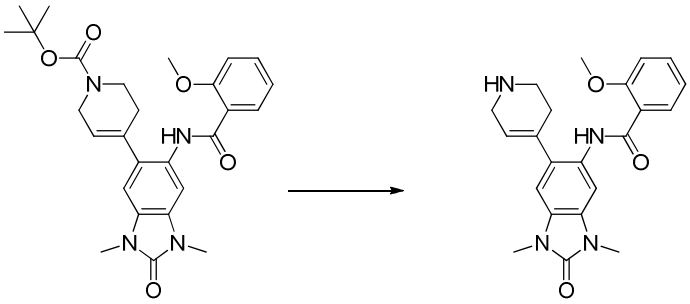
To *tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)piperidine-1-carboxylate (0.116 g, 0.235 mmol) was added trifluoroacetic acid (5 mL). After 85 minutes the mixture was concentrated *in vacuo* to give a residue which was taken up in DMSO:MeOH (1:1) and purified by MDAP (High pH) to give **30** (0.071 g, 0.18 mmol, 77%) as a white solid.

LCMS (High pH): Rt 0.76 min, $[M+H^+]^+$ 395.4.

δ_H NMR (400 MHz, DMSO-d_6, 393 K) 9.45 (br. s., 1H), 7.91 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.57 - 7.50 (m, 1H), 7.37 (s, 1H), 7.24 (d, *J* = 8.1 Hz, 1H), 7.15 - 7.09 (m, 1H), 7.02 (s, 1H), 4.05 (s, 3H), 3.36 (s, 3H), 3.32 (s, 3H), 3.14 - 3.06 (m, 2H), 3.01 - 2.92 (m, 1H), 2.66 (dt, *J* = 12.0, 2.8 Hz, 2H), 1.78 - 1.71 (m, 2H), 1.70 - 1.57 (m, 2H). One of the NH's can't be seen.

Compound 31

_N_-(1,3-Dimethyl-2-oxo-6-(1,2,3,6-tetrahydropyridin-4-yl)-2,3-dihydro-1_H_-benzo[_d_]imidazol-5-yl)-2-methoxybenzamide



To *tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-5,6-dihydropyridine-1(2*H*)-carboxylate (0.046 g, 0.093 mmol) was added trifluoroacetic acid (4 mL). After stirring for 25 minutes the mixture was concentrated *in vacuo* to give a residue which was taken up in DMSO:MeOH (1:1) and purified by HPLC (High pH) to give **31** (0.036 g, 0.092 mmol, 99%) as a white solid.

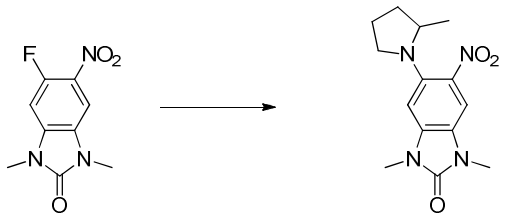
LCMS (High pH): Rt 0.81 min, $[M+H^+]^+$ 393.3.

δ_H NMR (400 MHz, DMSO-d_6, 393 K) 9.65 (br. s., 1H), 8.04 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.94 (s, 1H), 7.59 - 7.51 (m, 1H), 7.24 (d, *J* = 8.1 Hz, 1H), 7.17 - 7.10 (m, 1H), 6.89 (s, 1H), 5.84 - 5.76 (m, 1H), 4.07 (s, 3H), 3.47 - 3.40 (m, 2H), 3.34 (s, 6H), 2.99 (t, *J* = 5.6 Hz, 2H), 2.31 - 2.25 (m, 2H). One of the NH's can't be seen.

Compound 32-33

Step 1

1,3-Dimethyl-5-(2-methylpyrrolidin-1-yl)-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one



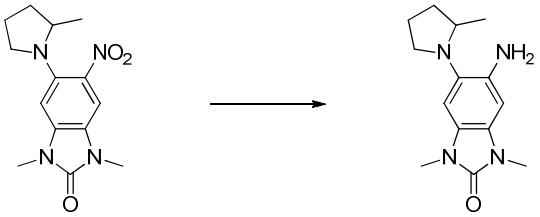
To a suspension of 5-fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (1.54 g, 6.83 mmol) in dimethyl sulfoxide (15 mL) in a 20 mL microwave vial was added *N*,*N*-diisopropylethylamine (2.386 mL, 13.66 mmol) and 2-methylpyrrolidine (1.046 mL, 10.25 mmol). The reaction mixture was heated to 120 ^{o}C in a Biotage Initiator for 1 h using high absorbance. The reaction mixture was cooled to room temperature and poured into a 20% (v/v) solution of saturated sodium bicarbonate in water (100 mL) and extracted with ethyl acetate (3 x 100 mL). The extractions were combined and dried with Na_2SO_4. The drying agent was filtered off and the filtrate was concentrated *in vacuo* to give a brown liquid. The liquid was redissolved in dichloromethane (100 mL) and washed with water (50 mL). The aqueous layer was extracted with dichloromethane (35 mL). The organic extracts were eluted through a hydrophobic frit and concentrated *in vacuo* to give the impure **title compound** (2.027 g) as a red crystalline solid, which was carried on to the next step without further purification.

LCMS (High pH): Rt 1.01 min, $[M+H^+]^+$ 291.2.

δ_H NMR (400 MHz, DMSO-d_6) 7.66 (s, 1H), 6.84 (s, 1H), 3.97 (dt, *J* = 9.0, 6.1 Hz, 1H), 3.51 (td, *J* = 10.3, 6.1 Hz, 1H), 3.31 (s, 3H), 3.34 (s, 3H), 2.67 - 2.59 (m, 1H), 2.30 - 2.21 (m, 1H), 1.98 - 1.87 (m, 1H), 1.71 (d, *J* = 11.0 Hz, 1H), 1.63 - 1.50 (m, 1H), 1.12 - 1.05 (m, 3H).

Step 2

5-Amino-1,3-dimethyl-6-(2-methylpyrrolidin-1-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one



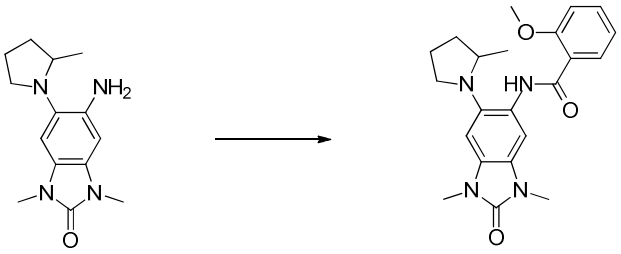
To a stirred suspension of 1,3-dimethyl-5-(2-methylpyrrolidin-1-yl)-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (2.03 g, 6.98 mmol) in ethanol (60 mL) and water (20 mL) was added iron powder 325 mesh (1.95 g, 34.9 mmol) and ammonium chloride (1.120 g, 20.94 mmol). The reaction mixture was heated to 90 ^{o}C for 4 h under nitrogen then was cooled to room temperature and filtered through celite (10 g cartridge) which was pre-washed with MeOH. The filter cake was washed with methanol (c. 120 mL) and the filtrate was concentrated *in vacuo*. The residue was dissolved in methanol and loaded onto a Biotage SCX-2 70 g cartridge which was pre-washed with methanol. The cartridge was then washed with methanol (c. 700 mL) followed by 2M methanolic ammonia (c. 400 mL). The basic wash was concentrated *in vacuo* and the residue purified by silica chromatography (0-55% [3:1 - EtOAc:EtOH] in cyclohexane) to give the **title compound** (1.43 g, 5.50 mmol, 81 % yield over 2 steps) as a brown powder.

LCMS (High pH): Rt 0.87 min, $[M+H^{+}]^{+}$ 261.3

δ_H NMR (400 MHz, DMSO-d_6) 6.85 (s, 1H), 6.46 (s, 1H), 4.60 (br. s., 2H), 3.23 (s, 3H), 3.21(s, 3H), 2.60 - 2.47 (m, 2H), 2.14 - 2.00 (m, 1H), 1.92 - 1.71 (m, 2H), 1.53 - 1.39 (m, 1H), 1.09 – 1.03 (m, 1H), 0.90 (d, *J* = 5.8 Hz, 3H).

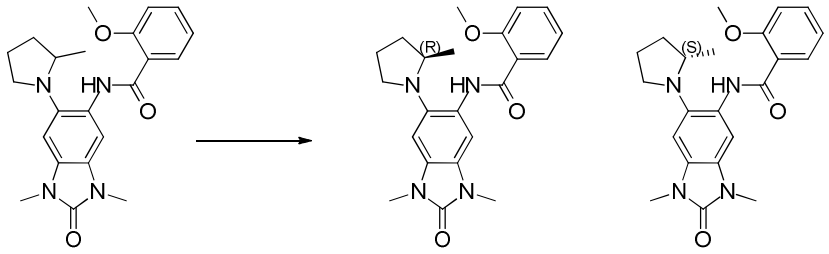
Step 3

***N*-(1,3-Dimethyl-6-(2-methylpyrrolidin-1-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide**



To a solution of 5-amino-1,3-dimethyl-6-(2-methylpyrrolidin-1-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.047 g, 0.18 mmol) in *N*,*N*-dimethylformamide (1 mL) were added pyridine (0.025 mL, 0.31 mmol) and 2-methoxybenzoyl chloride (0.055 mL, 0.37 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was purified by MDAP (High pH) to give the racemic **title compound** (0.044 g, 0.11 mmol, 61 % yield) as a light brown solid.

Step 4



Compound 32 Compound 33

Analytical Method: Approx 0.5 mg of the racemate was dissolved in 50% EtOH/Heptane (1 mL) 20 µL and injected on the column.

10% EtOH/Heptane, f=1.0 mL/min, wavelength 215 nm. Ref 550,100
Column 4.6 mm id x 25 cm Chiralcel OD-H
Lot No.ODH0CE-PD027

Prep Method: Approx 24 mg dissolved in 1 mL EtOH + Heat
Injection; 1 mL of the solution was injected onto the column.
10% EtOH/Heptane, f=30 mL/min, wavelength, 215 nm. Ref 550,100
Column 30 mm x 25 cm Chiralcel OD-H
Lot No ODH11158-01
Total number of injections = 1

The appropriate fractions were bulked, concentrated *in vacuo* and then transferred to a weighed flask for final analysis as described by the analytical method above. The final isomers were re-dissolved in dichloromethane then concentrated *in vacuo* in order to obtain a solid.

Fraction Collection: Fractions from 20.5-24.5 mins (0.010 g) = (*S*)-enantiomer
Fractions from 25-30 mins (0.010 g) = (*R*)-enantiomer

The absolute configuration of these enantiomers was elucidated by vibrational circular dichroism (VCD) analysis, a well-established technique for stereochemical analysis.*

Experimental:

VCD spectra were acquired using a BioTools Chiral-2X FT-VCD spectrometer operating at 4 cm^{-1} resolution. Spectra were measured for samples $CDCl_3$ contg. 20% ACN-d_3 (~ 0.17-M). Baseline artifacts were removed using the standard half-difference method (e.g., VCD_{E1} (corr'd) = (VCD_{E1} minus VCD_{E2})).

Computational Methods:

Low energy conformations were identified using the LowMode search tool in MOE'09 (Amber12:EHT force field) with Born solvation (ε=40). Harmonic vibrational frequencies and intensities (VCD + IR) were calculated using Gaussian'09 model chemistry B3LYP/dgdzvp2 with PCM solvent modeling (ethanol dielectric). IR and VCD spectra for each conformational model were synthesized by fitting the frequency vs intensity line spectra with Lorentzian band shapes using a resolution factor of 8 cm^{-1} (HWHH). Conformationally-averaged VCD and IR spectra were synthesized using the fractional populations predicted for major conformers by Boltzmann statistics (QM free energies). Frequency scales were then aligned with solution-phase data using a uniform scale factor (0.9825).

Results:

Stereochemical Analysis: Comparisons of VCD spectra are shown in Figure 1. The spectrum of compound **33** (top panel) is coincident with the spectrum calculated for a model with (*S*) absolute configuration, while the one for compound **32** is the mirror image of the model. These data are consistent with compound **33** being assigned with (*S*) absolute configuration and compound **32** with (*R*) absolute configuration. The confidence limit in these assignments was estimated to be ≥99% by CompareVOA™ (BioTools, Inc. Jupiter Beach, FLA, USA), based on similarity index statistics (ESI = 72.8; TNS = 70.9).

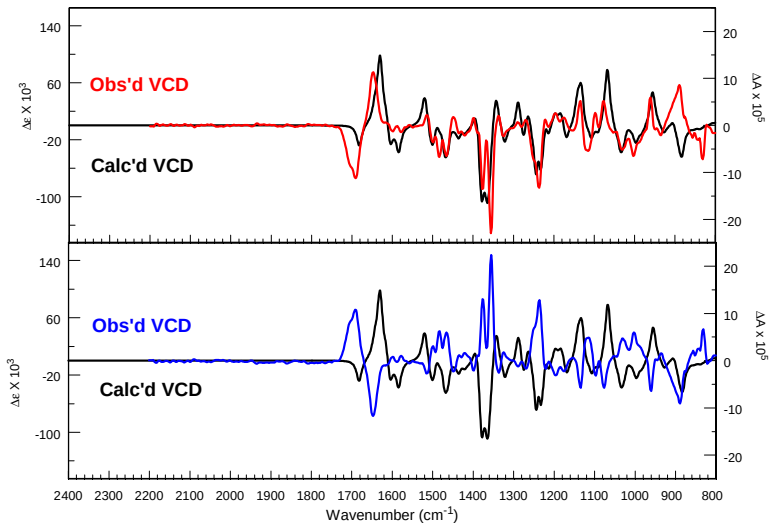


Figure 1: VCD Comparisons: compound **33** vs calc'd (top); compound **32** vs calc'd (bottom)

Structure Confirmation: The comparison of IR spectra in Figure 2 shows a close match between the model and compound **33** (compound **33** identical to compound **32**), confirming the structure of the isomers (molecular connectivity) and satisfactory coverage of their solution-phase conformational space by the computational analysis.

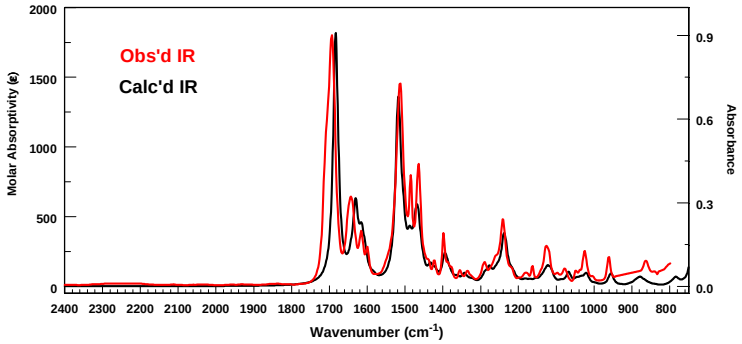


Figure 2: IR Comparison: Compound **33** *vs* model

* Yanan He, Bo Wang, Rina K. Dukor, and Laurence A. Nafie, Appl. Spectrosc. **65** (7), 669 (2011).

