New Synthetic Routes to Triazolo-benzodiazepine Analogues: Expanding the Scope of the Bump-and-Hole Approach for Selective Bromo and Extra-Terminal (BET) Bromodomain Inhibition

Matthias G. J. Baud,*†‡ Enrique Lin-Shiao,†‡‖ Michael Zengerle,† Cynthia Tallant,†⊥ and Alessio Ciulli‡†∥

INTRODUCTION

The 1,4-benzodiazepine scaffold occupies a place of choice in the toolbox of medicinal chemists and is often referred to as a “privileged scaffold” in drug discovery. A large number of biologically active small molecules containing a 1,4-benzodiazepine scaffold have been approved by the FDA for the treatment of various disease states, although most of them are well-known for their psychotropic effects.1 Well known examples include diazepam, alprazolam or prazepam. The therapeutic potential of 1,4-benzodiazepines has fueled the interest of synthetic chemists in developing new routes to a range of substituted analogues for biological evaluation.2 Interest of synthetic chemists in developing new routes to a range of substituted analogues for biological evaluation.2 In particular, compounds 1–4 represent molecules I-BET762 (1),3 JQ1 (2),4 GW841819X (3),5 OTX015 (4),6 and RVX-208 (5)7 (Figure 1A). In particular, compounds 1–4 are based on a triazoloaryl diazepine scaffold (aryl = methoxyphenyl or dimethylphenyl) and bind to the acetyl-lysine (KAc) pocket of BET bromodomains with high affinity (Kd of 1 = 50–370 nM).11 These compounds display activity in vivo12 against a number of disease states, including NUT-midline carcinoma,13 multiple myeloma,14 mixed-lineage leukemia,15 and acute myeloid leukemia.16,17 Despite selectively targeting the BET bromodomain family with high potency over other bromodomains, these compounds are pan-BET selective thus do not significantly discriminate between individual bromodomains of the four BET members. This lack of selectivity within the BET subfamily so far has prevented accurate deconvolution of the biological function of individual BET proteins and of their tandem bromodomains. To address this problem, we recently developed a chemical genetics approach to engineer the selectivity of the BET bromodomain inhibitor I-BET762/JQ1 within the BET proteins family.11 This so-called “bump and hole” approach is based on the generation of orthogonal and differential protein/ligand pairs and involves introducing a single point mutation (large to smaller amino acid, that is, the “hole”) onto the BET bromodomain of interest together with a synthetic modification (bulky substituent, that is, the “bump”) onto the parent BET bromodomain binder to complement the newly created protein subpocket (Figure...
1B). As a result, the bulky ligand is expected to bind with high affinity to the mutated BET protein, while exhibiting weak to no binding to wild-type (WT) proteins due to a steric clash occurring between the “bump” and the naturally occurring residue (Figure 1B). This approach was previously shown to aid selective targeting of protein kinases through engineering of the ATP binding site and ATP competitive inhibitors. In our study, we demonstrated for the first time that the bump and hole approach can be used to selectively disrupt protein−protein interactions within the BET family of proteins. Compound ET (6) (Figure 1C,D), a derivative of I-BET762/JQ1 bearing an ethyl functional group at the level of the side chain methylene moiety, bound to Brd4 (1) (PDB code 3PSO) surface representation; red indicates negative and blue positive electrostatic potential). W81, V87, and L94 are highlighted. (B, C) I-BET762 chemical structure and positions selected for derivatization to target the corresponding mutations.
of individual BET bromodomains is important for accurate and reliable target validation in the different disease states that are associated with unbalanced activity of BET proteins.

The ET−L/A orthogonal inhibitor−protein pair was discovered and optimized within the framework of an extended study in which we explored several mutations (“holes”) and I-BET762 substitution patterns (“bumps”). In the current manuscript, we report the full journey that led to that discovery. In doing so, we also describe our synthetic efforts toward 6 and other novel analogues aimed at targeting the mutant proteins. In particular, we report new synthetic routes that we developed toward this aim, including I-BET762 mutant proteins. In particular, we report new synthetic routes that we developed toward this aim, including I-BET762 analogues bearing substitution patterns at the level of the methoxyphenyl and chlorophenyl rings, in addition to the side chain methylene. Finally, we present biophysical evaluation of the compounds within the context of our bump-and-hole project, and highlight useful isoform selectivity criteria for the design of the next generation of BET bromodomain inhibitors.

