DOI: 10.1002/hlca.202000214

FULL PAPER

HELVETICA chimica acta

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

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Dedicated to Prof. *Antonio Togni* for his seminal contributions to organic chemistry and catalysis. Thanks, *Antonio*, for introducing me the delights of ferrocene chemistry (*J. S.*, ETH (1994–1996)) 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-[(9*S*)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.02,6]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamide, **2e**, showed excellent potency with an K_D = *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: benzodiazepines, bioinorganic chemistry, bioorganometallic chemistry, bromodomain, cancer, epigenetics..

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

Supporting information for this article is available on the WWW under https://doi.org/10.1002/hlca.202000214

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.

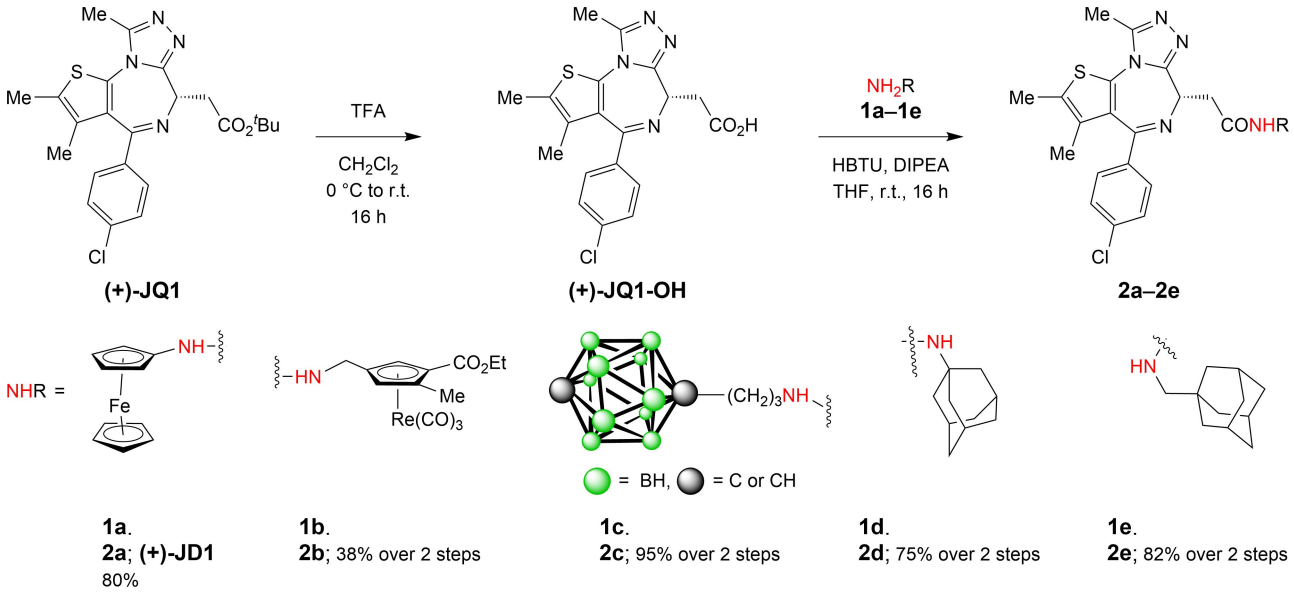
Recently, we disclosed the nM active **(+)-JD1 (2a)**, an analogue of **(+)-JQ1**, which has a bulky amino-ferrocene moiety in place of the ^{t}Bu ester.[11] Its activity was lower than that of its organic analogue, typically around 3–4-fold less active (*e.g. vs.* BRD4(1); **(+)-JQ1** *vs.* **(+)-JD1**: $K_D = 110$ nM *vs.* 400 nM, resp.) 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 versus bulky organic moieties.

