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

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Recently, we disclosed the nM active (+)-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analogues, we now report our findings on other series of related analogues, where, crucially, the Ot-Bu ester has been replaced by amides containing relatively large organometallic or metalloid groups. Notably, bulky, organic, escape from flatland,adamantylamine biosiosteres of ferrocenyamine,13[dl] were also synthesized (Scheme 1) as an extension of recent work from our group,[14] which showed similar activities of adamantyl vs ferrocenyl derivatives.

The synthesis of the new analogues was achieved using known protocols.[15] The 1-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),


dedicated to Prof. Antonio Togni for his seminal contributions to organic chemistry and catalysis. Thanks, Antonio, for introducing me the delights of ferrocene chemistry (US, ETH (1999–96)) 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-[(adamant-1-yl)methyl]-2-(5-[(S)-5-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatriacyclo[8.3.0.2.6]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamide, 2e, showed excellent potency with an K= ca. 130 nM vs BRD4(1) and a ca. 2-fold selectivity over BRD4(2) (K= ca. 260 nM). Its binding to the first bromodomain of BRD4 was determined by a protein cocystal 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.[1–3] 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).[4] One well-documented chemical probe is the BET BRD4 inhibitor (+)-JQ1, which has applications in cancer, inflammation and even in contraception.[5] 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 (+)-JQ2 (2a), an analogue of (+)-JQ1, which has a bulky aminoferrocene moiety in place of the t-Bu ester.[6] Its activity was lower than that of its organic analogue, typically around 3 - 4-fold less active (e.g. vs. BRD4(1): (+)-JQ1 vs (+)-JQ2: K= 110 nM vs 400 nM, respectively) due to the structural flexibility of the metalloocene 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 ferrocen-containing HDAC inhibitor.[6] We, therefore, wished to synthesise other metal-based analogues in order to explore structure activity relationships, steric bulk, versus bulky organic moities, 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 metalloid groups. Notably, bulky, organic, escape from flatland,adamantylamine biosiosteres of ferrocenyamine,13[dl] were also synthesized (Scheme 1) as an extension of recent work from our group,[14] which showed similar activities of adamantyl vs ferrocenyl derivatives.

The synthesis of the new analogues was achieved using known protocols.[15] The 1-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),

Scheme 1. Synthesis of (+)-JQ1 analogues.
carborane (2c), \[17\text{ -} 19\] 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 $^1$H, $^{13}$C NMR spectroscopy, HRMS and analysed by HPLC purity (Scheme 1). As a positive control, we also synthesised the reported iridium-containing BRD4 inhibitor 3a.\[20\]

BRD were obtained (Figure 2). Compared with (+)-JO1, 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 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(2), 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 appendix rigidly extends away from the binding site.

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).
Finally, we wished to explore the ability of 2e to suppress c MYC and S100A8 in THP-1 cells. 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 S100A8 have previously been shown to be suppressed by (+)-JQ1. These results have shown that 2e was even more effective than (+)-JQ1.

**Conclusions**

BRD inhibitors based on (+)-JQ1s and (+)-JQ2s, including rhenium and carborane analogues, with bulky amide groups have been synthesized and characterized. The related, sterically bulky, adamantanyl analogue (2e) displayed excellent binding (K<sub>d</sub> ca. 150 nM) to BRD4 with a ca. two-fold selectivity over BRD4 (K<sub>d</sub> 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.

**Synthetic Chemistry**

(+)-JQ1-1-OH. A mixture of (+)-JQ1 (200 mg, 0.048 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/Et-O. The solvent was removed under reduced pressure and the sequence repeated five times to give (+)-JQ1-1-OH as a yellow solid of sufficient purity to be utilised in subsequent reactions without further manipulation.

Rhenium Analogue of (+)-JQ1 (2b)

To (+)-JQ1-1-OH (113 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 (30 mg, 0.08 mmol) and the mixture stirred at ambient temperature for 16 h under an argon atmosphere. The reaction was cooled to 40 °C and stirred for 72 h under an argon atmosphere. The reaction was concentrated under reduced pressure and to the residue was added acetonitrile (10 ml) and 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 (10 ml) followed by brine (10 ml), dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure to give a yellow solid (49 mg). The resulting mixture was purified by flash column chromatography (EtOAc/MeOH, 100:0–70:30, 49 g SiO<sub>2</sub>). The appropriate fractions were combined and concentrated under reduced pressure to give the rhenium analogue of (+)-JQ1 as a yellow solid (9 mg, 38% over 2 steps). LCMS (UV, ESI) R<sub>t</sub> = 23.57 min, [M+H]+ m/z = 294.9, 92% purity. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ = 7.45-7.37 (2H, m), 7.35-7.29 (2H, m), 5.89 and 5.85 (8H, δ = J = 2.12 Hz, two conformations of the half-sandwich complex), 5.43 and 5.32 (4H, δ = J = 2.2 Hz, two conformations of the half-sandwich complex), 6.63-4.57 (17H, m), 4.32–3.13 (1H, m), 3.61-3.55 (1H, m), 3.42-3.36 (1H, m), 2.67 (3H, s), 2.41 (3H, s), 1.67 (3H, s), 1.32-1.28 (1H, m). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>): δ = insufficient material for an accurate spectrum. HRMS (ESI+[+H]+) m/z: Calcd for C<sub>21</sub>H<sub>25</sub>C<sub>11</sub>Cl<sub>13</sub>N<sub>8</sub>O<sub>13</sub>S<sub>2</sub> 1341.3743; Found 1341.3743.

**Carborane Analogue of (+)-JQ1 (2c)**

To (+)-JQ1-1-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), <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ = 7.29-7.14 (2H, m), 6.61 (2H, s), 3.65-3.55 (1H, m), 3.54-3.35 (1H, m), 2.68 (3H, s), 2.51 (3H, s), 1.86 (3H, s), 1.24-1.20 (1H, m). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>): δ = 179.7, 166.5, 129.7, 128.0, 126.3, 124.8, 121.6, 121.2, 131.0, 130.7, 130.0, 128.8, 54.8, 52.1, 40.3, 39.8, 37.0, 35.9, 28.3, 14.5, 15.3, 29.5.

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

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 S100A8 have previously been shown to be suppressed by (+)-JQ1. These results have shown that 2e was even more effective than (+)-JQ1.
To a mixture of dichlorotetrakis(2-(2-pyridyli)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 EtO. The resulting solution was concentrated under reduced pressure to give a yellow solid (32 mg, 95%). Data are in agreement with those previously reported.

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Supporting Information for this article is available on the WWW under (G.G.).

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. A.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.

References.

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(+)-JQ1 analogues containing bulky groups show good BRD4 affinity especially the adamantyl analogue 2e.

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