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Scale-up and optimization of the synthesis of dual CBP/BRD4 inhibitor ISOX-DUAL†

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ISOX-DUAL is a dual inhibitor of CBP/p300 (IC₅₀ = 0.65 μM) and BRD4 (IC₅₀ = 1.5 μM) bromodomains, and a useful chemical probe for epigenetic research. Aspects of the published synthetic route to this compound and its analogues are small-scale, poor-yielding or simply unamenable to scale-up without optimization. Herein we describe the development of a refined synthesis that circumvents the challenges of the original report, with notable improvements to several of the key synthetic transformations. Moreover, a general Suzuki Miyaura protocol for the late stage installation of alternative dimethyl-isoxazole acetyl-lysine (KAc) binding motifs is presented.

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Introduction

ISOX-DUAL ([3-[4-[2-[5-(dimethyl-1,2-oxazol-4-yl)-1-[2-(morpholin-4-yl)ethyl]-1H-1,3-benzodiazol-2-yl]ethyl]phenoxy]propyl]dimethylamine) **1** is a dual inhibitor of BRD4 and CBP/p300 bromodomains.¹ It is structurally related to a series of CBP/p300 and BRD4 bromodomain inhibitors through a benzimidazole central scaffold with an adjoining 3,5-dimethylisoxazole group, which acts as an acetylated lysine (KAc) mimic.^{2,3} In previous work, differential activity and overall potency were tuned through structure-based design, yielding compounds with selectivity for CBP/p300 over BRD4, together with a probe (ISOX DUAL) possessing balanced potency against both targets (Fig. 1). ISOX-DUAL is a useful tool compound for exploring transcriptional regulation.^{1,2,4}

Our interest in functionalised bromodomain inhibitors necessitated a feasible, scalable route to **1** and its analogues. However, the reported synthetic strategy (Scheme 1) is, in places, both small-scale in nature and inviting of optimisation.¹ The key transformation is an elegant, albeit low-yielding, one-pot nitro-reduction and *in situ* cyclisation sequence,

in which exposure of nitroaniline **4** to sodium dithionite in the presence of aldehyde **5** facilitated the installation of the benzimidazole framework in one synthetic operation. Of additional note, the late-stage alkylation of phenol **7** to furnish **1** was also of modest yield: these two crucial stages thereby being significant contributors to an overall isolated yield of just 1%.

Our modified retrosynthetic analysis identified that independent reduction of nitroaniline **4** to bis-aniline **8**, followed by an amide coupling with subsequent acid-catalysed benzimidazole formation, might furnish the target **1** in higher yield (Scheme 2). Employing carboxylic acid **9** instead of aldehyde **5** offers the prospect of shortening the synthetic route by two chemical stages in comparison to the original, and further provides the distinct advantage of enabling an alternative early-stage phenolic alkylation, the comparative transformation having been a significant limitation to the output of the published synthesis.

The initial stage in both synthetic approaches involves an S_NAr reaction between commercially available 4-bromo-1-fluoro-nitrobenzene **2** and 4-(2-aminoethyl)morpholine. In our hands, this transformation, to prepare bromide **3**, was readily replicated on large scale in near-quantitative yield, and further, was demonstrated to proceed rapidly under microwave (MW) conditions with commensurate output.

Next, in sequence, we turned to elaboration of **3** to install the requisite pendant isoxazole functionality. This necessitated examination of the Suzuki–Miyaura cross-coupling reaction, where we hypothesised that competing hydrodehalogenation and protodeboronation could be potential contributory factors to a drop-off in expected product yield in the original report.^{5–8} A screen for substrate scope was performed under microwave conditions using a range of palladium catalysts and bases,

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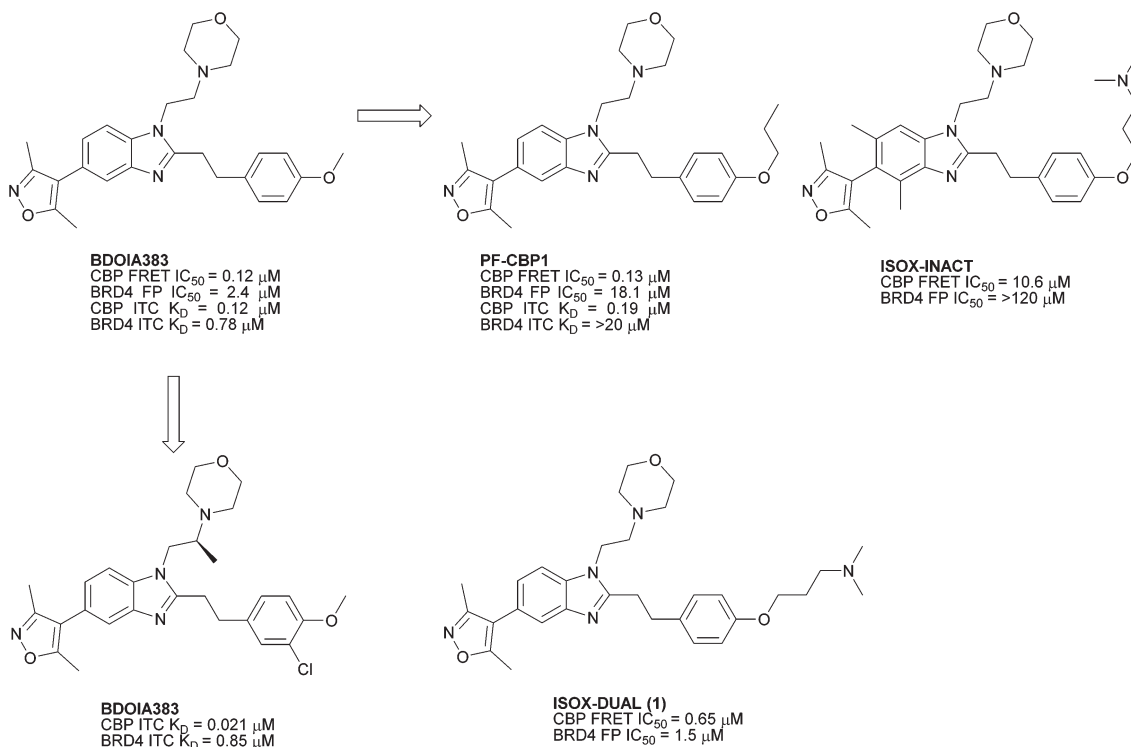
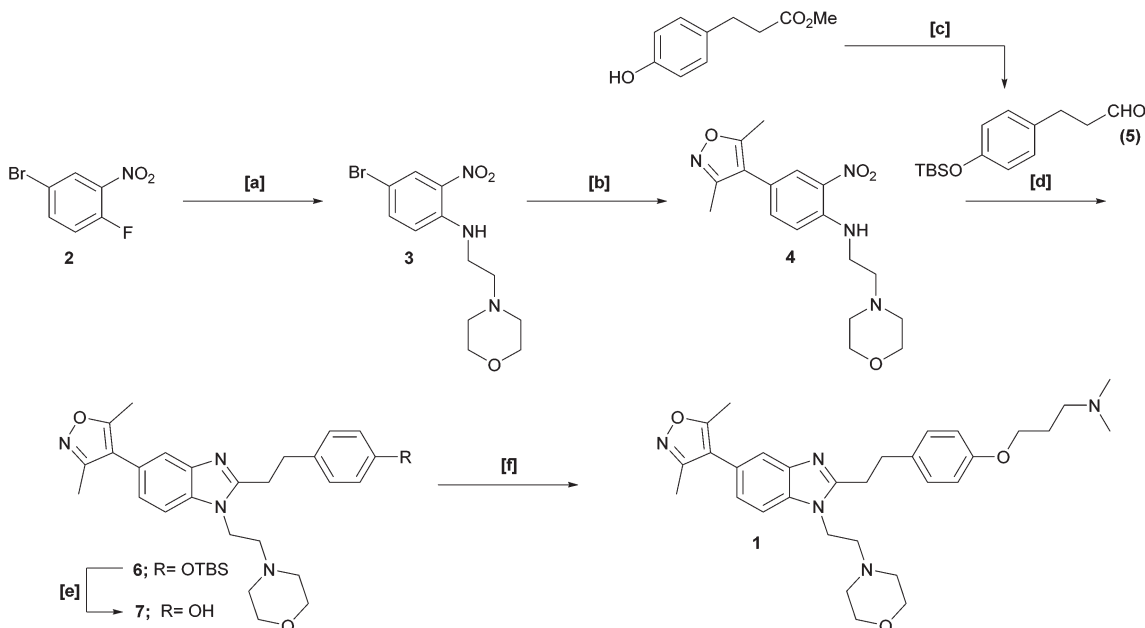