RESULTS AND DISCUSSION

Design of Engineered BET Bromodomain−Ligand Pairs and Synthetic Targets. Analyses of sequence alignments (Figure S1) and inhibitor-bound crystal structures11 guided us to focus on 11 residues that are strictly conserved within the BET subfamily and are in close contact with the ligand. Keeping in mind that the introduced mutations should not significantly disrupt protein stability and substrate binding, residues Y97, C136, Y139, and N140 (Brd4(1) numbering) were discarded, because they are known to be important for KAc recognition20−25 and for preserving a key network of bound water molecules.26 Buried residues P82 and F83 from the bottom of the so-called WPF shelf were also discarded because their mutation was predicted to destabilize the integrity of the hydrophobic core.27 Residues L92 and M149 looked promising but were not pursued further due to a lack of suitable vectors arising from the inhibitor scaffold that could be exploited to complement potential mutations. The remaining three residues, that is, the more peripheral hydrophobic W81 from the top of the WPF shelf and V87 and L94 from the ZA loop, were selected for site directed mutagenesis (Figure 2A). Mutants W/F, W/H, V/A, L/I, and L/A were constructed within Brd2(1), recombinantly expressed, purified from Escherichia coli and biophysically characterized in order to assess their stability and histone binding capacity (Table S1). All mutants maintained melting temperatures (Tm) above 37 °C, and most had comparable stabilities to the WT proteins, as assessed by differential scanning fluorimetry (DSF). Importantly, all mutants retained competence to bind a tetraacetylated H4 derived peptide32 as assessed by ITC albeit to varying degrees (Table S1). Most mutants exhibited comparable peptide binding affinities relative to WT, while the V/A proved the most disruptive mutation.

With three positions identified and corresponding mutants characterized, I-BET762 (1) was selected as the starting scaffold because it is more synthetically tractable and better suited to all required vectors than JQ1. Molecular modeling studies suggested that (i) a “bump” R1 originating from the methoxyphenyl ring could target the hole introduced by the V87A mutation, (ii) R2 functionalization at the level of the side chain methylene, in an (R) configuration, could target L94 mutations, and (iii) the p-chlorophenyl ring could provide suitable vectors for R3 substituents to explore W81 mutations (Figure 2B,C). A methyl group was selected as the bump of choice to explore the engineered holes because it represents the smallest hydrophobic functional group that at the same time introduces minimal alteration of the ligand scaffold in terms of electronics, conformation, and physicochemical properties. We therefore performed a “methyl scan” around the I-BET762 scaffold by synthesizing analogues functionalized with methyl groups at R1, R2, and R3 (Figure 2C) to target mutations at the respective positions.

Chemical Strategies To Target the V87A Mutation: R1 = Me. The ester derivative of I-BET762 was chosen as the parent scaffold for efficient enolate generation and substitution of the methylene side chain (R2, see later). Compound 14 was prepared as previously described, with significant yield improvement (55% overall) compared with those reported by Chung et al. (22% overall) (Scheme 1).8 Chlorination of protected acid 710 and N-acetylation of the appropriate amino-benzophenones 8 and 9, followed by Fmoc deprotection and subsequent cyclization afforded 10 and 11 in excellent yields in a four-step, one-pot sequence. Further thionation afforded thioamide derivatives 12 and 13. Treatment of thioamides 12 and 13 with hydrazine monohydrate, followed by acetylation and further cyclization in acidic conditions afforded triazoles 14 and 15 in high yield in a three-step, one-pot procedure. Starting from benzophenone derivative 9 allowed us to ultimately introduce the R1 methyl group (Figure 2C). While yields for the condensation and thionation reactions were excellent, the triazole formation toward 15 proceeded in only 38% yield, much lower than in the case of 14. This reflects the lower yield for the final cyclization (Scheme 1, step h), which only proceeded under reflux conditions, along with significant degradation. We attributed the latter to the steric demand

Scheme 1. Synthesis of 14 and Its Methylated Derivative 15a

| aConditions: | Conditions: | Conditions: | Conditions: | Conditions: | Conditions: | Conditions: | Conditions: |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| (a) SOCl2, CH2Cl2, reflux, 2.5 h | (b) benzophenone, CHCl3, reflux, 3 h | (c) Et3N, CHCl3, reflux, 16 h | (d) AcOH, 1,2-DCE, 60 °C, 3 h | (e) Lawesson’s reagent, toluene, reflux, 4 h | (f) hydrazine-H2O, THF, 0 °C, 5 h | (g) AcCl, Et3N, 0 °C to rt, 16 h | (h) AcOH, rt, 2 days (R = H) or reflux, 3 h (R = Me). |
imposed by the R₁ methyl group and the triazole methyl group in the cyclization process.

