[1,2,4]Triazolo[4,3-α]phthalazines: Inhibitors of Diverse Bromodomains

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Supporting Information

ABSTRACT: Bromodomains are gaining increasing interest as drug targets. Commercially sourced and de novo synthesized substituted [1,2,4]triazolo[4,3-α]phthalazines are potent inhibitors of both the BET bromodomains such as BRD4 as well as bromodomains outside the BET family such as BRD9, CECR2, and CREBBP. This new series of compounds is the first example of submicromolar inhibitors of bromodomains outside the BET subfamily. Representative compounds are active in cells exhibiting potent cellular inhibition activity in a FRAP model of CREBBP and chromatin association. The compounds described are valuable starting points for discovery of selective bromodomain inhibitors and inhibitors with mixed bromodomain pharmacology.

INTRODUCTION

The rapidly expanding field of epigenetics can be broadly divided into two levels of processes: DNA methylation and histone modification. Various post-translational modifications of histone proteins contribute to the epigenetic code including methylation, acetylation, phosphorylation, ubiquitinylation, and citrullination.1 Acetylation of lysine residues plays an important role in the regulation of chromatin structure and ultimately transcription due to the charge neutralization that occurs, leading to changes in protein conformation and protein–protein interactions. It is similar to phosphorylation in its prevalence and has been particularly studied on unstructured histone tails. Aberrant lysine acetylation frequently leads to a hydrogen bond acceptor which mimics the acetyl group of acetylated lysine. The structurally related thieno- and benzo-diazepine triazoles (+)-JQ1 and I-BET762 (Figure 1A) were shown to be potent inhibitors of the BET bromodomains and acetyl lysine. The rapidly expanding field of epigenetics can be broadly divided into two levels of processes: DNA methylation and histone modification. Various post-translational modifications of histone proteins contribute to the epigenetic code including methylation, acetylation, phosphorylation, ubiquitinylation, and citrullination.1 Acetylation of lysine residues plays an important role in the regulation of chromatin structure and ultimately transcription due to the charge neutralization that occurs, leading to changes in protein conformation and protein–protein interactions. It is similar to phosphorylation in its prevalence and has been particularly studied on unstructured histone tails. Aberrant lysine acetylation frequently leads to aberrant expression of bromodomain-containing proteins, and protein dysfunctions have been tightly linked to tumorigenesis,6 and new avenues for the development of antineoplastic drugs have recently been highlighted by the potent antitumor activity exhibited by inhibitors which selectively target bromodomains.7 Known bromodomain inhibitors mainly target the BET family of bromodomains, including BRD3 and BRD4, but many other bromodomain-containing proteins such as CREBBP, TIF1α, ATAD2, and SMARCA4 have been implicated in a variety of diseases.4

Potent BET inhibitors generally fall into three structural classes: isoxazoles,8 amides/ureas,9 and 1,2,4-triazoles.5 Most BET inhibitors described to date have a methyl group adjacent to a hydrogen bond acceptor which mimics the acetyl group of acetyl lysine. The structurally related thieno- and benzo-diazepine triazoles (+)-JQ1 and I-BET762 (Figure 1A) were shown to be potent inhibitors of the BET bromodomains and have potential for use in inflammatory disease,10 atherosclerosis,11 NUT-midline carcinoma,7 acute leukemia,12 lympho-
function and therapeutic potential.
containing proteins in order to investigate their biological
for inhibitors for the remaining subfamilies of bromodomain-
bromodomains in disease, it is clear that there is an urgent need
BET762 in deciphering the role of the BET subfamily of
triazoles (compounds 3, compound
Figure 1. (A) Triazole-containing BET inhibitors. (B) The
triadolophthalazines shared an amide or sulfonamide sub-
ative scanning fluorimetry (DSF) (Figure 1B).7,18 The
large italic).17 Family members screened in this work are shown in larger typeface.
ma,13 and HIV infection.14 Two structurally related fused
triazoles (compounds 3 and 4) have been exemplified in patents from GSK15 and Constellation16 as potent BET inhibitors.
Given the success of the chemical probes (+)-JQ1 and I-
BET762 in deciphering the role of the BET subfamily of
bromodomains in disease, it is clear that there is an urgent need for inhibitors for the remaining subfamilies of bromomain-containing proteins in order to investigate their biological function and therapeutic potential.

RESULTS AND DISCUSSION
The selectivity of (+)-JQ1 and I-BET762 for the BETs has
been attributed to the 4-chlorophenyl moiety which forms
hydrophobic interactions with residues on the edge of the
acetyl-lysine binding pocket including W81. It was hoped that
by keeping the 3-methyl-[1,2,4]-triazolo motif but varying the
fused ring and pendant substituents, novel compounds could be
found that would maintain bromodomain potency with altered
selectivity for non-BET proteins. To find new starting points
for bromodomains outside the BET family, a number of
triazole-containing commercial compounds were purchased and
profiled against 17 BRDs in the bromodomain family tree by
differential scanning fluorimetry (DSF) (Figure 1B).7,18 The
[1,2,4]triazolo[4,3-a]phthalazines shown in Figure 2 were
thought to be attractive potential BRD inhibitors due to the
presence of the 5-methyl group adjacent to the triazole H-bond
acceptor.19 It has been shown by Chung et al. that potent BRD
inhibitors can be discovered by focusing on privileged
substructures.20 By testing the potential inhibitors against
bromodomains from the entire protein family by the opera-
tionally simple DSF assay in a platform discovery approach, a
rapid assessment of BRD potency and selectivity was
established.

When tested in a panel of bromodomains, these initial
compounds showed hits for BRD4(1), BRD9, CECR2,
CREBBP, and TAF1L(2), with the greatest potencies against
BRD9 (compounds 7 and 15) and CREBBP (compounds 9,
14, and 17). Very little activity was observed against BAZ2A,
BAZ2B, PB1(5), and TIF1α. All of the commercially available
triazolophthalazines shared an amide or sulfonamide sub-
stituent at the meta-position (R₁) and a methyl group at the
para-position (R₂) of the pendant phenyl group. Sulfonamides
were observed to have greater potency than amides (e.g.,
compounds 7 and 15 vs compound 5). N- and S-linked
sulfonamides were found to have similar potencies (compound
7 vs 15). A variety of substituents were tolerated on the
sulfonamide, both aryl and alkyl. To examine the effect of the
omnipresent para-methyl group in the commercial compounds,
compound 13 was synthesized (Scheme 1). This methyl group
was shown to positively influence activity as the des-methyl
anologue 13 was less active in the DSF assay against all
bromodomains tested.

As these compounds showed binding to multiple bromodo-
mains, compound 17 was chosen as a representative for
docking into the bromodomain of CREBBP. Although there
were other compounds as potent as compound 17, it was
chosen for docking studies as it had a good combination of high
potency and rigidity which provided a small number of
calculated poses. All but the five water molecules previously
shown to be important in CREBBP²⁶ were removed from the
published complex of CREBBP with a fragment ligand (PDB
ID 3SVH), and compound 17 was docked into the protein
using the ligedit functionality of ICM-Pro.²³ As seen in Figure
3, compound 17 has an excellent fit to the bromodomain. The
triazole forms two hydrogen bonds via adjacent nitrogen atoms
to a structural water molecule and the conserved asparagine
residue (N1168) found in most bromodomains. The triazole’s
methyl group fits well into the cavity formed by the remaining
water molecules. The meta-sulfonamide formed two hydrogen
bonds to arginine (R1173) in CREBBP.

To confirm the potencies initially established by DSF,
AlphaScreen competition assays were used to test whether
representative compounds 6–8 and 15 could displace labeled
peptides from seven representative bromodomains (Table 1).²²
These compounds were chosen as representatives as they all
had similar structures but differed in their bromodomain
inhibition profiles. With one exception (compound 8 with

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CREBBP), the pIC50 values measured for the representative compounds were consistent with DSF Tm shifts (ΔTm). The coefficient of determination (R²) between the two assay formats was high enough that the general and operationally simple DSF assay was felt to be a useful surrogate for the more complex AlphaScreen in further efforts to increase the potency of the compounds.