Compound **32**

(slower running enantiomer)

LCMS (High pH): Rt 1.23 min, $[M+H^+]^+$ 395.4

δ_H NMR (400 MHz, CD_3OD) 8.42 (s, 1H), 8.22-8.16 (m, 1H), 7.61-7.55 (m, 1H), 7.27 (s, 1H), 7.22 (s, 1H), 7.16 (s, 1H), 4.13 (s, 3H), 3.49-3.40 (m, 8H), 2.91-2.82 (m, 1H), 2.33-2.23 (m, 1H), 2.10-1.99 (m, 2H), 1.77-1.67 (m, 1H), 1.02 (d, *J* = 6.1 Hz, 3H). NH not seen

Compound **33**

(faster running enantiomer)

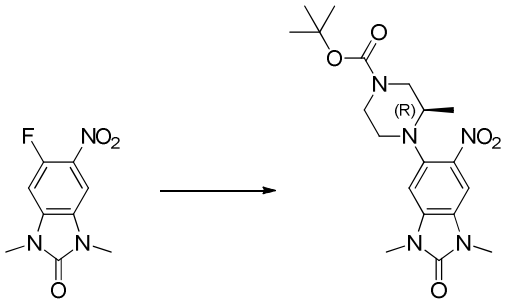
LCMS (High pH): Rt 1.23 min, $[M+H^+]^+$ 395.4

δ_H NMR (400 MHz, CD_3OD) 8.42 (s, 1H), 8.19 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.62-7.55 (m, 1H), 7.26 (d, *J* = 8.3 Hz, 1H), 7.22 (s, 1H), 7.16 (s, 1H), 4.13 (s, 3H), 3.49-3.38 (m, 8H), 2.91-2.83 (m, 1H), 2.33-2.23 (m, 1H), 2.10-2.00 (m, 2H), 1.77-1.65 (m, 1H), 1.02 (d, *J* = 5.9 Hz, 3H). NH not seen.

Compound 34

Step 1

(*R*)-*Tert*-butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate



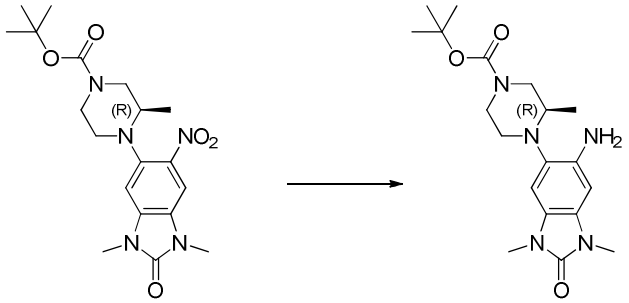
A stirred suspension of 5-fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[d]imidazol-2(3*H*)-one (0.924 g, 4.10 mmol), (*R*)-*tert*-butyl 3-methylpiperazine-1-carboxylate (1.23 g, 6.16 mmol), and *N*,*N*-diisopropylethylamine (1.43 mL, 8.21 mmol) in dimethylsulfoxide (4 mL) was heated to 120 ºC in a Biotage Initiator microwave reactor for 13 h, then to 130 ^{o}C for a further 10 h. The reaction mixture was cooled to room temperature and concentrated *in vacuo* then was partitioned between ethyl acetate and a saturated bicarbonate solution aqueous sodium. The layers were separated and the aqueous phase was extracted with ethyl acetate and the combined organics were dried (Na_2SO_4), filtered, and concentrated *in vacuo* to give a residue which was purified by silica chromatography (0-100% ethyl acetate in cyclohexane) to give the **title compound** as an orange/yellow solid (1.54 g, 3.80 mmol, 93%).

LCMS (Formic): Rt 1.17 min, $[M+H^+]^+$ 406.5.

δ_H NMR (400 MHz, $CDCl_3$) 7.36 (s, 1H), 6.83 (s, 1H), 4.04-3.87 (m,1H), 3.87 - 3.80 (m, 1H), 3.43 (s, 6H), 3.35 - 3.25 (m, 1H), 3.23 - 3.08 (m, 2H), 3.00 - 2.72 (m, 2H), 1.48 (s, 9H), 0.81 (d, *J* = 6.1 Hz, 3H)

Step 2

(*R*)-*Tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate



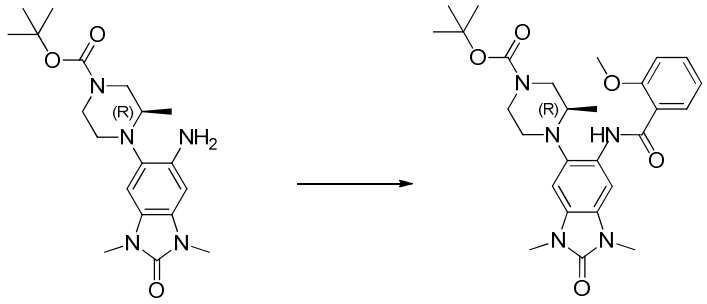
To (*R*)-*tert*-butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate (1.542 g) in *iso*-propanol (40 mL) was added palladium on carbon (5% w/w, 50% paste, 1.50 g) and the mixture was hydrogenated at room temperature and atmospheric pressure. After 4 h the mixture was filtered, the residue washed with ethanol and dichloromethane, and the combined filtrates concentrated *in vacuo* to give a residue which was purified by silica chromatography (50-100% ethyl acetate in cyclohexane) to afford the **title compound** (1.22 g, 3.25 mmol, 85%) as a cream solid.

LCMS (High pH): Rt 1.01 min, $[M+H^+]^+$ 376.4.

δ_H NMR (400 MHz, $CDCl_3$) 6.69 (s, 1H), 6.44 (s, 1H), 4.33 - 3.87 (m, 4H), 3.36 (s, 3H), 3.35 (s, 3H), 3.20 - 2.53 (m, 5H), 1.52 (s, 9H), 0.86 (d, *J* = 6.1 Hz, 3H).

Step 3

(*R*)-*Tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate



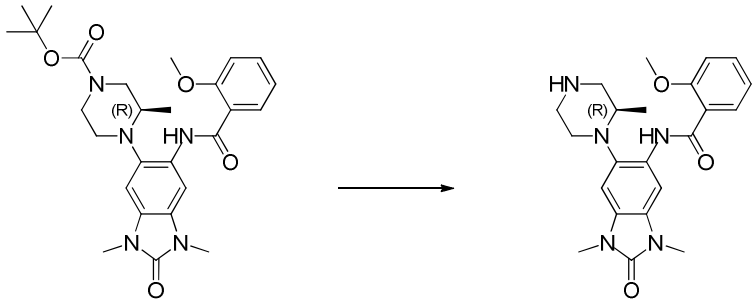
A stirred solution of (*R*)-*tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate (0.254 g, 0.675 mmol) and pyridine (0.164 mL, 2.02 mmol) in dichloromethane (2 mL) was treated 2-methoxybenzoyl chloride (0.182 mL, 1.35 mmol). After 1 h at room temperature the reaction mixture was concentrated *in vacuo* to give a residue which was taken up in DMSO:MeOH (1:1) and purified by MDAP (High pH) to give the **title compound** (0.302 g, 0.592 mmol, 88%) as a white solid.

LCMS (High pH): Rt 1.27 min, $[M+H^+]^+$ 510.5.

δ_H NMR (400 MHz, $CDCl_3$) 10.67 (s, 1H), 8.53 (s, 1H), 8.24 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.54 - 7.48 (m, 1H), 7.18 - 7.12 (m, 1H), 7.07 (d, *J* = 8.1 Hz, 1H), 6.82 (s, 1H), 4.27 - 3.94 (m, 2H), 4.08 (s, 3H), 3.45 (s, 3H), 3.40 (s, 3H), 3.18 - 2.99 (m, 2H), 2.92 - 2.70 (m, 3H), 1.50 (s, 9H), 0.87 (d, *J* = 6.1 Hz, 3H).

Step 4

(*R*)-*N*-(1,3-Dimethyl-6-(2-methylpiperazin-1-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide



A stirred solution of (*R*)-*tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate (0.302 g, 0.592 mmol) in dichloromethane (4 mL) was treated with trifluoroacetic acid (3 mL). After 15 minutes the mixture was concentrated *in vacuo* to give a residue which was loaded on a solid-phase cation exchange (SCX) cartridge (5 g), washed with MeOH, and then eluted with methanolic ammonia (2 M). The appropriate fractions were combined and concentrated *in vacuo* to give a white solid (0.240 g). Half of this material was taken up in DMSO:MeOH (1:1) and purified by MDAP (High pH) to give **34** (0.101 g, 0.246 mmol, 41%) as a white solid.

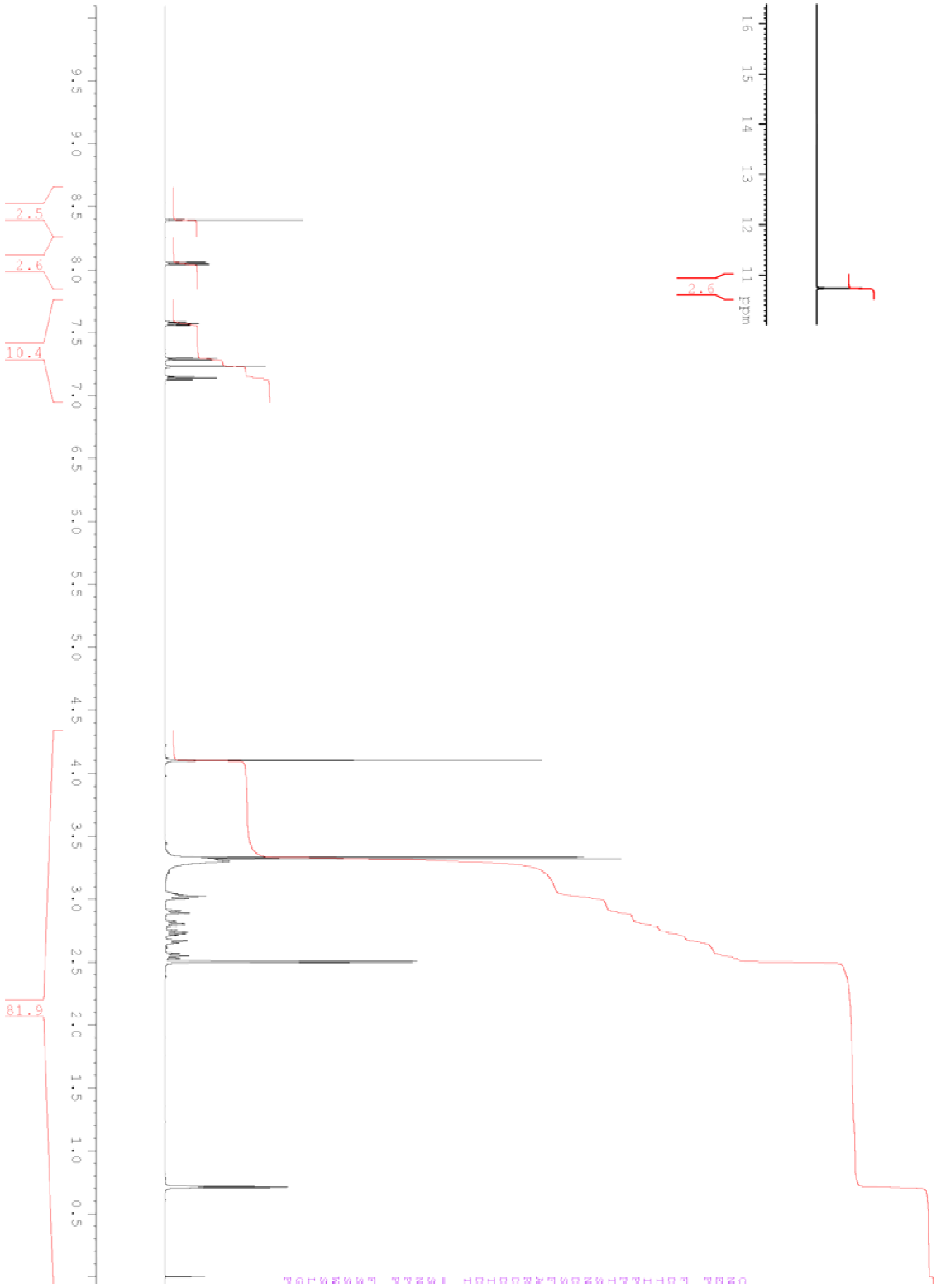
LCMS (High pH): Rt = 0.90 min, $[M+H^+]^+$ 410.5. HRMS (ES+) *m/z* (Found MH+, 410.2190, $C_{22}H_{28}N_5O_3$ requires 410.2187).

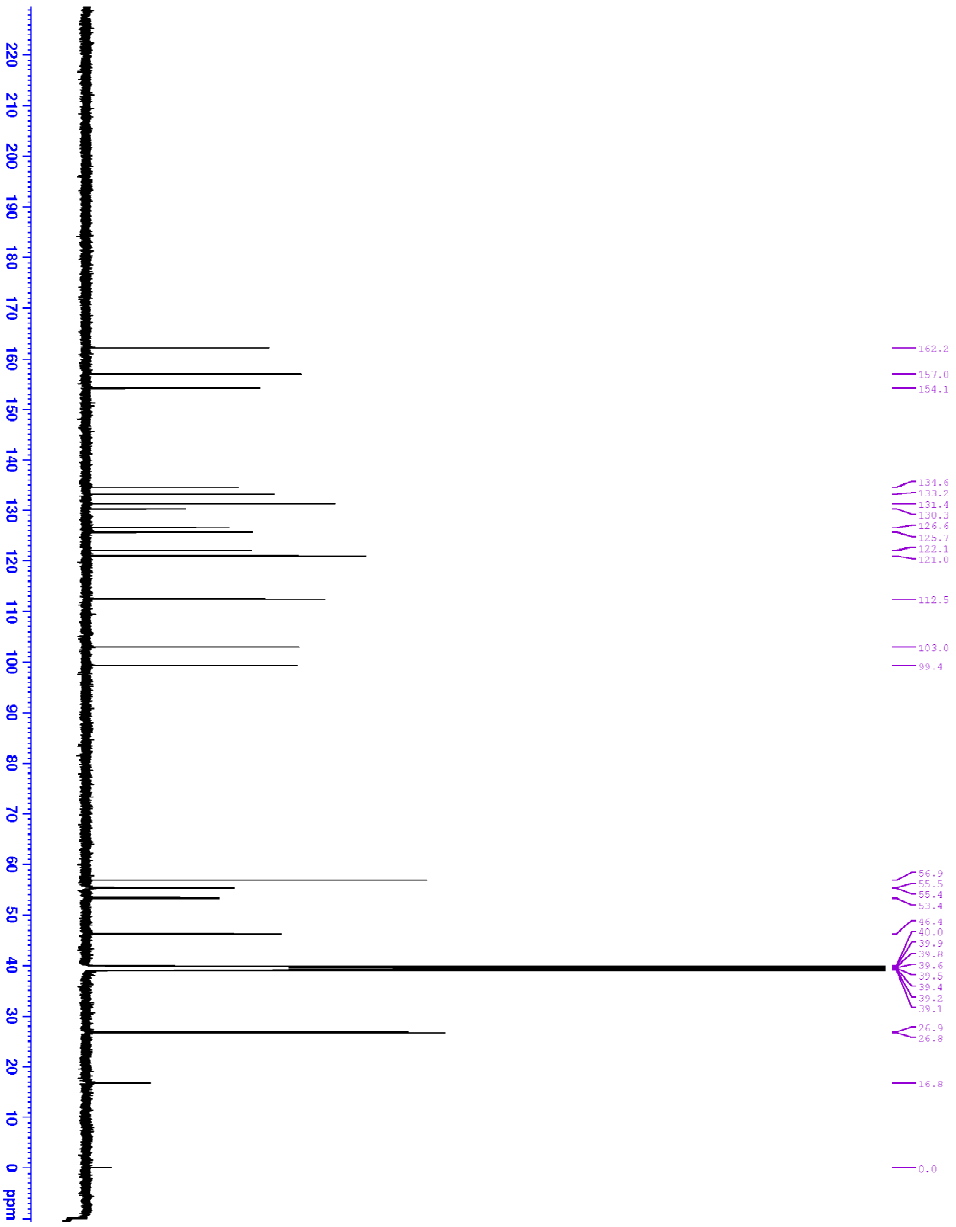
δ_H NMR (600 MHz, DMSO-d_6) 10.74 (s, 1H), 8.39 (s, 1H), 8.05 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.57 (ddd, *J* = 8.3, 7.2, 2.0 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.23 (s, 1H), 7.17 - 7.11 (m, 1H), 4.10 (s, 3H), 3.33 (s, 3H), 3.32 (s, 3H), 3.30 (br s, 1H), 3.07 - 3.02 (m, 1H), 3.02 - 2.99 (m, 1H), 2.92 - 2.87 (m, 1H), 2.80 (td, *J* = 11.3, 2.7 Hz, 1H), 2.73 (td, *J* = 11.0, 2.7 Hz, 1H), 2.68 - 2.63 (m, 1H), 2.55 (dd, *J* = 12.0, 9.8 Hz, 1H), 0.71 (d, *J* = 6.1 Hz, 3H).