Chemical Strategies To Target the L94 Mutations: R₂ = Me. We envisaged that introduction of alkyl substituents on the methylene side chain would be achievable through the generation of the enolate of 14 followed by reaction with an alkyl halide (Scheme 2). Among the various bases explored for enolate generation, including LDA, NaH, and KHMDS, the latter proved the most efficient and provided the cleanest reaction and best yields. Treatment of (±)-14 with 1.2 equiv of KHMD at −78 °C, followed by addition of methyl iodide, afforded a diastereomeric mixture of (±)-(1S,2S)-16 and (±)-(1S,2R)-16 (Scheme 2A). The reaction provided (±)-(1S,2S)-16 as the major alkylation product and proved to be highly diastereoselective, for example, up to 25:1 with MeI. Such selectivity was strongly dependent on the temperature gradient, in certain cases down to 3:1. The structure of the major diastereomer resulting from the alkylation of (±)-14 with MeI could be unambiguously assigned as (±)-(1S,2S)-16 on the basis of previous crystallographic studies. We refer to the active stereomer (±)-(1S,2R)-16 as ME for clarity. The reason for this observed diastereoselectivity is unclear. The potassium countercation might provide conformational restriction to the (Z)-enolate in a six-membered ring transition state via coordination to the sp² nitrogen of the diazepine ring (Scheme 2C). However, in such a transition state the observed preference for the attack on the pro-S face is not evident simply based on steric, suggesting that other factors come into play. Further mechanistic studies will be needed to address this point. Nevertheless, when a high diastereomeric ratio did not allow for the isolation of reasonable amounts of the desired active (±)-(1S,2R)-16 diastereomer in pure form, the diastereomeric mixture could be readily epimerized with sodium methoxide under microwave irradiation to afford a 1:1 mixture of diastereomers, which could then be separated by flash column chromatography.

Along with alkylation of the side chain, we observed minor albeit observable alkylation of the ternary C1 carbon of the diazepine ring, affording derivative (±)-17. This suggests that the C1 position of (±)-14 is deprotonated, at least partially, during addition of KHMD at −78 °C. This is consistent with the intense dark color observed following addition of KHMD, potentially reflecting the generation of a highly delocalized anionic species. During their study of the memory of chirality in related 1,4-benzodiazepin-2-one systems, the Carlier group documented the installment of quaternary centers at C1 using enolate alkylation chemistry. Notably, they also observed superior results when using KHMD as a base for enolate generation. Due to the “privileged” status attributed to the 1,4-benzodiazepin-2-one scaffold in drug discovery, this represented an important finding because it offered the first short and robust route toward novel, conformationally restricted 1,4-benzodiazepin-2-one analogues. We here show that installment of a quaternary center at C1 on a triazolo-benzodiazepine scaffold can indeed be achieved, although in low yield. This can be particularly attractive if one wants to develop structure–activity relationships of BET bromodomain binders (e.g., 1–4) through double functionalization at C1. In particular, this would represent a real advantage to the use of quaternary amino acid precursors, which are sterically hindered and likely to reduce the overall yield, in addition to being expensive and providing a narrow scope for substitution. Moreover, this would offer a late state divergent synthetic strategy toward library analogues. However, the usefulness and general applicability of such a strategy will be contingent on more robust reaction conditions and improved yields. Further optimization studies are ongoing in our laboratories in order to tune the regioselectivity, reaction times, and yields of this key reaction. Of note, the ¹H NMR spectrum of (±)-17 showed two species in a ca. 2:1 ratio. This is reflective of the slow conformational equilibrium imposed by the steric demand at C1, in line with Carlier’s results.
Chemical Strategies To Target the W81 Mutations: Derivatization of the Chlorophenyl Ring.

We envisaged that developing new synthetic routes toward the I-BET scaffold would be of particular interest to gain rapid access to libraries of analogues to address other mutational positions in the binding pocket. Specifically, we next explored the possibility to access I-BET762 analogues with diverse substitution patterns at the level of the chlorophenyl ring (Figure 2C). While this should be potentially achievable through previously reported routes (Scheme 1), the early stage introduction of the chlorophenyl ring makes this linear sequence very impractical for analogue generation. We therefore considered that a new route allowing the late stage introduction of the substituted phenyl moiety would be valuable. We hypothesized that such analogues would be obtained by Suzuki–Miyaura cross coupling of an imidoyl chloride with an appropriate phenylboronic acid derivative (Scheme 3). A wide variety of phenylboronic acid derivatives are commercially available, readily accessible, and affordable. The imidoyl chloride would be obtained by chlorination of the corresponding amide with an appropriate phenylboronic acid derivative. The triazole moiety would in turn be introduced from the corresponding amide, through a thionation/condensation/cyclization sequence. The diamide would be obtained through condensation of inexpensive 5-methoxyisatoic anhydride and aspartic acid dimethyl ester.