With an interesting series of inhibitors discovered through compound purchasing, additional analogues were designed and synthesized as shown in Scheme 1. Initial analogues focused on optimizing compounds 12 and 17 for CECR2 and CREBBP potency. Compound 12, although not the most potent analogue, had a preference for CECR2 and CREBBP by DSF Tm shift. Compound 17 was an attractive CREBBP lead due to its potency and synthetic accessibility. The fused methyl triazole ring was formed by substitution of 1,4-dichlorophthalazine 18 with acetyl hydrazide and in situ condensation. Suzuki coupling of the resulting aryl chloride with boronic ester 35 gave compound 13. Stepwise chloride displacement of compound 18 with hydrazine and subsequent condensation with carboxylic acids gave more elaborate substitution at the 2-position of the triazole in compounds 24–28 following Suzuki reaction. It was hoped that triazole substituents larger than methyl might compensate for the loss of activity on CECR2.

Figure 2. Commercial [1,2,4]triazolo[4,3-a]phthalazines are potent inhibitors of multiple bromodomains by DSF screening.
and CREBBP seen between compounds 12 and 13 by displacing conserved waters in the bromodomain pocket (blue sphere in Figure 3). The hydrophobic ethyl and cyclopropyl groups of compounds 24 and 25, and hydrogen bonding groups of compounds 26 and 28 provided no additional binding beyond the methyl group of compound 13 and could not rescue the loss of the para-methyl group from compound 12 (Figure 4).17

The next group of analogues explored alternative additional substituents to the aryl sulfonamide. Compound 29 was made from the commercially available sulfonamide-containing boronic ester 36. To examine the effect of a chlorine in the 6-position of the phenyl ring, 2-chloro-5-nitrophenylboronic acid 37 was coupled to intermediate 19. Reduction and sulfonylation gave compound 30. Although compound 29 showed only weak binding to all bromodomains, compound 30 had modest binding to BRD4(1) and CREBBP.

As highlighted in Figure 3, CREBBP has an arginine (R1173) at the mouth of the acetylated peptide binding pocket and it was thought that the arylsulfonamide (calculated pKₐ 8.1)²₃ of compound 15 might be interacting with this charged arginine. Carboxylic acid-containing compounds were synthesized to try to exploit a potential salt bridge to R1173 and create CREBBP selective inhibitors. Compounds 31–33 were synthesized from compound 19 via Suzuki reaction with boronic acids 38–40 and hydrolysis of the methyl esters if required. Of the three, compound 33 with its extended acid was the most active against CREBBP but did not show selectivity over BRD4(1).

Comparing compound 33 to compounds 12 and 13 showed that the acetic acid group could compensate to some extent for the loss of activity going from 12 to 13 and turn a CECR2 and CREBBP favoring inhibitor into a BRD4(1) and CREBBP inhibitor. Removing all substituents from the 6-phenyl group gave the simplest molecule described so far, compound 34, which, although it is only a modest inhibitor, has an intriguing selectivity for BRD9.²⁴

The structure–activity relationship (SAR) established thus far had highlighted the importance of a group at the 4-position of the 6-phenyl substituent and the utility of a sulfonamide at the 3-position. The potent and lipopholic analogues in Figure 1, Scheme 1. The Synthesis of Compounds 13 and 24−33

“Reagents and conditions: (a) NH₂NHC₆H₅, nBuOH, reflux (41%); (b) NH₂NH₂ THF; (c) R₆CO₂H, p-dioxane, reflux (32−42%); (d) Boc-glycine, THF, reflux (39%); (e) 35, Pd(PPh₃)₄, K₂CO₃, p-dioxane/H₂O (29−80%); (f) HCl, EtOAc (100%); (g) 36, Pd(PPh₃)₄, K₂CO₃, p-dioxane/H₂O (25%); (h) (i) 37, Pd(PPh₃)₄, K₂CO₃, p-dioxane/H₂O, (ii) SnCl₂, EtOH, reflux, (iii) PhSO₂Cl, pyridine, THF (16% over 3 steps); (i) 39, Pd(PPh₃)₄, K₂CO₃, p-dioxane/H₂O (35%); (j) (i) 38 or 40, Pd(PPh₃)₄, K₂CO₃, p-dioxane/H₂O, (ii) KOH, MeOH (39−79% over 2 steps).
Table 1. pIC₅₀ of Representative Compounds As Measured by AlphaScreen and ΔTₘ As Measured by DSF

| Target | ΔTₘ°C± | pIC₅₀ | ΔTₘ°C± | pIC₅₀ | ΔTₘ°C± | pIC₅₀ | ΔTₘ°C± | pIC₅₀ |
|--------|--------|-------|--------|-------|--------|-------|--------|-------|
| BRD4(1)| 1.4±0.40(3) | 4.7(6.4–4.8) | 2.2±0.15(3) | 5.7(6.5–5.8) | 1.7±0.08(3) | 5.9(5.9–5.9) | 4.3±0.16(3) | 5.3±0.54(2) |
| BRD9  | 2.1±0.56(3) | ND    | 3.6±0.31(3) | 5.9(5.7–6.1) | 1.8±0.22(3) | 5.8(5.6–6.7) | 5.6±0.30(3) | 6.3±0.12(2) |
| CECR2 | 1.5±0.54(3) | 5.3(5.1–5.5) | 2.4±0.73(3) | 5.5(4.9–6.0) | 3.2±0.87(3) | 6.6(6.5–6.7) | 3.3±0.14(3) | 6.3±0.17(2) |
| CREBBP| 1.6±0.10(3) | 5.2±0.48(2) | 1.9±0.12(3) | 5.8(5.5–6.1) | 0.7±0.1(3) | 5.9(5.7–6.2) | 2.8±0.13(3) | 4.8±0.06(1) |
| BAZ2B | 0.03±0.03(3) | <4d   | 0.23±0.09(3) | <4d   | 0.20±0.11(3) | <4d   | -0.03±0.18(3) | <4d   |
| PB1(5) | 0.16±0.03(3) | <4d   | -0.18±0.13(3) | <4d   | -0.33±0.05(3) | <4d   | -0.07±0.087(3) | <4d   |
| TFI1α | -0.14±0.04(3) | <4d   | -0.27±0.05(3) | <4d   | -0.19±0.06(3) | <4d   | -0.12±0.082(3) | <4d   |

R²  0.90  0.90  0.80  0.82

*pIC₅₀ (95% CI based on duplicate pIC₅₀ measurements). **Coefficient of determination based on a linear correlation between DSF Tm shift (abscissa) and AlphaScreen pIC₅₀ (ordinate).

**Mean ± standard error of the mean (number of determinations). *Compound concentration 10 μM. **Coefficient of determination based on a linear correlation between DSF Tm shift (abscissa) and AlphaScreen pIC₅₀ (ordinate).
in profile to compound 51, with less discrimination over CECR2. The only piperazine derivative, compound 55, had a slight preference for CECR2 over BRD4(1), but despite inclusion of polar substituents, poor solubility of this compound showed variable IC$_{50}$ determinations for BRD9 and CREBBP as shown by the high standard errors.