Results and Discussion

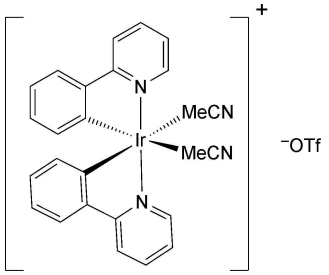
Given that steric bulk appears to be tolerated around the ester unit in **(+)-JQ1** analogues, we now report our findings on other series of related analogues, where, crucially, the O^{t}Bu ester has been replaced by amides containing relatively large organometallic or metalloid groups. Notably, bulky, 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] carborane (**2c**),[17–19] (in the present work, the *p*-carborane isomer was used) as well as adamantyl analogues (**2d**, **2e**) in moderate to good yields. All new compounds were characterised by ^{1}H- and ^{13}C-NMR spectroscopy, HR-MS and analysed by HPLC purity (*Scheme 1*). As a positive control, we also synthesised the reported iridium-containing BRD4 inhibitor **3a** (*Figure 1*).[20]

With these compounds in hand, we tested their biochemical activity against BRDs. Thermal melt (ΔTm) data of these compounds against BET and other BRDs were obtained (*Figure 2*). Compared with **(+)-JQ1**, the *p*-carborane and iridium complexes **2c**, **3a**, respec-



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



3a

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

tively, 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 for example with **RVX208**.[21] Unfortunately, we were unable to obtain crystals of **2e** in BRD4(2) or BRD2(2).