The synthesis of our library of analogues is shown in Scheme 4. 2-Amino-5-methoxybenzoic acid was converted to 5-methoxyisatoic anhydride in quantitative yield. Condensation of 19 with aspartic acid dimethyl ester afforded the bicyclic precursor 20 in 42% yield. Selective thionation could be achieved by treatment with Lawesson’s reagent in refluxing pyridine, affording thioamide 21 in 48% yield. We envisaged that a one-step procedure for the installment of the triazole would be particularly convenient compared with the three-step procedure employed previously (Scheme 1). A representative set of conditions for the installment of the triazole moiety is shown in Table 1. Reaction outcome was assessed by NMR of crude mixtures. Thioamide 21 was poorly soluble in a variety of solvents but was soluble in refluxing pyridine. Treatment of 21 with 2.5 equiv of acethydrazide for 1 day at reflux led to the formation of product 22, along with remaining unreacted 21 and significant formation of the exocyclized product arising from condensation of the intermediate acylhydrazone with the thioamide.

### Table 1. Representative Conditions for Triazole Formation (Conversion of 21 to 22)

| entry | conditions | outcome |
|-------|------------|---------|
| 1     | 2.5 equiv of AcNHNH₂, pyridine, reflux ∼1 d | mix. 21 (32%) + 22 (43%) + exocyclization (25%) |
| 2     | 6 equiv of AcNHNH₂, pyridine, reflux ∼1 d | mix. 21 (22%) + 22 (29%) + exocyclization (49%) |
| 3     | 3 equiv of AcNHNH₂, pyridine, rt, 1 d | 21 insoluble |
| 4     | 3 equiv of AcNHNH₂, 1.5 equiv of Hg(OAc)₂, pyridine, rt, 1 d | acylhydrazone (>95%) + 22 (<5%) |
| 5     | 3 equiv of AcNHNH₂, 1.5 equiv of Hg(OAc)₂, MeCN, rt, 6 d | acylhydrazone (87%) + 22 (15%) |
| 6     | 3 equiv of AcNHNH₂, 1.5 equiv of Hg(OAc)₂, THF/AcOH, rt, 24 h | 91% 22 |

*Yields for entries 1–5 were determined based on NMR spectra of crude samples. The yield for entry 6 is for the isolated, purified material.*
Table 2. “Methyl Scan”a

| bromodomain protein | I-BET762 (1) | 15 | 16b | MEc | 24 | 25 | 26 | 27 | 28 |
|---------------------|-------------|----|-----|-----|----|----|----|----|----|
| Brd2(1)             | 5.4 ± 0.5   | 0.7 ± 0.2 | −0.3 ± 0.2 | 3.2 ± 0.2 | 6.3 ± 0.1 | 1.5 ± 0.2 | 1.8 ± 0.2 | 1.2 ± 0.2 | 6.8 ± 0.6 |
| Brd2(1)_{P35A}      | 0.1 ± 0.6   | 0.5 ± 0.3 |
| Brd2(1)_{L110I}     | 6.7 ± 0.4   | 0.0 ± 0.5 | 5.7 ± 0.7 |
| Brd2(1)_{L110A}     | 3.1 ± 0.4   | 1.6 ± 0.2 | 7.9 ± 0.2 |
| Brd2(1)_{W47PF}     | 0.4 ± 0.2   | 1.4 ± 0.2 | −0.1 ± 0.2 | 0.1 ± 0.2 | 0.0 ± 0.2 | 1.9 ± 0.5 |
| Brd2(1)_{W47PH}     | 0.7 ± 0.2   | 0.9 ± 0.3 | 0.2 ± 0.2 | −0.4 ± 0.3 | −0.4 ± 0.2 | 0.6 ± 0.3 |
| Brd2(2)             | 8.3 ± 0.3   | 4.0 ± 0.1 | 0.2 ± 0.2 | 5.6 ± 0.1 | 6.6 ± 0.2 | 3.2 ± 0.1 | 3.5 ± 0.1 | 2.5 ± 0.2 | 7.7 ± 0.2 |
| Brd2(2)_{P35A}      | 1.1 ± 0.0   | 1.2 ± 0.1 |
| Brd2(2)_{L383I}     | 9.3 ± 0.3   | 0.3 ± 0.2 | 9.6 ± 0.1 |
| Brd2(2)_{L383A}     | 6.4 ± 0.2   | 0.8 ± 0.6 | 9.3 ± 0.2 |
| Brd2(2)_{W77AF}     | 2.1 ± 0.0   | 2.8 ± 0.1 | 1.5 ± 0.0 | 0.6 ± 0.1 | 0.3 ± 0.0 | 5.2 ± 0.1 |
| Brd2(2)_{W77AH}     | 1.7 ± 0.2   | 1.1 ± 0.2 | 1.0 ± 0.3 | −0.1 ± 0.1 | −0.4 ± 0.2 | 2.7 ± 0.4 |

