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CONCISE ARTICLE

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Supplementary Material for:

The design and synthesis of 5- and 6-isoxazolylbenzimidazoles as selective inhibitors of the BET bromodomains.

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Synthesis of Representative Compounds

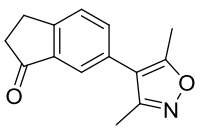
General experimental

Commercial reagents were used as received without further purification. Commercial anhydrous solvents were used in reactions and HPLC grade solvents were employed for work-up and chromatography. NMR spectra were recorded using a Varian Mercury 300 MHz or 400 MHz for ^{1}H and 75 MHz or 101 MHz for ^{13}C. The solvent was used as internal deuterium lock. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.5 Hz. Identical proton coupling constants are averaged in each spectrum and reported to the nearest 0.1 Hz. When peak multiplicities are reported, the following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broadened, dd = doublet of doublets, dt = doublet of triplets. LRMS employed an electrospray ionisation source acquiring in positive and negative ionisation mode. *m/z* values are reported in Daltons. Analytical HPLC was carried out on an Agilent 1100 equipped with photodiode-array detector (DAD), quaternary gradient pump and micro plate sampler (Agilent 220). Separation of the analytes was performed upon Centurysil C18-AQ+ 5µm, 50×4.6mm (Johnson). The flow rate of the mobile phase was kept at 3.5 mL/min. Mobile phases B and C were acetonitrile with 0.35% CF_3CO_2H and water with 0.35% CF_3CO_2H respectively. The gradient conditions were as follows: 0-0.5 min 1% B and 99% C, 3.7 min 90% B and 10% C, 5 min 99% B and 1% C. The injection volume was 10 µl. Compound purity was assessed at 254 nm and by evaporative light scattering detection (ELSD).

List of abbreviations

Boc	*tert*-Butyloxycarbonyl
DME	1,2-Dimethoxyethane
EtOAc	Ethyl acetate
HPLC	High Performance Liquid Chromatography
LRMS	Low Resolution Mass Spectrometry
NMR	Nuclear Magnetic Spectroscopy
Ph	Phenyl
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
t_r	Retention time

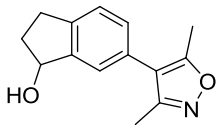
6-(3,5-Dimethyl-isoxazol-4-yl)-indan-1-one (6)



1 M aqueous $NaHCO_3$ (15 mL, 15 mmol) was added to a mixture of 6-bromo-indan-1-one **5a** (1.06 g, 5.00 mmol), 3,5-dimethylisoxazole-4-boronic acid pinacol ester (1.34 g, 6.00 mmol) and $Pd(dppf)Cl_2$ (73 mg, 0.10 mmol) in DME (25 mL). The mixture was degassed by evacuating and refilling with nitrogen several times then heated to 100 °C and left to stir at this temperature for 18 h. The resulting purple mixture was allowed to cool then treated with activated charcoal. The mixture was filtered through a Celite® plug then washed through with EtOAc. The resulting brown filtrate was concentrated *in vacuo* then partitioned between EtOAc (50 mL) and 10% aq. K_2CO_3 solution (50 mL). The organic phase was washed with water (50 mL) and brine (50 mL) then $MgSO_4$ and more activated

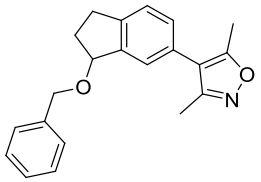
charcoal was added. The mixture was filtered then the resulting golden filtrate was evaporated to a cream solid. This was triturated with *tert*-butyl methyl ether (~15 mL) then filtered and dried under vacuum to yield the title product as a taupe solid (559 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.28 (s, 3 H) 2.42 (s, 3 H) 2.73 - 2.80 (m, 2 H) 3.18 - 3.24 (m, 2 H) 7.49 (dd, *J* = 2.0, 8.0 Hz, 1 H) 7.56 - 7.60 (m, 1 H) 7.64 - 7.66 (m, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 10.7 (s, 1C) 11.5 (s, 1C) 25.7 (s, 1C) 36.5 (s, 1C) 115.8 (s, 1C) 123.9 (s, 1C) 127.2 (s, 1C) 129.9 (s, 1C) 135.3 (s, 1C) 137.7 (s, 1C) 154.3 (s, 1C) 158.5 (s, 1C); 165.6 (s, 1C) 206.4 (s, 1C); LRMS (ESI$^+$) *m/z* 228 [MH$^+$]; HPLC t_r 2.9 min (254 nm: 97%, ELSD: >99%).