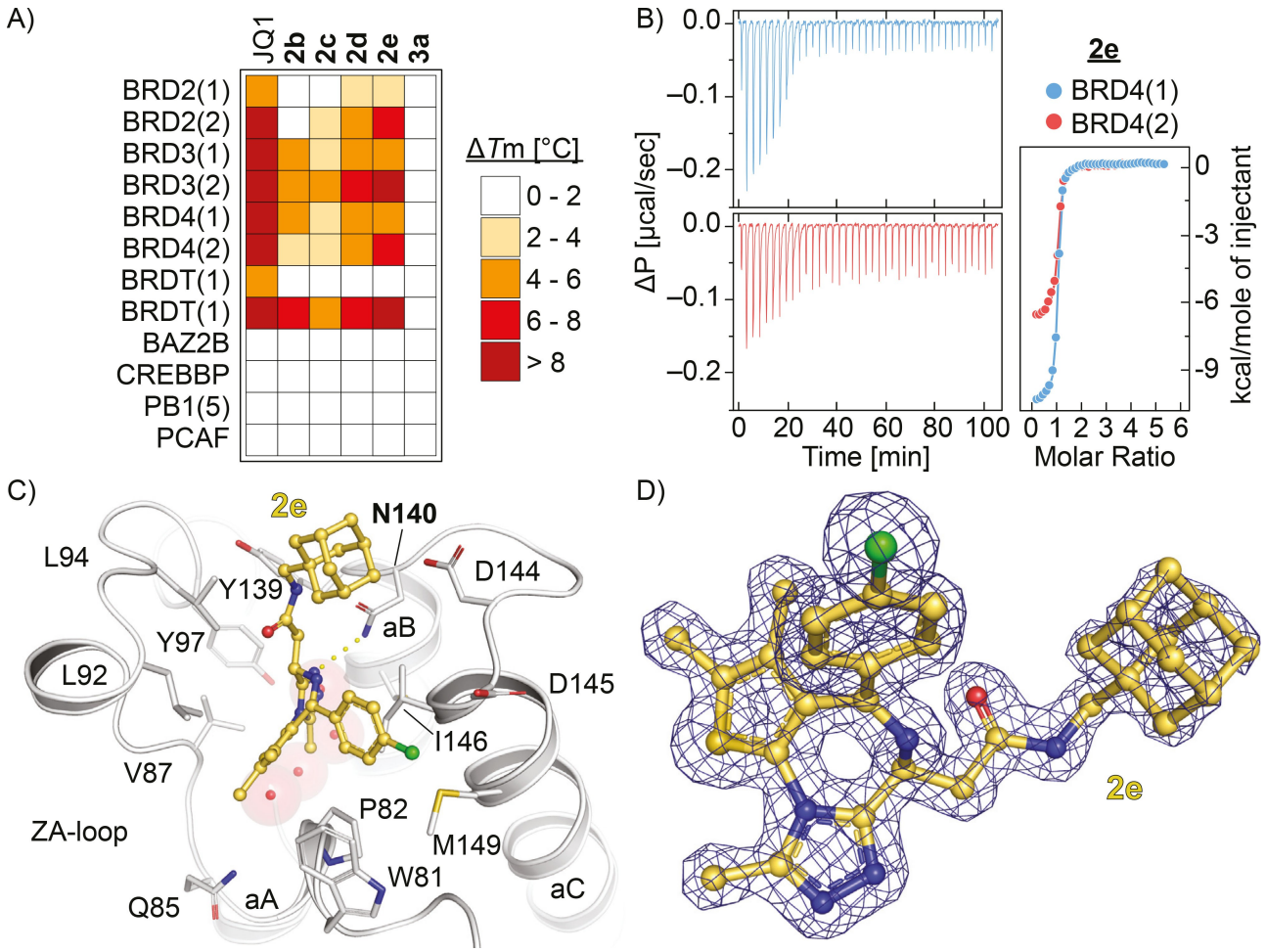


Figure 2. *In vitro* evaluation of compound **2** series. A) Thermal melt (Δ*T*m) data of **2b**–**2e** against BET and other BRDs using proteins at a final concentration of 2 μM and ligands at 10 μM. B) Thermodynamic evaluation of **2e** binding to BRD4 bromodomains, by isothermal titration calorimetry. 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.

Binding to the first bromodomain of BRD4 was established in a high resolution co-crystal structure (*Figure 2,c*) confirming its Kac-competitive binding mode. The ligand adopts the classical Kac mimetic pose previously found in other thieno- and benzodiazepine chemotypes, with the methyl-triazole moiety inserting into the hydrophobic recognition site and engaging the conserved asparagine (N140), while the adamantane appendix rigidly extends away from the binding site.

Finally, we wished to explore the ability of **2e** to suppresses *c MYC* and S100 A8 in THP-1 cells.[11] The acute myeloid leukaemia (AML) cell line THP-1 was treated with 1 μM **2e** or 1 μM **(+)-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 S1008 have previously been shown to be supressed by **(+)-JQ1**.[22] These results showed that **2e** was even more effective than **(+)-JQ1**.

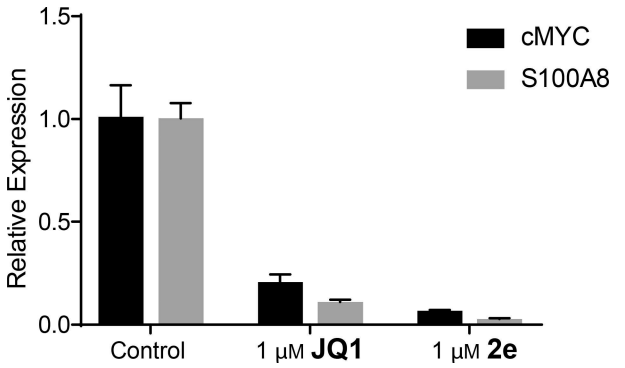


Figure 3. Comparison of effects of **2e** *vs.* **(+)-JQ1** in suppressing cMYC and S100A8 (triplicate data).

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 cMYC 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 CH_2Cl_2 (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 mixture was concentrated under reduced pressure and to the residue was added a 1:1 mixture of CH_2Cl_2/Et_2O. 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 μL, 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 CH_2Cl_2 (5 mL) and a solution of 2 M aqueous NaOH (5 mL). The resulting biphasic mixture was separated, and the resulting organic extract washed with a solution of 2 M HCl (5 mL) followed by brine (5 mL),