It is noteworthy that the compounds disclosed in this work are potent inhibitors of the CECR2, BRD4(1)/(2), CREBBP, BRD9, and TAF1L(2) bromodomains to varying degrees. The inhibited bromodomains do not cluster as expected from the sequence-based phylogenetic tree (Figure 1b). From the phylogenetic tree it would be expected that closely related BRDs such as PCAF (subfamily I shared with CECR2), PHIP(2) (subfamily III shared with CREBBP), BRPF3 and ATAD2 (subfamily IV shared with BRD9), and TAF1(1) (subfamily VII shared with TAF1L(2)) would also be affected by the inhibitors. It could be argued that the phylogenetic tree in Figure 1b described by Filippakopoulos et al.\textsuperscript{17} is based on a sequence alignment of the entire bromodomain, whereas only the residues in the ligand binding region are relevant to selectivity. A more ligand-focused phylogenetic tree has been described by Vidler et al. which clusters BRDs based on binding site residues.\textsuperscript{26} This refined analysis goes part of the way to explaining compound selectivity as it clusters PHIP(2) with PB1(5) (both not inhibited) and moves ATAD2 to its own branch, but it does not explain the remaining inconsistencies as PCAF still clusters with CECR2, BRPF3 with BRD9, and TAF1(1) with TAF1L(2). Calculated druggability is also insufficient to explain inhibitor preference as both PCAF and TAF1(1) have similar SiteMap D-scores to CECR2 and TAF1L(2).\textsuperscript{26}

Compound 51 was chosen as a representative of this series, and co-crystallization was attempted with multiple bromodomains. High resolution structures were obtained with BRD4(1) and BRD9 (Figure 6). The overall pose of the compound with the two bromodomains was exactly as expected from the initial docking studies of the predecessor 17 in CREBBP (Figure 3). The triazole moiety formed hydrogen bonds to the conserved asparagine N140 in BRD4(1) and N100 in BRD9 and to a conserved pocket water molecule in both proteins. The phthalazine ring system was sandwiched securely between L94 and I146 in BRD4 and I53 and Y106 in BRD9.

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**Figure 4.** Synthetic inhibitors of multiple bromodomains.
Scheme 2. The Synthesis of Compounds 49–57\textsuperscript{a}

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41 + a or b → 42
O

| R  | X  | Y  |
|----|----|----|
| NO₂ | O  | N-Me |
| NO₂ | N-Me | O  |

44 + c, d → 45
O

| R  | X  | Y  |
|----|----|----|
| NH₂ | N-Me | O  |
| NH₂ | O  | N-Me |

47 + e → 49
O

| R  | X  | Y  |
|----|----|----|
| Tol | O  | X  |
| Me  | X  | O  |
| NO₂ | X  | O  |
| Me  | X  | O  |
| 3-Ch-Ph | X  | O  |
| 4-Ch-Ph | X  | O  |
| 3,5-Cl₂-Ph | X  | O  |
| 4-Me-O-Ph | X  | N-Me |

44 + f → 51
O

| R  | X  | Y  |
|----|----|----|
| Nh₂ | N-Me | O  |

50 + 51 + 52 + 53 + 54 + 55

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\textsuperscript{a}Reagents and conditions: (a) morpholine, iPrOH (100%); (b) 4-methylpiperazine, Et₂N, iPrOH (100%); (c) (BOpin)₂, Pd(dppf)Cl₂, KOAc, p-dioxane/DMSO (47–72%); (d) 19, Pd(PPh₃)₄, K₂CO₃, p-dioxane/H₂O (32–50%); (e) SnCl₂, EtOH, reflux (26–100%); (f) RSO₂Cl, Et₂N, p-dioxane or RSO₂-Cl, pyridine, DCM (47–81%); (g) 22, Pd(PPh₃)₄, K₂CO₃, p-dioxane/H₂O (62%); (h) 23, Pd(PPh₃)₄, K₂CO₃, p-dioxane/H₂O, (ii) HCl, EtOAc (60%).

The ability of inhibitors to displace the bromodomain of CREBBP from chromatin was assessed using fluorescence recovery after photobleaching (FRAP). A construct consisting of the multimerised bromodomain of CREBBP as well as a similar construct in which the conserved asparagine responsible for binding of acetylated lysine has been mutated to a phenylalanine was transfected into U2OS cells. Cells were treated with the histone deacetylase (HDAC) inhibitor SAHA in order to globally increase lysine acetylation, resulting in a better assay window (Figure 7A). Treatment of the cells with compounds 50, 51, 53, and 55 significantly decreased FRAP recovery times (Figures 7B), indicative of displacement of the BRD construct from hyper-acetylated chromatin. The piperazine derivative, compound 55, showed a slightly decreased recovery time, indicating a stronger binding to the CREBBP bromodomain.

\section*{CONCLUSIONS}

A series of potent BRD inhibitor compounds has been developed. Initial SAR in this series shows the potential to develop selective inhibitors for individual bromodomads, with compounds showing some preference for the BRDs of CECR2, BRD4(1), CREBBP, BRD9, and TAF1L(2) over the likes of PCAF, PHIP(2), BRPF3, ATAD2, TIF1β, SP140, BAZ2A/B, TAF1(1), PB1(5), and SMARCA4. It is not clear from sequence- and structure-based clustering why these novel inhibitors have preference for some BRDs over others. Inhibitors with in vitro IC₅₀ <1 \(\mu\)M have been identified for the previously untargeted BRDs of BRD9 and CECR2. A modular synthetic route allows diversification of multiple positions of the core and will be used to further explore this scaffold to find more selective molecules to probe the biological function of the less well studied members of this epigenetic reader family. Using a FRAP assay, selected compounds have been shown to be cell active. By adopting a chemical probe approach\textsuperscript{27} rather than a preselected target approach and characterizing compounds across the entire BRD family, compounds with intriguing polypharmacology have also been uncovered such as compound 53, which selectively inhibits three bromodomain-containing proteins implicated in leukemia (BRD4, CREBBP, and BRD9).

\section*{EXPERIMENTAL SECTION}

\textbf{General Experimental.} Commercial reagents were used as received without further purification. Commercial anhydrous solvents were used in reactions, and HPLC grade solvents were employed for workup and chromatography. NMR spectra were recorded using a Varian Mercury 300 or 400 MHz for \(^1\)H and 75 or 101 MHz for \(^13\)C. The solvent was used as internal deuterium lock. Coupling constants (\(J\)) are quoted in Hz and are recorded to the nearest 0.5 Hz. Identical proton coupling constants are averaged in each spectrum and reported to the nearest 0.1 Hz. When peak multiplicities are reported, the following abbreviations are used: \(s =\) singlet, \(d =\) doublet, \(t =\) triplet, \(m =\) multiplet, \(br =\) broadened, \(dd =\) doublet of doublets, \(dt =\) doublet of
triplets. LRMS employed an electrospray ionization source acquiring in positive and negative ionization mode. \(m/z\) values are reported in Daltons. Analytical HPLC was carried out on an Agilent 1100 equipped with photodiode array detector (DAD), quaternary gradient pump, and micro plate sampler (Agilent 220). Separation of the analytes was performed upon Centurysil C18-AQ + 5 \(\mu m\), 50 mm \(\times\) 4.6 mm (Johnson). The flow rate of the mobile phase was kept at 3.5 mL/min. Mobile phases B and C were acetonitrile with 0.35% CF3CO2H and water with 0.35% CF3CO2H, respectively. The gradient conditions were as follows: 0–0.5 min 1% B and 99% C, 3.7 min 90% B and 10% C, 5 min 99% B and 1% C. The injection volume was 10 \(\mu L\).

All compounds tested in biological assays were \(\geq 95\%\) pure by HPLC at 254 nm and by evaporative light scattering detection (ELSD).

**Synthetic Procedure and Characterization of Compounds 13, 24–34, 49–57.** 6-Chloro-3-methyl-[1,2,4]triazolo[3,4-a]phthalazine (19). 1,4-Dichlorophthalazine 18 (5 g, 25.1 mmol) was mixed with \(n\)-butanol (100 mL) under argon, and acetic hydrazide (3.7 g, 50.2 mmol) was added. The reaction was stirred at reflux overnight. The mixture was cooled to room temperature, followed by filtration. The solid was washed with EtOAc and MeOH. The solid residue was purified by flash column chromatography (EtOAc:petroleum ether 1:3) to obtain title compound 19 (2.26 g, 41%). MS (ESI): \(m/z\) calcd for \((C_{10}H_7ClN_4 + H)^+\) 219.0, found 219.0. Purity (ELSD) >95%.

