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Probing BRD Inhibition Substituent Effects in Bulky Analogues of (+)-JQ1

Storm Hassell-Hart,^a Sarah Picaud,^b Raphael Lengacher,^c Joshua Csucker,^c Regis Millet,^d Gilles Gasser,^e Roger Alberto,^c Hannah Maple,^f Robert Felix,^f Zbigniew J. Leśnikowski,^g Helen J. S. Stewart,^h Timothy J. Chevassut,^h Simon Morley,ⁱ Panagis Filippakopoulos,^{*b} John Spencer^{*a}

^a Chemistry Department, School of Life Sciences, University of Sussex, Brighton BN1 9QJ, U.K,
email: j.spencer@sussex.ac.uk

^b Structural Genomics Consortium (SGC), University of Oxford, Oxford OX3 7DQ, U.K.

^c University of Zurich, Department of Chemistry, Winterthurerstrasse 190, CH-8057 Zurich

^d University of Lille, Inserm, INFINITE, U1286, ICPAL, 3 rue du Professeur Laguesse, BP-83, F-59006, Lille, France.

^e Chimie ParisTech, PSL University, CNRS, Institute of Chemistry for Life and Health Sciences, Laboratory for Inorganic Chemical Biology, 75005 Paris, France.

^f Bio-Techne (Tocris Bioscience), The Watkins Building, Atlantic Road, Avonmouth, Bristol BS11 9QD, U.K.

^g Laboratory of Medicinal Chemistry and Biological Chemistry, Institute of Medical Biology of the Polish Academy of Sciences, 92-232 Łódź, Poland

^h Brighton and Sussex Medical School, University of Sussex, Brighton BN1 9PS, U.K.

ⁱ Biochemistry, School of Life Sciences, University of Sussex, Brighton BN1 9QG, U.K.

Dedicated to Prof. Antonio Togni for his seminal contributions to organic chemistry and catalysis. Thanks, Antonio, for introducing me the delights of ferrocene chemistry (JS, ETH (1994-96)) and for your continued friendship, sense of humour and mentoring.

A series of bulky organometallic and organic analogues of the bromodomain (BRD) inhibitor **(+)-JQ1** have been prepared. The most potent, N-[(adamantan-1-yl)methyl]-2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamide, **2e**, showed excellent potency with an K_D of ca. 130 nM vs BRD₄(1) and a ca. 2-fold selectivity over BRD₄(2) (K_D = ca. 260 nM). Its binding to the first bromodomain of BRD₄ was determined by a protein cocrystal structure.

Keywords: Cancer • epigenetics • bioorganometallic chemistry • bromodomain • benzodiazepines

Introduction

The study of post-translational modifications (PTMs) is an area of current great scientific importance in medicine and biology, with bromodomains (BRDs) emerging as important acetylated lysine (Kac) epigenetic reader targets in medicinal chemistry.^[1-4] In total, there are 61 BRDs in the „write-read-erase“ „epigenetic code“ and inhibitors are being sought in order to elucidate their biological and clinical relevance. The BET (bromodomain and extra-terminal) BRD family includes BRD-containing proteins (BRD2, BRD3, BRD4) as well as the BRD testis-specific protein (BRDT). Each protein has two tandem N-terminal bromodomains as well as an extra-terminal protein interaction domain (ET).^[5-8] One well-documented chemical probe is the BET BRD₄ inhibitor **(+)-JQ1**, which has applications in cancer, inflammation and even in contraception.^[9,10] The crystal structure of **(+)-JQ1** showed it to bind in the Kac pocket of BRD₄(1) with the methyl-triazole unit acting as a Kac mimic. Its enantiomer **(-)-JQ1** was found to be significantly less active.

Recently, we disclosed the nM active **(+)-JD1 (2a)**, an analogue of **(+)-JQ1**, which has a bulky aminoferrocene moiety in place of the t-Bu ester.^[11] Its activity was lower than that of its organic analogue, typically around 3–4-fold less active (e.g. vs. BRD₄(1); **(+)-JQ1** vs **(+)-JD1**: K_D = 110 nM vs 400 nM, respectively) possibly due to the structural flexibility of the metallocene group, which was found to adopt different conformations in its protein crystal structure in BRD₄(1). The presence of the ferrocene group allowed for a marginal supplementary increase in cytotoxicity by the generation of reactive oxygen species (ROS) through formation of a ferrocenium species. However, this was much less pronounced than in a previous ferrocene-containing HDAC inhibitor.^[12] We, therefore, wished to synthesise other metal-based analogues in order to explore structure activity relationships, steric bulk, versus bulky organic moieties, where, obviously, steric bulk predominates.

