ACS Medicinal Chemistry Letters

Letter

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.7b00389 • Publication Date (Web): 14 Nov 2017

Just Accepted

ACS Publications

ACS Medicinal Chemistry Letters is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Design, Synthesis and Biological Activity of 1,2,3-Triazolobenzodiazepine BET Bromodomain Inhibitors

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KEYWORDS. bromodomain, JQ1, triazole, leukemia, benzodiazepine

ABSTRACT: A number of diazepines are known to inhibit bromo- and extra-terminal domain (BET) proteins. Their BET inhibitory activity derives from the fusion of an acetyl-lysine mimetic heterocycle onto the diazepine framework. Herein we describe a straightforward, modular synthesis of novel 1,2,3-triazolobenzodiazepines and show that the 1,2,3-triazole acts as an effective acetyl-lysine mimetic heterocycle. Structure-based optimization of this series of compounds led to the development of potent BET bromodomain inhibitors with excellent activity against AF9-MLL-driven leukemic cells, concomitant with a reduction in c-*MYC* expression. These novel benzodiazepines therefore represent a promising class of therapeutic BET inhibitors.

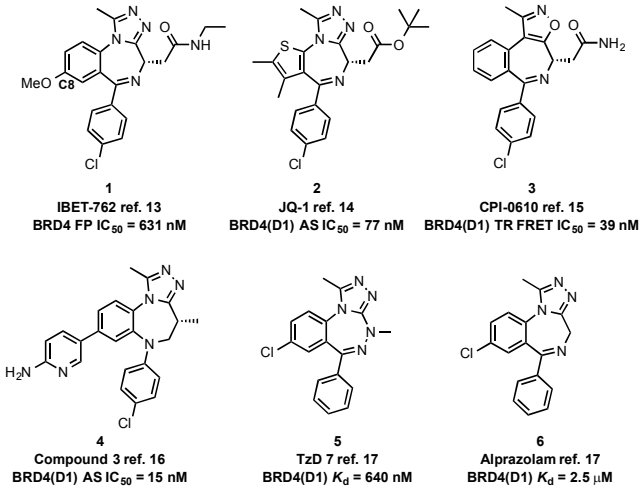
The bromo- and extra-terminal domain (BET) family of proteins are epigenetic reader proteins involved in transcription regulation and chromatin remodelling.[1,2] The family consists of BRD2, BRD3 and BRD4, which are ubiquitously expressed, and BRDT, which is expressed only in the testes. Each BET protein contains two bromodomain structural motifs, here designated as D1 and D2, which have been shown to bind acetylated lysines on histones H3 and H4, and existing data indicates that the biology of BET proteins is largely regulated through the first bromodomain.[3,4] The acetylated histone lysine residues bind into a hydrophobic pocket of the BET proteins making specific hydrogen bonding interactions with a conserved asparagine and tyrosine residue, the latter through a water molecule.[5] In the last six years a number of research teams have identified high affinity, small molecule ligands for this hydrophobic pocket that block binding to the cognate acetylated histones.[6–10] Such small molecule BET inhibitors, along with genetic deletion studies, have demonstrated that inhibition of the BET – histone interaction can result in profound disruption of transcriptional programs resulting in anticancer and anti-inflammatory activity, amongst others. For example, treatment of cancer cells dependent on the oncogene c-*MYC* with BET inhibitors, either in vitro or in vivo, can result in significant anti-proliferative and cytotoxic effects. As a consequence of these and other data, at least ten BET inhibitors have now entered clinical trials for the treatment of a range of hematological cancers (incl. leukemia, lymphoma and myeloma), certain solid tumors (including glioblastoma multiforme and NUT-midline carcinoma) and atherosclerosis.[2,11]

The first potent BET inhibitors described in the literature were the benzodiazepine IBET-762 (**1**)[12,13] and the related thienodiazepine (*S*)-JQ-1 (**2**) (Chart 1).[14] Co-crystal structures of these compounds with BRD4(D1) revealed that the 1,2,4-triazole within these inhibitors acts as an acetyl-lysine mimic by interacting with Asn140 and a conserved water molecule in the KAc recognition pocket. Constellation Pharmaceuticals have reported isoxazole azepines (e.g. **3**)[15] and benzotriazolo[4,3-*d*][1,4]diazepine **4**[16] that also inhibit BET bromodomains, while Knapp and coworkers reported that benzotriazepine **5** binds to BRD4.[17] Interestingly, based on structural similarities to IBET-762, a range of benzodiazepine drugs were retrospectively tested for inhibition of BRD4(D1).