δ_C NMR (151 MHz, DMSO-d_6) 162.1, 156.8, 154.1, 134.4, 133.2, 131.5, 130.1, 126.6, 125.7, 121.9, 121.0, 112.5, 103.0, 99.4, 56.8, 55.4, 55.3, 53.3, 46.3, 26.8, 26.6, 16.7.

$[\alpha_D]^{25\ °C}$ = -50.1 (c = 0.3, MeOH).

NMR spectra of Compound **34** (^{1}H 600MHz, ^{13}C 151 MHz)

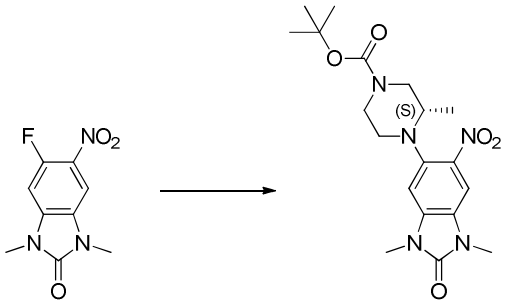



220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm
162.2
157.0
154.1
134.6
133.2
131.4
130.3
126.6
125.7
122.1
121.0
112.5
103.0
99.4
56.9
55.5
55.4
53.4
46.4
40.0
39.9
39.8
39.6
39.5
39.4
39.2
39.1
26.9
26.8
16.8
0.0

Compound 35

Step 1

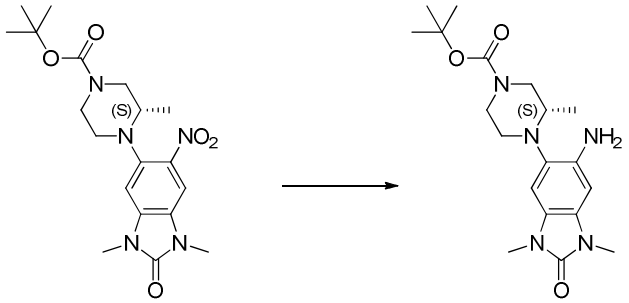
(*S*)-*Tert*-Butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)-3-methylpiperazine-1-carboxylate



A suspension of 5-fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[d]imidazol-2(3*H*)-one (0.400 g, 1.78 mmol), (*S*)-*tert*-butyl 3-methylpiperazine-1-carboxylate (0.391 g, 1.95 mmol), and *N*,*N*-diisopropylethylamine (0.621 mL, 3.55 mmol) in dimethylsulfoxide (2.5 mL) was heated to 120 ºC in a Biotage Initiator microwave reactor for 6 h. Further (*S*)-*tert*-butyl 3-methylpiperazine-1-carboxylate (0.100 g) was added and the reaction was heated to 120 ºC for 3 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was partitioned between ethyl acetate (15 mL) and water (30 mL) and the layers were separated. The aqueous phase was extracted with ethyl acetate (2 x 15 mL) and the combined organics were dried through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by silica chromatography (0-4% methanol in dichloromethane) to afford the **title compound** (0.580 g, 82%).LCMS (Formic): Rt 1.19 min, $[M+H^+]^+$ 406.1.

Step 2

(*S*)-*Tert*-Butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate



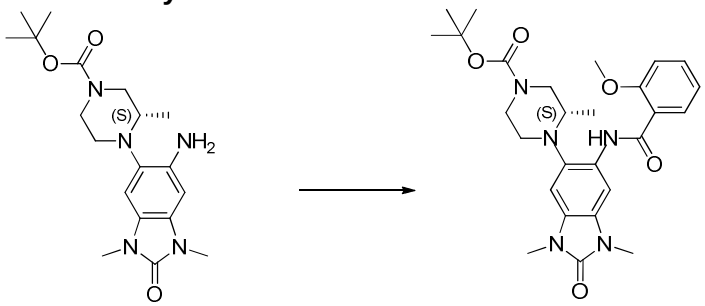
A mixture containing (*S*)-*tert*-Butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate (0.580 g) was dissolved in methanol (48 mL) and dichloromethane (2 mL) and the reaction was hydrogenated using a H-cube flow hydrogenation reactor (settings: room temperature, full H_2, 1 mL/min flow rate, 10% Pd/C CatCart 30). The solution was concentrated *in vacuo* and the residue was purified by silica chromatography (0-4% methanolic ammonia [2M] in dichloromethane) to afford the **title compound** (0.407 g, 1.08 mmol, 70%) as an off-white solid.

LCMS (Formic): Rt 0.68 min, $[M+H^+]^+$ 376.1.

δ_H NMR (400 MHz, $CDCl_3$) 6.67 (s, 1H), 6.43 (s, 1H), 4.22 - 3.95 (m, 4H), 3.34 (s, 3H), 3.33 (s, 3H), 3.11 - 2.90 (m, 2H), 2.89 - 2.78 (m, 1H), 2.77 - 2.58 (m, 2H), 1.50 (s, 9H), 0.84 (d, *J* = 6.1 Hz, 3H).

Step 3

(*S*)-*Tert*-Butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)-3-methylpiperazine-1-carboxylate



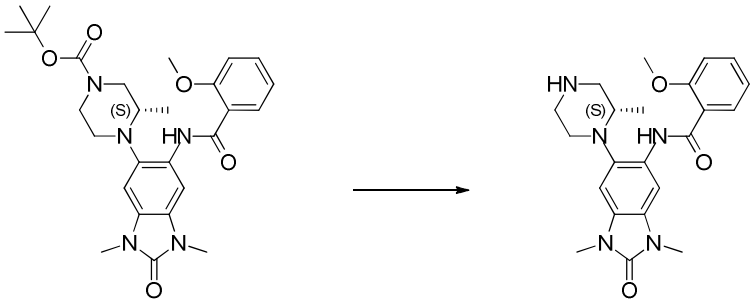
To a solution of (*S*)-*tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate (0.16 g, 0.43 mmol) and pyridine (0.069 mL, 0.85 mmol) in dichloromethane (5.5 mL) was added 2-methoxybenzoyl chloride (0.086 mL, 0.64 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was then partitioned between dichloromethane (10 mL) and a saturated $NaHCO_3$ aqueous solution (10 mL) and the layers were separated. The aqueous phase was extracted with dichloromethane (2 x 10 mL) and the combined organics were dried through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by silica chromatography (0-100% ethyl acetate in cyclohexane) to afford the **title compound** (0.17 g, 0.34 mmol, 80%) as an off-white solid.

LCMS (High pH): Rt 1.26 min, $[M+H^+]^+$ 510.4.

δ_H NMR (400 MHz, $CDCl_3$) 10.67 (s, 1H), 8.52 (s, 1H), 8.24 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.51 (ddd, *J* = 8.5, 7.5, 1.7 Hz, 1H), 7.18 - 7.12 (m, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.82 (s, 1H), 4.08 (s, 3H), 4.21 - 3.96 (m, 2H), 3.45 (s, 3H), 3.40 (s, 3H), 3.17 - 2.98 (m, 2H), 2.80 (d, *J* = 9.0 Hz, 3H), 1.50 (s, 9H), 0.87 (d, *J* = 6.1 Hz, 3H).

Step 4

(*S*)-*N*-(1,3-Dimethyl-6-(2-methylpiperazin-1-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide



To (*S*)-*tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate (0.173 g, 0.339 mmol) was added trifluoroacetic acid (3.5 mL) and the mixture was stirred at room temperature for 90 min. The reaction mixture was then loaded on a solid-phase cation exchange (SCX) cartridge (5 g), washed with methanol (4 CV) and eluted with methanolic ammonia [2M] (4 CV). The appropriate fractions were combined and concentrated *in vacuo* to afford **35** (0.135 g, 0.330 mmol, 97%) as a white solid.

LCMS (High pH): Rt 0.89 min, $[M+H^+]^+$ 410.4.

δ_H NMR (400 MHz, DMSO-d_6) 10.74 (s, 1H), 8.40 (s, 1H), 8.05 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.58 (ddd, *J* = 8.3, 7.5, 1.7 Hz, 1H), 7.30 (dd, *J* = 8.3, 0.7 Hz, 1H), 7.24 (s, 1H), 7.14 (ddd, *J* = 7.9, 7.5, 0.7 Hz, 1H), 4.11 (s, 3H), 3.33 (s, 3H), 3.32 (s, 3H), 3.10 - 2.99 (m, 2H), 2.96 - 2.88 (m, 1H), 2.86 - 2.63 (m, 3H), 2.56 (dd, *J* = 12.3, 10.4 Hz, 1H), 0.72 (d, *J* = 5.9 Hz, 3H).

δ_C NMR (101 MHz, DMSO-d_6) 162.7, 157.5, 154.7, 135.1, 133.7, 131.9, 130.8, 127.1, 126.2, 122.6, 121.5, 113.0, 103.6, 99.9, 57.4, 55.9, 55.8, 53.7, 46.8, 27.5, 27.3, 17.3.

$[\alpha_D]^{25\ °C}$ = +49.4 (c = 0.3, MeOH).

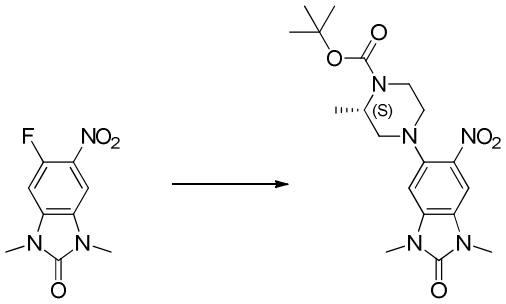
M.pt.: 207-209 °C

ν_{max} (neat): 3287, 2936, 1708, 1649, 1615, 1507, 1483, 1455, 1398, 1229, 1124, 1024, 975, 864, 847, 752, 738, 683 cm^{-1}.

Compound 36

Step 1

(*S*)-*tert*-Butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate



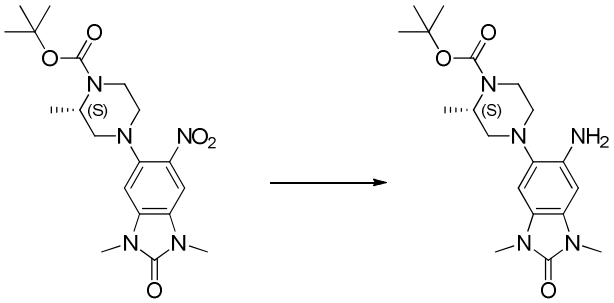
A mixture of 5-fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.20 g, 0.89 mmol), (*S*)-*tert*-butyl 2-methylpiperazine-1-carboxylate (0.196 g, 0.977 mmol), and *N*,*N*-diisopropylethylamine (0.31 mL, 1.8 mmol) in dimethylsulfoxide (1 mL) was heated to 120 ºC in the Biotage Initiator microwave reactor for 8 h. The reaction mixture was partitioned between ethyl acetate (15 mL) and water (30 mL) and the layers were separated. The aqueous phase was extracted with ethyl acetate (2 x 15 mL) and the combined organics were dried through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by silica chromatography (0-4% methanol in dichloromethane) to afford the **title compound** (0.358 g, 0.88 mmol, 99%) as an orange solid.

LCMS (Formic): Rt 1.19 min, $[M+H^+]^+$ 406.1.

δ_H NMR (400 MHz, $CDCl_3$) 7.53 (s, 1H), 6.71 (s, 1H), 4.40 - 4.30 (m, 1H), 3.95 - 3.87 (m, 1H), 3.43 (s, 6H), 3. 40 - 3.34 (m, 1H), 3.18 - 3.13 (m, 1H), 3.09 (dd, *J* = 11.5, 3.7 Hz, 1H), 3.00 (dt, *J* = 11.5, 1.7 Hz, 1H), 2.83 - 2.74 (m, 1H), 1.50 - 1.47 (m, 9H), 1.34 (d, *J* = 6.6 Hz, 3H).

Step 2

(*S*)-*Tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate



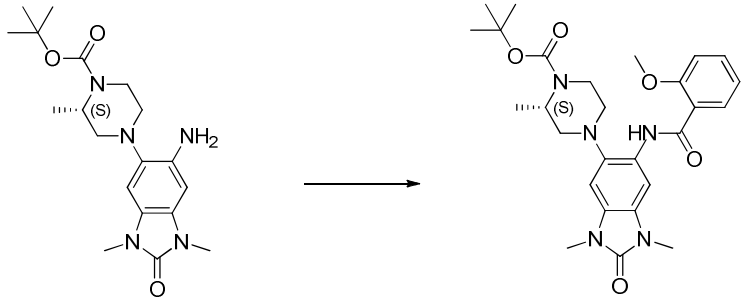
(*S*)-*tert*-Butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate (0.35 g, 0.86 mmol) was dissolved in methanol (29 mL) and the reaction was hydrogenated using the H-cube flow hydrogenation reactor (settings: room temperature, full H_2, 1 mL/min flow rate, 10% Pd/C CatCart 30). The solvent was evaporated *in vacuo* to afford the **title compound** (0.32 g, 0.85 mmol, 99%) as an off-white solid.

LCMS (Formic): Rt 0.79 min, $[M+H^+]^+$ 376.1.

δ_H NMR (400 MHz, $CDCl_3$) 6.65 (s, 1H), 6.44 (s, 1H), 4.44 - 4.28 (m, 1H), 4.22 - 3.76 (m, 3H), 3.34 (s, 3H), 3.32 (s, 3H), 3.30 - 3.20 (m, 1H), 3.02 - 2.94 (m, 1H), 2.91 - 2.80 (m, 2H), 2.73 (td, *J* = 11.7, 3.3 Hz, 1H), 1.50 (s, 9H), 1.40 (d, *J* = 6.8 Hz, 3H).

Step 3

(*S*)-*Tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate



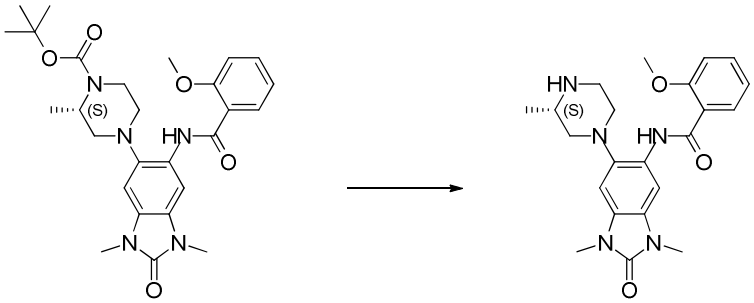
To a solution of (*S*)-*tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate (0.080 g, 0.21 mmol) and pyridine (0.035 mL, 0.43 mmol) in dichloromethane (3 mL) was added 2-methoxybenzoyl chloride (0.043 mL, 0.32 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was partitioned between dichloromethane (10 mL) and a saturated $NaHCO_3$ aqueous solution (10 mL) and the layers were separated. The aqueous phase was extracted with dichloromethane (2 x 10 mL) and the combined organics were dried through a hydrophobic frit and concentrated *in vacuo*. The residue obtained was purified by silica chromatography (40-100% ethyl acetate in cyclohexane) to afford the **title compound** (0.088 g, 0.17 mmol, 81%) as an off-white solid.

LCMS (High pH): Rt 1.22 min, $[M+H^+]^+$ 510.5.

δ_H NMR (400 MHz, $CDCl_3$) 10.01 (s, 1H), 8.24 (s, 1H), 8.14 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.53 - 7.46 (m, 1H), 7.17 - 7.11 (m, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 6.77 (s, 1H), 4.36 - 4.26 (m, 1H), 4.00 (s, 3H), 4.03 - 3.94 (m, 1H), 3.43 (s, 3H), 3.40 (s, 3H), 3.23 (td, *J* = 12.6, 3.2 Hz, 1H), 3.01 - 2.95 (m, 1H), 2.91 (dd, *J* = 11.5, 3.9 Hz, 1H), 2.81 - 2.71 (m, 2H), 1.47 (s, 9H), 1.23 (d, *J* = 6.6 Hz, 3H).