(±)-6-(3,5-Dimethylisoxazol-4-yl)-2,3-dihydro-1*H*-inden-1-ol



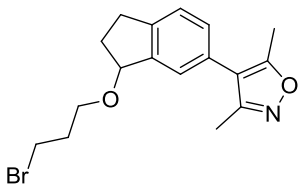
Compound **6** (50 mg, 0.22 mmol) was dissolved in ethanol (5 mL) and stirred for 10 min. $NaBH_4$ (5 mg, 0.11 mmol) was added then the mixture was heated to reflux. The reaction process was monitored by TLC. Once complete, the reaction mixture was concentrated to 1/3 the original volume *in vacuo*, diluted with water, and extracted with CH_2Cl_2 (20 mL×4). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash chromatography (petroleum ether:EtOAc, 2:1) to obtain the title compound (48 mg, 95% yield); ^{1}H NMR (300 MHz, CDCl3) δ 7.32-7.29 (m, 2H), 7.13 (dd, *J* = 1.5, 7.5 Hz, 1H), 5.29 (t, *J* = 6.5 Hz, 1H), 3.14-3.04 (m, 1H), 2.91-2.79 (m, 1H), 2.60-2.49 (m, 1H), 2.39 (s, 3H), 2.26 (s, 3H), 2.07-1.93 (m, 1H); LRMS (ESI$^+$) *m/z* 230 [MH$^+$]; HPLC t_r 2.6 min (254 nm: >99%, ELSD: >99%).

(±)-4-(3-(Benzyloxy)-2,3-dihydro-1*H*-inden-5-yl)-3,5-dimethylisoxazole (9)



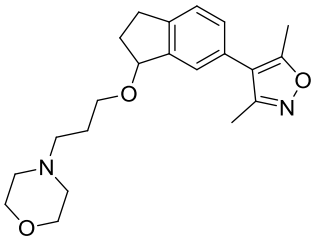
NaH (76.8 mg, 3.2 mmol) was added to a solution of (±)-6-(3,5-dimethylisoxazol-4-yl)-2,3-dihydro-1*H*-inden-1-ol (72 mg, 0.32 mmol) in THF (5 mL). The mixture was heated at reflux for 0.5 h. Benzyl bromide was added slowly to the reaction mixture at 60 °C. The reaction was allowed to stir at this temperature for 18 h then allowed to cool. Distilled water was added to the reaction mixture then this was extracted with diethyl ether. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash chromatography (petroleum ether:EtOAc, 5 : 1) to yield the title compound (78 mg, 76% yield); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41-7.28 (m, 7H), 7.14 (d, *J* = 6 Hz, 1H), 5.07 (m, 1H), 4.66 (m, 2H), 3.14 (m, 1H), 2.85 (m, 1H), 2.42 (m, 1H), 2.42 (s, 3H), 2.27 (s, 3H), 2.17 (m, 1H); LRMS (ESI$^+$): *m/z* 320 [MH$^+$]; HPLC t_r 3.7 min (254 nm: 90%, ELSD: >99%).