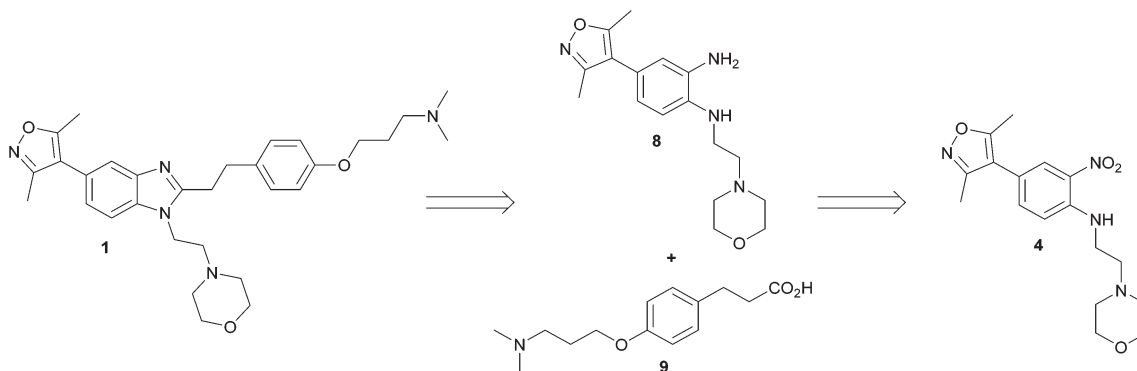
Fig. 1 Reported CBP BRD inhibitors including dual binding ISOX-DUAL (1).¹



exploring their effectiveness as boronic acid, boronate ester and MIDA boronate coupling partners, with selected results displayed in Table 1. From this screen, we found that employ-

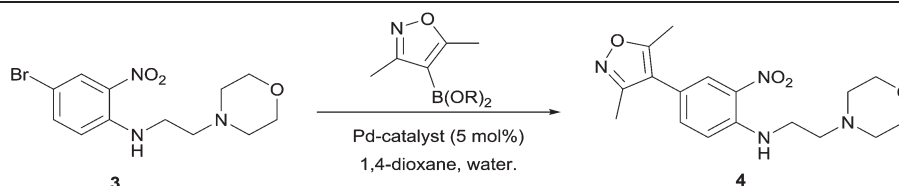
ment of a boronate ester bearing the isoxazole moiety performed the best, with potassium phosphate and PdCl₂(dppf)·DCM acting as base and catalyst, respectively.





Scheme 2 Retrosynthetic approach to ISOX-DUAL 1.

Table 1 Optimisation of the Suzuki–Miyaura reaction



Entry	Method ^a	(3) (mmol)	Boronate Species	Catalyst	Base	Yield ^{b,c} (%)
1	Thermal	30	Boronic acid	PdCl ₂ (dppf)·DCM	Cs ₂ CO ₃	53 ^b
2	MW	0.5	Boronic acid	PdCl ₂ (dppf)·DCM	Cs ₂ CO ₃	54 ^c
3	MW	0.5	Boronate ester	PdCl ₂ (dppf)·DCM	Cs ₂ CO ₃	74 ^c
4	MW	0.5	Boronate ester	PdCl ₂ (dtbpf)	Cs ₂ CO ₃	71 ^c
5	MW	0.5	Boronate ester	PdCl ₂ (dppf)·DCM	CsF	72 ^c
6	MW	0.5	Boronate ester	PdCl ₂ (dtbpf)	CsF	47 ^c
7	MW	0.5	Boronate ester	PdCl ₂ (dppf)·DCM	K ₃ PO ₄	72 ^c
8	MW	0.5	MIDA ester	PdCl ₂ (dppf)·DCM	K ₃ PO ₄	60 ^c
			 (CCDC 2110084)			
9	MW	0.5	MIDA ester	PdCl ₂ (dppf)·DCM	K ₃ PO ₄ ^d	51 ^c
10	MW	0.5	Boronate ester	PdCl ₂ (dppf)·DCM	CsF	74 ^c
11	MW	1	Boronate ester	PdCl ₂ (dppf)·DCM	Cs ₂ CO ₃	79 ^b
12	MW	1	Boronate ester	PdCl ₂ (dppf)·DCM	CsF	95 ^b
13	MW	1	Boronate ester	PdCl ₂ (dppf)·DCM	K ₃ PO ₄	95 ^b
14	MW	1	Boronate ester	PdCl ₂ (dppf)·DCM ^e	K ₃ PO ₄	66 ^b
15	Thermal	115	Boronate ester	PdCl ₂ (dppf)·DCM	K ₃ PO ₄	94 ^b

^a Typical method: 5 mol% Pd-catalyst, 2.6 eq. base (1 M solution in H₂O), 1,4-dioxane, MW, 150 °C, 3 h, or reflux, 16 h. ^b Isolated yield after purification. ^c Crude yield assessed by ¹H NMR. ^d 7.6 eq. base used. ^e 1 mol% catalyst.