aThermal stabilization (°C) of wild-type and mutant Brd2 bromodomains by I-BET derivatives 15, 16, 24–28, as assessed by DSF. 

| K_d (nM) | ΔH (kcal/mol) |
|---------|---------------|
| Brd2(1) | 1470 ± 180 | −8.6 ± 0.2 |
| Brd2(1)_{L110I} | 260 ± 40 | −8.5 ± 0.1 |
| Brd2(1)_{L110A} | 17 ± 4 | −16.8 ± 0.2 |
| Brd2(2) | 300 ± 80 | −5.4 ± 0.1 |
| Brd2(2)_{L383I} | 27 ± 12 | −9.8 ± 0.1 |
| Brd2(2)_{L383A} | 22 ± 4 | −12.6 ± 0.1 |

Some side chain ester (Table 1, entry 1). Increasing the number of equivalents of acetylhydrazide resulted in low formation of 22 and afforded the exocyclized byproduct as the major component of the reaction (Table 1, entry 2). We envisaged that exocyclization could be prevented by lowering the reaction temperature. However, the reaction did not proceed due to the poor solubility of 21 in pyridine at rt (Table 1, entry 3). Despite its poor solubility, activation of the thioamide with Lawesson’s reagent and a reference sample. This synthetic route proved to be robust and reasonably scalable, allowing preparation of imidoyl chloride in 91% yield after 22 h reaction. Other methods involving chlorophosphate reagents have been previously reported for the installment of the triazole unit of JQ1, although in those cases the amide derivatization step required cooling to −78 °C and the subsequent cyclization step required heating up to 90 °C.7,35 Despite the toxicity of the mercury reagent, our one-step procedure is particularly convenient because it is milder, lowers the reaction time by ca. 3-fold compared with previously published methods.7,35

Table 3. Affinities (K_d’s) and Binding Enthalpies (ΔH) Obtained by ITC for ME against Wild-Type and L/A and L/ I Mutant Brd2 Bromodomains at 25 °C

| bromodomain protein | K_d (nM) | ΔH (kcal/mol) |
|---------------------|----------|---------------|
| Brd2(1)             | 1470 ± 180 | −8.6 ± 0.2 |
| Brd2(1)_{L110I}     | 260 ± 40 | −8.5 ± 0.1 |
| Brd2(1)_{L110A}     | 17 ± 4 | −16.8 ± 0.2 |
| Brd2(2)             | 300 ± 80 | −5.4 ± 0.1 |
| Brd2(2)_{L383I}     | 27 ± 12 | −9.8 ± 0.1 |
| Brd2(2)_{L383A}     | 22 ± 4 | −12.6 ± 0.1 |

Highly potent against both leucine mutants, displaying K_d’s of 17 and 22 nM against Brd2(1)_{L110I} and Brd2(2)_{L383A}, and K_d’s of 260 and 27 nM against Brd2(1)_{L110A} and Brd2(2)_{L383I}, respectively. Crucially, ME showed between 11-fold and 8-fold weaker affinities to WT compared with the leucine mutant proteins. As we anticipated, the diastereoisomer (±)-(1S,2S)-
16 did not induce a significant stabilization of mutant or WT proteins (Table 2).

Synthetic optimization of ME led to the highly potent and L/A mutant selective compound ET (6) (Figure 1C), data that we have reported elsewhere. A complete binding selectivity profiling by DSF and ITC against all eight WT BET bromodomains and their L/A mutant counterparts showed that ET binds up to 540-fold more strongly and not less than 30-fold (average 160-fold) to L/A mutants compared with WT proteins, therefore validating our design strategy. Importantly, selective targeting of engineered L/A mutants could be achieved in a cellular context, as demonstrated using a fluorescence recovery after photobleaching assay.