Results and Discussion

Given that steric bulk appears to be tolerated around the ester unit in **(+)-JQ1** analogues, we now report our findings on other series of related analogues, where, crucially, the Ot-Bu ester has been replaced by amides containing relatively large organometallic or metalloidal groups. Notably, bulky, organic, „escape from flatland“,^[13] adamantylamine bioisosteres of ferrocenylamine,^[14] were also synthesized (Scheme 1) as an extension of recent work from our group,^[15] which showed similar activities of adamantyl vs ferrocenyl derivatives.

The synthesis of the new analogues was achieved using known protocols.^[11] The t-Bu ester of **(+)-JQ1** was treated with trifluoroacetic acid and the resulting acid **(+)-JQ1-OH** was reacted with various amines **1a–1e** using standard coupling reagents. We were able to synthesise rhenium (**2b**),^[16]

Scheme 1. Synthesis of **(+)-JQ1** analogues.

carborane (**2c**),^[17-19] (in the present work *p*-carborane isomer was used) as well as adamantyl analogues (**2d**, **2e**) in moderate to good yields. All new compounds were characterised by ¹H, ¹³C NMR spectroscopy, HRMS and analysed by HPLC purity (Scheme 1). As a positive control, we also synthesised the reported iridium-containing BRD₄ inhibitor **3a**.^[20]

Figure 1. Iridium-containing positive control (**3a**).

With these compounds in hand, we tested their biochemical activity against BRDs. Thermal melt (ΔT_m) data of these compounds against BET and other

BRDs were obtained (Figure 2). Compared with (+)-**JQ1**, the *p*-carborane and iridium complexes **2c**, **3a**, respectively, had relatively weak activity. The organic compound **2e** showed better affinity toward the second bromodomains of BETs, more so than the other adamantyl analogue, **2d**. Thermodynamic evaluation of binding using Isothermal titration calorimetry (ITC) of **2e** against the two BRDs of BRD₄ showed higher affinity for BRD₄(1), however, albeit with differences in entropic contributions between the two domains (Table 1). We selected **2e** for further investigation on the basis of its relatively high affinity for BRD₄(2) domains as with previous studies from our group e.g. with **RVX208**.^[21] Unfortunately, we were unable to obtain crystals of **2e** in BRD₄(2) or BRD₂(2). Binding to the first bromodomain of BRD₄ was established in a high resolution co-crystal structure (Figure 2c) confirming its Kac-competitive binding mode. The ligand adopts the classical Kac mimetic pose previously found in other thieno- and benzo-diazepine chemotypes, with the methyl-triazole moiety inserting into the hydrophobic recognition site and engaging the conserved asparagine (N140), while the adamantane appendage rigidly extends away from the binding site.

Figure 2. *In vitro* evaluation of compound **2** series. **A**) Thermal melt (ΔT_m) data of **2b-e** against BET and other BRDs using proteins at a final concentration of 2 μ M and ligands at 10 μ M. **B**) Thermodynamic evaluation of **2e** binding to BRD₄ bromodomains, by isothermal titration calorimetry. Raw injection heats for titrations of BRD₄(1) or BRD₄(2) into solutions of **2e** are shown in the left panels (reverse titrations). The right panel shows the normalized binding enthalpies corrected for the heat of protein dilution as a function of binding site saturation (symbols as indicated in the figure). Solid lines represent a nonlinear least squares fit using a single-site binding model. Titrations were performed in 50 mM HEPES pH 7.5 (at 25 °C), 150 mM NaCl and 15 °C while stirring at 750 rpm. (BRD₄(1): $K_D = 133 \pm 6$ nM; BRD₄(2): $K_D = 257 \pm 21$ nM). **C**) Crystal structure of **2e** bound onto BRD₄(1). (PDB: **7AJN**). Key residues of the Kac-binding pocket are annotated and conserved structural waters are shown. **D**) The ligand (**2e**) 2Fc-Fo map contoured at 2σ is shown in detail.