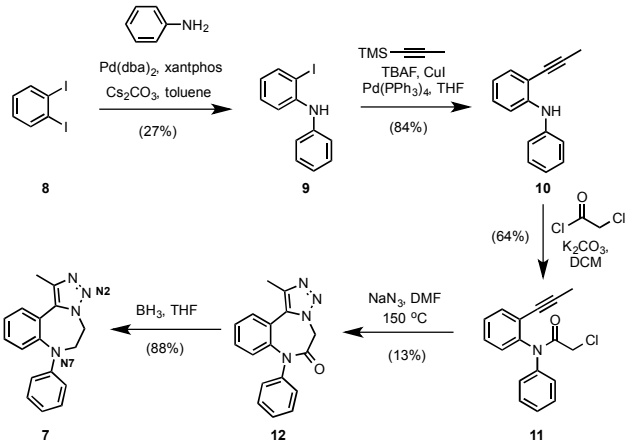
Of these, alprazolam (**6**) was found to be a weak inhibitor (K_d = 2.5 μM) and X-ray crystallography confirmed the 1,2,4-triazole acts as an acetyl lysine mimetic. Other closely related analogues of alprazolam that were devoid of the 1,2,4-triazole motif were found to be inactive,[17] underscoring the importance of the acetyl lysine mimicking heterocycle for BET activity.

We have been interested in understanding the impact of the KAc mimicking pharmacophore on bromodomain inhibition. Based on previous studies[18] we designed and tested compounds based on a *5H*-benzo[*f*][1,2,3]triazolo[1,5-*d*][1,4]diazepine nucleus. The 1,2,3-triazole motif was predicted to have appropriate hydrogen-bond accepting vector at N2 to interact with the conserved asparagine [Asn140 in BRD4(D1)] and molecular modeling indicated that a phenyl substituent at N7 of the diazepine ring would project into the WPF shelf, closely mapping over chlorophenyl (C6) of *3H*-benzo-1,4-diazepines JQ-1 and IBET-762. We found that this is an excellent BET bromodomain-binding framework and, through subsequent structure-based optimization, developed a series of 1,2,3-triazolobenzodiazepines that display potent BET bromodomain inhibition and excellent cellular activity.

Chart 1. Example azepine BET inhibitors



Initially, we prepared 1,2,3-triazole-containing benzodiazepine **7** via the route shown in Scheme 1. Starting from 1,2-diiodobenzene (**8**), Buchwald coupling with aniline afforded diarylaniline **9**. The propyne was introduced at the remaining iodinated position using Sonogashira cross-coupling and the product (**10**) was acetylated with chloroacetyl chloride providing chloroacetamide **11**. Based on a report by Majumdar *et. al.* and others,[19-21] we performed a one-pot S_N2/Hüigsen 1,3-dipolar cycloaddition reaction cascade to directly prepare the 1,2,3-triazolobenzodiazepine framework by heating **11** to 150 °C in the presence of sodium azide. While this strategy provided triazole **12** as expected, the un-optimized yield in this case was low (13%). Nevertheless, with the diazepinone in hand, selective deletion of the carbonyl oxygen with borane provided the desired 1,2,3-triazolobenzodiazepine **7**. Using an optimized version of this general strategy, followed by additional late-stage cross-coupling reactions and other functional group interconversions, we synthesized a range of analogs shown in Table 1 (see electronic supporting information for full synthetic details).



Scheme 1. Synthesis of 1,2,3-triazolobenzodiazepines: Synthesis of **7**.

Initially we assessed the BET inhibitory activity of **7** using AlphaScreen™, a well-validated competition binding assay. Gratifyingly, **7** showed good activity in this assay against all bromodomains tested with the strongest activity against BRD4(D2) (IC_{50} = 22 nM), but significantly weaker activity against BRD2(D1) (IC_{50} = 264 nM) (Figure 1 and Table 1). Control compound, JQ-1, gave BRD4(D2) IC_{50} = 12 nM and BRD2(D1) IC_{50} = 26 nM (Figure 1). We also tested the diazepinone (**12**) but this was essentially inactive (IC_{50} greater than 10 μM, data not shown).