Step 4

(*S*)-*N*-(1,3-Dimethyl-6-(3-methylpiperazin-1-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide



To (*S*)-*tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate (0.088 g, 0.17 mmol) was added trifluoroacetic acid (1.7 mL) and the mixture was stirred at room temperature for 90 minutes. The reaction mixture was then loaded on a solid-phase cation exchange (SCX) cartridge (1 g), washed with methanol (4 CV), then eluted with methanolic ammonia [2M] (4 CV). The appropriate fractions were combined and concentrated *in vacuo.* The residue was purified by MDAP (High pH). The solvent was evaporated *in vacuo* to give **36** (0.059 g, 0.14 mmol, 83%) as a white solid.

LCMS (High pH): Rt 0.89 min, $[M+H^+]^+$ 410.4.

δ_H NMR (400 MHz, DMSO-d_6) 10.52 (s, 1H), 8.29 (s, 1H), 8.07 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.62 - 7.53 (m, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.18 - 7.12 (m, 2H), 4.07 (s, 3H), 3.34 (s, 3H), 3.31 (s, 3H), 3.02 - 2.88 (m, 3H), 2.84 - 2.75 (m, 2H), 2.72 - 2.62 (m, 1H), 2.49 - 2.42 (m, 1H), 1.01 (d, *J* = 6.4 Hz, 3H). One NH not observed.

δ_C NMR (101 MHz, DMSO-d_6) 162.7, 157.5, 154.6, 137.4, 133.8, 131.9, 128.4, 126.4, 126.2, 122.3, 121.6, 113.1, 101.8, 100.6, 60.2, 57.3, 53.4, 51.2, 46.1, 27.5, 27.3, 19.8.

$[\alpha_D]^{25\,°C}$ = -3.9 (c = 0.3, MeOH)

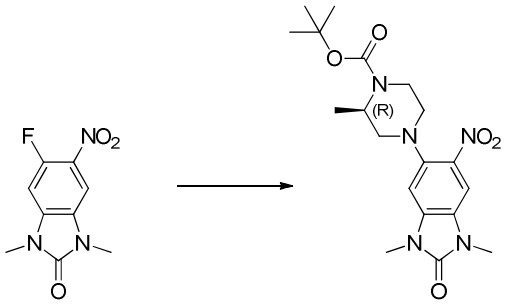
M.pt.: 184-186 °C

ν_{max} (neat): 3464, 3293, 2936, 2836, 1689, 1642, 1508, 1481, 1459, 1396, 1234, 1126, 1009, 856, 789, 739, 682 cm^{-1}.

Compound 37

Step 1

(*R*)-*Tert*-butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate



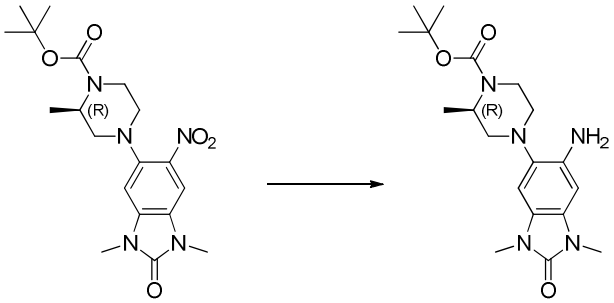
A mixture of 5-fluoro-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.20 g, 0.89 mmol), (*R*)-*tert*-butyl 2-methylpiperazine-1-carboxylate (0.20 g, 0.98 mmol), and *N*,*N*-diisopropylethylamine (0.31 mL, 1.8 mmol) in dimethylsulfoxide (1 mL) was heated to 120 ºC in the Biotage Initiator microwave reactor for 8 h. The reaction mixture was partitioned between ethyl acetate (15 mL) and water (30 mL) and the layers were separated. The aqueous phase was extracted with ethyl acetate (2 x 15 mL) and the combined organics were dried through a hydrophobic frit and concentrated *in vacuo*. The residue obtained was purified by silica chromatography (0-4% methanol in dichloromethane) to afford the **title compound** (0.355 g, 0.88 mmol, 99%) as an orange solid.

LCMS (Formic): Rt 1.19 min, $[M+H^+]^+$ 406.1.

δ_H NMR (400 MHz, $CDCl_3$) 7.53 (s, 1H), 6.71 (s, 1H), 4.40 - 4.30 (m, 1H), 3.95 - 3.87 (m, 1H), 3.43 (s, 6H), 3. 40 - 3.34 (m, 1H), 3.18 - 3.13 (m, 1H), 3.09 (dd, *J* = 11.5, 3.7 Hz, 1H), 3.00 (dt, *J* = 11.5, 1.7 Hz, 1H), 2.83 - 2.74 (m, 1H), 1.50 - 1.47 (m, 9H), 1.34 (d, *J* = 6.6 Hz, 3H).

Step 2

(*R*)-*Tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[d]imidazol-5-yl)-2-methylpiperazine-1-carboxylate



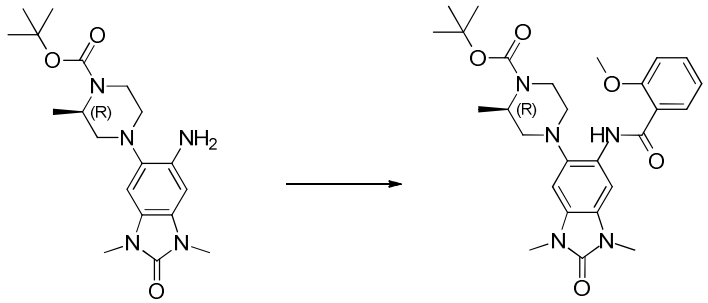
(*R*)-*tert*-Butyl 4-(1,3-dimethyl-6-nitro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate (0.35 g, 0.88 mmol) was dissolved in methanol (29 mL) and the reaction was hydrogenated using the H-cube flow hydrogenation reactor (settings: room temperature, full H_2, 1 mL/min flow rate, 10% Pd/C CatCart 30). The solvent was evaporated *in vacuo* to afford the **title compound** (0.33 g, 0.88 mmol, 100%) as an off-white solid.

LCMS (Formic): Rt 0.79 min, $[M+H^+]^+$ 376.1.

δ_H NMR (400 MHz, $CDCl_3$) 6.66 (s, 1H), 6.50 (s, 1H), 4.48 - 4.25 (m, 1H), 4.07 - 3.90 (m, 1H), 3.35 (s, 3H), 3.34 (s, 3H), 3.23 - 3.21 (m, 1H), 3.02 - 2.94 (m, 1H), 2.91 - 2.80 (m, 2H), 2.79 - 2.69 (m, 1H), 1.49 (s, 9H), 1.40 (d, *J* = 6.6 Hz, 3H). 2 NHs not observed.

Step 3

(*R*)-*Tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate



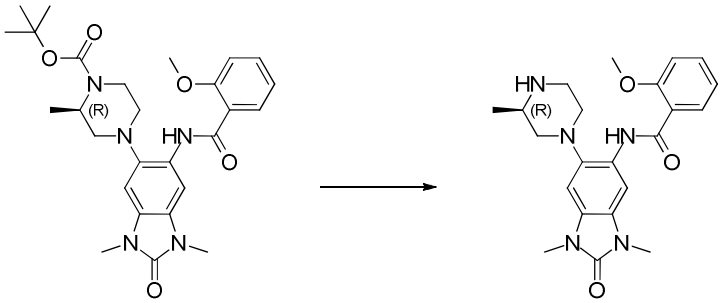
To a solution of (*S*)-*tert*-butyl 4-(6-amino-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate (0.080 g, 0.21 mmol) and pyridine (0.035 mL, 0.43 mmol) in dichloromethane (3 mL) was added 2-methoxybenzoyl chloride (0.043 mL, 0.32 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with dichloromethane (10 mL) and a saturated $NaHCO_3$ aqueous solution (10 mL) and the layers were separated. The aqueous phase was extracted with dichloromethane (2 x 10 mL) and the combined organics were dried through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by silica chromatography (40-100% ethyl acetate in cyclohexane) to afford the **title compound** (0.085 g, 0.17 mmol, 78%) as an off-white solid.

LCMS (High pH): Rt 1.22 min, $[M+H^+]^+$ 510.5.

δ_H NMR (400 MHz, $CDCl_3$) 10.01 (s, 1H), 8.24 (s, 1H), 8.14 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.53 - 7.46 (m, 1H), 7.17 - 7.11 (m, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 6.77 (s, 1H), 4.36 - 4.26 (m, 1H), 4.00 (s, 3H), 4.03 - 3.94 (m, 1H), 3.43 (s, 3H), 3.40 (s, 3H), 3.23 (td, *J* = 12.6, 3.2 Hz, 1H), 3.01 - 2.95 (m, 1H), 2.91 (dd, *J* = 11.5, 3.9 Hz, 1H), 2.81 - 2.71 (m, 2H), 1.47 (s, 9H), 1.23 (d, *J* = 6.6 Hz, 3H).

Step 4

(*R*)-*N*-(1,3-Dimethyl-6-(3-methylpiperazin-1-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methoxybenzamide



To (*S*)-*tert*-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2-methylpiperazine-1-carboxylate (0.085 g, 0.17 mmol) was added trifluoroacetic acid (1.7 mL) and the mixture was stirred at room temperature for 90 min. The reaction mixture was loaded on a solid-phase cation exchange (SCX) cartridge (1 g), washed with methanol (4 CV), then eluted with methanolic ammonia [2M] (4 CV). The appropriate fractions were combined and concentrated *in vacuo.* The residue was purified by MDAP (High pH). The solvent was evaporated *in vacuo* to give **37** (0.064 g, 0.16 mmol, 94%) as a white solid.

LCMS (High pH): Rt 0.89 min, $[M+H^+]^+$ 410.5.

δ_H NMR (400 MHz, DMSO-d_6) 10.52 (s, 1H), 8.29 (s, 1H), 8.07 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.62 - 7.53 (m, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.18 - 7.12 (m, 2H), 4.07 (s, 3H), 3.34 (s, 3H), 3.31 (s, 3H), 3.02 - 2.88 (m, 3H), 2.84 - 2.75 (m, 2H), 2.72 - 2.62 (m, 1H), 2.49 - 2.42 (m, 1H), 1.01 (d, *J* = 6.4 Hz, 3H). One NH not observed.

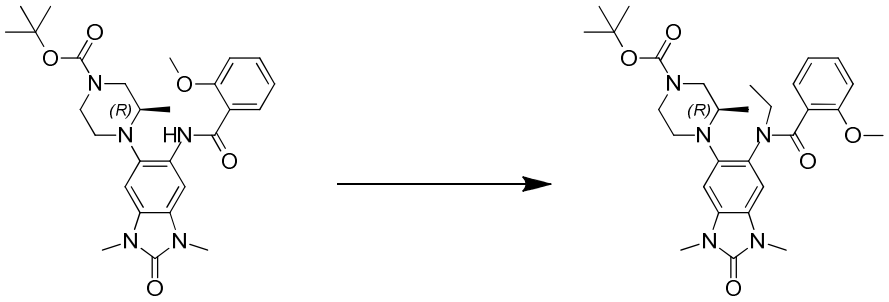
$[\alpha_D]^{25\,°C}$ = +6.0 (c = 0.3, MeOH).

Compound 38

(GSK9311)

Step 1

(*R*)-Tert-butyl 4-(6-(*N*-ethyl-2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate



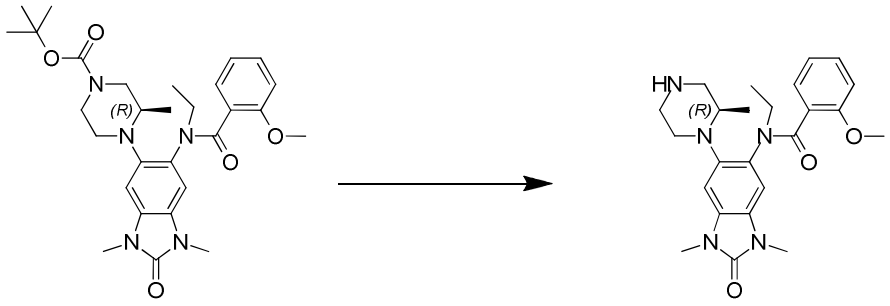
To a solution of (*R*)-tert-butyl 4-(6-(2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate (0.100 g, 0.196 mmol) in *N*,*N*-dimethylformamide (4 mL) at room temperature was added sodium hydride (60% w/w in mineral oil, 0.024 g, 0.59 mmol). The reaction mixture was stirred at room temperature for 15 minutes, then iodoethane (0.024 mL, 0.30 mmol) was added. The reaction was stirred at room temperature for 1 h, then quenched with water. The reaction mixture was diluted with water (30 mL) and extracted with ethyl acetate (3 x 15 mL). The combined organics were dried through a hydrophobic frit and evaporated to dryness. The residue was purified by silica chromatography (0-20% ethanol and ethyl acetate) to afford the **title compound** (0.089 g, 0.17 mmol, 84%) as a white solid.

LCMS (High pH): Rt 1.24 min, $[M+H^+]^+$ 538.5.

δ_H NMR (400 MHz, 393 K, DMSO-d_6) 7.43 - 7.21 (m, 2H), 7.12 - 6.84 (m, 4H), 3.86 (br. s., 3H), 3.81 - 3.57 (m, 3H), 3.32 (br. s., 8H), 3.21 - 3.00 (m, 2H), 2.71 - 2.54 (m, 2H), 1.48 (s, 9H), 1.12 - 0.93 (m, 3H), 0.87 (d, *J* = 5.1 Hz, 3H).

Step 2

(R)-*N*-(1,3-Dimethyl-6-(2-methylpiperazin-1-yl)-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-*N*-ethyl-2-methoxybenzamide



To a stirred solution of (*R*)-*tert*-butyl 4-(6-(*N*-ethyl-2-methoxybenzamido)-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-3-methylpiperazine-1-carboxylate (0.046 g, 0.085 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (2 mL). After 25 minutes the mixture was concentrated *in vacuo* to give a residue which was taken up in DMSO:MeOH (1:1) and purified by MDAP (High pH) to give **38** (0.031 g, 0.070 mmol, 83%) as a white solid.

LCMS (High pH): Rt 0.87 min, $[M+H^+]^+$ 438.5. HRMS: ($C_{24}H_{31}N_5O_3$) $[M+H^+]^+$ requires 438.2500, found $[M+H]^+$ 438.2509.

δ_H NMR (400 MHz, DMSO-d_6, 150^{o}C) 7.36 - 7.31 (m, 1H), 7.29 - 7.24 (m, 1H), 7.10 - 7.01 (m, 1H), 6.96 (br. s, 1H), 6.90 (s, 2H), 3.87 (s, 3H), 3.84 - 3.77 (m, 1H), 3.74 - 3.44 (m, 2H), 3.32 (s, 3H), 3.30 (br. s, 3H), 3.26 – 3.18 (m, 1H), 3.17 - 3.10 (m, 1H), 3.05 (br. dd, *J* = 12.0, 3.0 Hz, 1H), 2.99 - 2.84 (m, 2H), 2.62 (dd, *J* = 12.0, 8.0 Hz, 1H), 1.04 (t, *J* = 6.5 Hz, 3H), 0.86 (br. d, *J* = 6.3 Hz, 3H).

$[\alpha_D]^{25\ °C}$ = +93 (c = 0.3, MeOH)

M.pt.: 165-170 °C (decomp)

ν_{max} (neat): 3423, 2928, 2829, 1694, 1626, 1598, 1508, 1453, 1396, 1246, 1120, 1020, 947, 740 cm^{-1}.