**N-Benzyl-3-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)benzenesulfonamide (13).** A mixture of compound 19 (45 mg, 0.21 mmol), boronate 35 (50 mg, 0.17 mmol), Pd(PPh3)4 (20 mg), and K2CO3 (58 mg, 0.43 mmol) in dioxane and water was stirred under argon at 120°C. The reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined, washed with brine, and dried (Na2SO4). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 30:1) to give title compound 13 (30 mg, 33%). MS (ESI): \(m/z\) calcd for \((C_{23}H_{19}N_5O_2S + H)^+\) 430.1, found 429.9. \(^1H\) NMR (DMSO-\(d_6\)) \(\delta\) 8.60 (1H, \(d, J = 7.8\)), 8.11–8.02 (3H, m), 7.96–7.79 (3H, m), 7.66 (1H, \(d, J = 8.1\)), 7.24–7.11 (5H, m), 4.10 (2H, s), 2.71 (3H, s).

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**Figure 5.** Triazolophthalazines with para-aminophenyl substituents are potent bromodomain inhibitors.
N-Benzyl-3-(3-ethyl-[1,2,4]triazolo[3,4-a]pyrazin-6-yl)-benzenesulfonamide (24). 1-Chloro-4-hydrazi-pyridazinylbenzenesulfonamide (25) (300 mg, 1.54 mmol) was dissolved in propanoic acid (3 mL), and the solution was heated to reflux. The reaction was monitored by TLC. Upon completion, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 20:1) to provide the intermediate 1-chloro-4-hydrazi-pyridazinylbenzenesulfonamide (26) (300 mg, 1.54 mmol), boronate 35 (50 mg, 0.17 mmol), Pd(PPh3)4 (20 mg), and K2CO3 (58 mg, 0.43 mmol) in dioxane and water was heated and stirred under argon at 120 °C. The reaction was monitored by TLC. Upon completion, water was added and the mixture was extracted with DCM. The organic layers were combined, washed with brine, and dried (Na2SO4). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 30:1) to give the title compound 24 (30 mg, 33%). MS (ESI): m/z calcld for (C23H20N6O2S+H)+ 445.1, found 445.9.1H NMR (DMSO-d6) δ 8.62 (1H, d, J = 7.8), 8.12–7.80 (6H, m), 7.69 (1H, d, J = 8.4), 7.25–7.18 (5H, m), 4.12 (2H, s), 3.14 (2H, q, J = 7.5), 1.42 (3H, t, J = 7.8). HPLC retention time 3.088 min.

N-Benzyl-3-(3-cyclopropyl-[1,2,4]triazolo[3,4-a]pyrazin-6-yl)-benzenesulfonamide (25). Following the same procedure as for 24, the title compound was obtained in 80% yield via compound 21. MS (ESI): m/z calcld for (C24H21N5O2S+H)+ 447.1, found 447.0.1H NMR (DMSO-d6) δ 8.59 (1H, d, J = 7.2), 8.10–8.04 (3H, m), 7.95 (1H, d, J = 7.8), 7.91–7.80 (2H, m), 7.66 (1H, d, J = 7.8), 7.25–7.17 (5H, m), 4.11 (2H, s), 2.45 (4H, m), 1.21–1.15 (4H, m).

N-Benzyl-3-[3-(hydroxymethyl)[1,2,4]triazolo[3,4-a]pyrazin-6-yl]-benzenesulfonamide (26). Following the same procedure as for 24, title compound 26 was obtained in 37% yield via compound 21. MS (ESI): m/z calcld for (C24H21N5O2S+H)+ 446.1, found 446.1.1H NMR (DMSO-d6) δ 8.65 (1H, d, J = 8.1), 8.14–8.04 (3H, m), 7.99–7.90 (2H, m), 7.83 (1H, t, J = 7.8), 7.69 (1H, d, J = 7.8), 7.25–7.18 (5H, m), 4.96 (2H, s), 4.11 (2H, s).

tert-Butyl [6-(3-(N-Benzylsulfamoyl)phenyl)[1,2,4]triazolo[3,4-a]pyrazin-6-yl]methyl]carbamate (27). 1-Chloro-4-hydrazi-pyridazinylbenzenesulfonamide (28) (1.6 g, 8.24 mmol) and Boc-glycine (7.2 g, 40 mmol) were dissolved in THF (150 mL), and the mixture was stirred at reflux. The reaction was monitored by TLC. Upon completion, the mixture was concentrated in vacuo, followed by dilution with H2O (50 mL) and extraction with DCM (3 × 20 mL). The combined organic layers were dried (Na2SO4), concentrated in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 30:1) to provide the intermediate 23 (237 mg, 39%).

A mixture of tricyclic triazole 23 (169 mg, 0.51 mmol), boronate 35 (162 mg, 0.56 mmol), Pd(PPh3)4 (58 mg, 0.1 equiv), and K2CO3 (175 mg, 1.27 mmol) in dioxane (3 mL) and water (0.5 mL) was stirred at reflux. The reaction was monitored by TLC. Upon completion, water (20 mL) was added and the mixture was extracted with DCM (3 × 20 mL). The combined organic layers were dried (Na2SO4), concentrated in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 40:1) to give the title compound 27 (80 mg, 29%). MS (ESI): m/z calcld for (C25H25N7O5S+H)+ 545.2, found 545.1.1H NMR (CDCl3): δ 8.70 (1H, d, J = 7.8), 8.17 (1H, m), 8.10–8.07 (2H, m), 8.01–7.98 (2H, m), 7.85 (1H, m), 7.75 (1H, d, J = 7.8), 7.27–7.22 (2H, m), 4.65 (2H, s), 4.12 (2H, s).

N-(3-Chloro-5-(3-methyl-[1,2,4]triazolo[3,4-a]pyrazin-6-yl)phenyl)benzenesulfonamide (29). Benzenesulfonaryl chloride (0.26 mL, 2 mmol) was added to a mixture of 3-bromo-5-chloroaniline (350
mg, 1.7 mmol) and pyridine (0.16 mL, 2 mmol) in THF (8 mL), and the resulting mixture was stirred at room temperature for 6 h. Water was added, and the aqueous layer was extracted with DCM. The organic layers were combined, washed with brine, and dried (Na2SO4). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (EtOAc:petroleum ether 8:1) to give the intermediate N-[(3-bromo-5-chlorophenyl)-benzene sulfonamide (380 mg).

A mixture of the sulfonamide from the preceding reaction (180 mg, 0.52 mmol), bis(pinacolato)diboron (145 mg, 0.57 mmol), KOAc (101 mg, 1.04 mmol), and Pd(dppf)Cl2 (11 mg, 0.016 mmol) in dioxane and water was stirred and heated under argon at 120 °C for 26 h. The reaction was monitored by TLC. Upon completion, water was added, and the aqueous layer was extracted with DCM. The organic layers were combined, washed with brine, and dried (Na2SO4). The solvents were removed in vacuo, and the residue was puriﬁed by flash column chromatography (EtOAc:petroleum ether 20:1) to give the title compound (100 mg, 95%). MS (ESI): m/z calcd for (C16H10ClN5O2 + H)⁺ 340.0 (35Cl) and 342.0 (37Cl), found 339.9 (35Cl) and 341.9 (37Cl). 1H NMR (CDCl3) δ 8.79 (1H, d, J = 7.8), 8.46−8.42 (2H, m), 7.99 (1H, m), 7.82 (1H, d, J = 9.6), 7.74 (1H, m), 7.43 (1H, d, J = 8.1), 2.84 (3H, s).

Step 2: 4-Chloro-3-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)benzenesulfonylamide (30). A mixture of tricyclic triazole from step 1 (115 mg, 0.34 mmol) and SnCl2 (381 mg, 1.69 mmol) in ethanol was stirred under argon at 85 °C for 10 min at room temperature. The mixture was extracted with EtOAc, 6 M HCl was added to the aqueous layer to adjust the pH to 3−4, and it was then extracted with EtOAc. The organic layer was washed with brine, dried (Na2SO4), concentrated in vacuo, and puriﬁed by flash column chromatography to afford the product 36 (126 mg).