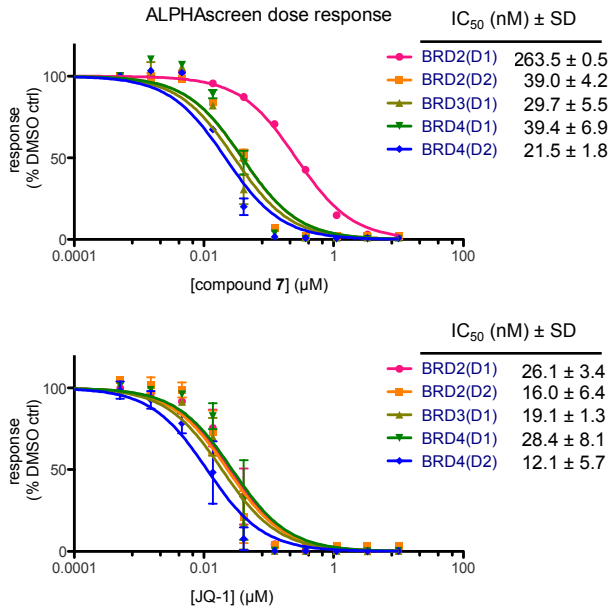


Figure 1. Dose response plots for AlphaScreen™ assays. Results of at least 2 independent experiments reported as mean ± SD.

The good ligand efficiency [0.51 kcal.mol^{-1} per non-hydrogen atom for BRD4(D2)] of **7** prompted us to expand this series and explore structure-activity relationships with the view to improve the overall BET activity. First we briefly examined modifications to the D-ring. Benzyl substitution at N7 (**13**) led to a reduction in activity by greater than 50-fold against all bromodomains tested, while addition of either *meta* or *para* substituents on the D-ring were tolerated in terms of overall activity with the 4-chlorophenyl-substituted analog **15** provided a modest improvement over the activity of parent compound **7** [about 2-fold improvement against BRD2(D1)], consistent with previous studies on BET inhibitors, and we therefore retained this modification in subsequent compounds. Next we investigated modifications to the A-ring. Crystal structures of both IBET-762 (pdb code 2YEK) or alprazolam (pdb code 3U5J) bound to BET bromodomains indicated that substitution at C9 (C8 in IBET-762 numbering) projected into the ZA channel and we extrapolated that our series of benzodiazepines would also accommodate functionalization at this position. A set of compounds was therefore designed to incorporate a substituent from the C9 vector. From the activity of the set of C9 analogs it was clear that sp^3 substitution provided no improvement in activity (compounds **16**, **17** and **27**), whereas heteroaryl substituents significantly improved activity, with pyrazole **18** and aminopyridine **20** giving IC_{50} values as low as 7 and 6 nM respectively against BRD4(D2). Interestingly, tetrazole **22** showed the greatest activity across each bromodomain with IC_{50} values ranging from 7 nM against BRD2(D2) to 2 nM against BRD4(D2). At this point we established the binding mode of the aminopyridyl-substituted compound (**20**) in BRD2(D2) by single crystal X-ray analysis (Figure 2). The structure confirmed that the 1,2,3-triazole motif acts as an acetyl-lysine mimetic, accepting a hydrogen bond from both Asn429 and the conserved water molecule coordinated to Tyr386, as expected. Additionally, the chlorophenyl ring lies within the WPF shelf and the aminopyridine ring extends through the ZA channel and towards solvent exposed space. The positioning of the aminopyridine towards solvent indicated that modifications to this position should be well tolerated. Based on these structural insights we reasoned that other sp^2-hybridized moities, such as carbonyls, might also improve potency and allow for greater diversity of functionalization. Initially we compared carboxylic acids, esters and simple amides. In general, these derivatives showed good activity. Of the initial carbonyl series, primary amide **25** was significantly more potent (~3-fold) against all BET bromodomains tested compared with ester **23** and prompted us to explore the amide derivatives further. The benzyl amide series (compounds **29** to **33**) were also exceptionally potent, particularly chiral benzyl amides **29**, **30** and **31**. Further, in the amide series the *S*-configured amides **29** and **30** displayed significant domain selectivity [BRD2(D2) vs BRD2(D1)] realtive to the *R*-configured analog **31**. In light of the potent activity of the tetrazole, **22**, we explored bioisosteric acylsulfonamides at the C9 position. These compounds, although somewhat less active than the tetrazole, also displayed low nanomolar activity against BRD4(D2) despite the significantly increased steric bulk, further attesting to the accomodation of sp^2-modifications at C9. Lastly, we investigated substitutions to the benzodiazepine ring. Methyl substitution at C5 (Compounds 36, 37) improved activity against BRD4(D1) and the *R*-stereogenic configuration was preferred consistent with similar modification of previously reported benzodiazepine BET inhibitors.[16]