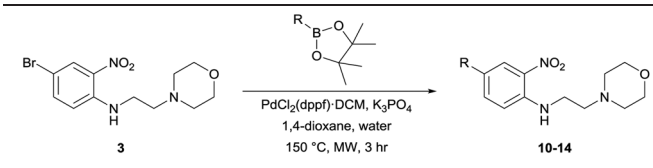
This reaction could be conducted on small scale in a micro-wave and was successfully demonstrated thermally on a larger scale to give almost 40 g of product, in near quantitative yield.


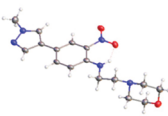

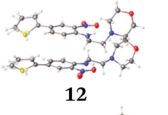
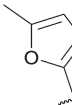
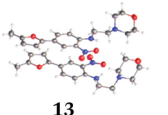

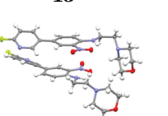

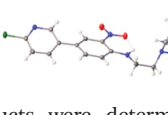
To demonstrate the synthetic utility and reliability of this now optimised procedure, a small series of analogues (**10–14**) were synthesised employing a variety of heterocyclic boronate esters (Table 2). This demonstrates the potential for late-stage KAc bio-isostere modification to these and similar scaffolds, which is of interest to ongoing work in our laboratory. Yields

tend to be good to excellent and all analogues were further characterised in the solid state by X-ray studies.

With key precursor nitro-aniline **4** in-hand, we turned our attention to the reduction of the nitro group, whereupon standard exposure to palladium on carbon under a balloon of hydrogen, the desired product **8** was readily obtained in reasonably high isolated yield. However, analysis of the profile of the reaction indicated the formation of a minor by-product, presumed to be generated by cleavage of the labile isoxazole N–O bond.^{10,11} This was established by repetition of the hydro-



Table 2 Analogues synthesised using the optimised Suzuki–Miyaura reaction


Entry	R	Product ^a	Yield (%)
1			85
2			90
3			80
4			74
5			81

^a Solid state structures of products were determined. Some have >1 molecule in unit cell.

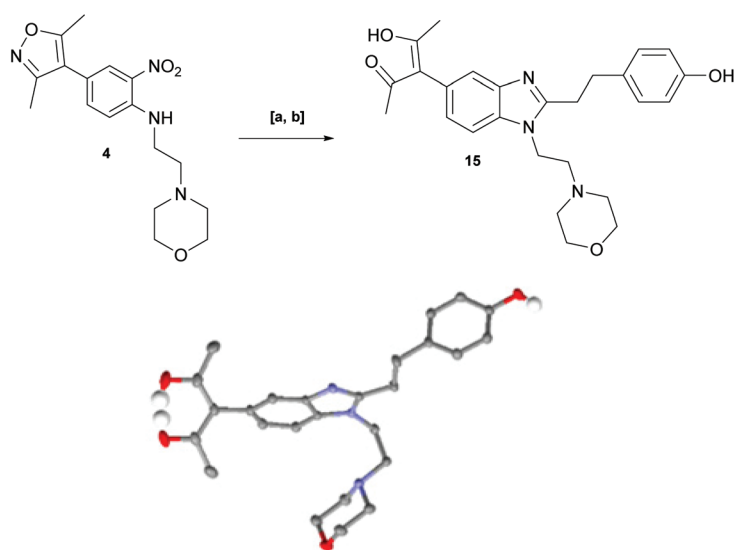
generation reaction under pressure, which, solely delivered the ring-opened product. Indicative confirmation was obtained by the changes observed in the ¹H NMR spectrum where the signals corresponding to the two methyl groups moved upfield from $\delta = 2.26$ and 2.40 ppm to $\delta = 1.72$ and 1.87 ppm respectively. The crude product was then carried forward through an amide coupling and cyclisation sequence to afford benzimidazole **15**, whose structure was solved by single crystal X-ray analysis (Scheme 3).

We opted to replicate and scale-up the reported reduction which can be induced by employment of sodium dithionite as the reducing agent² affording aniline **8** in high yield (81%) and purity (Scheme 4).

Independent construction of the requisite amide coupling partner **9** successfully allowed circumvention of the low yielding alkylation step from the original synthesis. As such, after conventional phenolic alkylation to give methyl ester **16** in high yield (87%), a subsequent ester hydrolysis gave rise to carboxylate **9** in quantitative yield, which proved most convenient to isolate and manipulate as the lithium salt (Scheme 4). To complete the synthesis, a HATU-mediated amide coupling between **8** and **9**, followed by acetic acid treatment of the crude product induced cyclisation to **1** in a one-pot procedure with good yield (55%) and high purity.