Supplementary Methods

Expression & purification of BET and BRPF1 proteins. BET proteins were produced using protocols given in the literature.[1] 6H-Flag-TEV-BRPF1 (622-738) was expressed in *E.coli* BL21 (DE3) cells. The pellet from the *E.coli* culture was resuspended in buffer A (50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM imidazole, and 0.5 mM TCEP) plus 0.1 mg/mL lysozyme (Sigma 92971-50G-F) and 1 μL/mL Protease Inhibitor Cocktail (Sigma P8340). Cells were lysed by sonication, on ice, and centrifuged at 100,000 x g for 90 minutes at 4 ˚C. The supernatant was applied to a HisTRAP HP Column (GE Healthcare 17-5248-02) equilibrated with buffer A, washed with ten column volumes buffer A, and the bound protein eluted using a linear gradient of 10-500 mM imidazole over twenty column volumes. Eluted BRPF1 protein was cleaved with rTEV protease at a ratio of 1:200, and dialysed overnight at 4°C against buffer B (50 mM HEPES pH 7.5, 150 mM NaCl and 0.5 mM TCEP) using SnakeSkin Dialysis Tubing 3.5K MWCO (Thermo Scientific #68035). Cleaved protein was applied to a HiLoad 26/60 Superdex 75 prep grade size exclusion column (GE Healthcare 45-002-489), equilibrated with buffer B. Fractions containing BRPF1 were pooled, concentrated to 5.9 mg/mL using an Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-3 membrane (Millipore UFC900308), aliquoted and stored at -80°C. Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by Liquid Chromatography/Mass Spectrometry.

Expression and purification of BRPF2 protein.

6His-Flag-Tev-BRPF2 (551-673) was expressed in *E.coli* BL21 (DE3) and grown in LB medium containing 50 μg/mL Kanamycin, at 37 °C, until an optical density at 600 nm of 1.0 was obtained. Expression was induced by the addition of 0.5 mM isopropyl-β-D-thiogalactoside (IPTG) and the temperature was reduced to 18°C. The cells were harvested post overnight incubation. The pellet from the *E.coli* culture was resuspended with 4 mL/g buffer A (50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM Imidazole and 5 % (v/v) glycerol). Cells were lysed by sonication, on ice, and centrifuged at 15,000 rpm for 30 minutes at 4 °C. The supernatant was loaded on to Ni-NTA resin pre-equilibrated with buffer A. The column was washed with 10 column volumes of buffer A, and the bound BRD1 protein was eluted with 5 column volumes buffer B (50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM Imidazole and 5 % (v/v) glycerol). Eluted BRD1 was concentrated to 10 mL and loaded on to a HiLoad 26/60 Superdex 75 prep grade size exclusion column equilibrated with buffer C (50 mM HEPES pH 7.5, 300 mM NaCl and 0.5 mM TCEP). Fractions containing BRD1 were pooled and concentrated to 4.6 mg/mL, aliquoted and stored at -80 °C. Protein identity was confirmed by peptide mass fingerprinting and a molecular weight consistent with expected minus N-terminal methionine was obtained by LC/MS.

Expression and purification of BRPF3 protein.

6His-Flag-Tev-BRPF3 (579-706) was expressed in *E.coli* BL21 (DE3) and grown in LB medium containing 50 μg/mL Kanamycin, at 37 °C, until an optical density at 600 nm of 1.0 was obtained. Expression was induced by the addition of 0.5 mM isopropyl-β-D-thiogalactoside (IPTG) and the temperature was reduced to 18°C. The cells were harvested post overnight incubation. The pellet from the *E.coli* culture was resuspended with 4 mL/g buffer A (50 mM HEPES pH 7.5, 500mM NaCl, 5 mM Imidazole and 5 % (v/v) glycerol). Cells were lysed by sonication, on ice, and centrifuged at 15,000 rpm for 30 minutes at 4 °C. The supernatant was loaded on to Ni-NTA resin pre-equilibrated with buffer A. The column was washed with 10 column volumes of buffer A, and the bound BRPF3 protein was eluted with 5 column volumes buffer B (50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM Imidazole and 5 % (v/v) glycerol). Eluted BRPF3 was further purified using a HiLoad 26/60 Superdex 75 prep grade size exclusion column equilibrated with buffer C (25 mM HEPES pH 7.5, 300 mM NaCl and 0.5 mM TCEP). Fractions containing BRPF3 were pooled and concentrated to 8.3 mg/mL, aliquoted and stored at -80 °C. Protein identity was confirmed by peptide mass fingerprinting and a molecular weight consistent with expected minus N-terminal methionine was obtained by LC/MS.

BRFP1 Bromodomain Crystallography. *E.coli* expressed His-tagged BRPF1 622-738 was purified to homogeneity, using a Ni-NTA Superflow column, cleavage by TEV protease and size exclusion chromatography. The protein was concentrated to 5.9-10 mg/mL in 50 mM HEPES pH7.5, 150 mM NaCl, 0.5 mM TCEP.

Compounds were added to the protein in 3-fold excess and spun prior to co-crystallisation in 120 nL +120 nL sitting drops @ 4°C and 20°C within MRC plates. The successful co-crystallisation conditions are given in Figure S4. Crystals were flash frozen straight from the Morpheus well conditions by plunge freezing into liquid nitrogen. Data was processed using XDS and SCALA.[2] The structures were solved by molecular replacement using PHASER (CCP4)[3] starting from a previously determined in house structure, model-building was performed using Coot[4] and refinement carried out using REFMAC (CCP4).[5] Data collection and refinement statistics are given in Supplementary Information (Figure S4). The final models have been deposited in the protein data back under the accession codes *(to be added upon acceptance for publication)*.

For Compound **34** data from a single frozen crystal at 100 K was collected on ID23-1 at the European Synchotron Facilities (Grenoble). The P1 cell contains four molecules in the asymmetric unit. Clear difference density for the ligands in the acetyl-lysine binding could be unambiguously modelled in all four chains (Fo-Fc and 2Fc-Fc OMIT maps are in Supplementary Information, Figure S5). For Compound **38** data from a single frozen crystal at 100 K was collected on ID29 at the European

Synchotron Facilities (Grenoble). The $P4_32_12$ cell contains a single molecule in the asymmetric unit. Clear difference density for the ligands in the acetyl-lysine binding site could be unambiguously modelled (2Fo-Fc and Fo-Fc OMIT maps in Supplementary Information, Figure S6).

BRD4 TR-FRET assays. Compounds were screened against either 6H-Thr BRD4 (1-477) (Y390A) (BRD4 BD2 mutation to monitor compound binding to BD1) or 6H-Thr BRD4 (1-477) (Y97A) (BRD4 BD1 mutation to monitor compound binding to BD2) in a dose-response format in a TR-FRET assay measuring competition between test compound and an Alexa Fluor 647 derivative of I-BET762.[1] Compounds were titrated from 10 mM in 100% DMSO and 50 nL transferred to a low volume black 384 well micro titre plate using a Labcyte Echo 555. A Thermo Scientific Multidrop Combi was used to dispense 5 μL of 20 nM protein in an assay buffer of 50 mM HEPES, 150 mM NaCl, 5% glycerol, 1 mM DTT and 1 mM CHAPS, pH 7.4, and in the presence of 100 nM fluorescent ligand (~K_d concentration for the interaction between BRD4 BD1 and ligand). After equilibrating for 30 mins in the dark at rt, the bromodomain protein:fluorescent ligand interaction was detected using TR-FRET following a 5 μL addition of 3 nM europium chelate labelled anti-6His antibody (Perkin Elmer, W1024, AD0111) in assay buffer. Time resolved fluorescence (TRF) was then detected on a TRF laser equipped Perkin Elmer Envision multimode plate reader (excitation = 337 nm; emission 1 = 615 nm; emission 2 = 665 nm; dual wavelength bias dichroic = 400 nm, 630 nm). TR-FRET ratio was calculated using the following equation: Ratio = ((Acceptor fluorescence at 665 nm) / (Donor fluorescence at 615 nm)) * 1000. TR-FRET ratio data was normalised to high (DMSO) and low (compound control derivative of I-BET762) controls and IC_{50} values determined for each of the compounds tested by fitting the fluorescence ratio data to a four parameter model: y = a + ((b – a) / (1 + (10 ^ x / 10 ^ c) ^ d) where ‘a’ is the minimum, ‘b’ is the Hill slope, ‘c’ is the IC_{50} and ‘d’ is the maximum.

BRPF1, 2 and 3 TR-FRET assays. Compounds were screened against 6H-Flag-Tev-BRPF1 (622-738), 6HisFlag-Tev-BRPF2 (also known as BRD1) (551-673) or 6His-Flag-Tev-BRPF3 (579-706) protein in dose-response format in a TR-FRET assay measuring competition between test compound and a synthetic fluorescent ligand. Compounds were titrated from 10 mM in 100% DMSO and 100 nL transferred to a low volume black 384 well micro titre plate using a Labcyte Echo 555. A Thermo Scientific Multidrop Combi was used to dispense 5 μL of 4 nM BRPF1, 20 nM BRPF2 or 40 nM BRPF3 protein respectively in an assay buffer of 50 mM HEPES, 150 mM NaCl, 5% glycerol, 1mM DTT and 1 mM CHAPS, pH 7.4, and in the presence of the appropriate fluorescent ligand concentration (~K_d concentration for the interaction between protein and ligand). After equilibrating for 30 mins in the dark at rt, the bromodomain protein:fluorescent ligand interaction was detected using

TR-FRET following a 5 μL addition of either 3 nM Lanthascreen Elite Tb-anti His antibody (Invitrogen PV5863) for the Alexa 488 ligands, or 3 nM europium chelate labelled anti-6His antibody (Perkin Elmer, W1024, AD0111) for the Alexa 647 ligand, in assay buffer. Time resolved fluorescence energy transfer (TR-FRET) was then detected on a time-resolved fluorescence laser equipped Perkin Elmer Envision multimode plate reader using the appropriate protocol (excitation = 337 nm; emission 1 Alexa 488 = 495 nm; emission 2 Alexa 488 = 520 nm, emission 1 Alexa 647 = 615 nm; emission 2 Alexa 647= 665 nm). TR-FRET ratio was calculated using the following equation: Ratio = ((Acceptor fluorescence at 520 or 665 nm) / (Donor fluorescence at 495 or 615 nm)) * 1000. Data were analysed as for the BRD4 assay.

BRPF1	BRPF2	BRPF3
Alexa Fluor 488 labelled ligand	Alexa Fluor 488 labelled ligand	Alexa Fluor 647 labelled ligand
100 nM	200 nM	400 nM

NanoBRET assays. HEK293 cells (8 x 10^5) were plated in each well of a 6-well plate and co-transfected with Histone H3.3-HaloTag (NM_002107) and NanoLuc-BRPF1 isoform 1 (P55201-1) bromodomain amino acids 625-735 or isoform 2 (P55201-2) bromodomain amino acids 625-741. Isoform 2 has an insertion S660 -> SEVTELD in the bromodomain. Twenty hours post-transfection cells were collected, washed with PBS, and exchanged into media containing phenol red-free DMEM and 4% FBS in the absence (control sample) or the presence (experimental sample) of 100 nM NanoBRET 618 fluorescent ligand (Promega). Cell density was adjusted to 2 x 10^5 cells/ml and then re-plated in a 96-well assay white plate (Corning Costar #3917). Inhibitors were then added directly to media at final concentrations between 0-33 μM and the plates were incubated for 18hrs at 37 ^oC in the presence of 5% CO_2. NanoBRET furimazine substrate (Promega) was added to both control and experimental samples at a final concentration of 10 μM. Readings were performed within 5 minutes using the CLARIOstar (BMG) equipped with 450/80 nm bandpass and 610 nm longpass filters with a 0.5sec reading setting. A corrected BRET ratio was calculated and is defined as the ratio of the emission at 610 nm/450 nm for experimental samples (i.e. those treated with NanoBRET fluorescent ligand) subtracted by the emission at 610 nm/450 nm for control samples (not treated with NanoBRET fluorescent ligand). BRET ratios are expressed as milliBRET units (mBU), where 1 mBU corresponds to the corrected BRET ratio multiplied by 1000.

Artificial membrane permeability measurement. Permeability across a lipid membrane was measured using the published protocol.[6]

CLND solubility measurement. Solubility was determined by precipitation of 10 mM DMSO stock concentration to 5% DMSO pH7.4 phosphate buffered saline, with quantification by ChemiLuminescent Nitrogen Detection.

Method description for chemoproteomic profiling of BRPF1 inhibitor

Preparation of cell fractions

Nuclear extract was produced from fresh HuT78 cells grown at $5 \times 10^6 – 1 \times 10^6$ cells/mL in spinner flasks. Cells were collected by centrifugation, washed with PBS and resuspended in 3 volumes with hypotonic buffer A (10 mM Tris-Cl, pH 7.4, 1.5 mM $MgCl_2$, 10 mM KCl, 25 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, and 1 Roche protease inhibitor tablet per 25 mL). After approximately 15 min cells were homogenized with a Dounce homogenizer. Nuclei were collected by centrifugation (2500xg), washed with hypotonic buffer A and homogenized in one volume of extraction buffer B (50 mM Tris-Cl, pH 7.4, 1.5 mM $MgCl_2$, 20 % glycerol, 420 mM NaCl, 25 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, 400 Units/mL DNase I, and 1 Roche protease inhibitor tablet per 25 mL). Extraction was allowed to proceed under agitation for 30 min at 4°C before the extract was clarified by centrifugation at 13000xg. The extract was diluted in buffer D (50 mM Tris-Cl, pH 7.4 (RT), 1.5 mM $MgCl_2$, 25 mM NaF, 1 mM Na_3VO_4, 0.6 % NP40, 1 mM DTT, and Roche protease inhibitors), and aliquots were snap frozen in liquid nitrogen and stored at -80°C.

Tight chromatin-associated proteins enriched fractions were prepared by resuspending the remaining pellet in 10 volumes of high salt extraction buffer (20 mM Hepes pH7.4, 1.5 mM $MgCl_2$, 1000 mM KCl, 10% glycerol, 0.1% NP40, 0.5 mM DTT, and Roche protease inhibitors) with 6 cycles of 10 sec on – 50 sec off sonication, with an ultrasound homogenizer (Bandelin Sonoplus). After sonication and incubation for 45 min at 4 °C, the homogenate was clarified at 8000xg. The salt concentration was adjusted to 150 mM KCl through stepwise dialysis before ultracentrifugation at 100,000xg for 20 min. Aliquots were snap frozen in liquid nitrogen and stored at -80 °C.

Chemoproteomic assay with dose-dependent competition

Affinity profiling assays were performed as described previously.[7]

Compound **34** was spiked into HuT78 nuclear and chromatin extracts and incubated for 45 min at 4 °C. Derivatized sepharose beads (35 μL beads per sample) were equilibrated in lysis buffer and incubated with cell extract pre-incubated with compound. Beads were washed with lysis buffer containing 0.2 % NP-40 and eluted with 2x SDS sample buffer supplemented with DTT. Proteins were alkylated with iodoacetamide, separated on 4–12 % NuPAGE (Invitrogen), and stained with colloidal Coomassie.

Western Blot

Aliquots of the eluates from chemoproteomic assays were used for Western Blot analysis and BRPF1 (novus biologicals, NBP2-15620) and BRD3 (santa cruz, sc-81202) detection. Quantification was performed using the Odyssey infrared imager system (Li-Cor).

Supplementary References.