From this chemical series we selected compounds **18** and **20** for further study because they displayed potent activity and were predicted to have good cell permeability (for example the tetrazole **22**, while potent, is likely an efflux transporter substrate).[22] First, we assessed the bromodomain selectivity of **20** across a panel of 32 recombinant human bromodomains in a *bromo*MAX^{TM} assay (see electronic supporting information for full data). In these assays, **20** showed excellent selectivity for the BET family bromodomains. We also assessed the binding kinetics of **20** against individual bromodomains using surface plasmon resonance (SPR). The dissociation-rate measured for compound **20** against BRD2 was approximately 3-fold slower for domain 2 than domain 1 (k_d = 0.039 s^{-1} vs k_d = 0.13 s^{-1}, respectively), whereas association rates were similar (k_a = 2.90 x10^5 M^{-1}s^{-1} vs k_a = 2.62 x10^5 M^{-1}s^{-1}, respectively) (see electronic supporting information for full data).

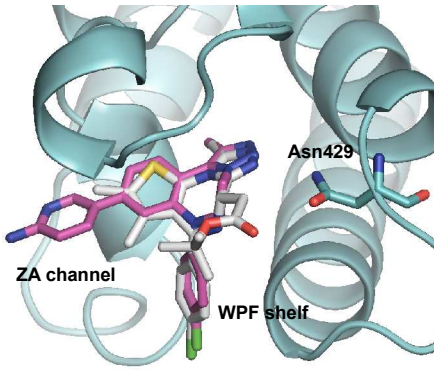
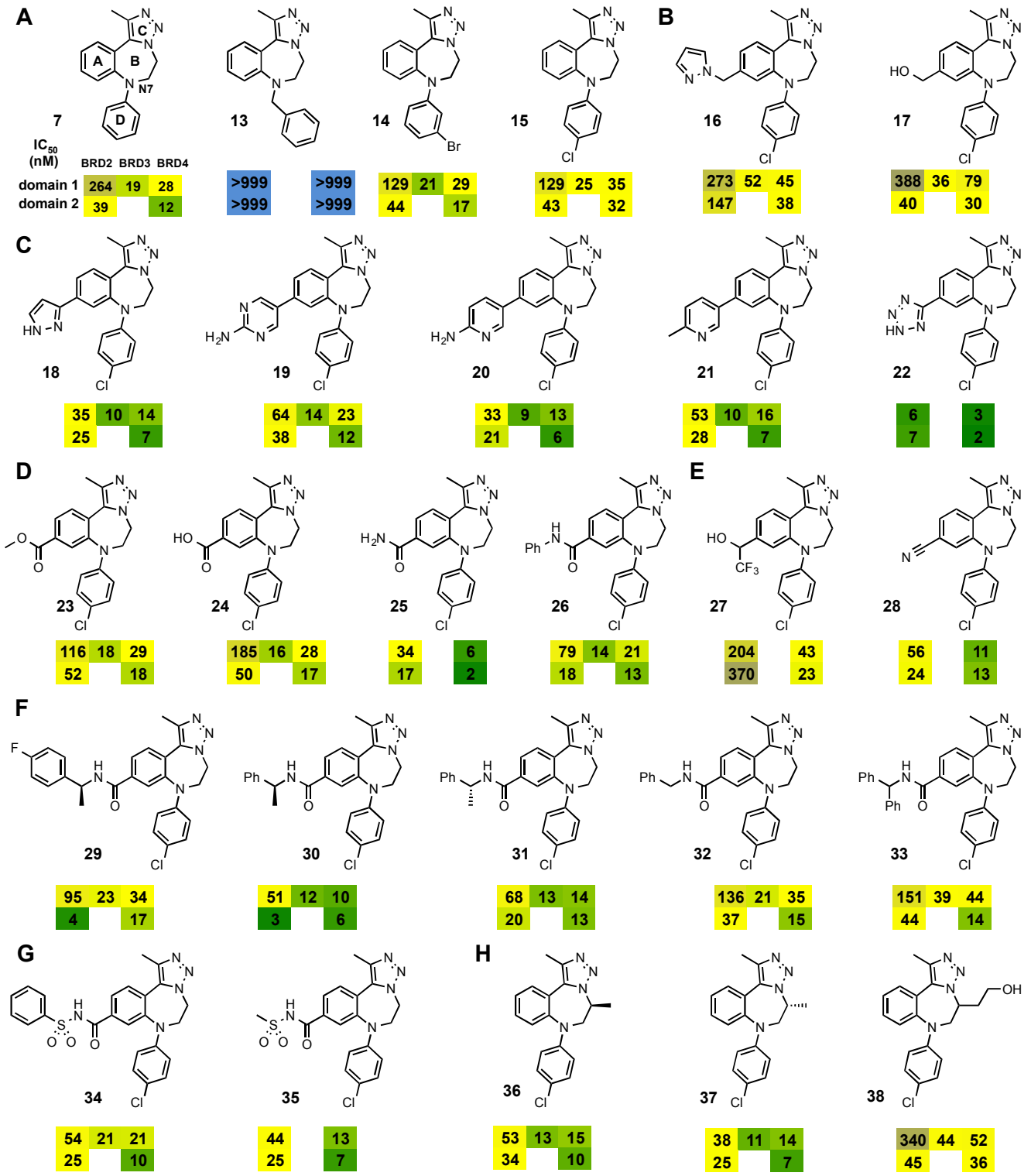