Table 1 – Isothermal Titration Calorimetry of human BRD₄ bromodomains with compound **2e**. Titration was carried out in 50 mM HEPES pH 7.5 (at 25 °C), 150 mM NaCl and 15 °C while stirring at 750 rpm. The protein was titrated into the compound solution (reverse titration).

Protein	Ligand	[P] (μ M)	[L] (μ M)	K_D (nM)	N	ΔH^{obs} (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)
BRD ₄ (1)	2e	550	22	133 ± 6	0.96 ± 0.002	-10.20 ± 0.03	-1.135	-9.07
BRD ₄ (2)	2e	586	25	257 ± 21	0.97 ± 0.004	-6.53 ± 0.05	2.154	-8.69

Finally, we wished to explore the ability of **2e** to suppresses *c MYC* and *S100A8* in THP-1 cells.^[11] The acute myeloid leukaemia (AML) cell line THP-1 was treated with 1 μ M **2e** or 1 μ M (+)-**JQ1** (positive control) for 24 h prior to RNA extraction (Figure 3). Gene expression was determined by using quantitative Real Time PCR (qPCR) and normalised using the housekeeping gene GAPDH. Both *cMYC* and *S100A8* have previously been shown to be suppressed by (+)-**JQ1**.^[22] These results showed that **2e** was even more effective than (+)-**JQ1**.

Figure 3. Comparison of effects of **2e** vs (+)-**JQ1** in suppressing *c MYC* and *S100A8* (triplicate data).

Conclusions

BRD inhibitors based on (+)-**JQ1** and (+)-**JD1**, including rhenium and carborane analogues, with bulky amide groups have been synthesized and characterized. The related, sterically bulky, adamantyl analogue (**2e**) displayed excellent binding (K_D = ca. 130 nM) to BRD4(1) with a ca. two-fold selectivity over BRD4(2) (K_D = ca. 260 nM) and its crystal structure in the first bromodomain of BRD4 was determined. The use of **2e**, which also suppresses *c MYC* in THP-1 cells, as a tool compound in cell-based systems, is currently underway in our laboratory and will be disclosed in due course.

Experimental Section

Synthetic chemistry details, including NMR, mass spectrometry, HPLC, are as reported recently.^[11]

Synthetic Chemistry

(+)-JQ-1-OH. A mixture of (+)-**JQ-1** (200 mg, 0.438 mmol) and anhydrous DCM (50 mL) was cooled to 0 °C. To the mixture was added dropwise trifluoroacetic acid (20 mL) and the resulting mixture was warmed to ambient temperature and stirred for 16 h under an argon atmosphere. The reaction was concentrated under reduced pressure and to the residue was added a 1:1 mixture of DCM:Et₂O. The solvent was removed under reduced pressure and the sequence repeated five times to give (+)-**JQ-1-OH** as a yellow solid of sufficient purity to be utilised in subsequent reactions without further manipulation.

Rhenium Analogue of (+)-**JQ-1** (**2b**)

To **(+)-JQ-1-OH** (11.3 mg, 0.0283 mmol) was added anhydrous THF (1.25 mL) and anhydrous DIPEA (9.9 μ L, 0.0566 mmol) under an argon atmosphere. To the resulting mixture was added HBTU (21.5 mg, 0.0566 mmol) and rhenium complex **1b** (15.6 mg, 0.0283 mmol). The resulting mixture was heated to 40 °C and stirred for 72 h under an argon atmosphere. The reaction was cooled to ambient temperature and to the mixture was added DCM (5 mL) and a solution of 2M aqueous NaOH (5 mL). The resulting biphasic mixture was separated and the resulting organic extract washed with a solution of 2M HCl (5 mL) followed by brine (5 mL), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to give an orange gum (59 mg). The resulting residue was purified by automated flash column chromatography (EtOAc/MeOH, 100:0 – 80:20, 4 g SiO₂). The appropriate fractions were combined and concentrated under reduced pressure to give the Rhenium analogue of **JQ-1** as a yellow solid (9 mg, 38% over 2 steps). LCMS (UV, ESI) R_t = 23.57 min, $[M-H]^+$ m/z = 294.9, 92% purity. ¹H NMR (600 MHz, CDCl₃): δ = 7.45–7.37 (2H, m), 7.35–7.29 (2H, m), 5.89 and 5.85 (1H, d, J = 2.1 Hz, two conformations of the half-sandwich complex), 5.43 and 5.32 (1H, d, J = 2.2 Hz, two conformations of the half-sandwich complex), 4.63–4.57 (1H, m), 4.32–4.13 (4H, m), 3.61–3.55 (1H, m), 3.43–3.36 (1H, m), 2.67 (3H, s), 2.41 (3H, s), 1.67 (3H, s), 1.32–1.28 (3H, m). ¹³C NMR (151 MHz, CDCl₃): δ = insufficient material for an accurate spectrum. HRMS (ESI-[+H]) m/z : Calcd for C₃₂H₃₀ClN₅O₆ReS 834.1163; Found 834.1198.