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] [μM]	[L] [μM]	K_D [nM]	N	ΔH^{obs} [kcal/mol]	$T\Delta S$ [kcal/mol]	ΔG [kcal/mol]
BRD4(1)	**2e**	550	22	133 ± 6	0.96 ± 0.002	−10.20 ± 0.03	−1.135	−9.07
BRD4(2)	**2e**	586	25	257 ± 21	0.97 ± 0.004	−6.53 ± 0.05	2.154	−8.69

dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give an orange gum (59 mg). The resulting residue was purified by automated flash column chromatography (AcOEt/MeOH, 100:0–80:20, 4 g SiO_2). 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 two steps). LC/MS (UV, ESI): t_R = 23.57 min, $[M-H]^+$ m/z = 294.9, 92% purity. ^{1}H-NMR (600 MHz, $CDCl_3$): 7.45–7.37 (*m*, 2 H); 7.35–7.29 (*m*, 2 H); 5.89, 5.85 (*d*, *J* = 2.1, 1 H, two conformations of the half-sandwich complex); 5.43, 5.32 (*d*, *J* = 2.2, 1 H, two conformations of the half-sandwich complex); 4.63–4.57 (*m*, 1 H); 4.32–4.13 (*m*, 4 H); 3.61–3.55 (*m*, 1 H); 3.43–3.36 (*m*, 1 H); 2.67 (*s*, 3 H); 2.41 (*s*, 3 H); 1.67 (*s*, 3 H); 1.32–1.28 (*m*, 3 H). ^{13}C-NMR (151 MHz, $CDCl_3$): insufficient material for an accurate spectrum. HR-ESI-MS: 834.1198 ($[M+H]^+$, $C_{32}H_{30}ClN_5O_6ReS^+$; calc. 834.1163).

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 μL, 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 was stirred at ambient temperature for 16 h under an argon atmosphere. To the mixture was added CH_2Cl_2 (10 mL) and a solution of 2 M aqueous NaOH (10 mL). The resulting biphasic mixture was separated, and the resulting organic extract washed with a solution of 2 M HCl (10 mL) followed by brine (10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give a yellow solid (94 mg). The resulting residue was purified by automated flash column chromatography (AcOEt/MeOH, 100:0–70:30, 12 g SiO_2). The appropriate fractions were combined and concentrated under reduced pressure to give **2c** as a white solid (72 mg, 95% over two steps). LC/MS (UV, ESI): t_R = 25.90 min, $[M-H]^+$ m/z = 585.2, 95% purity. ^{1}H-NMR (600 MHz, (D_6)DMSO): 8.17 (*t*, *J* = 5.8, 1 H); 7.51–7.47 (*m*, 2 H); 7.44–7.40 (*m*, 2 H); 4.46 (*dd*, *J* = 8.5, 5.7, 2 H); 3.40–3.31 (*m*, 11 H); 3.20 (*dd*, *J* = 14.9, 5.7, 1 H); 3.11 (*dd*, *J* = 14.9, 5.7, 1 H); 3.03–2.96 (*m*, 1 H); 2.91–2.84 (*m*, 1 H); 2.59 (*s*, 3 H); 2.41 (*s*, 3 H); 1.68–1.64 (*m*, 2 H); 1.63 (*s*, 3 H); 1.30–1.23 (*m*, 2 H). ^{13}C-NMR (151 MHz, (D_6)DMSO): 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. HR-ESI-MS: 586.3196 ($[M+H]^+$, $C_{24}H_{35}B_{10}ClN_5OS^+$; calc. 586.3181).

***N*-(Adamantan-1-yl)-2-[(6*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 μL, 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 was stirred at ambient temperature for 16 h under an argon atmosphere. To the mixture was added CH_2Cl_2 (5 mL) and a solution of 2 M aqueous NaOH (5 mL). The resulting biphasic mixture was separated, and the resulting organic extract washed with a solution of 2 M HCl (5 mL) followed by brine (5 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give a yellow solid (40 mg). The resulting residue was purified by automated flash column chromatography (AcOEt/MeOH, 100:0–70:30, 40 g SiO_2). 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). LC/MS (UV, ESI): t_R = 24.09 min, $[M-H]^+$ m/z = 534.1, 97% purity. ^{1}H-NMR (600 MHz, $CDCl_3$): 7.40 (*d*, *J* = 8.1, 2 H); 7.35–7.31 (*m*, 2 H); 5.99 (*s*, 1 H); 4.60 (*t*, *J* = 7.0, 1 H); 3.45 (*dd*, *J* = 14.3, 7.1, 1 H); 3.26 (*dd*, *J* = 14.4, 6.9, 1 H); 2.67 (*s*, 3 H); 2.39 (*s*, 3 H); 2.09–2.04 (*m*, 3 H); 2.04–2.01 (*m*, 6 H); 1.71–1.63 (*m*, 9 H). ^{13}C-NMR (151 MHz, $CDCl_3$): 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. HR-ESI-MS: 534.2089 ($[M+H]^+$, $C_{29}H_{32}ClN_5OS^+$; calc. 534.2094).