Figure 2. A X-ray crystal structure of **20** (magenta) in complex with BRD2(D2) PDB code = 5U6V overlayed with co-crystal structure of JQ-1 (grey) bound to BRD2(D2) PDB = 3ONI.

BET inhibition has been shown to have a remarkable effect on certain primary cancer cells and cell lines, which is thought to be a consequence of downregulation of oncogenes c-*MYC*, *BCL-2* and others by displacement of BRD4 from hyperacetylated histone tails near their respective promoters and enhancers.[23,24] To test the anti-leukemic effect of this chemical series we profiled **18** and **20** against a cancer cell panel (Table 2). Compound **20** potently inhibited proliferation of known BRD4-dependant leukemic line MLL-AF9, and showed significant inhibition of leukemic macrophage cell line MV4-11, (EC_{50} = 140 nM), whereas B cell lymphoma lines DOHH2, SU-DHL-4 and Raji, and myeloma cell line RPMI-8226 were less sensitive (EC_{50} > 500 nM). Consistent with BET-targeted anti-proliferative effects, BCR-Abl-driven cell line K562 was completely resistant to **20** (>1000 nM).

Table 1. Inhibition of BET bromodomains as determined by AlphaScreen™ assays. Numbers represent mean IC_{50} in nanomolar (nM) of at least two independent experiments. **A**. D-ring modifications. **B** C9 sp3 functionalized derivatives. **C** C9 sp2-functionalized derivatives. **D** C9 carbonyl derivatives. **E** miscellaneous C9 derivatives. **F** C9 benzylamide derivatives. **G** Acylsulphonamides. **H** C5 Alkyl derivatives.



Osteosarcoma (OS) cells are also known to be sensitive to BET inhibition, but through a MYC-independent mechanism.[25] We were therefore interested to characterize the effect of **18** and **20** on primary mouse OS cells (fibroblastic 494H and osteoblastic 148I). Both compounds **18** and **20** inhibited proliferation of both primary OS cell types, serving to highlight the broad utility of 1,2,3-triazolobenzodiazepines derivatives in cancer studies. The cellular potency profile of **18** and **20** also closely correlated with positive control JQ-1.

Table 2. Treatment of various cancer cell lines with **18**, **20** or JQ-1 for 72 h. Cell viability measure by Cell-TiterGlo™

	EC_{50} (nM)		
Cell line	**20**	**18**	JQ-1
MLL-AF9	77	111	64
HL-60	152	139	51
MV4-11	140	200	39
DOHH2	561	285	279
RPMI-8226	631	495	258
SU-DHL-4	977	570	660
CCRF-CEM	1365	2221	2037
Raji	560	780	330
HEL92.1.7	400	720	180
K562	>10000	>10000	>10000
494H	166	194	122
148I	1285	973	284

We used RNA-seq to compare the effect of **20** and JQ-1 on global gene expression in THP-1 leukemia cells. These experiments revealed that the effect on gene expression between these two compounds is highly correlated for both up and down regulated genes (Figure 3A). In particular, BRD4-dependent oncogene *c-MYC* demonstrated robust downregulation on treatment with **20** (Figure 3B) whilst *HEXIM1* levels were upregulated.[26] Similar effects on *c-MYC* and *BCL-2* downregulation were observed for JQ-1, **18**, **20** and non-diazepine BET inhibitor IBET-151 in MV4-11 cells (data not shown, see electronic supporting information for full data).