A mixture of tricyclic triazole (40 mg, 0.19 mmol), boronate 36 (70 mg, 0.18 mmol), Pd(PPh3)4 (20 mg), and KEt3 (6 mg, 0.43 mmol) in dioxane and water was stirred and heated under argon at 120 °C. The reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined and washed with brine, and dried (Na2SO4). The solvents were removed in vacuo, and the residue was puriﬁed by flash column chromatography (DCM:MeOH 30:1) to give title compound 29 (20 mg, 25%). MS (ESI): m/z calcd for (C16H12ClN5O2S + H)⁺ 450.1 (35Cl) and 452.1 (37Cl), found 449.8 (35Cl) and 451.9 (37Cl). 1H NMR (DMSO-d6) δ 8.57 (1H, d, J = 7.2), 8.06 (1H, t, J = 7.2), 7.81−7.77 (3H, m), 7.64−7.51 (5H, m), 7.34−7.27 (2H, m), 2.69 (3H, s).

Figure 6. (A) Compound 51 (green stick) in complex with BRD4(1) (PDB ID: 4NQM, blue ribbon and stick, top loop removed for clarity in first image) shows H-bonds (dashed lines) between the triazole moiety, the conserved asparagine (N140), and a pocket water (red sphere). The anionic sulfonamide forms an additional hydrogen bond to tryptophan (W81). (B) In complex with BRD9 (PDB ID: 4NQN, yellow ribbon and stick (the ZA loop was removed for clarity in first image)), compound 51 forms H-bonds to N100 and water but acts as an H-bond acceptor via a sulfonamide oxygen to Y106. The electron density map from the X-ray reﬁnement is shown as a dark-blue mesh around the ligand.
the reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined, washed with brine, and dried (Na$_2$SO$_4$). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 30:1) to give title compound 30 (30 mg, 42%). MS (ESI): m/z calcd for (C$_{22}$H$_{16}$ClN$_5$O$_2$S+H$^+$) 450.1 (35Cl) and 452.1 (37Cl), found 449.9 (35Cl) and 451.9 (37Cl).

$^1$H NMR (DMSO-$d_6$) $\delta$ 8.58 (1H, d, $J = 7.8$), 8.07 (1H, t, $J = 7.8$), 7.85– 7.79 (3H, m), 7.71– 7.57 (4H, m), 7.39 (1H, dd, $J = 8.7$, 2.7), 7.32 (1H, d, $J = 2.7$), 7.19 (1H, d, $J = 8.1$), 2.69 (3H, s).

3-(3-Methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)benzoic Acid (31). A mixture of tricyclic triazole 19 (24 mg, 0.11 mmol), boronic acid 38 (21 mg, 0.12 mmol), Pd(PPh$_3$)$_4$ (13 mg, 0.1 equiv), and K$_2$CO$_3$ (37 mg, 0.27 mmol) in dioxane and water was stirred and heated under argon at 120 °C. The reaction was monitored by TLC. Upon completion, water was added and the aqueous layers were extracted with DCM. The organic layers were combined and dried (Na$_2$SO$_4$). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 30:1) to give the Suzuki adduct methyl 3-(3-methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)benzoate (29 mg, 83%). The intermediate ester (29 mg, 0.09 mmol) was dissolved in methanol (14 mL), and KOH (99 mg, 1.76 mmol) was added. The reaction was monitored by TLC, and upon completion water (21 mL) was added. The mixture was adjusted to pH 3–4 with 6 M HCl and extracted with DCM. The combined organic layers were dried (Na$_2$SO$_4$), and the solvent was removed in vacuo to give the title compound 31 (26 mg, 95%). MS (ESI): m/z calcd for (C$_{17}$H$_{12}$N$_4$O$_2$ +H$^+$) 305.1, found 305.1.

$^1$H NMR (DMSO-$d_6$) $\delta$ 8.60 (1H, d, $J = 7.8$), 8.25– 8.19 (2H, m), 8.08 (1H, m), 7.98 (1H, m), 7.88 (1H, m), 7.80– 7.76 (2H, m), 2.72 (3H, s).

3-(3-Methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)-5-nitrobenzoic Acid (32). Following the same procedure as for compound 31 step 1, the methyl ester hydrolyzed under the reaction conditions to yield title compound 32 directly (35%). MS (ESI): m/z calcd for (C$_{17}$H$_{11}$N$_5$O$_4$ +H$^+$) 350.1, found 350.0.

$^1$H NMR (DMSO-$d_6$) $\delta$ 8.84– 8.79 (2H, m), 8.64– 8.60 (2H, m), 8.09 (1H, m), 7.90– 7.80 (2H, m), 2.73 (3H, s).

2-(3-(3-Methyl-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)phenyl)acetic Acid (33). Tricyclic triazole 19 and boronic acid 40 were coupled and hydrolyzed using the same procedures as for compound 31 to give the desired compound (Suzuki, 53%; hydrolysis, 73%). MS (ESI): m/z calcd for (C$_{18}$H$_{14}$N$_4$O$_2$ +H$^+$) 319.1, found 319.1.

$^1$H NMR (DMSO-$d_6$) $\delta$ 8.58 (1H, d, $J = 7.8$), 8.21 (1H, m), 7.94– 7.76 (2H, m), 7.69– 7.46 (4H, m), 3.75 (2H, s), 2.72 (3H, s).

3-Methyl-6-phenyl-[1,2,4]triazolo[3,4-a]phthalazine (34). 1-Hydradino-4-phenylphthalazine (300 mg, 1.27 mmol) was dissolved in acetic acid (3 mL) and refluxed for 2 h. The reaction mixture was concentrated in vacuo and the residue purified by flash column chromatography (EtOAc:petroleum ether 1:3) to give title compound 34 (269 mg, 81% yield). MS (ESI): m/z calcd for (C$_{24}$H$_{17}$N$_4$O$_2$ +H$^+$) 381.1, found 381.0.

$^1$H NMR (CDCl$_3$) $\delta$ 8.67 (1H, d, $J = 6.0$), 7.89– 7.82 (2H, m), 7.64– 7.49 (6H, m), 2.77 (3H, s).

Figure 7. (A) Cells transfected with a trimerized CREBBP-BRD-GFP construct show rapid recovery of fluorescent intensity after photobleaching (FRAP) (black). Recovery time is increased by pretreating cells with 2.5 μM SAHA$^*$ (green) and restored by transfecting with incompetent mutant protein (N1168F, red). (B) Cells treated with SAHA$^*$ (2.5 μM) and compounds 50 (blue), 51 (yellow), 53 (purple), and 55 (red) (1 μM) show increased recovery rates. (C) Recovery half-lives of transfected (black), SAHA treated (green), and SAHA plus compound treated cells. (D) Fluorescent images of cells show rapid recovery of photobleached area (red circle) after compound treatment. $^*$SAHA treated cells; $^\dagger$N1168F, mutation of N1168 to Phe.
A mixture of aryl chloride 19 (59 mg, 0.27 mmol), boronate from 20 (0.25 mL, 3.09 mmol). The mixture was stirred at room temperature, and the reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The combined organic layers were dried with brine, and dried (NaSO₄). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 1:1) to give the title compound 49 (50 mg, 50%). MS (ESI): m/z calculated for (C₂₆H₂₃ClN₆O₄S⁺H)⁺ 551.1, found 550.1. 1H NMR (CDCl₃) δ 8.37 (1H, d, J = 8.1), 7.71 (1H, t, J = 7.8), 7.17 (1H, m), 7.05–7.04 (2H, m), 3.92 (4H, t, J = 4.2), 3.04 (4H, t, J = 4.2), 2.83 (3H, s).

4-Methyl-N-(5-(3-methyl-1H-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)-2-morpholino-phenyl)benzenesulfonamide (50). Methanesulfonyl chloride (14 mg, 0.12 mmol) was added to a solution of 48 (22 mg, 0.06 mmol) in DCM (1.6 mL), followed by addition of pyridine (15 μL, 0.03 mmol). The resulting mixture was stirred at room temperature, and the reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined and dried (Na₂SO₄). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 60:1) to give title compound 49 (27 mg, 63%). MS (ESI): m/z calculated for (C₂₆H₂₃ClN₆O₄S⁺H)⁺ 515.1, found 514.9. 1H NMR (CDCl₃) δ 8.71 (1H, d, J = 7.8), 8.01–7.70 (7H, m), 7.39–7.25 (3H, m), 3.86 (4H, t, J = 4.2), 2.74 (4H, t, J = 4.2), 2.40 (3H, s).