***N*-[(Adamantan-1-yl)methyl]-2-[(6*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 μL, 0.08 mmol) under an argon atmosphere. To the resulting mixture was added HBTU (30 mg, 0.08 mmol) and 1-adamantanemethylamine (14 μL, 0.08 mmol), and the mixture was stirred at ambient temperature for 16 h under an argon atmosphere. To the mixture was added CH_2Cl_2 (5 mL) and a solution of 2 M aqueous NaOH (5 mL). The resulting biphasic mixture was separated, and the resulting organic extract washed with a solution of 2 M HCl (5 mL) followed by brine (5 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to give a yellow solid (49 mg). The resulting residue was purified by automated flash column chromatography (AcOEt/MeOH, 100:0–70:30, 4 g SiO_2). 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). LC/MS (UV, ESI): $t_R = 24.64$ min, $[M-H]^+$ $m/z = 548.2$, 96% purity. ^{1}H-NMR (600 MHz, $CDCl_3$): 7.41 (*d*, *J* = 8.2, 2 H); 7.35–7.29 (*m*, 2 H); 6.50 (*t*, *J* = 6.4, 1 H); 4.60 (*dd*, *J* = 7.4, 6.4, 1 H); 3.60 (*dd*, *J* = 14.1, 7.4, 1 H); 3.35 (*dd*, *J* = 14.1, 6.5, 1 H); 3.01 (*dd*, *J* = 13.4, 6.4, 1 H); 2.95 (*dd*, *J* = 13.4, 6.4, 1 H); 2.67 (*s*, 3 H); 2.39 (*s*, 3 H); 1.97–1.92 (*m*, 3 H); 1.71–1.67 (*m*, 3 H); 1.66 (*s*, 3 H); 1.61–1.56 (*m*, 3 H); 1.50–1.46 (*m*, 3 H); 1.46–1.41 (*m*, 3 H). ^{13}C-NMR (151 MHz, $CDCl_3$): 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. HR-ESI-MS: 548.2231 ($[M+H]^+$, $C_{30}H_{34}ClN_5OS^+$; calc. 548.2251).

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 Et_2O. 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]

Acknowledgements

EPSRC (EP/P026990/1, *S. H.-H.*, *J. S.*), MRC (MR/N010051/1, *P. F.*) and NSC Poland (2014/13/B/NZ1/03989, *Z. J. L.*) are thanked for funding. The authors thank the Diamond Light Source for beam time (proposal mx19301) and the staff of beamlines I03 for assistance with crystal testing and data collection. The SGC is a registered charity (number 1097737) that receives funds from *AbbVie*, *Bayer Pharma AG*, *Boehringer Ingelheim*, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada, Innovative Medicines Initiative (EU/EFPIA) [ULTRA-DD grant no. 115766], *Janssen*, *Merck KgaA* Darmstadt Germany, *MSD*, *Novartis Pharma AG*, Ontario Ministry of Economic Development and Innovation, *Pfizer*, FAPDF, CAPES, CNPq, São Paulo Research Foundation-FAPESP, *Takeda*, and *Wellcome* [106169/ZZ14/Z]. This work was funded by the Swiss National Science Foundation (Grant Sinergia CRSII5_173718) and has received support under the program 'Investissements d'Avenir' launched by the French Government and implemented by the ANR with the reference ANR-10-IDEX-0001-02 PSL (*G. G.*).

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

Author Contribution Statement

J. S. and *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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Received November 15, 2020
Accepted January 14, 2021

