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The Bromodomain: From Epigenome Reader to Druggable Target

Roberto Sanchez, **Jamel Meslamani**, and **Ming-Ming Zhou**
Department of Structural and Chemical Biology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Abstract

Lysine acetylation is a fundamental post-translational modification that plays an important role in control of gene transcription in chromatin in an ordered fashion. The bromodomain, the conserved structural module present in transcription-associated proteins, functions exclusively to recognize acetyl-lysine on histones and non-histone proteins. The structural analyses of bromodomains' recognition of lysine-acetylated peptides derived from histones and cellular proteins provide detailed insights into the differences and unifying features of biological ligand binding selectivity by the bromodomains. Newly developed small molecule inhibitors targeting bromodomain proteins further highlight the functional importance of bromodomain/acetyl-lysine binding as a key mechanism in orchestrating molecular interactions and regulation in chromatin biology and gene transcription. These new studies argue that modulating bromodomain/acetyl-lysine interactions with small-molecule chemicals offer new opportunities to control gene expression in a wide array of human diseases including cancer and inflammation.

Introduction

Gene transcriptional activation or repression in the human genome is closely coupled to changes the structure of chromatin comprising DNA and histone proteins. This complex and tightly coordinated relationship is made possible through the post-translational modifications of DNA-packing histones present in the chromatin. Chromatin contains the entire genomic DNA present in eukaryotic cells, and functions as the primary regulator that controls global dynamic changes in gene expression and silencing. Nucleosomes that function as the building blocks of chromatin pack 147-bp lengths of DNA in two super-helical turns around a histone octamer, which consists of a histone-3-histone-4 (H3–H4) tetramer and two H2A–H2B dimers. These nucleosome core particles are connected by short lengths of DNA between the linker histones H1 and H5 to form a nucleosomal filament, which then fold into the higher-order structure of the chromatin fiber. Within the chromatin structure, the structurally flexible N- and C-termini of the core histone octamers protrude out from the nucleosome particles and are subject to a wide array of post-translational modifications,

including acetylation, methylation, phosphorylation, ubiquitination, ribosylation, biotinylation, citrullination, crotonylation, and SUMOylation [1–3]. These site-and state-specific modifications may act collectively in orchestrating genomic stability and gene expression or repression in the cell nucleus [4–6]. Lysine acetylation [7] is highly dynamic modification that impacts broadly chromatin structure and function as well as gene transcription [8–10]. Further, lysine acetylation has been shown not to be limited to histones, but also take place on different types of transcription-associated proteins, including histone modifying enzymes, transcription factors as well as chromatin regulators [11, 12] suggesting that it may act as a more general regulator of protein function likley beyond transcriptional regulation, akin to phosphorylation [13]. Not surprisingly, changes in lysine acetylation among such transcription-associated proteins has been linked to different human diseases [14].

The dynamic role of lysine acetylation is, to some extent, attributed to the bromodomain (BrD), which is the only protein domain whose conserved activity is to function as an acetyl-lysine binding domain [15]. Some of BrD-containing proteins have been functionally implicated in disease processes, including cancer, inflammation and viral replication [16–19]. The development of small-molecule inhibitors of BrDs in recent years has enabled a number of chemical biology guided studies of BrD function and strongly suggests that they are *bona fide* druggable targets for various human diseases [19, 20]. This review describes the current status of the description of the bromodomain family from a structural and chemical biology point of view.

The bromodomain fold and acetyl-lysine recognition

The available structures of BrDs reveal that they all share an evolutionary conserved structural fold of a left-handed four-helix bundle (α_Z, α_A, α_B and α_C), termed the ‘BrD fold’ [21–23]. The inter-helical α_Z-α_A (ZA) and α_B-α_C (BC) loops constitute a pocket that recognizes the acetyl-lysine modification (Figure 1A). Despite the conserved BrD fold, the overall sequence similarity between members of the BrD family is not high, and there are significant variations in the sequences of the ZA and BC loops [24]. Nevertheless, the amino acid residues that are engaged in acetyl-lysine recognition are among the most conserved residues in the large BrD family, and correspond to Tyr^{1125}, Tyr^{1167} and Asn^{1168} in CREBBP (or CBP) (Figure 1B) [25–27]. The acetyl-lysine residue forms a specific hydrogen bond between the oxygen of the acetyl carbonyl group and the side-chain amide nitrogen of the conserved asparagine residue (Asn^{1168} in CBP) [28] (Figure 1B). However some BrDs, such as that of TRIM28 or the sixth BrD in the human Polybromo protein, contain a different residue (Tyr, Thr, or Glu) at the position of the conserved Asn [23]. The TRIM28 BrD does not bind to lysine-acetylated histones [29], whereas the BrD6 of Polybromo does, suggesting that there may be alternative modes of acetyl-lysine recognition by the BrD fold. The cooperative binding of two acetylation marks by a single BrD has also been described. This binding mode was first observed in BrD1 of BRDT [30] where both Kac residues of the H4K5acK8ac peptide occupy a single binding pocket. More recently BrD1 of BRD3 [31] and BrD1 of BRD4 [23] have been shown to also have the ability to recognize two acetylation marks with a single binding pocket. The recognition of di-acetylated peptides is also mediated by the conserved Asn residue, which interacts with one