N-(5-(3-methyl-1H-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)-2-morpholino-phenyl)methanesulfonamide (50). Methanesulfonyl chloride (14 mg, 0.12 mmol) was added to a solution of 48 (22 mg, 0.06 mmol) in DCM (1.6 mL), followed by addition of pyridine (15 μL, 0.03 mmol). The resulting mixture was stirred at room temperature, and the reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined and dried (Na₂SO₄). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 60:1) to give title compound 49 (27 mg, 63%). MS (ESI): m/z calculated for (C₂₆H₂₃ClN₆O₄S⁺H)⁺ 515.1, found 514.9. 1H NMR (CDCl₃) δ 8.71 (1H, d, J = 7.8), 8.01–7.70 (7H, m), 7.39–7.25 (3H, m), 3.86 (4H, t, J = 4.2), 2.74 (4H, t, J = 4.2), 2.40 (3H, s).

N-(5-(3-methyl-1H-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)-2-morpholino-phenyl)methanesulfonamide (50). Methanesulfonyl chloride (14 mg, 0.12 mmol) was added to a solution of 48 (22 mg, 0.06 mmol) in DCM (1.6 mL), followed by addition of pyridine (15 μL, 0.03 mmol). The resulting mixture was stirred at room temperature, and the reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined and dried (Na₂SO₄). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 60:1) to give title compound 49 (27 mg, 63%). MS (ESI): m/z calculated for (C₂₆H₂₃ClN₆O₄S⁺H)⁺ 515.1, found 514.9. 1H NMR (CDCl₃) δ 8.71 (1H, d, J = 7.8), 8.01–7.70 (7H, m), 7.39–7.25 (3H, m), 3.86 (4H, t, J = 4.2), 2.74 (4H, t, J = 4.2), 2.40 (3H, s).

N-(5-(3-methyl-1H-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)-2-morpholino-phenyl)methanesulfonamide (50). Methanesulfonyl chloride (14 mg, 0.12 mmol) was added to a solution of 48 (22 mg, 0.06 mmol) in DCM (1.6 mL), followed by addition of pyridine (15 μL, 0.03 mmol). The resulting mixture was stirred at room temperature, and the reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined and dried (Na₂SO₄). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 60:1) to give title compound 49 (27 mg, 63%). MS (ESI): m/z calculated for (C₂₆H₂₃ClN₆O₄S⁺H)⁺ 515.1, found 514.9. 1H NMR (CDCl₃) δ 8.71 (1H, d, J = 7.8), 8.01–7.70 (7H, m), 7.39–7.25 (3H, m), 3.86 (4H, t, J = 4.2), 2.74 (4H, t, J = 4.2), 2.40 (3H, s).

N-(5-(3-methyl-1H-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)-2-morpholino-phenyl)methanesulfonamide (50). Methanesulfonyl chloride (14 mg, 0.12 mmol) was added to a solution of 48 (22 mg, 0.06 mmol) in DCM (1.6 mL), followed by addition of pyridine (15 μL, 0.03 mmol). The resulting mixture was stirred at room temperature, and the reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined and dried (Na₂SO₄). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 60:1) to give title compound 49 (27 mg, 63%). MS (ESI): m/z calculated for (C₂₆H₂₃ClN₆O₄S⁺H)⁺ 515.1, found 514.9. 1H NMR (CDCl₃) δ 8.71 (1H, d, J = 7.8), 8.01–7.70 (7H, m), 7.39–7.25 (3H, m), 3.86 (4H, t, J = 4.2), 2.74 (4H, t, J = 4.2), 2.40 (3H, s).

N-(5-(3-methyl-1H-[1,2,4]triazolo[3,4-a]phthalazin-6-yl)-2-morpholino-phenyl)methanesulfonamide (50). Methanesulfonyl chloride (14 mg, 0.12 mmol) was added to a solution of 48 (22 mg, 0.06 mmol) in DCM (1.6 mL), followed by addition of pyridine (15 μL, 0.03 mmol). The resulting mixture was stirred at room temperature, and the reaction was monitored by TLC. Upon completion, water was added and the aqueous layer was extracted with DCM. The organic layers were combined and dried (Na₂SO₄). The solvents were removed in vacuo, and the residue was purified by flash column chromatography (DCM:MeOH 60:1) to give title compound 49 (27 mg, 63%). MS (ESI): m/z calculated for (C₂₆H₂₃ClN₆O₄S⁺H)⁺ 515.1, found 514.9. 1H NMR (CDCl₃) δ 8.71 (1H, d, J = 7.8), 8.01–7.70 (7H, m), 7.39–7.25 (3H, m), 3.86 (4H, t, J = 4.2), 2.74 (4H, t, J = 4.2), 2.40 (3H, s).
550.1 and 552.0. 1H NMR (300 MHz, CDCl3/CD3OD) δ 8.65 (1H, d, J = 8.1), 8.02 (1H, m), 7.84 (2H, d, J = 4.2), 7.64 (3H, m), 7.43 (1H, d, J = 8.1), 7.36–7.32 (3H, m), 4.64 (2H, s), 3.75 (4H, br s), 2.73 (4H, br s).

**Biological Evaluation. Protein Expression and Purification.** Proteins were cloned, expressed, and purified as previously described. Peptides, HA44 peptide (BRD4 and CECR2 assays), H3K9Ac (13)K15Ac peptide (BRD9 assay), H3K4Ac peptide (BAZ2B, PB1(5) and TIF1gamma assays), H3-N-QTARKSTGGK(Ac)APRKQLATKA-K(biotin)-CO2H were synthesized by Tufts University Core Facility, Pepsciences, or Alta Biosciences.

**DSF Tm Shift Assays.** Bromodomain DSF σm shift assays were carried out as previously described.αβ

**AlphaScreen Peptide Displacement Assay.** Bromodomain AlphaScreen assays were carried out as previously described.αβ All experiments were carried out in duplicate on the same plate. **CREBBP Fluorescence Recovery After Photobleaching (FRAP) Assay.** FRAP studies were performed using a protocol modified from previous studies.αβ In brief, U2OS cells were transfected (Lipofectamine 2000, Life Technologies) with mammalian overexpression constructs encoding a GFP chimera with three tandem repeats of the fusion method at 4°C. Crystals of BRD4(1) with compound 51 were grown by mixing 100 nL of protein (14.9 mg/mL and 5 mM H3K14(Ac) peptide (BAZ2B, PB1(5) and TIF1gamma assays), H3-N-QTARKSTGGK(Ac)APRKQLATKA-K(biotin)-CO2H were synthesized by Tufts University Core Facility, Pepsciences, or Alta Biosciences.

**Data Collection and Structure Solution.** BRD9 crystals were cryoprotected using the well solution supplemented with additional ethylene glycol and were flash-frozen in liquid nitrogen. BRD4 crystals were frozen without any additional cryoprotection. Data were collected in-house on a Rigaku FRE rotating anode system equipped with a RAXIS-IV detector at 1.52 Å. Indexing and integration was carried out using MOSFLM,αβ and scaling was performed with SCALA.αβ Initial phases were calculated by molecular replacement with Phaserαβ using the known models of BRD4(1) (PDB ID 2OSS) and BRD9 (PDB ID 3HME). Initial models were built by ARP/wARP,αβ followed by manual building in COOT.αβ Refinement was carried out in REFMAC5.αβ In all cases, thermal motions were analyzed using TLSMDαβ and hydrogen atoms were included in late refinement cycles. Data collection and refinement statistics can be found in Table 3. The models and structure factors have been deposited with PDB accession codes: 4NQM (BRD4(1)/compound 51), 4NQN (BRD9/compound 51).