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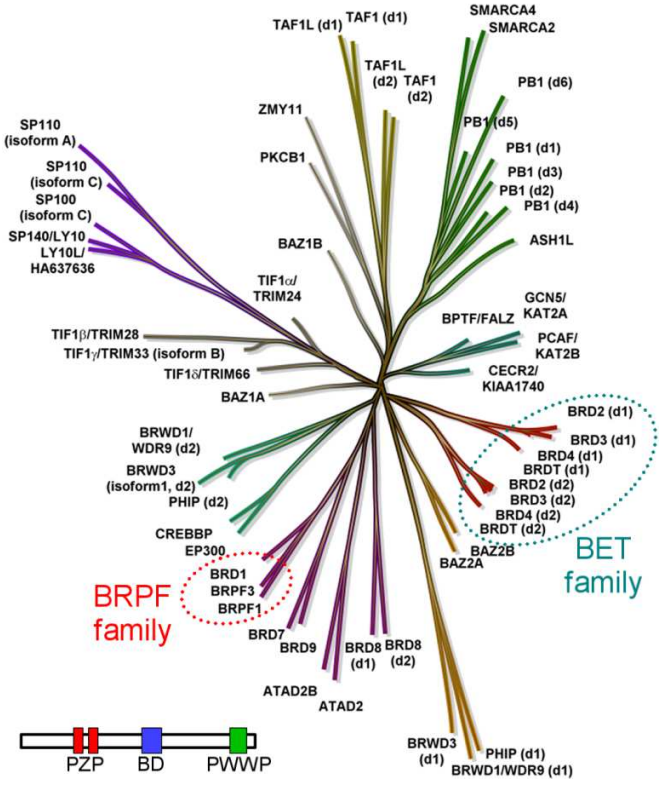
(7) Werner, T.; Becher, I.; Sweetman, G.; Doce, C.; Savitski, M. M.; Bantscheff, M. High-Resolution Enabled TMT 8-Plexing. *Anal. Chem.* **2012**, *84*, 7188–7194.

Figure S1: A) Bromodomain phylogenetic tree, with BRPF1 domain structure inset. B) Aligned acetyl-lysine site residues of the BET family BD1 and BRPF family bromodomains. C) Sequence alignment of the BET and BRPF family bromodomains. The BET WPF motif is coloured blue, the water-binding tyrosine red, the conserved asparagine green, and the gatekeeper magenta.

A)



B)

BRD2 BD1	BRD3 BD1	BRD4 BD1	BRPF1	BRPF2 / BRD1	BRPF3	Comment
W97	W57	W81	N651	R585	H612	WPF motif
P98	P58	P82	I652	I586	I613	WPF motif
F99	F59	F83	F653	F587	F614	WPF motif
R100	Y60	Q84	S654	A588	A615	ZA channel
Q101	Q61	Q85	E655	Q589	E616	ZA channel
P102	P62	P86	P656	P590	P617	ZA channel
V103	V63	V87	V657	V591	V618	ZA channel
D104	D64	D88	P658	S592	N619	ZA loop
A105	A65	A89	L659	L593	L620	ZA loop
K107	K67	K91				ZA loop
L108	L68	L92				ZA loop
G109	N69	N93	E661	E595	E622	ZA loop
L110	L70	L94	V662	V596	V623	ZA loop
Y113	Y73	Y97	Y665	Y599	Y626	Water-binding Tyr
H114	H74	Y98	L666	L600	L627	
I117	I77	I101	I669	I603	I630	
M148	M108	M132	I700	I634	I661	
N151	N111	N135	N703	N637	N664	
C152	C112	C136	C704	C638	C665	Conserved Cys
Y155	Y115	Y139	Y707	Y641	Y668	Conserved Tyr
N156	N116	N140	N708	N642	N669	Conserved Asn
D160	D120	D144	T712	T646	T673	
D161	D121	D145	I713	V647	I674	
I162	I122	I146	F714	F648	F675	Gatekeeper
V163	V123	V147	Y715	Y649	H676	
M165	M125	M149	A717	A651	A678	
A166	A126	A150	A718	A652	A679	

C)

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BRPF1 631 TPFLILLRKTLEQLQE...KDTGNIFSEPVPLS..EVPDYLDHIKKPMDFFTMKQNLE
  BRD1 565 TPLTVLLRSVLDQLQD...KDPARIFAQPVSLK..EVPDYLDHIKHPMDFATMRKRLE
 BRPF3 592 MPFNVLLRTTLDLLQE...KDPAHIFAEPVNLS..EVPDYLEFISKPMDFSTMRRKLE
BRD2_1  77 NQLQYLHKVVMKALWK...HQFAWPFRQPVDAVKLGLPDYHKIIKQPMDMGTIKRRLE
BRD3_1  37 NQLQYMQNVVVKTLWK...HQFAWPFYQPVDAIKLNLPDYHKIIKNPMDMGTIKKRLE
BRD4_1  61 NQLQYLLRVVLKTLWK...HQFAWPFQQPVDAVKLNLPDYYKIIKTPMDMGTIKKRLE
BRD2_2 347 SEQLKHCNGILKELLSKKHAAYAWPFYKPVDASALGLHDYHDIIKHPMDLSTVKRKME
BRD3_2 309 SEHLRYCDSILREMLSKKHAAYAWPFYKPVDAEALELHDYHDIIKHPMDLSTVKRKMD
BRD4_2 351 SEQLKCCSGILKEMFAKKHAAYAWPFYKPVDVEALGLHDYCDIIKHPMDMSTIKSKLE
           <-     αZ     ->          ZA loop                <-  αA ->