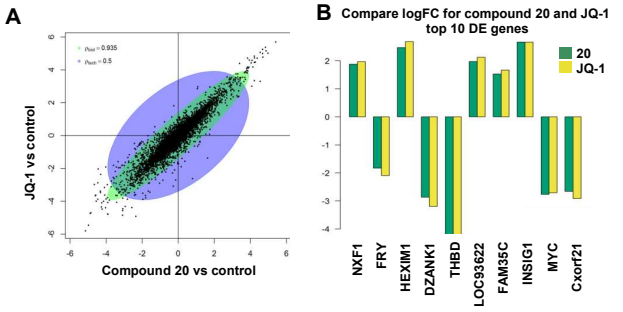


Figure 3. **A**. GENAS plot comparing effects of **20** and JQ-1 on THP-1 cells. **B**. Identity of 10 highest up and down regulated genes by **20** and **JQ-1**.

activity relationship studies and crystal structure-guided optimization led to the development of analogs with single-digit nanomolar activity. Compound **20**, showed good BET bromodomain selectivity and potent activity against a number of BRD4-dependant leukaemia cell lines and caused down-regulation of oncogene *MYC*. Taken together these data show that the benzodiazepine scaffold is an useful framework on which to base BET bromodomain inhibtors.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental methods, synthesis, and characterization (PDF)

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Funding Sources

Our work is supported by the NHMRC (fellowship to PEC) and the Victorian State Government Operational Infrastructure Support, and the Australian Government NHMRC IRIISS.

Notes

The authors declare no competing financial interests.

ACKNOWLEDGMENT

We thank beamline staff at the Australian Synchrotron, where diffraction data were collected. Crystallization experiments were performed at the C3 Collaborative Crystallization Centre.

ABBREVIATIONS

BET, bromo- and extra C-terminal domain containing protein.

REFERENCES

(1) Belkina, A. C.; Denis, G. V. BET Domain Co-Regulators in Obesity, Inflammation and Cancer. Nat. Rev. Cancer 2012, 12 (7), 465–477.

(2) Filippakopoulos, P.; Knapp, S. Targeting Bromodomains: Epigenetic Readers of Lysine Acetylation. Nat. Rev. Drug Discov. 2014, 13 (5), 337–356.

(3) Picaud, S.; Wells, C.; Felletar, I.; Brotherton, D.; Martin, S.; Savitsky, P.; Diez-Dacal, B.; Philpott, M.; Bountra, C.; Lingard, H.; Fedorov, O.; Müller, S.; Brennan, P. E.; Knapp, S.; Filippakopoulos, P. RVX-208, an Inhibitor of BET Transcriptional Regulators with Selectivity for the Second Bromodomain. Proc. Natl. Acad. Sci. U. S. A. 2013, 110 (49), 19754–19759.

(4) Baud, M. G. J.; Lin-Shiao, E.; Cardote, T.; Tallant, C.; Pschibul, A.; Chan, K.-H.; Zengerle, M.; Garcia, J. R.; Kwan, T. T.-L.; Ferguson, F. M.; Ciulli, A. A Bump-and-Hole Approach to

Engineer Controlled Selectivity of BET Bromodomain Chemical Probes. Science 2014, 12 (7), 465–477.

(5) Filippakopoulos, P.; Picaud, S.; Mangos, M.; Keates, T.; Lambert, J. P.; Barsyte-Lovejoy, D.; Felletar, I.; Volkmer, R.; Müller, S.; Pawson, T.; Gingras, A. C.; Arrowsmith, C. H.; Knapp, S. Histone Recognition and Large-Scale Structural Analysis of the Human Bromodomain Family. Cell 2012, 149 (1), 214–231.

(6) Garnier, J.-M.; Sharp, P. P.; Burns, C. J. BET Bromodomain Inhibitors: A Patent Review. Expert Opin. Ther. Pat. 2013, 24 (2), 1–15.