### Table 3. Data Collection and Refinement Statistics for BRD4(1) and BRD9 Complexes

| Data Collection | PDB ID | protein | ligand | space group | cell dimensions (Å, deg) | completeness (%) | redundancy | Rmerge (%) | Rwork (%) | Rfactor (%) |
|-----------------|--------|---------|--------|-------------|--------------------------|-----------------|------------|------------|-----------|-------------|
| 4NQM            | BRD4(1)| compd 51| compd 51| P2_2_2 | a: 45.15, 46.57, 62.38; b: 90.00, 90.00, 90.00; c: 1.58 (1.66–1.58) | 98.7 (93.5) | 3.9 (2.8) | 0.049 (0.439) | 15.7 (2.0) | 1.58 |
| 4NQN            | BRD9   | compd 51| compd 51| P2_2_2 | a: 45.15, 46.57, 62.38; b: 90.00, 90.00, 90.00; c: 1.58 (1.66–1.58) | 98.7 (93.5) | 3.9 (2.8) | 0.049 (0.439) | 15.7 (2.0) | 1.58 |

Reeflection Statistics:

| resolution (Å) | Rmerge (%) | Rwork (%) | Rfactor (%) |
|----------------|------------|-----------|-------------|
| 1.58           | 16.9/20.3  | 190/24.5  | 30.7/37.4   |
| 1.73           | 16.9/20.3  | 190/24.5  | 30.7/37.4   |

**ASSOCIATED CONTENT**

1. Supporting information
2. DSF Tm shifts with replicates and errors (XLSX). This material is available free of charge via the Internet at http://pubs.acs.org.

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**Author Contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.
Notes
The authors declare no competing financial interest.

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ABBREVIATIONS USED
HAT, histone acetyl transferase; HDAC, histone deacetylase; BET, bromodomain and extra terminal domain; BRD, bromodomain-containing protein or bromodomain; DSF, differential scanning fluorimetry; BRD4(1)/(2), first/second bromodomain of BRD4; BRDT, bromodomain, testis-specific; CREBBP, CREB (cyclic-AMP response element binding) binding protein; BRD9, bromodomain-containing protein 9; CECR2, cat eye syndrome chromosome region, candidate 2; TAF1, TBP-associated factor RNA polymerase 1; TAF1L, TAF1-like; TIF1α, transcription intermediary factor 1-alpha; ATAD2, ATPase family, AAA domain containing protein 2; SMARCA4, SWI/SNF related, matrix associated, actin dependent, TAF1, TBP-associated factor RNA polymerase 1; TAF1L, TAF1-like; P300/CBP-associated factor; BRPF3, bromodomain-containing protein 3; PHIP, pleckstrin homology domain interacting protein; FRAP, fluorescence recovery after photo-bleaching

REFERENCES
(1) Zettner, G. E.; Henikoff, S. Regulation of nucleosome dynamics by histone modifications. Nature Struct. Mol. Biol. 2013, 20 (3), 259–266.
(2) Dawson, M. A.; Kouzarides, T. Cancer Epigenetics: From Mechanism to Therapy. Cell 2012, 150 (1), 12–27.
(3) Brennan, P.; Filippakopoulos, P.; Knapp, S. The therapeutic potential of acetyl-lysine and methyl-lysine effector domains. Drug Discovery Today: Ther. Strategies 2012, 9 (2–3), e101–e110.
(4) Muller, S.; Filippakopoulos, P.; Knapp, S. Bromodomains as therapeutic targets. Expert Rev. Mol. Med. 2011, 13, e29/1–e29/21.
(5) Hewings, D. S.; Rooney, T. P. C.; Jennings, L. E.; Hay, D. A.; Schofield, C. J.; Brennan, P. E.; Knapp, S.; Conway, S. J. Progress in the Development and Application of Small Molecule Inhibitors of Bromodomains—Acetyl-lysine Interactions. J. Med. Chem. 2012, 55 (22), 9393–9413.
(6) Muller, S.; Lingard, H.; Knapp, S. Selective Inhibition of Acetyl-Lysine Effector Domains of the Bromodomain Family in Oncology. In Nuclear Signaling Pathways and Targeting Transcription in Cancer; Kumar, R., Ed.; Springer: New York, 2014; pp 279–298.
(7) Filippakopoulos, P.; Qi, J.; Picaud, S.; Shen, Y.; Smith, W. B.; Fedorov, O.; Morse, E. M.; Keates, T.; Hickman, T. T.; Flettet, I.; Philpot, 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.; West, O.; Kung, A. L.; Knapp, S.; Bradner, J. E. Selective inhibition of BET bromodomains. Nature 2010, 468 (7327), 1067–1073.
(8) Hewings, D. S.; Wang, M.; Philpott, M.; Fedorov, O.; Uttarkar, S.; Filippakopoulos, P.; Picaud, S.; Vuppucett, C.; Marsden, B.; Knapp, S.; Conway, S. J.; Heightman, T. D. 3,5-Dimethylxazolones Act As Acetyl-lysine-mimetic Bromodomain Ligands. J. Med. Chem. 2011, 54 (19), 6761–6770.
(9) Dawson, M. A.; Pinaia, R. K.; Dittmann, A.; Giotopoulos, G.; Bantscheff, M.; Chan, W. H.; Robson, S. C.; Chung, C.-w.; Hopf, C.; Savitski, M. M.; Huthmerchen, C.; Gudgin, E.; Lugo, D.; Beinke, S.; Chapman, T. D.; Roberts, E. J.; Soden, P. E.; Auger, K. R.; Mireguet, O.; Doehnner, K.; Delwel, R.; Burnett, A. K.; Jeffrey, P.; Drewes, G.; Lee, K.; Huntly, B. J. P.; Kouzarides, T. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 2011, 478 (7370), 529–533.
(10) Bamborough, P.; Diao, H.; Goodacre, J. D.; Gordon, L.; Lewis, A.; Seal, J. T.; Wilson, D. M.; Woodrow, M. D.; Chung, C.-w. Fragment-Based Discovery of Bromodomain Inhibitors Part 2: Optimization of Phenylxazolone Sulfonamides. J. Med. Chem. 2011, 55 (2), 587–596.
(11) Hewings, D. S.; Fedorov, O.; Filippakopoulos, P.; Martin, S.; Philpott, M.; Picaud, S.; Hewings, D. S.; Uttarkar, S.; Heightman, T. D.; Conway, S. J.; Knapp, S.; Brennan, P. E. The design and synthesis of 5- and 6-isoxazolobenzimidazoles as selective inhibitors of the BET bromodomains. MedChemComm 2013, 4 (1), 140–144.
(12) Hewings, D. S.; Fedorov, O.; Filippakopoulos, P.; Martin, S.; Picaud, S.; Tumber, A.; Wells, C.; Oicina, M. M.; Freeman, K.; Gill, A.; Ritchie, A. J.; Sheppard, D. W.; Russell, A. J.; Hammond, E. M.; Knapp, S.; Brennan, P. E.; Conway, S. J. Optimization of 3,5-Dimethylxazolone Derivatives as Potent Bromodomain Ligands. J. Med. Chem. 2013, 56 (8), 3217–3227.
(13) Albrecht, B. K.; Audia, J. E.; Cote, A.; Gehling, V. S.; Harmange, J.-C.; Hewitt, M. C.; Leblanc, Y.; Navesukh, C. G.; Mayer, A. T.; Vasmaw, R. G. Preparation of compounds containing azepine-based ring systems as bromodomain-containing protein inhibitors and therapeutic uses thereof. WO2012075383A2, 2012.
(14) Picaud, S.; Daha, D.; Thanasaopoulou, A.; Filippakopoulos, P.; Fish, P. V.; Philpott, M.; Fedorov, O.; Brennan, P.; Bunnage, M. E.; Owen, D. R.; Bradner, J. E.; Taniere, P.; O’Sullivan, B.; Muller, S.; Schwall, S.; Stankovic, T.; Knapp, S. PFI-1, a Highly Selective Protein Interaction Inhibitor, Targeting BET Bromodomains. Cancer Res. 2013, 73 (11), 3336–3346.
(15) Nicodeme, E.; Jeffrey, K. L.; Schaefer, U.; Beinke, S.; Dewell, S.; Chung, C.-w.; Chandwani, R.; Marazzi, I.; Wilson, P.; Coste, H.; White, J.; Kirlisloy, J.; Rice, C. M.; Lora, J. M.; Pinaia, R. K.; Lee, K.; Tarakhovsky, A. Suppression of inflammation by a synthetic histone mimic. Nature 2010, 468 (7327), 1119–1123.
(16) Mireguet, O.; Lamotte, Y.; Donche, F.; Tourn, J.; Gellibert, F.; Bouillot, A.; Gosmini, R.; Nguyen, V.-L.; Delannée, D.; Seal, J.; Blandel, F.; Boullay, A.-B.; Boursier, E.; Martin, S.; Brusq, J.-M.; Krysa, G.; Ricou, A.; Tellier, R.; Costaz, A.; Huet, P.; Dudit, Y.; Trottet, L.; Kirlisloy, J.; Nicodeme, E. From ApoA1 upregulation to BET family bromodomain inhibition: Discovery of I-BET151. Bioorg. Med. Chem. Lett. 2012, 22 (8), 2963–2967.
(17) Zuber, J.; Shi, J.; Wang, E.; Rappaport, A. R.; Herrmann, H.; Sison, E. A.; Magoon, D.; Qi, J.; Platt, K.; Wunderlich, M.; Taylor, M. J.; Johns, C.; Chicas, A.; Muller, J. C.; Kogan, S. C.; Brown, P.; Valient, P.; Bradner, J. E.; Lowe, S. W.; Vakoc, C. R. RNAi screen identify BRD4 as a therapeutic target in acute myeloid leukemia. Nature 2011, 478 (7410), 524–528.
(18) Wu, S.-Y.; Lee, A. Y.; Lai, H.-T.; Zhang, H.; Chiang, C.-M. Phospho Switch Triggers BRD4 Chromatin Binding and Activator Recruitment for Gene-Specific Targeting. Mol. Cell 2013, 49 (5), 843–857.
(19) Banerjee, C.; Archin, N.; Michaels, D.; Belkina, A. C.; Denis, G. V.; Bradner, J.; Sebastiani, P.; Margolis, D. M.; Montano, M. BET bromodomain inhibition as a novel strategy for reactivation of HIV-1. J. Leukoc. Biol. 2012, 92 (6), 1147–1154.
(20) Luo, Z.; Guo, J.; Wu, Y.; Zhou, Q. The BET bromodomain inhibitor JQ1 activates HIV latency through antagonizing BRD4 inhibition of Tat-transactivation. Nucleic Acids Res. 2013, 41 (1), 277–287.
(21) Chung, C.-W.; Nicodeme, E.; Quinoline, azoloquinoline, triazolobenzodiazepine derivatives as bromodomain inhibitors for
treating autoimmune and inflammatory diseases and their preparation.