Carborane Analogue of **JQ-1** (**2c**)

To **(+)-JQ-1-OH** (44 mg, 0.110 mmol) was added anhydrous THF (5 mL) and anhydrous DIPEA (38 μ L, 0.219 mmol) under an argon atmosphere. To the resulting mixture was added HBTU (83 mg, 0.219 mmol) and (aminopropyl-p-carborane),^[23] (52 mg, 0.219 mmol), and the mixture stirred at ambient temperature for 16 h under an argon atmosphere. To the mixture was added DCM (10 mL) and a solution of 2M aqueous NaOH (10 mL). The resulting biphasic mixture was separated and the resulting organic extract washed with a solution of 2M HCl (10 mL) followed by brine (10 mL), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to give a yellow solid (94 mg). The resulting residue was purified by automated flash column chromatography (EtOAc/MeOH, 100:0 – 70:30, 12 g SiO₂). The appropriate fractions were combined and concentrated under reduced pressure to give **2c** as a white solid (72 mg, 95% over two steps). LCMS (UV, ESI) R_t = 25.90 min, $[M-H]^+$ m/z = 585.2, 95% purity. ¹H NMR (600 MHz, *d*₆-DMSO): δ = 8.17 (1H, t, J = 5.8 Hz), 7.51–7.47 (2H, m), 7.44–7.40 (2H, m), 4.46 (1H, dd, J = 8.5, 5.7 Hz), 3.40–3.31 (11H, m), 3.20 (1H, dd, J = 14.9, 5.7 Hz), 3.11 (1H, dd, J = 14.9, 5.7 Hz), 3.03–2.96 (1H, m), 2.91–2.84 (1H, m), 2.59 (3H, s), 2.41 (3H, s), 1.68–1.64 (2H, m), 1.63 (3H, s), 1.30–1.23 (2H, m). ¹³C NMR (151 MHz, *d*₆-DMSO): δ = 169.5, 163.0, 155.1, 149.9, 136.7, 135.3, 132.3, 130.8, 130.2, 129.8, 129.6, 128.5, 59.8, 53.9, 37.7, 37.6, 35.6, 29.5, 20.8, 14.1, 12.7, 11.3. HRMS (ESI-[+H]) m/z : Calcd for C₂₄H₃₅B₁₀ClN₅O₅ 586.3181; Found 586.3196.

N-(1*s*,3*R*)-Adamantan-1-yl)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamide (**2d**).

To **(+)-JQ-1-OH** (16 mg, 0.04 mmol) was added anhydrous THF (2 mL) and anhydrous DIPEA (14 μ L, 0.08 mmol) under an argon atmosphere. To the resulting mixture was added HBTU (30 mg, 0.08 mmol) and 1-adamantanamine (12 mg, 0.08 mmol) and the mixture stirred at ambient temperature for 16 h under an argon atmosphere. To the mixture was added DCM (5 mL) and a solution of 2M aqueous NaOH (5 mL). The resulting biphasic mixture was separated and the resulting organic extract washed with a solution of 2M HCl (5 mL) followed by brine (5 mL), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to give a yellow solid (40 mg). The resulting residue was purified by automated flash column chromatography (EtOAc/MeOH, 100:0 – 70:30, 40 g SiO₂). The appropriate fractions were combined and concentrated under reduced pressure to give the title compound as a white solid (16 mg, 75% over two steps). LCMS (UV, ESI) R_t = 24.09 min, $[M-H]^+$ m/z = 534.1, 97% purity. ¹H NMR (600 MHz, CDCl₃): δ = 7.40 (2H, d, J = 8.1 Hz), 7.35–7.31 (2H, m), 5.99 (1H, s), 4.60 (1H, t, J = 7.0 Hz), 3.45 (1H, dd, J = 14.3, 7.1 Hz), 3.26 (1H, dd, J = 14.4, 6.9 Hz), 2.67 (3H, s), 2.39 (3H, s), 2.09–2.04 (3H, m), 2.04–2.01 (6H, m), 1.71–1.63 (9H, m). ¹³C NMR (151 MHz, CDCl₃): δ = 169.6, 164.0, 155.8, 150.0, 137.9, 136.9, 132.3, 131.0, 130.8, 130.5, 129.9, 128.9, 54.6, 52.3, 41.7, 40.4, 36.5, 29.6, 14.6, 13.2, 12.0. HRMS (ESI-[+H]) m/z : Calcd for C₂₉H₃₃ClN₅O₅ 534.2094; Found 534.2089.