(7) Müller, S.; Knapp, S. Discovery of BET Bromodomain Inhibitors and Their Role in Target Validation. Medchemcomm 2014, 5 (3), 288.

(8) Brand, M.; Measures, A. M.; Wilson, B. G.; Cortopassi, W. A.; Alexander, R.; Höss, M.; Hewings, D. S.; Rooney, T. P. C.; Paton, R. S.; Conway, S. J. Small Molecule Inhibitors of Bromodomain–Acetyl-Lysine Interactions. ACS Chem. Biol. 2015, 10 (1), 22–39.

(9) Zhang, G.; Smith, S. G.; Zhou, M. M. Discovery of Chemical Inhibitors of Human Bromodomains. Chem. Rev. 2015, 115 (21), 11625–11668.

(10) Liu, Z.; Wang, P.; Chen, H.; Wold, E. A.; Tian, B.; Brasier, A. R.; Zhou, J. Drug Discovery Targeting Bromodomain-Containing Protein 4. J. Med. Chem. 2017, 60 (11), 4533–4558.

(11) Gelato, K. A.; Shaikhibrahim, Z.; Ocker, M.; Haendler, B. Targeting Epigenetic Regulators for Cancer Therapy: Modulation of Bromodomain Proteins, Methyltransferases, Demethylases, and microRNAs. Expert Opin. Ther. Targets 2016, 20 (7), 783–799.

(12) Nicodeme, E.; Jeffrey, K. L.; Schaefer, U.; Beinke, S.; Dewell, S.; Chung, C.; Chandwani, R.; Marazzi, I.; Wilson, P.; Coste, H.; White, J.; Kirilovsky, J.; Rice, C. M.; Lora, J. M.; Prinjha, R. K.; Lee, K.; Tarakhovsky, A. Suppression of Inflammation by a Synthetic Histone Mimic. Nature 2010, 468 (7327), 1119–1123.

(13) Mirguet, O.; Gosmini, R.; Toum, J.; Clément, C. A.; Barnathan, M.; Brusq, J. M.; Mordaunt, J. E.; Grimes, R. M.; Crowe, M.; Pineau, O.; Ajakane, M.; Daugan, A.; Jeffrey, P.; Cutler, L.; Haynes, A. C.; Smithers, N. N.; Chung, C. W.; Bamborough, P.; Uings, I. J.; Lewis, A.; Witherington, J.; Parr, N.; Prinjha, R. K.; Nicodème, E. Discovery of Epigenetic Regulator I-bet762: Lead Optimization to Afford a Clinical Candidate Inhibitor of the BET Bromodomains. J. Med. Chem. 2013, 56 (19), 7501–7515.

(14) Filippakopoulos, P.; Qi, J.; Picaud, S.; Shen, Y.; Smith, W. B.; Fedorov, O.; Morse, E. M.; Keates, T.; Hickman, T. T.; Felletar, I.; Philpott, M.; Munro, S.; McKeown, M. R.; Wang, Y.; Christie, A. L.; West, N.; Cameron, M. J.; Schwartz, B.; Heightman, T. D.; La Thangue, N.; French, C. A.; Wiest, O.; Kung, A. L.; Knapp, S.; Bradner, J. E. Selective Inhibition of BET Bromodomains. Nature 2010, 468 (7327), 1067–1073.

(15) Gehling, V. S.; Hewit, M. C.; Vaswani, G. R.; et al.; Hewitt, M. C.; Vaswani, R. G.; Leblanc, Y.; Coîté, A.; Nasveschuk, C. G.; Taylor, A. M.; Harmange, J. C.; Audia, J. E.; Pardo, E.; Joshi, S.; Sandy, P.; Mertz, J. A.; Sims, R. J.; Bergeron, L.; Bryant, B. M.; Bellon, S.; Poy, F.; Jayaram, H.; Sankaranarayanan, R.; Yellapantula, S.; Bangalore Srinivasamurthy, N.; Birudukota, S.; Albrecht, B. K. Discovery, Design, and Optimization of Isoxazole Azepine BET Inhibitors. ACS Med. Chem. Lett. 2013, 4 (9), 835−840.