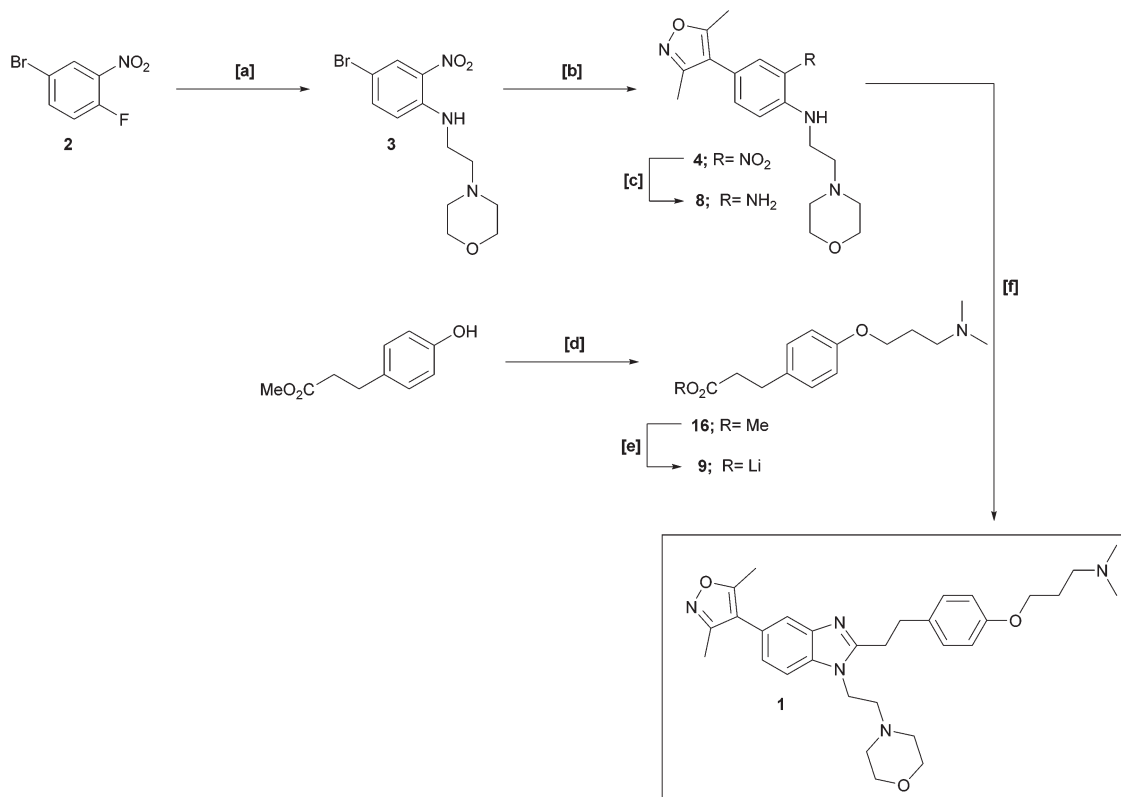
Conclusions

In summary, we detail an optimised synthetic route to ISOX-DUAL (**1**) and related analogues, with improved yields at every stage. By repositioning the phenolic alkylation to earlier in the plan, and by implementing a redesigned stepwise construction approach to the benzimidazole core, we now report



Scheme 3 Forced generation and structural confirmation of open chain side-product. Reagents & Conditions: [a] 10% Pd/C, EtOAc/MeOH (5 : 1), COware (with Zn and 7 M HCl in 2nd chamber) (quant.); [b] 3-(4-hydroxyphenyl)propanoic acid, HATU, TEA, DMF, rt, 16 h, then AcOH, reflux, 2 h (51%).





Scheme 4 Scaled-up and optimised ISOX-DUAL (**1**) synthesis. Reagents & Conditions: [a] 4-(2-aminoethyl)morpholine, TEA, DMSO, 80 °C, 2 h (quant.); or 4-(2-aminoethyl)morpholine, TEA, 120 °C, MW, 10 mins (quant.); [b] 3,5-dimethylisoxazole-4-boronic acid pinacol ester, PdCl₂(dppf)·DCM, K₃PO₄, 1,4-dioxane/H₂O, reflux, 16 h (94%); [c] (i) 1 M Na₂S₂O₄ (aq.), EtOH, 80 °C, 1 h; (ii) 10% aq. NH₃ solution (81%); [d] Cl(CH₂)₃NMe₂·HCl, Cs₂CO₃, NaI, MeCN, reflux, 16 h (87%); [e] LiOH·H₂O, THF/H₂O, rt, 16 h (quant.); [f] (i) HATU, DMF, rt, 16 h; (ii) AcOH, reflux, 2 h (55%).

an improved synthesis, shortened from eight stages to six, with a significant overall isolated yield increase from 1% to 42%. Additionally, development of the Suzuki coupling invoking a boronate ester establishes prospective in-roads to structural modification and opportunity to employ late stage Pd coupling process in the installation of alternative KAc bioisosteres in bioactive scaffolds.

Experimental

General methods

All reagents and solvents were purchased from commercial sources and used without further purification. Nuclear magnetic resonance spectra were recorded on a Bruker Avance III HD spectrometer operating at 400 MHz or a Varian VNMRS 500 or VNMRS 600 spectrometer operating at 500 MHz or 600 MHz for ¹H NMR and 126 MHz or 151 MHz for ¹³C NMR, respectively. ¹⁹F NMR spectra were recorded on a Varian VRMS 400 spectrometer operating at 376 MHz. ¹H NMR and ¹³C NMR chemical shifts (δ) are reported in parts per million (ppm) and are referenced to residual protium in solvent and to the carbon resonances of the residual solvent peak, respectively. DEPT and correlation spectra were run in conjunction to

aid assignment. ¹⁹F NMR chemical shifts are reported in ppm and are uncorrected. Coupling constants (J) are quoted in Hertz (Hz), and the following abbreviations were used to report multiplicity: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet.

Purification by flash column chromatography was carried out using Fisher Scientific silica gel 60 Å (35–70 μ m), or with silica gel or C18 columns using a Combi flash RF 75 PSI or 150 PSI, Teledyne ISCO unit. Analytical thin layer chromatography was performed on glass plates pre-coated with silica gel (250 μ m/UV254), with visualization being achieved using UV light (254 nm) and/or by staining with alkaline potassium permanganate dip. Reaction monitoring LC-MS analyses were conducted using a Shimadzu LC-MS 2020, on a Gemini 5 m C18 110 Å column. High resolution mass spectral (HRMS) data was collected in the laboratories of the University of Sussex Chemistry Department using a Bruker Daltonics Apex III (Apollo ESI ion source). Single crystal X-ray measurements were recorded in the laboratories of the UK National Crystallography Service at the University of Southampton.

4-Bromo-N-(2-morpholinoethyl)-2-nitroaniline (**3**)

Microwave method. A CEM Explorer microwave vial was equipped with a magnetic flea and 4-bromo-1-fluoro-2-nitrobenzene **2** (3.10 mL, 25 mmol, 1 eq.). Triethylamine (15 mL,



100 mmol, 4 eq.) and 4-(2-aminoethyl)morpholine (3.34 mL, 25.25 mmol, 1.01 eq.) were added successively and the vessel was sealed and heated using the dynamic heating method, with max power set to 300 W, max pressure 300 psi, max temperature 120 °C, high stirring throughout and power max turned off. This method was used to hold the method at 120 °C for 10 min. After cooling, the reaction mixture was partitioned between EtOAc (250 mL) and water (300 mL). The organic layer was collected and the aqueous was extracted with EtOAc (4 × 150 mL). The combined organic extracts were washed with NaHCO₃ (sat. aq.) and brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give the title compound as an orange solid (7.95 g, 24.07 mmol, quant.).