While indole derivative 28 could only induce moderate stabilization of W/F and W/H mutants, we noted that 28 greatly stabilized WT Brd2(1) and Brd2(2) (Table 2). We therefore decided to further characterize 28 and determined its binding affinity to Brd2(1) and Brd2(2) by ITC (Table 4). Compound 28 exhibited $K_d$'s of 800 and 40 nM against Brd2(1) and Brd2(2), respectively, corresponding to ca. 20-fold selectivity for the second over the first bromodomain. The same trend in selectivity was observed with the two bromodomains of Brd4 (Table 4). This selectivity of 28 for the second BET bromodomain could result at least in part from amino acid changes in the BC loop flanking the inhibitor binding site. In particular, an aspartate residue in the BC loop (Asp160 in Brd2(1)) is conserved among all first BET bromodomains and conservatively replaced by a histidine residue in the second BET bromodomains (His433 in Brd2(2), highlighted in Figure S1). To test this hypothesis, we solved the X-ray crystal structures of Brd2(2)$_{W370F}$ in its apo form and with both 28 and the parent I-BET762 (1) bound (Figure 3B). All structures, as shown in Figure S1, data highlight that such isoform selectivity can be achieved with the I-BET762/JQ1 scaffold via careful substitution of the parent chlorophenyl ring. This adds a useful isoform selectivity criterion that can be exploited for the design of next generation triazolo-benzodiazepine probes targeting BET proteins.

![Figure 3](image-url)

**Figure 3.** (A) Co-crystal structure of Brd2(2)$_{W370F}$ (transparent surface representation) in complex with 28 (PDB code 5DFD, stick representation, orange carbons) superimposed with the cocrystal structure of Brd2(2)$_{W370F}$ in complex with I-BET762 (1) (PDB code 5DFD, stick representation, yellow carbons). The side chain of His433 switches from an “open” conformation when bound to I to a “closed” conformation when bound to 28, engaging in an edge-to-face interaction with 28. (B) Co-crystal structure of Brd2(2)$_{W370F}$ in complex with 28 (PDB code 5DFD, stick representation, orange carbons) superimposed with the cocrystal structure of Brd2(1) (transparent surface representation) in complex with I-BET762 (1) (PDB code 2YEK, stick representation, pink carbons). All structures show a conserved scaffold binding mode in the K(Ac) pocket.
Here, we have described novel synthetic analogues of the triazolo-aryldiazepine-based bromodomain inhibitor I-BET762. We were able to introduce substitutions at the level of the methoxyphenyl ring, the ternary carbon center, the side chain methylene, and the chlorophenyl moiety. The design and development of the analogue series was aimed at targeting a number of specific BET bromodomain mutants with high selectivity compared with wild-type via a bump-and-hole approach. Among the “bumped” compounds reported, ME and ET achieved the highest selectivity levels targeting mutations at the Leu94 position. Several interesting chemistries were developed in the process that will potentially see useful applications. For example, we showed that alkylation at the ternary center and the side chain methylene could be achieved, and that a high level of stereocentre could be achieved during enolate alkylation. We also developed a new route allowing late stage diversity introduction at the level of the chlorophenyl ring. An indole analogue (28) was highly potent and displayed a marked BD2 selectivity profile by exploiting the aspartate/histidine substitution in the bromodomain BC loop. Taken together, we anticipate that our findings should be of broad interest, not only to other researchers working in the field of epigenetics and bromodomain inhibition but also to medicinal chemists focusing on related benzodiazepine systems.

CONCLUSIONS

Acknowledgments

This work was supported by awards to A.C. from the UK Biotechnology and Biological Sciences Research Council (BB/SRC, Grants BB/J001201/1 and BB/J001201/2 and David Phillips Fellowship BB/G023123/1 and BB/G023123/2). E.L.S. was supported by a European Commission Erasmus work placement grant. Biophysics and drug discovery activities at Dundee are supported by a Wellcome Trust strategic award 100476/Z/12/Z to the Division of Biological Chemistry and Drug Discovery. We thank Stefan Knapp and his team at the Oxford Structural Genomics Consortium for providing expression vectors and the staff at the Diamond Light Source synchrotron for providing support on beamlines I02 and I03.