(±)-4-(3-(3-Bromopropoxy)-2,3-dihydro-1*H*-inden-5-yl)-3,5-dimethylisoxazole



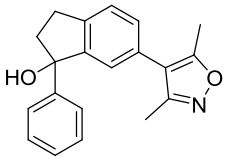
p-Toluene sulfonic acid (12 mg, 0.063 mmol) was added to a solution of (±)-6-(3,5-dimethylisoxazol-4-yl)-2,3-dihydro-1H-inden-1-ol (144 mg, 0.63 mmol) in CH_2Cl_2 (3 mL). The solution was stirred at room temperature for 5 min then 3-bromopropan-1-ol (0.28 mL, 3.15 mmol) was added dropwise to the reaction mixture. The mixture was then heated to reflux and monitored by TLC. After completion of reaction, distilled water (10 mL) was added then the mixture was extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was then purified by flash chromatography (petroleum ether:EtOAc 9:1) and the desired alkylated product was obtained (100 mg, 45% yield); ^{1}H NMR (300 MHz, CDCl3) δ 7.33 (1H, d, *J* = 9.0 Hz), 7.27 (1H, d, *J* = 3.0 Hz), 7.1 (1H, dd, *J* = 3.0, 6.0 Hz), 4.97 (1H, m), 3.72 (2H, m), 3.54 (2H, t, *J* = 6.0 Hz), 3.11 (1H, m), 2.85 (1H, m), 2.44 (1H, m), 2.41 (3H, s), 2.28 (3H, s), 2.21-2.04 (3H, m); LRMS (ESI$^+$) *m/z* 352 [M(^{81}Br)H$^+$], 350 [M(^{79}Br)H$^+$]; HPLC t_r 3.6 min (254 nm: 76%, ELSD: 98%).

(±)-4-(3-((6-(3,5-Dimethylisoxazol-4-yl)-2,3-dihydro-1*H*-inden-1-yl)oxy)propyl)morpholine (13)



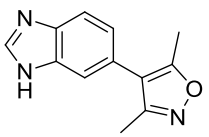
(±)-4-(3-(3-Bromopropoxy)-2,3-dihydro-1H-inden-5-yl)-3,5-dimethylisoxazole (70 mg, 0.20 mmol), morpholine (35 mg, 0.40 mmol) and triethylamine (0.05 mL, 0.4 mmol) were dissolved in THF (2 mL). The reaction mixture was stirred under reflux and monitored by TLC. After the reaction was complete, the mixture was diluted with CH_2Cl_2, washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was then purified by column chromatography (CH_2Cl_2:methanol, 10:1) to obtain the title compound (71 mg, quant.); ^{1}H NMR (300 MHz, CDCl3) δ 7.31 (1H, d, *J* = 9.0 Hz), 7.24 (1H, d, *J* = 3.0 Hz), 7.12 (1H, dd, *J* = 3.0, 9.0 Hz), 4.93 (1H, m), 3.70-3.53 (6H, m), 3.08 (1H, m), 2.84 (1H, m), 2.44-2.39 (7H, m), 2.39 (3H, s), 2.26 (3H, s), 2.07 (1H, m), 1.80 (2H, m); LRMS (ESI$^+$) *m/z* 357 [MH$^+$]; HPLC t_r 2.2 min (254 nm: >99%, ELSD: >99%).

(±)-6-(3,5-Dimethyl-isoxazol-4-yl)-1-phenyl-indan-1-ol (15)