Thermal method. To a stirred solution of 4-bromo-1-fluoro-2-nitrobenzene (**2**) (29.9 g, 136 mmol, 1 eq.) in DMSO (300 mL) at ambient temperature was added triethylamine (56 mL, 408 mmol, 3 eq.) followed by 4-(2-aminoethyl)morpholine (18.7 mL, 143 mmol, 1.05 eq.) in a dropwise fashion. The reaction mixture was then heated to 80 °C for 2 hours. Upon completion of the reaction, the mixture was cooled to ambient temperature and partitioned between EtOAc (500 mL) and water (500 mL). The organic layer was collected and the aqueous was extracted with EtOAc (3 × 750 mL). The combined organic extracts were washed successively with NaHCO₃ (sat. aq.) and brine (1 L), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give the title compound as an orange solid (40.6 g, 123 mmol, quant.). δ_{H} (400 MHz, CDCl₃) 2.52 (s, 4H), 2.72 (t, $J = 6.3$ Hz, 2H), 3.34 (q, $J = 5.8$ Hz, 2H), 3.76 (s, 4H), 6.73 (d, $J = 9.2$ Hz, 1H), 7.49 (dd, $J = 8.6, 1.6$ Hz, 1H), 8.33 (d, $J = 2.2$ Hz, 1H), 8.53 (s, 1H). LC-MS (5-95 MeCN in 5 min) $t_{\text{R}} = 3.178$, purity = >99%, $m/z = 332.1$ [M + H]⁺. Data in accordance with literature.¹

General Suzuki reaction microwave method

A 35 mL microwave vessel was charged with **3** (1 eq.), 3,5-dimethylisoxazole-4-boronate species (1.12 eq.), and palladium catalyst (5 mol%) before dissolution in 1,4-dioxane (12 mL mmol⁻¹). The solution was sealed, degassed with argon (×3) before the addition of base (2.6 eq.) as a 1 M solution in water. The vessel was then heated using the dynamic heating method, with max power set to 300 W, max pressure 300 psi, max temperature 150 °C, high stirring throughout and power max turned off. This method was used to hold the method at 150 °C for 3 hours. The reaction mixture was cooled and filtered through Celite™. The filter cake was washed with EtOAc (3 × 50 mL), and the filtrates were concentrated under reduced pressure.

4-(3,5-Dimethylisoxazol-4-yl)-N-(2-morpholinoethyl)-2-nitroaniline (4). The title compound was synthesised following the General Suzuki Reaction Microwave Method using 1 mmol of (**3**). Purification by flash column chromatography, eluting with 0–100% EtOAc in hexane, afforded the title compound as an orange solid (328.7 mg, 0.95 mmol, 95%). δ_{H} (600 MHz, CDCl₃) 2.25 (s, 3H), 2.39 (s, 3H), 2.53 (s, 4H), 2.74 (t, $J = 6.1$ Hz, 2H), 3.76 (t, $J = 4.6$ Hz, 4H), 6.90 (d, $J = 8.8$ Hz, 1H), 7.33 (dd, $J = 8.8, 2.2$ Hz, 1H), 8.08 (d, $J = 2.1$ Hz, 1H), 8.57 (s, 1H).

Thermal method. A stirred solution of 4-bromo-*N*-(2-morpholinoethyl)-2-nitroaniline (**3**) (38 g, 115 mmol, 1 eq.), potassium phosphate (63.5 g, 299 mmol, 2.6 eq.) and 3,5-dimethylisoxazole boronic acid pinacol ester (25.6 g, 115 mmol, 1 eq.) in 1,4-dioxane (1.2 L) and water (120 mL) was degassed with argon (×3) before the addition of PdCl₂(dppf)-DCM (4.7 g, 5.75 mmol, 0.05 eq.). The reaction mixture was then degassed and refilled with argon once further, heated to reflux and stirred overnight under a stream of N₂. The reaction mixture was then cooled to ambient temperature and filtered through a pad of Celite™ before concentrating under reduced pressure to approximately 300 mL. The residue was then partitioned between water (600 mL) and EtOAc (600 mL), the organic phase was collected, and the aqueous phase extracted with EtOAc (3 × 250 mL). The combined organic extracts were washed with brine (3 × 400 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Purification by flash column chromatography, eluting with 0–80% EtOAc in hexane, afforded the title compound as an orange solid (37.4 g, 108 mmol, 94%). δ_{H} (400 MHz, CDCl₃) 2.26 (s, 3H), 2.40 (s, 3H), 2.55 (d, $J = 4.6$ Hz, 3H), 2.75 (t, $J = 6.1$ Hz, 2H), 3.38–3.44 (m, 2H), 3.78 (d, $J = 4.6$ Hz, 4H), 6.91 (d, $J = 8.8$ Hz, 1H), 7.34 (dd, $J = 8.8, 2.2$ Hz, 1H), 8.09 (d, $J = 2.2$ Hz, 1H), 8.58 (s, 1H). LC-MS (5-95 MeCN in 5 min) $t_{\text{R}} = 3.266$, purity = >99%, $m/z = 347.2$ [M + H]⁺. Data in accordance with literature.¹

4-(3,5-Dimethylisoxazol-4-yl)-N1-(2-morpholinoethyl)benzene-1,2-diamine (8). To a stirred suspension of 4-(3,5-dimethylisoxazol-4-yl)-*N*-(2-morpholinoethyl)-2-nitroaniline (**4**) (17.4 g, 50 mmol, 1 eq.) in EtOH (800 mL) was added 1 M aqueous sodium dithionite solution (800 mL), and the resulting mixture was heated at 80 °C for 1 hour. The reaction mixture was then cooled and partitioned between 10% aqueous ammonia solution (800 mL), and EtOAc (400 mL). The organic phase was separated, and the aqueous phase was extracted with EtOAc (4 × 400 mL). The combined organic extracts were washed with brine (2 × 500 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure to afford the title compound as beige solid (12.70 g, 40.14 mmol, 81%). δ_{H} (400 MHz, CDCl₃) 2.25 (s, 3H), 2.38 (s, 3H), 2.50 (s, 4H), 2.71 (t, $J = 5.9$ Hz, 2H), 3.21 (d, $J = 5.8$ Hz, 2H), 3.45 (s, 2H), 3.69–3.76 (m, 4H), 4.08 (s, 1H), 6.59 (s, 1H), 6.68 (s, 2H). LC-MS (5-95 MeCN in 5 min) $t_{\text{R}} = 2.364$, purity = >99%, $m/z = 317.2$ [M + H]⁺. Data in accordance with literature.²

4-(1-Methyl-1H-pyrazol-4-yl)-N-(2-morpholinoethyl)-2-nitroaniline (10). Compound (**10**) was synthesised following the General Microwave Method on a 0.5 mmol scale. Purification by flash column chromatography, eluting with 0–10% MeOH in EtOAc, afforded the title compound as a red solid (113 mg, 0.414 mmol, 85%). δ_{H} (600 MHz, CDCl₃) 2.53 (s, 4H), 2.74 (t, $J = 6.1$ Hz, 2H), 3.40 (q, $J = 5.8$ Hz, 2H), 3.79–3.74 (m, 4H), 3.94 (s, 3H), 6.85 (d, $J = 8.9$ Hz, 1H), 7.57 (dd, $J = 8.8, 2.1$ Hz, 1H), 7.71 (s, 1H), 7.58 (s, 1H), 8.27 (d, $J = 2.1$ Hz, 1H), 8.50 (s, 1H). δ_{C} (151 MHz, CDCl₃) 39.3, 39.7, 53.4, 56.3, 67.2, 77.2, 114.7, 120.8, 121.7, 122.9, 126.6, 132.2, 134.1, 136.4, 144.1 LC-MS (30-95 MeCN in 20 min) $t_{\text{R}} = 3.29$ min, purity = >99%, $m/z =$



332.00 $[M + H]^+$. HR-MS (m/z): $[M + H]^+$ calculated for $C_{16}H_{22}N_5O_3$, 332.1723; found, 332.1714.