Abbreviations

ΔT_m, thermal shift; AcOH, acetic acid; ATP, adenosine triphosphate; BET, bromodomain and extra-terminal; Brd, bromodomain; DSF, differential scanning fluorimetry; FDA, Food and Drug Administration; ITC, isothermal titration calorimetry; K(ΔC), acetyl-lysine; K_d, dissociation constant; KHMD5, potassium hexamethyldisilazane; MeOH, methanol; NMR, nuclear magnetic resonance; NUT, nuclear protein in testis; THF, tetrahydrofuran; T_m, melting temperature; WT, wild-type

References

1. Sternbach, L. H. The benzodiazepine story. J. Med. Chem. 1979, 22, 1–7.
2. Sternbach, L. H.; Fryer, R. L.; Metlesics, W.; Reeder, E.; Sach, G.; Saury, G.; Stempel, A. Quinazolines and 1,4-Benzodiazepines. VI.1a Halo-, Methyl-, and Methoxy-substituted 1,3-Dihydro-5-phenyl-2H-1,4-benzodiazepin-2-ones1b,c. J. Org. Chem. 1962, 27, 3788–3796.
3. Sternbach, L. H. The Benzodiazepines; Raven Press: New York, 1973.
4. Ellman, J. A. Design, Synthesis, and Evaluation of Small-Molecule Libraries. Acc. Chem. Res. 1996, 29, 132–143.
5. Filipppakopoulos, P.; Knapp, S. Targeting bromodomains: epigenetic readers of lysine acetylation. Nat. Rev. Drug Discovery 2014, 13, 337–356.
6. Nicodeme, E.; Jeffrey, K. L.; Schafer, U.; Benke, S.; Dewell, S.; Chung, C.; Chandwani, R.; Marazzi, I.; Wilson, P.; Coste, H.; White, J.; Kirilovsky, J.; Rice, C. M.; Lora, J. M.; Pinjha, R. K.; Lee, K.; Tarakhovsky, A. Suppression of inflammation by a synthetic histone mimic. Nature 2010, 468, 1119–1123.
7. Filipppakopoulos, P.; Qi, J.; Picaud, S.; Shen, Y.; Smith, W. B.; Fedorov, O.; Morse, E. M.; Keates, T.; Hickman, T. T.; Fattler, 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, 1067–1073.
8. Chung, C. W.; Coste, H.; White, J. H.; Mirguet, O.; Wilde, J.; Gosmini, R. L.; Delves, C.; Magny, S. M.; Woodward, R.; Hughes, S. A.; Bouris, E. V.; Flynn, H.; Boullot, A. M.; Bamborough, P.; Brusq, J. M.; Gellibert, F. J.; Jones, E. J.; Riou, A. M.; Homes, P.; Martin, S. L.; Uings, I. J.; Toum, J.; Clement, C. A.; Boullay, A. B.; Grimley, R. L.; Blandel, F. M.; Pinjha, R. K.; Lee, K.; Kirilovsky, J.; Nicodeme, E. Discovery and characterization of small molecule inhibitors of the BET family bromodomains. J. Med. Chem. 2011, 54, 3827–3838.
9. Herait, P.; Dombret, H.; Thieblemont, C.; Facon, T.; Stathis, A.; Cunningham, D.; Palumbo, A.; Vey, N.; Michallet, M.; Recher, C.; Retai, K.; Preudhomme, C. OTX015: Final results of the dose-finding part of a phase I study in hematologic malignancies. Ann. Oncol. 2015, 26 (suppl2), ii10.
10. McLure, K. G.; Gesner, E. M.; Tsukikawa, L.; Kharenko, O. A.; Attwell, S.; Campeau, E.; Wasia, S.; Stein, A.; White, A.; Fontano, E.;
(11) Baud, M. G.; Lin-Shiao, E.; Cardote, T.; Tallant, C.; Pechibur, A.; Chan, K.-H.; Zengerle, M.; Garcia, J. R.; Kwan, T. T. L.; Ferguson, F. M.; Ciulli, A. A Bump-and-Hole Approach to Engineered Controlled Selectivity of BET Bromodomain Chemical Probes. Science 2014, 346, 638–641.

(12) Prinjha, R. K.; Witherington, J.; Lee, K. Place your BETs: the therapeutic potential of bromodomains. Trends Pharmacol. Sci. 2012, 33, 146–153.

(13) A study to investigate the safety pharmacokinetics, pharmacodynamics, and clinical activity of GSK525762 in subjects with NUT midline carcinoma (NMC). https://clinicaltrials.gov/identiferNCT01587703.