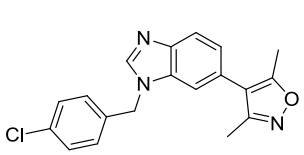
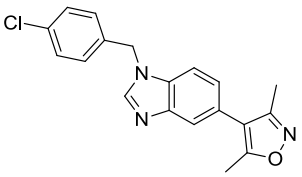
Phenylmangesium bromide (3.0 M in Et_2O, 0.98 mL, 2.9 mmol) was added drop-wise to a cooled (ice bath) suspension of 6-(3,5-dimethyl-isoxazol-4-yl)-indan-1-one (**6**) (555 mg, 2.44 mmol) in THF (10 mL). The mixture was allowed to warm to room temperature slowly then left to stir for 16 h. More phenylmangesium bromide (3.0 M in Et_2O, 0.407 mL, 1.22 mmol) was added at 0 °C then the mixture was allowed to warm to room temperature, left to stir for 3 h then more phenylmangesium bromide (3.0 M in Et_2O, 0.40 mL, 1.2 mmol) was added. Saturated aq. NH_4Cl solution (25 mL) was added then the mixture was stirred for 5 min, transferred to a separating funnel then extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with water (25 mL) and brine (25 mL) then dried over Na_2SO_4 and evaporated. The crude was purified by column chromatography, eluting with EtOAc:heptane and increasing the gradient linearly from 20:80 to 100:0 over 12 column volumes. The desired fractions were combined and evaporated to yield the title compound as a pale yellow gum (746 mg, 41%); ^{1}H NMR (300 MHz, CDCl3) δ 2.19 (s, 3 H) 2.33 (s, 3 H) 2.51 - 2.59 (m, 2 H) 2.94 - 3.03 (m, 1 H) 3.16 - 3.26 (m, 1 H) 6.96 - 7.00 (m, 1 H) 7.18 (dd, *J* = 8.0, 2.0 Hz, 1 H) 7.27 - 7.43 (m, 6 H); ^{13}C NMR (CDCl3) δ 10.7 (s, 1 C) 11.5 (s, 1 C) 29.8 (s, 1 C) 45.0 (s, 1 C) 85.1 (s, 1 C) 116.5 (s, 1 C) 123.9 (s, 1 C) 124.7 (s, 1 C) 125.5 (s, 1 C) 126.0 (s, 1 C) 127.2 (s, 1 C) 129.4 (s, 1 C) 129.5 (s, 1 C) 129.6 (s, 1 C) 134.2 (s, 1 C) 143.5 (s, 1 C) 148.2 (s, 1 C) 148.3 (s, 1 C) 158.6 (s, 1 C) 165.1 (s, 1 C); LRMS (ESI$^+$) *m/z* 306 [MH$^+$]; HPLC t_r 3.2 min (254 nm: >99%, ELSD: >99%).

4-(1H-Benzo[*d*]imidazol-6-yl)-3,5-dimethylisoxazole (8)



Et_3N (3 mL, 20 mmol) was added to a solution of 5-bromobenzimidazole (2.0 g, 10 mmol) in CH_2Cl_2 (110 mL). After stirring for 2 min, di-*tert*-butyl dicarbonate (4.4 g, 20 mmol) was added to the reaction mixture at 0 °C, then the reaction was stirred for 4 h at rt. The mixture of the reaction was washed with H_2O and brine, dried over Na_2SO_4 then concentrated in vacuo. The crude product was purified by silica gel column chromatography (petroleum ether:EtOAc 5:1) to yield a mixture of the regioisomers of the Boc-protected imidazole as a yellow oil (4.4 g). This material was dissolved in dioxane (50 mL) then $Pd(PPh_3)_4$ (1.7 g, 1.5 mmol), 3,5-dimethylisoxazol-4-ylboronic acid (4.23 g, 30 mmol), and aqueous Na_2CO_3 (2 M, 20 mL) were added. The reaction mixture was heated to reflux then stirred for 4 h. After cooling to rt, the mixture was concentrated *in vacuo*. The residue was diluted in EtOAc then washed with H_2O and brine then dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (CH_2Cl_2:EtOAc 1:1) to yield the title compound as a yellow solid (1.48 g, 68 %). ^{1}H NMR (300 MHz, DMSO-d6) δ 8.28 (1H, s), 7.67 (1H, d, *J* = 8.0 Hz), 7.57 (1H, d, *J* = 1.5 Hz), 7.18 (1H, dd, *J* = 1.5 Hz, 8.0 Hz), 2.41 (3H, s), 2.23 (3H, s); LRMS (ESI$^+$) *m/z* 214 [MH$^+$]; HPLC t_r 1.6 min (254 nm: 98%, ELSD: 95%).

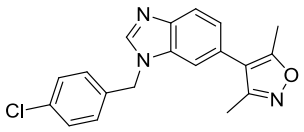
4-(1-(4-Chlorobenzyl)-1*H*-benzo[d]imidazol-6-yl)-3,5-dimethylisoxazole (23a) and 4-(1-(4-chlorobenzyl)-1*H*-benzo[*d*]imidazol-5-yl)-3,5-dimethylisoxazole (23b)