***N*-(2-Morpholinoethyl)-2-nitro-4-(thiophen-2-yl)aniline (11).** Compound (11) was synthesised following the General Microwave Method on a 0.5 mmol scale. Purification by flash column chromatography, eluting with 0–100% EtOAc in hexane, afforded the title compound as an orange solid (150 mg, 0.450 mmol, 90%). δ_H (600 MHz, $CDCl_3$) 2.56 (t, $J = 4.5$ Hz, 4H), 2.76 (t, $J = 6.1$ Hz, 2H), 3.42 (q, $J = 5.6$ Hz, 2H), 3.79 (s, 4H), 6.88 (d, $J = 8.9$ Hz, 1H), 7.07–7.10 (m, 1H), 7.25 (m, 2H), 7.71 (d, $J = 10.4$ Hz, 1H), 8.44 (d, $J = 1.7$ Hz, 1H), 8.60 (s, 1H). δ_C (151 MHz, $CDCl_3$) 25.1, 53.4, 56.2, 67.2, 114.7, 122.4, 122.5, 123.5, 124.3, 128.2, 132.0, 134.2, 142.7, 144.5. LC-MS (30–95 MeCN in 20 min) $t_R = 5.52$ min, purity = 95%, $m/z = 334.35[M + H]^+$. HR-MS (m/z): $[M + H]^+$ calculated for $C_{16}H_{20}N_3O_3S$, 334.1225; found, 334.1214.

4-(5-Methylfuran-2-yl)-*N*-(2-morpholinoethyl)-2-nitroaniline (12). Compound (12) was synthesised following the General Microwave Method on a 0.5 mmol scale. The crude material was then purified by flash chromatography using a C18 reverse phase column, eluting with 5–95% MeCN in water with 0.5% formic acid over 20 min, to afford the title compound as a red solid (132 mg, 0.400 mmol, 80%). δ_H (600 MHz, $CDCl_3$) 2.36 (s, 3H), 2.53 (s, 4H), 2.73 (t, $J = 6.0$ Hz, 2H), 3.39 (q, $J = 5.4$ Hz, 2H), 3.76 (s, 4H), 6.03 (s, 1H), 6.42 (s, 1H), 6.83 (d, $J = 8.9$ Hz, 1H), 7.69 (d, $J = 8.8$ Hz, 1H), 8.42 (s, 1H), 8.56 (s, 1H). δ_C (151 MHz, $CDCl_3$) 13.8, 39.6, 53.3, 56.2, 67.1, 104.9, 107.8, 114.5, 119.6, 121.0, 131.9, 132.0, 144.2, 150.7, 151.8. LC-MS (30–95 MeCN in 20 min) $t_R = 5.86$ min, purity = >99%, $m/z = 332.05[M + H]^+$. HR-MS (m/z): $[M + H]^+$ calculated for $C_{17}H_{22}N_3O_4$, 332.1610; found, 332.1605.

4-(6-Chloropyridin-3-yl)-*N*-(2-morpholinoethyl)-2-nitroaniline (13). Compound (13) was synthesised following the General Microwave Method on a 0.5 mmol scale. Purification by flash column chromatography, eluting with 0–10% MeOH in EtOAc, afforded the title compound as an orange solid (134.2 mg, 0.370 mmol, 74%). δ_H (600 MHz, $CDCl_3$) 2.54 (s, 4H), 2.75 (t, $J = 6.1$ Hz, 2H), 3.42 (q, $J = 5.6$ Hz, 2H), 3.77 (t, $J = 4.6$ Hz, 4H), 6.95 (d, $J = 8.9$ Hz, 1H), 7.38 (d, $J = 8.3$ Hz, 1H), 7.66 (dd, $J = 8.9, 2.2$ Hz, 1H), 7.81 (dd, $J = 8.3, 2.6$ Hz, 1H), 8.41 (d, $J = 2.2$ Hz, 1H), 8.57 (d, $J = 2.5$ Hz, 1H), 8.64 (t, $J = 4.5$ Hz, 1H). δ_C (151 MHz, $CDCl_3$) 39.6, 53.3, 56.1, 67.1, 115.3, 123.5, 124.5, 125.0, 132.4, 133.7, 134.7, 136.4, 136.3, 145.1, 147.2, 150.2. LC-MS (30–95 MeCN in 20 min) $t_R = 4.08$ min, purity = 94%, $m/z = 363.25[M + H]^+$. HR-MS (m/z): $[M + H]^+$ calculated for $C_{17}H_{20}ClN_4O_3$, 363.1224; found, 363.1221.

4-(6-Fluoropyridin-3-yl)-*N*-(2-morpholinoethyl)-2-nitroaniline (14). Compound (14) was synthesised following the General Microwave Method on a 0.5 mmol scale. Purification by flash column chromatography, eluting with 0–100% EtOAc in hexane, afforded the title compound as an orange oil (138 mg, 0.398 mmol, 81%). δ_H (600 MHz, $CDCl_3$) 2.55 (s, 4H), 2.76 (t, $J = 6.1$ Hz, 2H), 3.43 (q, $J = 5.7$ Hz, 2H), 3.77 (t, $J = 4.6$ Hz, 4H), 6.95 (d, $J = 8.8$ Hz, 1H), 7.00 (dd, $J = 8.5, 2.9$ Hz, 1H), 7.65 (d, $J = 8.8$ Hz, 1H), 7.94 (td, $J = 8.0, 2.5$ Hz, 1H), 8.39 (s, 2H), 8.61 (s, 1H). δ_C (151 MHz, $CDCl_3$) 39.6, 53.3, 56.1, 67.1, 109.8 (d, $J =$

37.6 Hz), 115.3, 123.8, 125.0, 132.4, 132.9 (d, $J = 4.4$ Hz), 134.6, 139.0 (d, $J = 7.9$ Hz), 144.9, 145.2 (d, $J = 15.3$ Hz), 162.3, 163.9. LC-MS (30–95 MeCN in 20 min) $t_R = 6.98$ min, purity = 96%, $m/z = 347.20[M + H]^+$. HR-MS (m/z): $[M + H]^+$ calculated for $C_{17}H_{20}FN_4O_3$, 347.1519; found, 347.1501.