 BRPF1 684 AYRYLNFDDFEEDFNLIVSNCLKYNAKDTIFYRAAVRLREQGGAVLRQA
   BRD1 618 AQGYKNLHEFEEDFDLIIDNCMKYNARDTVFYRAAVRLRDQGGVVLRQA
  BRPF3 645 SHLYRTLEEFEEDFNLIVTNCMKYNAKDTIFHRAAVRLRDLGGAILRHA
BRD2_1 132 NNYYWAASECMQDFNTMFTNCYIYNKPTDDIVLMAQTLEKIFLQKVASMPQEEQ
BRD3_1  92 NNYYWSASECMQDFNTMFTNCYIYNKPTDDIVLMAQALEKIFLQKVAQMPQEEV
BRD4_1 116 NNYYWNAQECIQDFNTMFTNCYIYNKPGDDIVLMAEALEKLFLQKINELPTEET
BRD2_2 405 NRDYRDAQEFAADVRLMFSNCYKYNPPDHDVVAMARKLQDVFEFRYAKMPDEPL
BRD3_2 367 GREYPDAQGFAADVRLMFSNCYKYNPPDHEVVAMARKLQDVFEMRFAKMPDEPV
BRD4_2 409 AREYRDAQEFGADVRLMFSNCYKYNPPDHEVVAMARKLQDVFEMRFAKMPDEPE
           <-     αB     ->     <-     αC     ->
```

Figure S2: The Cambridge Structure Database (CSD http://www.ccdc.cam.ac.uk/products/csd/) and the GSK internal database were searched with the two queries shown below. Torsions of interest are highlighted by the red arrow. Polar histograms of torsions are shown with the radius representing the frequency:

A) For the phenyl ring unsubstituted in the 2- and 6-positions the preferred torsions are spread around zero and 180 degrees, with the most populated torsions being between -23 and -35 degrees. This means that in the database of structures the phenyl ring is out of plane compared with the amide moiety.

B) For the 2-pyridyl, the polar histogram the majority of torsions spread around zero degrees. For this substructure, the amide and the pyridine sit in the same plane.

A third search was performed for the 2-OMe phenyl. However, only 3 results were returned and, therefore, not reported here due to the low statistical significance of so few examples.

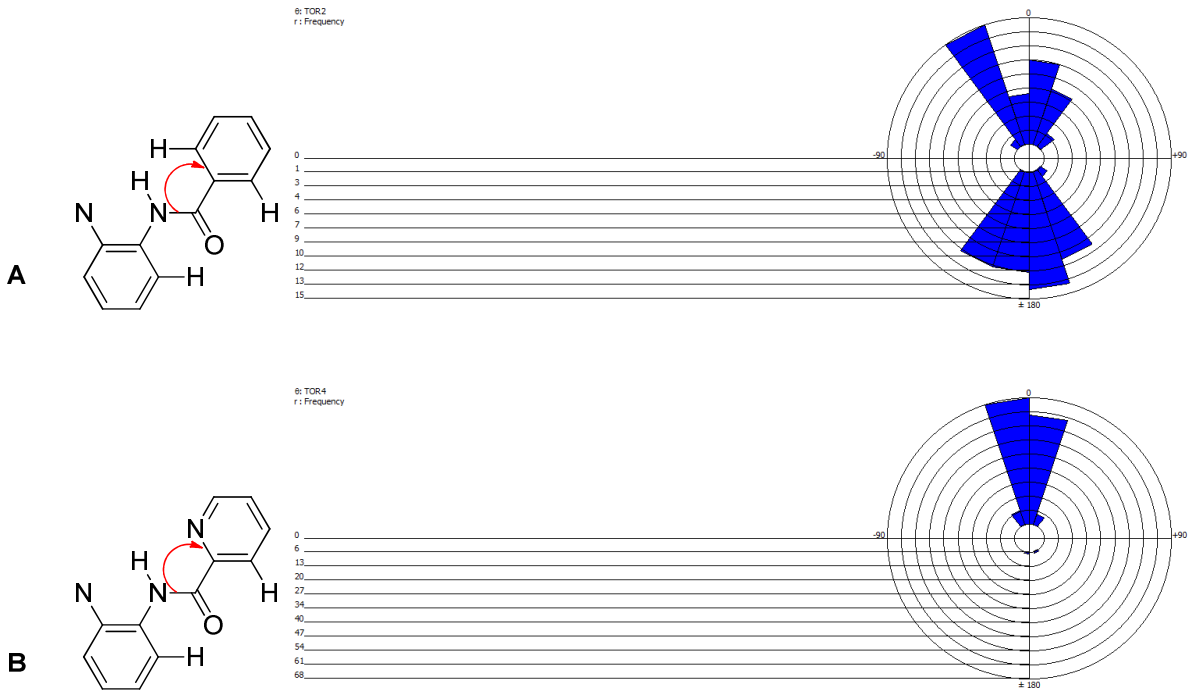
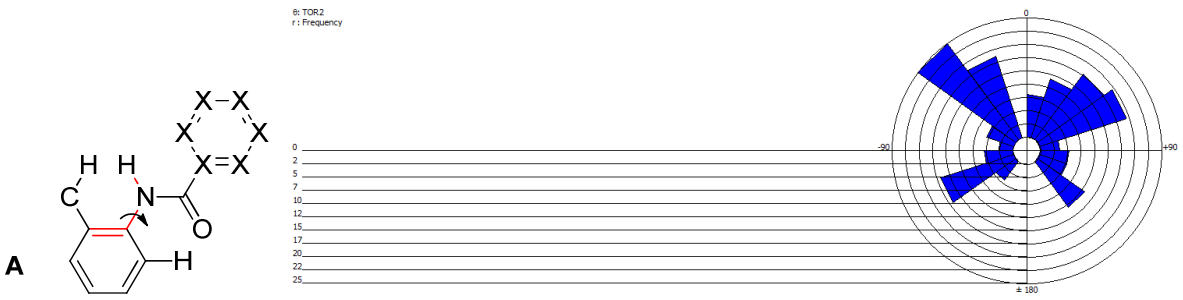


Figure S3: The Cambridge Structure Database (CSD) and the GSK internal database were searched with the two queries shown below. Torsions of interest are highlighted by the black arrow. Polar histograms of torsions are shown with the radius representing the frequency:

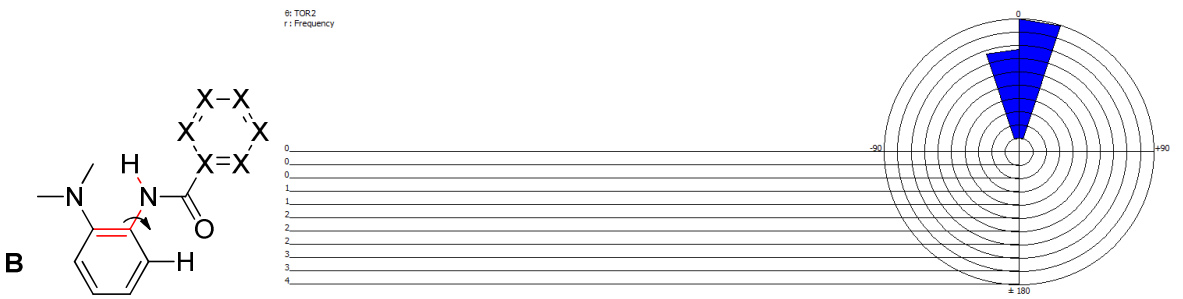
A) The CH group has a tendency to lie at an angle compared to the amide NH (roughly at a 45 degrees compared to the plane of the amide). Therefore, it would suggest a steric or electrostatic repulsion between the two moieties. This would verify the assumption that there is a difference in conformation between the *C*-linked and the *N*-linked molecules.

B) In the case of the *N*-linked molecules, the Nitrogen, and the NH of the amide would tend to lie coplanar, which would support the hypothesis that there is a favourable electrostatic interaction between these two groups.



A

44 examples



B

6 examples

Figure S4: Data collection and refinement statistics for BRPF1 crystal structures. Each dataset was collected from a single crystal. Value in parentheses are for the highest resolution shell.

	BRPF1 **Compound 34**	BRPF1 **Compound 38**
Crystallisation conditions	Morpheus 100nL+100nL , 30% P550MMe_P20K, 0.1M buffer 3, pH8.5, 10% ethylene glycols	0.1M Morpheus buffer 1, pH 6.5 37.5% MPD_P1K_P3350 10% morpheus nitrate-phosphate-sulphate
Data collection		
Space Group	P1	$P4_32_12$
Cell Dimensions		
a,b,c (Å)	47.444,50.703,61.981	60.530, 60.530, 85.140
α, β, γ ()	72.61 71.36 66.64	90.00 90.00 90.00
Resolution (Å)	1.96 (2.07)	1.60 (1.69)
R_{merge} [b]	0.059 (0.178)	0.059 (0.362)
Average *I*/σ*I*	7.2 (2.3)	26.4 (8.0)
Completeness (%)	93.9 (93.9)	100.0 (100.0)
Redundancy	1.8 (1.9)	12.6 (12.3)
No. Reflections	61107(8983)	271126 (38094)
No. Unique Reflections	33077(4841)	21557 (3087)
Wilson Bfactor	34.842	20.60
Refinement		
Resolution (Å)	57.57-1.96	49.33-1.60
R_{work}/R_{free}	0.240/0.202	0.187/0.193
No. Reflections	31410	20440
No. atoms	4197	1254
Protein	3677	957
Ligand/ion	120	64/4
Water	400	229
B-factors		
Protein	40.294	24.502
Ligand/ion	32.135	27.320/ 114.750
Water	40.648	40.197
R.M.S deviations		
Bond lengths (Å)	0.0101	0.007
Bond angles ()	1.3527	1.082

Figure S5: 2Fo-Fc and Fo-Fc OMIT maps for the BRPF1 + Compound 34 contoured at sigma levels indicated.

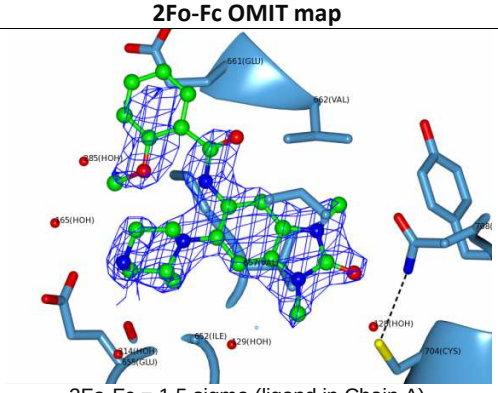
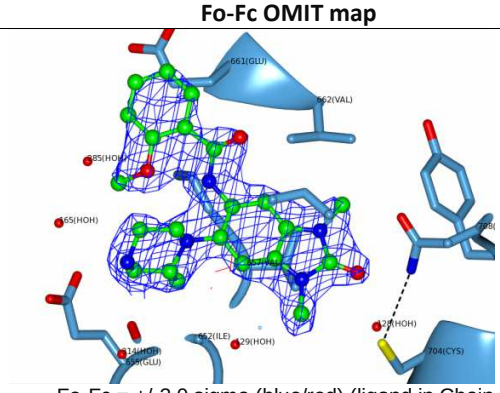
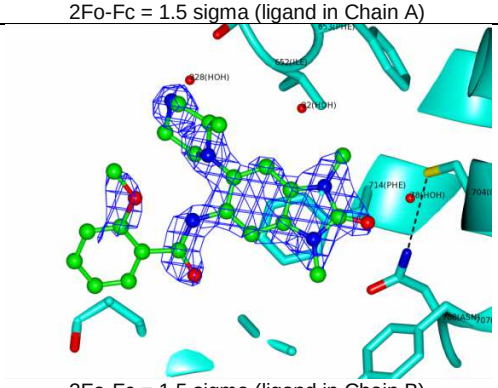
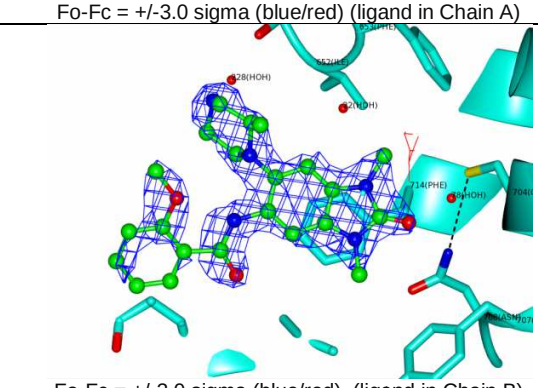
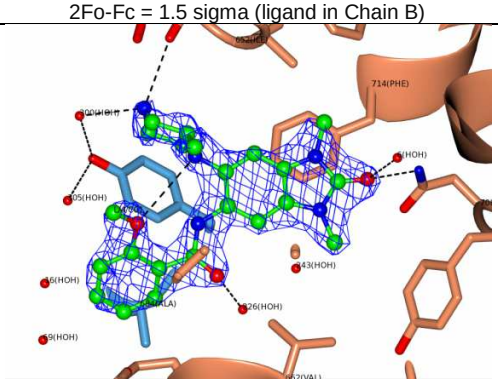
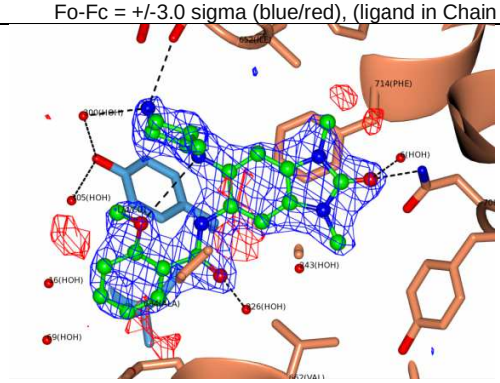
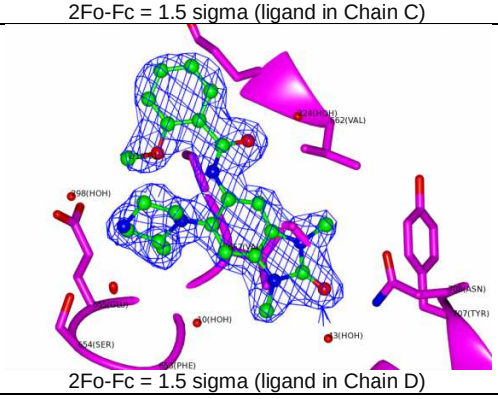
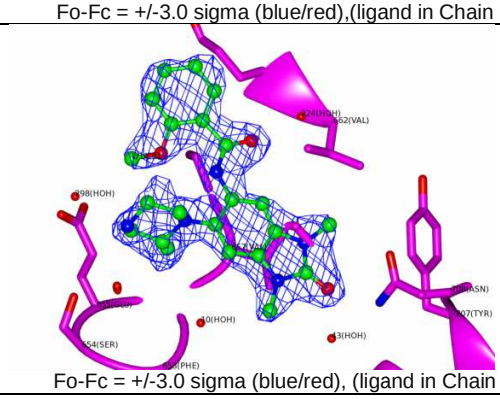
BRPF1 + compound 34 2Fo-Fc OMIT map	BRPF1 + compound 34 Fo-Fc OMIT map
 2Fo-Fc = 1.5 sigma (ligand in Chain A)	 Fo-Fc = +/-3.0 sigma (blue/red) (ligand in Chain A)
 2Fo-Fc = 1.5 sigma (ligand in Chain B)	 Fo-Fc = +/-3.0 sigma (blue/red), (ligand in Chain B)
 2Fo-Fc = 1.5 sigma (ligand in Chain C)	 Fo-Fc = +/-3.0 sigma (blue/red),(ligand in Chain C)
 2Fo-Fc = 1.5 sigma (ligand in Chain D)	 Fo-Fc = +/-3.0 sigma (blue/red), (ligand in Chain D)

Figure S6: 2Fo-Fc and Fo-Fc OMIT maps for the BRPF1 + **Compound 38** contoured at sigma levels indicated.

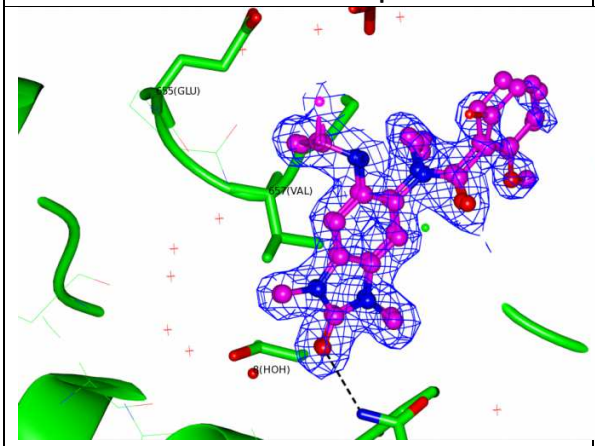
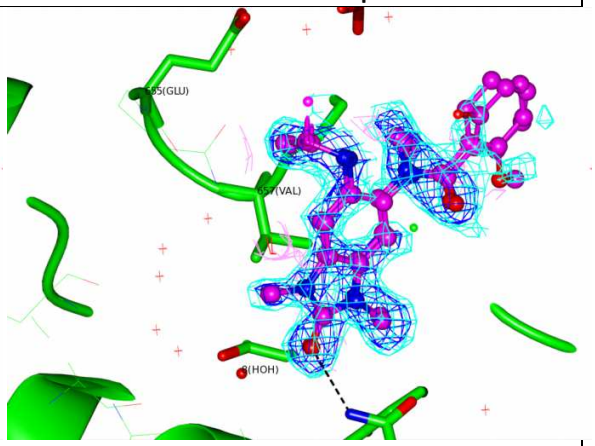
BRPF1 + compound 38 **2Fo-Fc OMIT map**	**BRPF1 + compound 34** **Fo-Fc OMIT map**
	
2Fo-Fc = 1.0 sigma (ligand in Chain A)	Fo-Fc = +/-3.0 sigma (cyan/pink), +/-2.0 (blue/red) (ligand in Chain A)

Figure S7: (A,B) Dose response curve NanoBRET experiments, showing inhibition of the NanoLuc-BRPF1 bromodomain interaction with Histone H3.3-HaloTag in HEK293 cells with A) compound **34**, as compared to B) the less active control, analogue **38**. A cellular IC_{50} value of 20 nM was calculated for **34**, and a cellular IC_{50} value of 3.7 μM was calculated for **38**. (C,D) NanoBRET experiments in HEK293 between the BRPF1 bromodomain isoform 2, which contains a 6 amino acid insert close to the acetyl-lysine site, and Histone H3.3. The dose response curves with compound **34** (C) enabled a cellular IC_{50} value of 7.6 μM to be calculated. Compound **38** (D) showed no change in BRET signal, indicating this compound did not inhibit the interaction between BRPF1 bromodomain isoform 2 and Histone H3.3. See Supplementary Methods, p81.

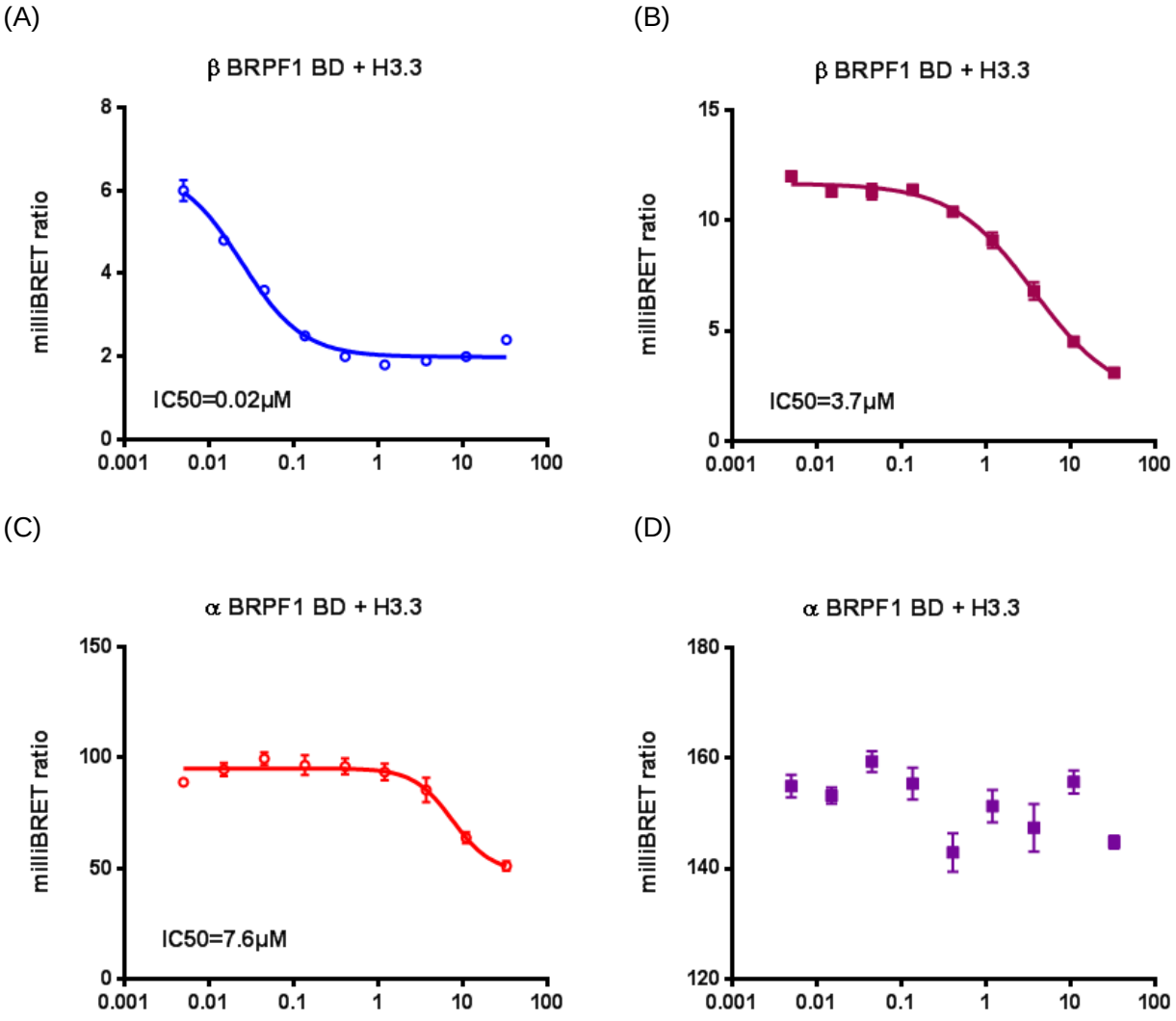


Table S1: TR-FRET pIC_{50} data for BRPF1-3 and BRD4 BD1 (N-terminal) and BD2 (C-terminal) bromodomains, including mean, standard deviation and number of test occasions.

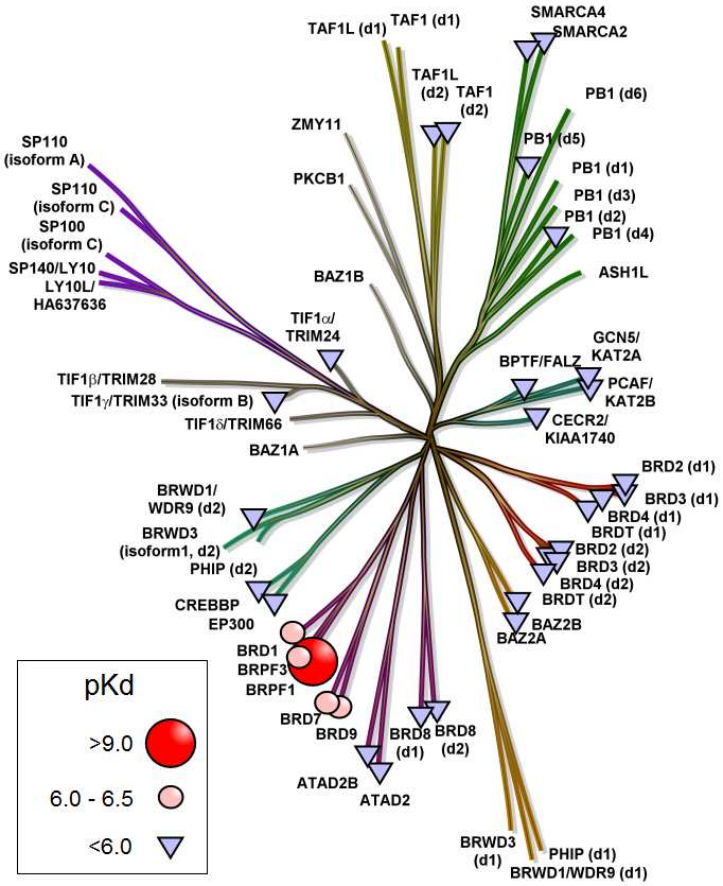
ID	BRPF1	BRPF2	BRPF3	BRD4 BD1	BRD4 BD2	CLND SOLUBILITY MEAN µg/mL	CHROM LogD pH74
1	7.1 ± 0.16 (7)	5.2 ± 0.24 (4)	4.5 (2) and <4.0 (2)	4.7 (1) and <4.3 (9)	4.6 (1) and <4.3 (9)	7.5	6.0
2	7.3 ± 0.19 (6)	5.4 ± 0.12 (4)	4.5 ± 0.09 (4)	5.0 ± 0.12 (6)	4.5 ± 0.03 (2) and <4.3 (4)	115	4.6
3	5.8 ± 0.16 (6)	4.8 ± 0.01 (2)	<4.0 (2)	<4.3 (3)	<4.3 (3)	110	3.9
4	6.9 ± 0.18 (9)	5.2 ± 0.09 (3) and <5.0 (1)	4.6 (1) and <4.0 (3)	5.0 ± 0.19 (3) and <4.3 (1)	<4.3 (4)	11	4.1
5	7.5 ± 0.22 (6)	5.6 ± 0.08 (4)	4.8 ± 0.04 (2) and <4.0 (2)	5.2 (1) and <4.3 (5)	4.7 (1) and <4.3 (5)	9	5.0
6	6.9 ± 0.08 (4)	5.6 ± 0.06 (4)	5.0 ± 0.04 (4)	4.4 ± 0.06 (3) and <4.3 (1)	4.5 ± 0.06 (2) and <4.3 (2)	156	3.5
7	7.0 ± 0.07 (4)	5.6 ± 0.06 (4)	4.8 ± 0.32 (3) and <4.0 (1)	4.3 (1) and <4.3 (3)	<4.3 (4)	19	2.6
8	6.5 ± 0.12 (4)	5.4 ± 0.07 (4)	4.9 ± 0.05 (4)	<4.3 (4)	<4.3 (4)	164	3.6
9	6.8 ± 0.15 (3)	5.2 ± 0.02 (2) and <4.0 (2)	4.5 ± 0.26 (2) and <4.0 (2)	4.5 (1) and <4.3 (3)	<4.3 (4)	3	4.2
10	7.0 ± 0.16 (4)	5.6 ± 0.09 (4)	5.1 ± 0.08 (4)	4.4 ± 0.03 (4)	<4.3 (4)	174	4.7
11	7.0 ± 0.16 (4)	5.4 ± 0.07 (4)	4.8 ± 0.04 (4)	4.4 ± 0.03 (4)	<4.3 (4)	163	3.4
12	6.5 ± 0.07 (4)	5.3 ± 0.05 (4)	4.8 ± 0.05 (4)	4.6 (1) and <4.3 (3)	<4.3 (4)	187	3.4
13	5.8 ± 0.01 (2)	4.8 ± 0.06 (2)	4.2 ± 0 (2)	4.4 (1) and <4.3 (1)	4.5 (1) and <4.3 (1)	114	3.7
14	6.0 ± 0.06 (4)	4.9 ± 0.07 (4)	4.3 ± 0.1 (4)	<4.3 (4)	<4.3 (4)	123	2.2
15	5.9 ± 0.2 (4)	4.9 ± 0.04 (4)	4.3 ± 0.08 (4)	<4.3 (4)	<4.3 (4)	145	3.7
16	6.1 ± 0.13 (4)	4.8 ± 0.05 (4)	4.5 ± 0.05 (4)	<4.3 (4)	<4.3 (4)	135	0.6
17	5.8 ± 0.12 (4)	4.8 ± 0.04 (4)	4.3 ± 0.07 (4)	<4.3 (4)	<4.3 (4)	141	2.7
18	6.3 ± 0.07 (6)	5.5 ± 0.08 (4)	4.8 ± 0.05 (4)	<4.3 (2)	<4.3 (2)	164	4.7