Separation method

K_2CO_3 (129 mg, 0.93 mmol) was added to a solution of compound **8** (100 mg, 0.47 mmol) in $CHCl_3$ (5 mL) followed by dropwise addition of 1-(bromomethyl)-4-chlorobenzene (116 mg, 0.56 mmol). The reaction mixture was heated under reflux and monitored by TLC. After completion, the reaction was allowed to cool to rt,then the mixture was filtered. The filtrate was diluted with CH_2Cl_2, washed with H_2O and brine then dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (CH_2Cl_2:EtOAc, 25:1) to yield **23a** (54 mg, 34%); ^{1}H NMR (300 MHz, CDCl3) δ 8.06 (1H, s), 7.88 (1H, d, *J* = 8.5 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 7.17-7.13 (3H, m), 7.04 (1H, s), 5.37 (2H, s), 2.32 (3H, s), 2.18 (3H, s). LRMS (ESI$^+$): *m/z* 340 [M(^{37}Cl)H$^+$], 338 [M(^{35}Cl)H$^+$]; HPLC t_r 2.4 min (254 nm: 93%, ELSD: >99%). and **23b** (38 mg, 24%); ^{1}H NMR (CDCl3) δ 8.18 (1H, s), 7.74 (1H, m), 7.38-7.32 (3H, m), 7.20-7.15 (3H, m), 5.41 (2H, s), 2.42 (3H, s), 2.28 (3H, s). LRMS (ESI$^+$) *m/z* 340 [M(^{35}Cl)H$^+$], 338 [M(^{35}Cl)H$^+$]; HPLC t_r 2.4 min (254 nm: 95%, ELSD: >99%).

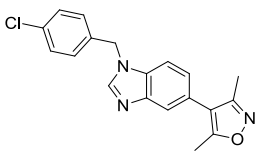
4-(1-(4-Chlorobenzyl)-1*H*-benzo[*d*]imidazol-6-yl)-3,5-dimethylisoxazole (23a)



K_2CO_3 (222 mg, 1.60 mmol) was added to a solution of 2,4-dibromo-1-nitrobenzene (300 mg, 1.07 mmol) and (4-chlorophenyl)methanamine (144 µL, 1.17 mmol) in DMSO. The mixture was stirred at 76 °C for 18 h. The reaction mixture was partitioned between EtOAc (30 mL) and water (10 mL). The organic phase was washed with saturated $NaHCO_3$ (×3), dried over Na_2SO_4 then the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether:EtOAc 50:1) to yield 5-bromo-*N*-(4-chlorobenzyl)-2-nitroaniline (85 mg, 23%). Iron powder (328 mg, 5.88 mmol) was added to a suspension of 5-bromo-*N*-(4-chlorobenzyl)-2-nitroaniline (100 mg, 0.29 mmol) in HCOOH (3 mL). The reaction mixture was stirred at 85 °C for 18 h then allowed to cool to room temperature. The reaction mixture was diluted with methanol then the insoluble material was filtered and washed with methanol. The filtrate was concentrated *in vacuo*, then the residue was purified by silica gel (petroleum ether:EtOAc 5:1 to 1:1) to yield 6-bromo-1-(4-chlorobenzyl)-1H-benzo[*d*]imidazole (49 mg, 52%); LRMS (ESI$^+$) *m/z* 323, [M(^{37}Cl)H$^+$], 321 [M(^{35}Cl)H$^+$].

A mixture of 6-bromo-1-(4-chlorobenzyl)-1H-benzo[*d*]imidazole (49 mg, 0.15 mmol), 3,5-dimethylisoxazole-4-yl boronic acid (107 mg, 0.72 mmol), $Pd(PPh_3)_4$ (18 mg, 10%) and K_2CO_3 (53 mg, 0.38 mmol) in dioxane and water was stirred under Ar at 120 °C. The reaction was monitored by TLC. On completion the reaction mixture was allowed to cool then water was added. The mixture was extracted with CH_2Cl_2, then the organic phase was dried over Na_2SO_4. The solvent was evaporated under vacuum then the residue was purified by silica gel column chromatography (CH_2Cl_2) to yield **23a** (33 mg, 65%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (1H, s), 7.88 (1H, d, *J* = 8.5 Hz), 7.34 (2H, d, *J* = 8.0 Hz), 7.18-7.13 (3H, m), 7.04 (1H, s), 5.37 (2H, s), 2.32 (3H, s), 2.18 (3H, s); LRMS (ESI$^+$) *m/z* 340 [M(^{37}Cl)H$^+$], 338 [M(^{35}Cl)H$^+$]; HPLC t_r 2.4 min (254 nm: 98%, ELSD: >99).