(14) Delmore, J. E.; Issa, G. C.; Lemieux, M. E.; Rahi, P. B.; Shi, J.; Jacobs, H. M.; Kastritis, E.; Cardote, T.; Tallant, C.; Paranal, R. M.; Qi, J.; Chesi, M.; Schinzel, A. C.; McKeown, M. R.; Heffernan, T. P.; Zengerle, M.; Garcia, J. R.; Kwan, T. T. L.; Ferguson, RVX-208, an Inducer of ApoA-I in Humans, Is a BET Bromodomain Pharmacodynamics, and Clinical Activity of GSK525762 in Subjects,identi

(15) Dawson, M. A.; Prinjha, R. K.; Dittmann, A.; Girotopoulos, G.; Bantscheff, M.; Chan, W.; Robson, S. C.; Chung, C.; Hopf, C.; Savitski, M. M.; Huthmacher, C.; Gudgin, E.; Lugo, D.; Beinke, S.; Chapman, T. D.; Roberts, E. J.; Soden, P. E.; Auger, K. R.; Mieguet, O.; Doehner, K.; Dehel, R.; Burnett, A. K.; Jeffrey, P.; Drewes, G.; Lee, K.; Hunty, B. J. P.; Kouzarides, T. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 2011, 478, 529–533.

(16) Zuber, J.; Shi, J.; Wang, E.; Rappaport, A. R.; Herrmann, H.; Sison, E. A.; Magoon, D.; Qi, J.; Blatt, K.; Wunderlich, M.; Taylor, M. J.; Johns, C.; Chicas, A.; Mulloy, J. C.; Kogan, S. C.; Brown, P.; Valet, P.; Bradner, J. E.; Lowe, S. W.; Vaclav, C. R. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature 2011, 478, 524–528.

(17) Mertz, J. A.; Conery, A. R.; Bryant, B. M.; Sandy, P.; Balasubramanian, S.; Mele, D. A.; Bergeron, L.; Sim, R. J. Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc. Natl. Acad. Sci. U. S. A. 2011, 108, 16669–16674.

(18) Shah, K.; Liu, Y.; Deirmengian, C.; Shokat, K. M. Engineering unnatural nucleotide specificity for Rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. Proc. Natl. Acad. Sci. U. S. A. 1997, 94, 3565–3570.

(19) Bishop, A. C.; Ubersax, J. A.; Petsch, D. T.; Mathews, D. P.; Gray, N. S.; Blethow, J.; Shimizu, E.; Tsien, J. Z.; Schultz, P. G.; Rose, M. D.; Wood, J. L.; Morgan, D. O.; Shokat, K. M. A chemical switch for inhibitor-sensitive alleles of any protein kinase. Nature 2000, 407, 395–401.

(20) Dhalluin, C.; Carlson, J.; Zeng, L.; He, C.; Aggarwal, A.; Zhou, M. Structure and ligand of a histone acetyltransferase bromodomain. Nature 1999, 399, 491–496.

(21) Mujtaba, S.; He, Y.; Zeng, L.; Farooq, A.; Carlson, J.; Ott, M.; Verdin, E.; Zhou, M. Structural basis of lysine-acetylated HIV-1 Tat recognition by PCAF bromodomain. Mol. Cell 2002, 9, 575–586.

(22) Mujtaba, S.; He, Y.; Zeng, L.; Yang, T.; Plotnikov, O.; Sachidanand; Sanchez, R.; Zeleznik-Le, N.; Ronai, Z.; Zhou, M. Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation. Mol. Cell 2004, 13, 251–263.

(23) Huang, H.; Zhang, J.; Shen, W.; Wang, X.; Wu, J.; Wu, J.; Shi, Y. Solution structure of the second bromodomain of Brd2 and its specific interaction with acetylated histone tails. BMC Struct. Biol. 2007, 7, 57.

(24) Shen, W.; Xu, C.; Huang, W.; Zhang, J.; Carlson, J.; Tu, X.; Wu, J.; Shi, Y. Solution structure of human Brd1 bromodomain and its specific binding to acetylated histone tails. Biochemistry 2007, 46 (8), 2100–2110.

(25) Liu, Y.; Wang, X.; Zhang, J.; Huang, H.; Ding, B.; Wu, J.; Shi, Y. Structural basis and binding properties of the second bromodomain of Brd4 with acetylated histone tails. Biochemistry 2008, 47, 6403–6417.