(*Z*)-4-Hydroxy-3-(2-(4-hydroxyphenethyl)-1-(2-morpholinoethyl)-1H-benzo[d]imidazol-5-yl)pent-3-en-2-one (15). **Part A:** COware™ vessel was equipped with a magnetic flea in one chamber and flushed with argon. 10 wt% Pd/C (5.02 g, 4.72 mmol, 0.2 eq.), and 4-(3,5-dimethylisoxazol-4-yl)-*N*-(2-morpholinoethyl)-2-nitroaniline (4) (8.17 g, 23.6 mmol, 1 eq.) were added to the same chamber in EtOAc:MeOH (5:1, 20 mL). Zinc (10 g) was added to the other chamber and the vessel was flushed with argon for a further 5 mins before sealing. 7 M HCl (aq.) (14.22 mL, 99.5 mmol) was then added to the zinc chamber. The reaction mixture was left to stir at room temperature overnight behind a blast shield, with the addition of more 7 M HCl (aq.) after 16 h (14.2 mL, 99.5 mmol). The reaction vessel was carefully vented, and the organic component was filtered over Celite, washing with EtOAc (3 × 50 mL) and the filtrate was concentrated under reduced pressure to afford the crude product as a brown oil (7.5 g, 23.6 mmol, 99%) which was used directly in the next stage without further purification. δ_H (600 MHz, $CDCl_3$) 1.72 (s, 3H), 1.87 (s, 3H), 2.51 (s, 4H), 2.70 (t, $J = 5.7$ Hz, 2H), 3.19 (t, $J = 5.7$ Hz, 2H), 6.51 (s, 1H), 3.73 (s, 4H), 6.62–6.53 (m, 2H), 10.45 (s, 1H). LC-MS (30–95 MeCN in 20 min) $t_R = 3.05$ min, purity = 90%, $m/z = 319.00[M + H]^+$.

Part B: To a stirred solution of 4-hydroxyphenyl propionic acid (914 mg, 5.05 mmol, 1.01 eq.) and HATU (2.465 g, 6.5 mmol, 1.3 eq.) in DMF (15 mL) at ambient temperature was added triethylamine (1.4 mL, 10 mmol, 2 eq.) followed by a solution of the crude di-aniline (1.732 g, 5 mmol, 1 eq.) in DMF (15 mL). After stirring overnight at ambient temperature, the reaction mixture was partitioned between DCM (50 mL) and water (50 mL) and the organic phase was separated. The aqueous phase was extracted with DCM (3 × 25 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was dissolved in AcOH (50 mL) and heated to reflux for 2 hours. The reaction mixture was then cooled to ambient temperature and concentrated under reduced pressure. The residue was treated with DCM (50 mL) followed by neutralisation with saturated aqueous $NaHCO_3$. The organic phase was collected, and the aqueous phase was extracted with DCM (4 × 50 mL). The combined organic extracts were washed with brine (250 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Purification by flash column chromatography, eluting with 0–15% MeOH in DCM with 0.5% NH_4OH , afforded the title compound as a yellow oil (1.14 g, 2.50 mmol, 51%). δ_H (600 MHz, $CDCl_3$) 1.87 (s, 6H), 2.50 (s, 4H), 2.67 (t, $J = 7.1$ Hz, 2H), 3.21–3.11 (m, 5H), 3.69 (s, 4H), 4.18 (t, $J = 7.1$ Hz, 2H), 6.74 (d, $J = 8.0$ Hz, 2H), 7.00 (d, $J = 8.1$ Hz, 2H), 7.05 (d, $J = 8.2$ Hz, 1H), 7.32 (d, $J = 8.2$ Hz, 1H), 7.51 (s, 1H). δ_C (151 MHz,



CDCl₃) 24.3, 29.7, 33.1, 41.5, 54.0, 57.6, 66.7, 109.5, 115.3, 115.8, 121.4, 125.5, 129.3, 131.0, 131.5, 134.0, 142.4, 155.3, 155.4, 191.20. LC-MS (30–95 MeCN in 20 min) ^tR = 3.36 min, purity = 99%, *m/z* = 450.10. [M + H]⁺. HR-MS (*m/z*): [M + H]⁺ calculated for C₂₆H₃₂N₃O₄, 450.2393; found, 450.2397.

Methyl 3-(4-(3-(dimethylamino)propoxy)phenyl)propanoate (16). To a stirred solution of methyl 3-(4-hydroxyphenyl)propanoate (9.00 g, 50 mmol, 1 eq.) in acetonitrile (200 mL) at ambient temperature was added cesium carbonate (48.9 g, 150 mmol, 3 eq.), followed by sodium iodide (3.75 g, 25 mmol 0.5 eq.) and 3-chloro-*N,N*-dimethylpropan-1-amine hydrochloride (9.3 g, 60 mmol 1.2 eq.). The reaction mixture was placed under argon and heated to reflux for 48 h. Upon reaction cooling, the mixture was concentrated under reduced pressure, and the residue was partitioned between DCM (300 mL) and water (300 mL). The organic phase was washed with 1 M NaOH (300 mL) and brine (300 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure, affording the title compound as a colourless oil (11.6 g, 43 mmol, 87%), which was used directly in the next step without further purification. δ_{H} (600 MHz, CDCl₃) 1.93 (p, *J* = 6.4 Hz, 2H), 2.24 (s, 6H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.59 (t, *J* = 7.8 Hz, 2H), 2.88 (t, *J* = 7.8 Hz, 2H), 3.66 (s, 3H), 3.98 (t, *J* = 6.4 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 4H), 7.09 (d, *J* = 8.3 Hz, 4H).

Lithium 3-(4-(3-(dimethylamino)propoxy)phenyl)propanoate (9). To a stirred solution of methyl 3-(4-(3-(dimethylamino)propoxy)phenyl)propanoate (16) (11.6 g, 43 mmol, 1 eq.) in a mixture of THF: water (5:1, 250 mL) at ambient temperature was added lithium hydroxide monohydrate (2.22 g, 52.8 mmol, 1.1 eq.), and the resulting solution was stirred overnight. The reaction mixture was concentrated under reduced pressure, and then sequentially reconcentrated from THF (5 × 50 mL) to afford the title compound as a white solid which was used directly in the next stage without further purification (11.06 g, 43 mmol, quant.).