4-(1-(4-Chlorobenzyl)-1*H*-benzo[d]imidazol-5-yl)-3,5-dimethylisoxazole (23b)

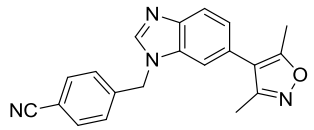


Na_2CO_3 (75 mg, 0.71 mmol) was added to a solution of 1,4-dibromo-2-nitrobenzene (100 mg, 0.36 mmol) and (4-chlorophenyl)methanamine (108 mg, 0.89 mmol) in EtOH. The mixture was stirred at 80 °C for 66 h. The reaction mixture was concentrated *in vacuo* then partitioned between EtOAc (30 mL) and water (10 mL). The organic phase was dried over Na_2SO_4 then the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (petroleum ether:EtOAc 50:1) to yield 4-bromo-*N*-(4-chlorobenzyl)-2-nitroaniline (110 mg, 90%). Iron powder (557 mg 9.94 mmol) was added to a suspension of 4-bromo-*N*-(4-chlorobenzyl)-2-nitroaniline (566 mg, 1.66 mmol) in EtOH:H_2O (4:1, 10 mL). The reaction mixture was stirred at room temperature for 0.5 h then ammonium chloride (89 mg, 1.66 mmol) was added. The resulting mixture was heated at 93 °C for 18 h then allowed to cool to room temperature. The reaction mixture was diluted with EtOAc then the insoluble material was filtered and washed with methanol. The filtrate was concentrated *in vacuo* then the residue was dissolved in EtOAc. The resulting solution was washed with water and brine then dried over Na_2SO_4 and evaporated. The residue was purified by silica gel (petroleum ether:EtOAc 25:1 to 10:1) to yield 4-bromo-*N*1-(4-chlorobenzyl)benzene-1,2-diamine (429 mg, 83%); LRMS (ESI$^+$) *m/z* 311, 313 [MH$^+$].

Triethoxymethane (1.67 mL, 4.42 mmol) and CF_3CO_2H (48 μL, 0.64 mmol) were added to a solution of 4-bromo-*N*1-(4-chlorobenzyl)benzene-1,2-diamine (400 mg, 1.28 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred at room temperature for 18 h. The mixture was diluted with EtOAc then washed with 10% aq. $NaHCO_3$, H_2O (×3), brine (×2) then concentrated *in vacuo*. The residue was purified by silica gel chromatography (petroleum ether:EtOAc, 10:1 to 2:1) to yield 5-bromo-1-(4-chlorobenzyl)-1H-benzo[*d*]imidazole (370 mg, 90%). LRMS (ESI^+) *m/z* 321, 323 [MH^+].

A mixture of 5-bromo-1-(4-chlorobenzyl)-1H-benzo[*d*]imidazole (49 mg, 0.15 mmol), 3,5-dimethylisoxazole-4-yl boronic acid (107 mg, 0.72 mmol), $Pd(PPh_3)_4$ (18 mg, 10%) and K_2CO_3 (53 mg, 0.38 mmol) in dioxane and water was stirred under Ar at 120 °C. The reaction was monitored by TLC. On completion the reaction mixture was allowed to cool then water was added. The mixture was extracted with CH_2Cl_2, then the organic phase was dried over Na_2SO_4. The solvent was evaporated under vacuum then the residue was purified by silica gel column chromatography (CH_2Cl_2) to yield **23a** (50 mg, 90%); ^1H NMR (300 MHz, CDCl3) δ 8.18 (1H, s), 7.74 (1H, m), 7.38-7.32 (3H, m), 7.20-7.14 (3H, m), 5.41 (2H, s), 2.42 (3H, s), 2.28 (3H, s); LRMS (ESI^+) *m/z* 340 [$M(^{35}Cl)H^+$], 338 [$M(^{35}Cl)H^+$]; HPLC t_r 2.4 min (254 nm: 97%, ELSD: >99).