(16) Taylor, A. M.; Vaswani, R. G.; Gehling, V. S.; Hewitt, M. C.; Leblanc, Y.; Audia, J. E.; Bellon, S.; Cummings, R. T.; Coîté, A.; Harmange, J. C.; Jayaram, H.; Joshi, S.; Lora, J. M.; Mertz, J. A.; Neiss, A.; Pardo, E.; Nasveschuk, C. G.; Poy, F.; Sandy, P.; Setser, J. W.; Sims, R. J.; Tang, Y.; Albrecht, B. K. Discovery of Benzotriazolo[4,3-d][1,4]diazepines as Orally Active Inhibitors of BET Bromodomains. ACS Med. Chem. Lett. 2016, 7 (2), 145–150.

(17) Filippakopoulos, P.; Picaud, S.; Fedorov, O.; Keller, M.; Wrobel, M.; Morgenstern, O.; Bracher, F.; Knapp, S. Benzodiazepines and Benzotriazepines as Protein Interaction Inhibitors Targeting Bromodomains of the BET Family. Bioorganic Med. Chem. 2012, 20 (6), 1878–1886.

(18) Sharp, P. P.; Garnier, J.-M.; Huang, D. C. S.; Burns, C. J. Evaluation of Functional Groups as Acetyl-Lysine Mimetics for BET Bromodomain Inhibition. Medchemcomm 2014, 5, 1834–1842.

(19) Majumdar, K. C.; Ray, K.; Ganai, S.; Ghosh, T. Catalyst-Free 1,3-Dipolar Cycloaddition: An Efficient Route for the Formation of the 1,2,3-Triazole-Fused Diazepinone Framework. Synthesis (Stuttg). 2010, No. 5, 858–862.

(20) Hooyberghs, G.; De Coster, H.; Vachhani, D. D.; Ermolat'Ev, D. S.; Van Der Eycken, E. V. Synthesis of [1,2,3]-triazolo[1,5-a][1,4]benzodiazepines via an Unprecedented One-Pot Cu-Catalyzed Azidation-Cyclization Reaction. Tetrahedron 2013, 69 (21), 4331–4337.

(21) Sudhapriya, N.; Nandakumar, A.; Arun, Y.; Perumal, P. T.; Balachandran, C.; Emi, N. An Expedient Route to Highly Diversified [1,2,3]triazolo[1,5-a][1,4]benzodiazepines and Their Evaluation for Antimicrobial, Antiproliferative and in Silico Studies. RSC Adv. 2015, 5 (81), 66260–66270.

(22) Young, A. M.; Audus, K. L.; Proudfoot, J.; Yazdanian, M. Tetrazole Compounds: The Effect of Structure and pH on Caco-2 Cell Permeability. J. Pharm. Sci. 2006, 95 (4), 717–725.

(23) Chapuy, B.; McKeown, M. R.; Lin, C. Y.; Monti, S.; Roemer, M. G.; Qi, J.; Rahl, P. B.; Sun, H. H.; Yeda, K. T.; Doench, J. G.; Reichert, E.; Kung, a L.; Rodig, S. J.; Young, R. a; Shipp, M. a; Bradner, J. E. Discovery and Characterization of Super-Enhancer-Associated Dependencies in Diffuse Large B Cell Lymphoma. Cancer Cell 2013, 24 (6), 777–790.

(24) Lovén, J.; Hoke, H. A.; Lin, C. Y.; Lau, A.; Orlando, D. A.; Vakoc, C. R.; Bradner, J. E.; Lee, T. I.; Young, R. A. Selective Inhibition of Tumor Oncogenes by Disruption of Super-Enhancers. Cell 2013, 153 (2), 320–334.

(25) Baker, E. K.; Taylor, S.; Gupte, A.; Sharp, P. P.; Walia, M.; Walsh, N. C.; Zannettino, A. C.; Chalk, A. M.; Burns, C. J.; Walkley, C. R. BET Inhibitors Induce Apoptosis through a MYC Independent Mechanism and Synergise with CDK Inhibitors to Kill Osteosarcoma Cells. Sci. Rep. 2015, 5 (May), 10120.

(26) Devaraj, S. G. T.; Fiskus, W.; Shah, B.; Qi, J.; Sun, B.; Iyer, S. P.; Sharma, S.; Bradner, J. E.; Bhalla, K. N. HEXIM1 Induction Is Mechanistically Involved in Mediating Anti-AML Activity of BET Protein Bromodomain Antagonist. Leukemia 2016, 30 (2), 504–508.

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