3-(4-(2-(5-(3,5-Dimethylisoxazol-4-yl)-1-(2-morpholinoethyl)-1H-benzod[*d*]imidazol-2-yl)ethyl)phenoxy)-*N,N*-dimethylpropan-1-amine, ISOX-DUAL (1). To a stirred suspension of lithium 3-(4-(3-(dimethylamino)propoxy)phenyl)propanoate (9) (283 mg, 1.1 mmol, 1.1 eq.) in DMF (5 mL) at ambient temperature was added HATU (494.3 mg, 1.3 mmol, 1.3 eq.) and DIPEA (179 μ L, 1.5 mmol, 1.5 eq.). After stirring at ambient temperature for 1 hour, a solution of 4-(3,5-dimethylisoxazol-4-yl)-*N*1-(2-morpholinoethyl)benzene-1,2-diamine (8) (316.4 mg, 1.0 mmol, 1 eq.) in DMF (3 mL) was added, and the reaction mixture was left to stir overnight before being partitioned between DCM (50 mL) and water (50 mL). The organic phase was separated, and the aqueous phase was extracted with DCM (3 × 25 mL). The combined organic extracts were washed with saturated aqueous NaHCO₃ (100 mL) and brine (100 mL), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was dissolved in AcOH (50 mL) and heated to reflux for 2 hours. The reaction mixture was then cooled to ambient temperature and concentrated under reduced pressure. The residue was treated with DCM (50 mL) followed by neutralisation with saturated aqueous NaHCO₃.

The organic phase was collected, and the aqueous phase was extracted with DCM (4 × 50 mL). The combined organic extracts were washed with brine (250 mL), dried over anhydrous magnesium sulfate and concentrated under reduced pressure. Purification by flash column chromatography, eluting with 0–10% MeOH in DCM with 0.5% NH₄OH, afforded the title compound as a yellow oil (292.7 mg, 0.55 mmol, 55%). δ_{H} (600 MHz, CDCl₃) 1.98 (p, *J* = 6.6 Hz, 2H), 2.29 (s, 9H), 2.42 (s, 3H), 2.45 (s, 4H), 2.50 (t, *J* = 7.0 Hz, 2H), 2.59 (t, *J* = 6.8 Hz, 2H), 3.19–3.14 (m, 2H), 3.25–3.20 (m, 2H), 3.67 (s, 4H), 3.99 (t, *J* = 6.3 Hz, 2H), 4.11 (t, *J* = 6.8 Hz, 2H), 6.84 (d, *J* = 8.1 Hz, 2H), 7.12 (t, *J* = 8.3 Hz, 3H), 7.34 (d, *J* = 8.2 Hz, 1H), 7.62 (s, 1H). δ_{C} (151 MHz, CDCl₃) 11.0, 11.7, 27.5, 30.1, 33.2, 41.6, 45.5, 54.2, 57.8, 66.3, 67.0, 109.5, 114.8, 117.3, 120.1, 123.5, 124.3, 129.4, 133.0, 134.4, 143.2, 155.6, 157.8, 159.2, 165.2, LCMS (5–95 MeCN in 20 mins) ^tR = 3.09 min, purity = 98%, *m/z* = 532.30 [M + H]⁺. Data in accordance with literature.¹

Abbreviations

CBP	CREB-binding protein
HATU	Hexafluorophosphate azabenzotriazole tetramethyl uronium
MW	Microwave
PdCl ₂ (dppf)	[1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane
DCM	
PdCl ₂ (dtbpf)	1,1'-Bis(di- <i>tert</i> -butylphosphino)ferrocene] palladium(II)

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest in this study.

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References

- 1 E. L. P. Chekler, J. A. Pellegrino, T. A. Lanz, R. A. Denny, A. C. Flick, J. Coe, J. Langille, A. Basak, S. Liu, I. A. Stock, P. Sahasrabudhe, P. D. Bonin, K. Lee, M. T. Pletcher and L. H. Jones, *Chem. Biol.*, 2015, **22**, 1588–1596.



- 2 D. A. Hay, O. Fedorov, S. Martin, D. C. Singleton, C. Tallant, C. Wells, S. Picaud, M. Philpott, O. P. Monteiro, C. M. Rogers, S. J. Conway, T. P. C. Rooney, A. Tumber, C. Yapp, P. Filippakopoulos, M. E. Bunnage, S. Müller, S. Knapp, C. J. Schofield and P. E. Brennan, *J. Am. Chem. Soc.*, 2014, **136**, 9308–9319.
- 3 D. S. Hewings, O. Fedorov, P. Filippakopoulos, S. Martin, S. Picaud, A. Tumber, C. Wells, M. M. Olcina, K. Freeman, A. Gill, A. J. Ritchie, D. W. Sheppard, A. J. Russell, E. M. Hammond, S. Knapp, P. E. Brennan and S. J. Conway, *J. Med. Chem.*, 2013, **56**, 3217–3227.
- 4 D. S. Hewings, M. Wang, M. Philpott, O. Fedorov, S. Uttarkar, P. Filippakopoulos, S. Picaud, C. Vuppusetty, B. Marsden, T. D. Heightman, S. Knapp, P. Brennan and S. J. Conway, *RSC Advances in Synthesis and Medicinal Chemistry*, 2012, vol. 243.
- 5 N. A. Isley, M. S. Hageman and B. H. Lipshutz, *Green Chem.*, 2015, **17**, 893–897.
- 6 Z. Ahmadi and J. S. McIndoe, *Chem. Commun.*, 2013, **49**, 11488–11490.
- 7 P. A. Cox, A. G. Leach, A. D. Campbell and G. C. Lloyd-Jones, *J. Am. Chem. Soc.*, 2016, **138**, 9145–9157.
- 8 J. Sherwood, J. H. Clark, I. J. S. Fairlamb and J. M. Slattery, *Green Chem.*, 2019, **21**, 2164–2213.
- 9 A. J. Close, P. Kemmitt, M. K. Emmerson and J. Spencer, *Tetrahedron*, 2014, **70**(47), 9125–9131.
- 10 P. Vitale and A. Scilimati, *Recent Developments in the Chemistry of 3-Arylisoxazoles and 3-Aryl-2-isoxazolines*, Elsevier Ltd, 2017, vol. 122.
- 11 J. A. Joule and K. Mills, *Heterocyclic Chemistry*, Wiley, 5th edn., 2010.
- 12 S. J. Coles and P. A. Gale, *Chem. Sci.*, 2012, **3**, 683–689.