WO2011054843A1, 2011.

(16) Albrecht, B. K.; Harmange, J.-C.; Cote, A.; Taylor, A. M. Bromodomain inhibitors for cancer therapy. WO2012174487A2, 2012.

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

(18) See Supporting Information for complete DSF $T_m$ shifts with replicates and errors.

(19) Compounds 5–12 and 14–17 were purchased from Interbioscreen (http://www.ibscreen.com).

(20) Chung, C.-w.; Dean, T. W.; Woolven, J. M.; Bamborough, P. Fragment-based discovery of bromodomain inhibitors part 1: inhibitor binding modes and implications for lead discovery. J. Med. Chem. 2011, 55 (2), 576–586.

(21) Totrov, M.; Abagyan, R. Flexible protein–ligand docking by global energy optimization in internal coordinates. Proteins 1997, Suppl 1, 215–220.

(22) Philpott, M.; Yang, J.; Tumber, T.; Fedorov, O.; Uttarkar, S.; Filipakopoulos, P.; Picaud, S.; Keates, T.; Felletar, I.; Ciulli, A.; Knapp, S.; Heightman, T. D. Bromodomain–peptide displacement assays for interactome mapping and inhibitor discovery. Mol. BioSyst. 2011, 7 (10), 2899–2908.

(23) Calculated using the free ACID/I-Lab calculator at http://ilab.cds.rsc.org.

(24) Compound 34 was synthesized from the commercially available 1-hydrazinyl-4-phenylphthalazine in a manner analogous to compound 20.

(25) Mullighan, C. G.; Zhang, J.; Kasper, L. H.; Lerach, S.; Payne-Turner, D.; Phillips, L. A.; Heatley, S. L.; Holmfield, L.; Collins-Underwood, J. R.; Ma, J.; Buetow, K. H.; Pui, C.-H.; Baker, S. D.; Brindle, P. K.; Downing, J. R. CREBBP mutations in relapsed acute lymphoblastic leukemia. Nature 2011, 471 (7337), 235–239.

(26) Vidler, L. B.; Brown, N.; Knapp, S.; Hoelder, S. Druggability Analysis and Structural Classification of Bromodomain Acetyl-lysine Binding Sites. J. Med. Chem. 2012, 55 (17), 7346–7359.

(27) Edwards, A. M.; Bountra, C.; Kerr, D. J.; Willson, T. M. Open access chemical and clinical probes to support drug discovery. Nature Chem. Biol. 2009, 5 (7), 436–440.

(28) Sternfeld, F.; Carling, R. W.; Jelley, R. A.; Ladduwahetty, T.; Merchant, K. J.; Moore, K. W.; Reeve, A. J.; Street, L. J.; O'Conner, D.; Sohal, B.; Atack, J. R.; Cook, S.; Seabrook, G.; Wafford, K.; Tattersall, F. D.; Collins, N.; Dawson, G. R.; Castro, J. L.; MacLeod, A. M. Selective, Orally Active $\gamma$-Aminobutyric AcidA $\alpha$5 Receptor Inverse Agonists as Cognition Enhancers. J. Med. Chem. 2004, 47 (9), 2176–2179.

(29) Garino, C.; Tomita, T.; Pietrancosta, N.; Laras, Y.; Rosas, R.; Herbette, G.; Maigret, B.; Quêléver, G.; Iwatsubo, T.; Kraus, J.-L. Naphthyl and Coumarinyl Biarylpyperazin Derivatives as Highly Potent Human $\beta$-Secretase Inhibitors. Design, Synthesis, and Enzymatic BACE-1 and Cell Assays. J. Med. Chem. 2006, 49 (14), 4275–4285.

(30) French, C. A.; Ramirez, C. I.; Kolmakaov, J.; Hickman, T. T.; Cameron, M. J.; Thyne, M. E.; Koot, J. L.; Toretsky, J. A.; Tadavarthy, A. K.; Kees, U. R.; Fletcher, J. A.; Aster, J. C. BRD-NUT oncoproteins: a family of closely related nuclear proteins that block epithelial differentiation and maintain the growth of carcinoma cells. Oncogene 2007, 27 (15), 2237–2242.

(31) Kedersha, N.; Tisdale, S.; Hickman, T.; Anderson, P. Real-time and quantitative imaging of mammalian stress granules and processing bodies. Methods Enzymol. 2008, 448, 521–552.

(32) Phair, R. D.; Gorski, S. A.; Misteli, T. Measurement of dynamic protein binding to chromatin in vivo, using photobleaching microscopy. Methods Enzymol. 2004, 375, 393–414.

(33) Leslie, A. G. W.; Powell, H. MOSFLM, 7.01; MRC Laboratory of Molecular Biology: Cambridge, 2007.

(34) Evans, P. SCALA—scale Together Multiple Observations of Reflections, 3.3;0; MRC Laboratory of Molecular Biology: Cambridge, 2007.

(35) McCoy, A. J.; Grosse-Kunstleve, R. W.; Storoni, L. C.; Read, R. J. Likelihood-enhanced fast translation functions. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2005, 61, 458–464.

(36) Filippakopoulos, P.; Morris, R.; Lamzin, V. S. Automated protein model building combined with iterative structure refinement. Nature Struct. Biol. 1999, 6 (5), 458–463.

(37) Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2004, 60, 2126–2132.

(38) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr., Sect. D: Biol. Crystallogr. 1997, 53, 240–255.

(39) Painter, J.; Merritt, E. A. Optimal description of a protein structure in terms of multiple groups undergoing TLS motion. Acta Crystallogr., Sect. D: Biol. Crystallogr. 2006, 62, 439–450.