19	7.2 ± 0.25 (4)	5.4 ± 0.06 (4)	4.6 ± 0.07 (4)	5.0 ± 0.13 (4)	4.5 (1) and <4.3 (3)	126	4.7
20	5.9 ± 0.03 (2)	5.3 ± 0.02 (2)	4.5 ± 0.02 (2)	<4.3 (2)	<4.3 (2)	154	2.7
21	6.1 ± 0.01 (2)	5.3 ± 0.01 (2)	4.8 ± 0.04 (2)	<4.3 (2)	<4.3 (2)	157	2.7
22	6.9 ± 0.17 (4)	5.4 ± 0.16 (4)	4.6 ± 0.12 (3) and <4.0 (1)	4.6 ± 0.14 (4)	4.6 (1) and <4.3 (3)	82	3.7
23	7.5 ± 0.17 (4)	5.5 ± 0.04 (3)	4.8 ± 0 (2) and <4.0 (2)	5.1 ± 0.18 (4)	4.5 (1) and <4.3 (3)	129.5	4.8
24	7.0 ± 0.12 (4)	5.2 ± 0.1 (4)	4.5 ± 0.13 (4)	4.4 ± 0.1 (3) and <4.3 (1)	<4.3 (4)	167	3.4
25	5.7 ± 0.28 (4)	5.0 ± 0.09 (4)	4.3 ± 0.07 (4)	<4.3 (4)	<4.3 (4)	167	4.1
26	5.9 ± 0.01 (2)	4.2 ± 0.08 (2)	<4.0 (2)	<4.3 (2)	<4.3 (2)	187	4.1
27	7.3 ± 0.19 (7)	5.5 ± 0.15 (4)	4.7 ± 0.12 (4)	4.7 (1) and <4.3 (3)	<4.3 (4)	71.5	5.4
28	7.1 ± 0.16 (4)	5.0 ± 0.14 (4)	4.7 ± 0.13 (4)	<4.3 (4)	<4.3 (4)	135	1.6
29	6.7 ± 0.02 (2)	4.8 ± 0.04 (2)	4.7 ± 0.04 (2)	<4.3 (2)	<4.3 (2)	149	3.0
30	5.5 ± 0.18 (4)	5.1 ± 0.05 (4)	4.9 ± 0.06 (4)	<4.3 (4)	<4.3 (4)	107	1.2
31	5.9 ± 0.24 (4)	5.4 ± 0.04 (4)	4.8 ± 0.07 (4)	<4.3 (4)	<4.3 (4)	110	1.3
32	8.3 ± 0.27 (4)	5.7 ± 0.08 (4)	4.9 ± 0.11 (4)	5.0 ± 0.06 (3) and <4.3 (1)	4.3 (1) and <4.3 (3)	3.5	5.9
33	7.8 ± 0.31 (4)	5.4 ± 0.11 (4)	4.6 ± 0.18 (3) and <4.0 (1)	4.9 ± 0.01 (2) and <4.3 (1)	<4.3 (4)	2.5	5.9
34	8.1 ± 0.45 (4)	5.1 ± 0.06 (4)	4.8 ± 0.11 (4)	4.7 ± 0.15 (5) and <4.3 (1)	<4.3 (6)	140	2.0
35	6.9 ± 0.04 (2)	4.9 ± 0.02 (2)	5.0 ± 0 (2)	<4.3 (2)	<4.3 (2)	134	1.9
36	7.1 ± 0.08 (2)	4.8 ± 0.01 (2)	4.7 ± 0.07 (2)	<4.3 (2)	<4.3 (2)	141	1.9
37	6.9 ± 0 (2)	4.7 ± 0.08 (2)	4.6 ± 0.09 (2)	4.7 (1) and <4.3 (1)	4.8 (1) and <4.3 (1)	139	1.9
38	6.0 ± 0.01 (2)	4.3 ± 0.19 (2)	4.9 ± 0.03 (2)	<4.3 (2)	<4.3 (2)	174	1.8

Table S2: A) Bromodomain selectivity of **34** in the BROMO*scan*™ panel (DiscoveRx Corp., Fremont, CA, USA, http://www.discoverx.com). This screen measures competition against reference immobilized ligands for 35 DNA-tagged bromodomains. Results are mean, n=2. B) Graphical representation of data in A. C) Duplicate BRPF1 dose-response curves for compound **34**.

A)

Bromodomain		pK*d*
ATAD2	<	4.52
ATAD2B	=	4.59
BAZ2A	=	5.43
BAZ2B	=	5.37
BRD2 (BD1)	=	5.46
BRD2 (BD2)	<	4.52
BRD3 (BD1)	=	5.96
BRD3 (BD2)	<	4.52
BRD4 (BD1)	=	5.62
BRD4 (BD2)	<	4.52
BRD7	=	6.09
BRD8 (BD1)	=	5.54
BRD8 (BD2)	<	4.52
BRD9	=	6.00
BRDT (BD1)	=	4.92
BRDT (BD2)	<	4.52
BRPF1	=	9.51
BRPF2 / BRD1	=	6.29
BRPF3	=	6.00
CECR2	=	5.25
CREBBP	<	4.52
EP300	<	4.52
FALZ / BPTF	=	5.77
GCN5L2	<	4.52
PBRM1 (BD2)	<	4.52
PBRM1 (BD5)	<	4.52
PCAF	=	4.80
SMARCA2	<	4.52
SMARCA4	<	4.52
TAF1 (BD2)	=	5.02
TAF1L (BD2)	<	4.52
TIF1α / TRIM24 (bromodomain)	=	4.92
TIF1α / TRIM24 (PHD/bromodomain)	=	5.02
TIF1γ / TRIM33 (PHD/bromodomain)	=	5.57
WDR9 (2)	<	4.52

B)



C)

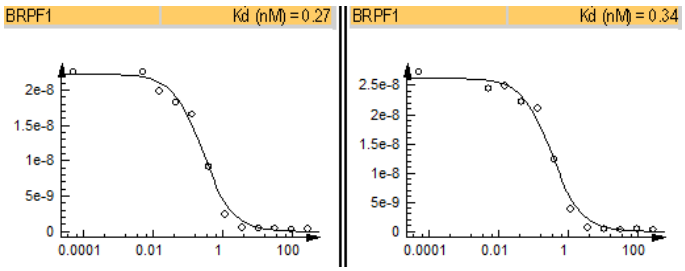


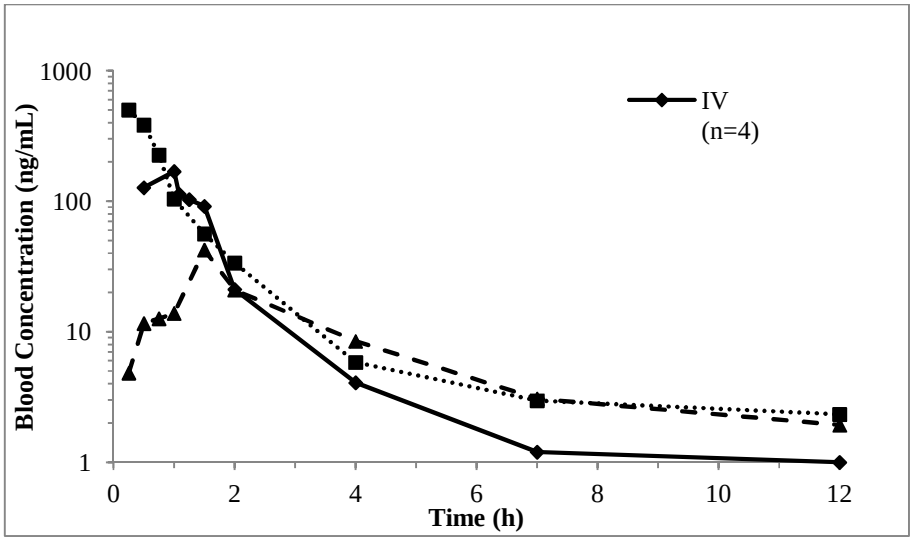
Table S3:
Activity Data for Compound 34 in 48 unrelated assays
Compound **34** was screened against a range of protein types using the specified assay. (n) is the number of replicates

TARGET CLASS	TARGET	ASSAY	pXC_{50} (n)
Ion Channel	Human hERG Barracuda	Electrophysiology	<4.3 (2)
Ion Channel	Human CaV1.2 Barracuda	Electrophysiology	4.1 (2)
Ion Channel	Human NaV1.5	Electrophysiology	<4.0 (3)
Ion Channel	Human Kv1.5	Electrophysiology	<4.3 (2)
Ion Channel	Human KCNQ1/minK	Electrophysiology	4.7 (2)
Ion Channel	Human Serotonin (5-HT3) Receptor opening	Intracellular Ca (Fluoresence)	<4.3 (2)
Ion Channel	Human Serotonin (5-HT3) Receptor blocking	Intracellular Ca (Fluoresence)	5.3 (2)
Ion Channel	Human NMDA/NR2B receptor antagonism	Intracellular Ca (Fluoresence)	<4.3 (2)
Ion Channel	Human Alpha 1 nicotinic AChR opening	Intracellular Ca (Fluoresence)	<4.3 (2)
Ion Channel	Human Alpha 1 nicotinic AChR blocking	Intracellular Ca (Fluoresence)	5.1 (2)
GPCR	Human Adenosine 2a receptor agonism	TR-FRET	<4.0 (2)
GPCR	Human Alpha 1b adrenergic receptor antagonism	Intracellular Ca (Fluoresence)	5.3 (2)
GPCR	Human Alpha 2c adrenergic receptor agonism	TR-FRET	<4.0 (2)
GPCR	Human Beta 2 adrenergic receptor agonism	TR-FRET	4.1 (2)
GPCR	Human Beta 2 adrenergic receptor antagonism	TR-FRET	<4.0 (2)
GPCR	Human Dopamine (D1) receptor antagonism	TR-FRET	<4.0 (2)
GPCR	Human Dopamine (D2) receptor agonism	LEADseeker SPA	<4.0 (2)
GPCR	Human Dopamine (D2) receptor antagonism	LEADseeker SPA	4.2 (2)
GPCR	Human Histamine 1 (H1) receptor antagonism	Intracellular Ca (Fluoresence)	<4.6 (2)
GPCR	Human Muscarinic 1 receptor agonism	Intracellular Ca (Fluoresence)	<4.3 (2)
GPCR	Human Muscarinic 1 receptor antagonism	Intracellular Ca (Fluoresence)	<4.3 (2)
GPCR	Human Muscarinic 2 receptor agonism	Intracellular Ca (Fluoresence)	<4.3 (2)
GPCR	Human Muscarinic 2 receptor antagonism	Intracellular Ca (Fluoresence)	<4.3 (2)
GPCR	Human Mu Opioid receptor agonism	LEADseeker SPA	<4.0 (2)
GPCR	Human Kappa Opioid agonism	LEADseeker SPA	<4.0 (2)
GPCR	Human Vasopressin 1a antagonism	Intracellular Ca (Fluoresence)	<4.3 (2)

GPCR	Human CB2 agonism	Yeast FDLu	<4.0 (2)
GPCR	Human Serotonin (5HT1B) receptor agonism	LEADseeker SPA	<4.0 (2)
GPCR	Human Serotonin (5HT1B) receptor antagonism	LEADseeker SPA	4.9 (2)
GPCR	Human Serotonin (5HT2A) receptor agonism	Intracellular Ca (Fluoresence)	<4.6 (2)
GPCR	Human Serotonin (5HT2A) receptor antagonism	Intracellular Ca (Fluoresence)	<4.6 (2)
GPCR	Human Serotonin (5HT2C) receptor agonism	Intracellular Ca (Fluoresence)	<4.6 (2)
GPCR	Human Serotonin (5HT2C) receptor antagonism	Intracellular Ca (Fluoresence)	5.4 (2)
GPCR	Human Neurokinin Type 1 (NK1) receptor antagonism	Intracellular Ca (Fluoresence)	<4.6 (2)
Nuclear Receptor	Human PXR (NR1I2) Agonist	Luciferase Reporter	<4.3 (2)
Transporter	Human Norepinephrine (NE) transporter antagonism	LEADseeker SPA	4.3 (2)
Transporter	Human Serotonin (SERT) transporter antagonism	LEADseeker SPA	<4.0 (2)
Transporter	Human Organic Anion Transport Polypeptide C OATP1B1 antagonism	Imaging	<4.3 (2)
Enzyme	Human Monoamine oxidase (MAO-A) inhibition	FLINT	<4.0 (2)
Enzyme	Human Monoamine oxidase (MAO-B) inhibition	FLINT	<4.0 (2)
Enzyme	Human Phosphodiesterase type 3 (PDE3A) inhibition	Luminescence	<4.0 (2)
Enzyme	Human Phosphodiesterase type 4 (PDE4B) inhibition	Luminescence	4.2 (2)
Enzyme	Human Cyclooxygenase 2 (COX-2) inhibition	FLINT	<4.0 (2)
Enzyme	Human Acetylcholinesterase inhibition	Mass Spectrometry	<4.0 (2)
Enzyme	CYP3A4 (substrate preincubation) inhibition	Mass Spectrometry	<4.4 (2)
Kinase	Human AuroraB antagonism	IMAP	<4.5 (3)
Kinase	Human PI3Kγ antagonism	TR-FRET	<4.5 (1)
Kinase	Human LCK antagonism	IMAP	<4.5 (3)

Table S4: Pharmacokinetic properties of Compound 34

The pharmacokinetic properties of **Compound 34** have been determined in the male CD1 mouse to assess the potential for this molecule to be used as an *in vivo* probe, allowing further understanding of the therapeutic benefit of $BRPF_1$ inhibition in established *in vivo* pharmacokinetic pharmacodynamic (PKPD) models. Following IV administration (1 mg/kg), **Compound 34** demonstrated a high blood clearance of 107 mL/min/kg, a moderate volume of distribution (5.5 L/kg) and a moderate terminal half-life of 1.7 h. Oral administration (PO, 3 mg/kg) achieved a moderate systemic exposure, with a C_{max} of 42 ng/mL and T_{max} of 1.5 h, resulting in a bioavailability of 22%. The intraperitoneal route of administration (IP, 3 mg/kg) reached a C_{max} of 469 ng/mL and Tmax of 0.25 h, resulting in a bioavailability of 85%. The results indicate that the IP route of administration would be suitable for dosing this molecule in potential PKPD models. In order to be able to compare biochemical potency measurements to free blood concentrations, the fraction unbound (fu_b) in the CD1 mouse was also determined with a resulting value of 7.9%.



IV pharmacokinetics (n=4)	CLb (mL/min/kg)	107
	Vd (L/kg)	5.5
	$t_{1/2}$ (h)	1.7
PO pharmacokinetics (n=3)	Cmax (ng/mL)	42
	Tmax (h)	1.5
	F%	22
IP pharmacokinetics (n=3)	Cmax (ng/mL)	469
	Tmax (h)	0.25
	F%	85

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.