4-(1-(4-Chlorobenzyl)-1*H*-benzo[d]imidazol-5-yl)-3,5-dimethylisoxazole (28a)



The title compound was prepared in a manner analogous to compound **23a** (separation method). ^1H NMR ($CDCl_3$) δ 8.08 (1H, s), 7.90 (1H, d, *J* = 8.4 Hz), 7.66 (2H, d, *J* = 8.1 Hz), 7.29 (2H, d, *J* = 8.1 Hz), 7.19 (1H, d, *J* = 8.4 Hz), 7.01 (1H, s), 5.48 (2H, s), 2.32 (3H, s), 2.17 (3H, s); LRMS (ESI^+) *m/z* 329 [MH^+]; HPLC t_r 2.1 min (254 nm: 98%, ELSD: >99).

X-ray Crystallography

Cloning, Protein Expression and Purification - cDNA encoding human BRD4 (NCBI accession numbers NP 055114.1) was obtained from FivePrime and was used as template to amplify the N-terminal bromodomain region of the protein. Protein expression and purification was carried out as previously described[1].

Crystallization: Aliquots of the purified proteins were set up for crystallization using a mosquito® crystallization robot (TTP Labtech, Royston UK). Coarse screens were typically setup onto Greiner 3-well plates using three different drop ratios of precipitant to protein per condition (100+50 nl, 75+75 nl and 50+100 nl). Initial hits were optimized further using Greiner 1-well plates and scaling up the drop sizes in steps. All crystallizations were carried out using the sitting drop vapor diffusion method at 4 °C. BRD4(1) crystals with compound **15** (5 mM final concentration) were grown by mixing 200 nl of the protein (8.3 mg/ml) with 100 nl of reservoir solution containing 0.1 M Bis-Tris-propane pH 6.5, 0.2 M Na_2SO_4, 20 % PEG3350 and 10 % ethylene glycol

Data Collection and Structure solution: Crystals were cryo-protected using the well solution supplemented with additional ethylene glycol and were flash frozen in liquid nitrogen. Data were collected at a Rigaku FRE Superbright using an RAXIS-VI detector at 1.52 Å. Indexing and integration was carried out using MOSFLM[2] and scaling was performed with SCALA[3]. Initial phases were calculated by molecular replacement with PHASER[4] using an ensemble of known bromodomain models (PDB IDs 2OSS, 2OUO, 2GRC, 2OO1, 3DAI, 3D7C, 3DWY). Initial models were built by ARP/wARP[5] and building was completed manually with COOT[6]. Refinement was carried out in REFMAC5[7]. Thermal motions were analyzed using TLSMD[8] and hydrogen atoms were included in late refinement cycles. Data collection and refinement statistics can be found in Supplemental Table 1. The model and structure factors have been deposited with PDB accession codes: XXXX (BRD4(1)/compound **15** complex).

Supplemental Table 1 Data collection and refinement statistics for BRD4(1)/compound **15** complex.

Data Collection	
Protein	BRD4(1)
Ligand	Compound **15**
PDB ID	XXXX
Space group	$P2_12_12_1$
Cell dimensions: a, b, c (Å)	37.29 44.38 79.12
α, β, γ (deg)	90.00 90.00 90.00
Resolution[a] (Å)	1.60 (1.69-1.60)
Unique observations[a]	17668 (2411)
Completeness[a] (%)	98.4 (94.3)
Redundancy[a]	4.6 (4.1)
Rmerge[a]	0.085 (0.298)
I/ σI[a]	11.3 (3.6)
Refinement Statistics	
Resolution (Å)	1.60
R_{work} / R_{free} (%)	16.52/20.42
Number of atoms (protein/other/water)	1065/32/163
B-factors (Å^2) (protein/other/water)	16.35/17.67/26.69
r.m.s.d bonds (Å)	0.015
r.m.s.d angles (°)	1.627
Ramachadran Favoured (%)	98.40
Allowed (%)	1.60
Disallowed (%)	0.00

[a] Values in parentheses correspond to the highest resolution shell.

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