N-(1*s*,3*R*)-Adamantan-1-ylmethyl)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetamide (**2e**).

To **(+)-JQ-1-OH** (16 mg, 0.04 mmol) was added anhydrous THF (2 mL) and anhydrous DIPEA (14 μ L, 0.08 mmol) under an argon atmosphere. To the resulting mixture was added HBTU (30 mg, 0.08 mmol) and 1-adamantanemethylamine (14 μ L, 0.08 mmol) and the mixture stirred at ambient temperature for 16 h under an argon atmosphere. To the mixture was added DCM (5 mL) and a solution of 2M aqueous NaOH (5 mL). The resulting biphasic mixture was separated and the resulting organic extract washed with a solution of 2M HCl (5 mL) followed by brine (5 mL), dried over anhydrous MgSO₄, filtered, and concentrated under reduced pressure to give a yellow solid (49 mg). The resulting residue was purified by automated flash column chromatography (EtOAc/MeOH, 100:0 – 70:30, 4 g SiO₂). The appropriate fractions were combined and concentrated under reduced pressure to give the title compound as a white solid (18 mg, 82% over two steps). LCMS (UV, ESI) R_t = 24.64 min, $[M-H]^+$ m/z = 548.2, 96% purity. ¹H NMR (600 MHz, CDCl₃): δ = 7.41 (2H, d, J = 8.2 Hz), 7.35–7.29 (2H, m), 6.50 (1H, t, J = 6.4 Hz), 4.60 (1H, dd, J = 7.4, 6.4 Hz), 3.60 (1H, dd, J = 14.1, 7.4 Hz), 3.35 (1H, dd, J = 14.1 Hz, 6.5 Hz), 3.01 (1H, dd, J = 13.4, 6.4 Hz), 2.95 (1H, dd, J = 13.4, 6.4 Hz), 2.67 (3H, s), 2.39 (3H, s), 1.97–1.92 (3H, m), 1.71–1.67 (3H, m), 1.66 (3H, s), 1.61–1.56 (3H, m), 1.50–1.46 (3H, m), 1.46–1.41 (3H, m). ¹³C NMR (151 MHz, CDCl₃): δ = 170.7, 164.0, 155.9, 150.0, 136.9, 136.7, 132.2, 131.1, 131.0, 130.7, 130.0, 128.8, 54.8, 51.2, 40.3, 39.8, 37.0, 33.9, 28.3, 14.5, 13.2,

12.0. HRMS (ESI-[+H]) *m/z*: Calcd for C₃₀H₃₄ClN₅OS 548.2251; Found 548.2231.

Iridium (III) Complex (3a)

To a mixture of dichlorotetrakis(2-(2-pyridinyl)phenyl)diiridium(III) (25 mg, 0.023 mmol) and anhydrous acetonitrile (2.5 mL) was added silver triflate (12 mg, 0.046 mmol) and the resulting mixture stirred at ambient temperature for 16 h under an argon atmosphere. The resulting mixture was filtered through celite®, washing with Et₂O. The resulting solution was concentrated under reduced pressure to give a yellow solid (32 mg, 95%). Data are in agreement with those previously reported. [20]

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Supplementary Material

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/MS-number>.

Abbreviations. HBTU (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate. DIPEA: N,N-Diisopropylethylamine.

Author Contribution Statement

J.S., P.F. designed the project and wrote the manuscript with writing and editing help from G.G., R.M., H.M., R.F. and S.M. S.H.-H. synthesized the final compounds and intermediates with help from J.C., R.L., Z.J. L. and R. A.. S.P., T. J. C., H.J.S.S. and P.F. performed biological evaluation of the final compounds as well as x-ray structural analysis.

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