of the Kac residues, while the second Kac is bound at the edge of the canonical acetyl-lysine binding pocket, establishing hydrogen bonds with the peptide backbone (Figure 1C) [30]. While BRDT, BRD3, and BRD4 all belong to the BET (bromodomain and extra-terminal) family of BrD-containing proteins, sequence and structural analysis suggests that BrDs outside of the BET family might also have the ability to recognize two acetylation marks [30, 32]. An additional characteristic feature of the acetyl-lysine recognition pocket in BrDs is the presence of a network of water molecules that form hydrogen bonds with carbonyl groups of the protein backbone at the base of the pocket [28]. These water molecules, which are an integral part of the acetyl-lysine binding pocket, and the residues in the deeper part of the acetyl-lysine binding pocket are relatively conserved over most of the BrD family (Figure 1D). The selective recognition of acetyl-lysine in the context of different sequences is due to differences in key residues at the peptide-binding site [23, 27, 31, 33, 34]. For example, the residues participating in the recognition of H3K14ac by the second BrD of the human Polybromo protein are very different in the BrDs of PCAF and CBP explaining their different specificities for this specific mark [33].

The association of bromodomains with other chromatin modules

Most bromodomains are part of large multi-domain proteins with varying domain architectures [35, 36], as such, BrDs are often found in tandem with other domains. More than 15 different domain types have been identified to occur within the same proteins as BrDs, including the PHD, PWWP, B-box type zinc finger, ring finger, SAND, FY Rich, SET, TAZ zinc Finger, helicase, ATPase, BAH (bromo adjacent homolog) domain, WD40 repeat and MBD (methyl-CpG binding domain) [37, 38]. The most frequent association is that of a BrD with a PHD domain [37]. In many of these proteins the PHD and BrD are separated by a short amino acid sequence (< 30 residues) and in some cases have been shown to form structurally interdependent tandem PHD/BrD arrangements such as that observed in TRIM28 [29] and TRIM33 [39]. The TRIM28 structure contains a distinct scaffold that unifies the two protein modules, in which the Z helix of the BrD forms a hydrophobic core that anchors the other three helices of the BrD on one side and the PHD finger on the other (Figure 2A). A comprehensive analysis correlating transcriptional repression, UBC9 binding and SUMOylation showed that the PHD and BrD of cooperate as a single functional unit to facilitate lysine SUMOylation [29, 40]. This SUMO ligase activity is a divergent function for the BrD, which does not bind to lysine-acetylated histones in this form. The structure of the TRIM33 tandem PHD/BrD shows that the PHD finger binds to a methylated lysine, while the BrD bind to an acetylated lysine on the same peptide (H3K9me3K18ac), thus acting as a combinatorial reader of histone marks [39] (Figure 2B). In contrast to TRIM28 and TRIM33, the structure of BPTF, which also contains a PHD finger and a BrD separated by a short linker [41], does not demonstrate any significant structural interactions between the two domains. In BPTF, the PHD domain recognizes the methylated lysine 4 residue of histone H3 (H3K4me3) [41, 42].

Less is known about the functional consequences of tandem BrD/BrD combinations (the second most common domain association for the BrD). The structures of BrD1 of BRD4 with H4K8acK12ac [23] and of BrD2 of BRD2 with H4K5acK12ac [43], showed that two distinct BrDs can bind to the two marks simultaneously, suggesting the possibility that a

tandem BrD/BrD combination might recognize di-acetylated peptides. The linker sequence between Kac marks and the length and flexibility of the linker between the tandem BrDs is likely to affect the ability of tandem BrD/BrD to bind the diacetylated peptide [32]. In the transcription initiation factors TAF1 and TAF1L (as well as in some BrD pairs in Polybromo) the two BrDs are separated by short sequences (< 20 residues). The structure of the TAF1 BrDs suggests that they form a tandem arrangement that binds selectively to multiple acetylated histone H4 peptides [23, 44]. Another tandem BrD arrangement has been observed in the yeast Rsc4 protein, which is related to human Polybromo [45]. The Rsc4 structure contains a compact BrD tandem that binds H3K14ac in BrD2 and the acetylated lysine 25 of Rsc4 itself in BrD1, suggesting an autoregulatory mechanism [45]. Because the arrangements of the tandem BrDs in TAF1 and Rsc4 are different it is not clear whether the arrangement of tandem BrDs are protein-specific or evolutionarily conserved.

Structural Coverage of the Bromodomain Family

The human genome encodes 46 BrD-containing proteins (Table 1), each of which contains between one and six BrDs [38]. Most of the BrD-containing proteins function as chromatin modifying enzymes or transcriptional regulators. Their biological functions have been reviewed recently [46]. The total number of individual human BrDs is 61. This can be contrasted with the yeast genome, which encodes only nine BrD-containing proteins and a total of 14 BrDs [38]. Depending on the exact alignment used, a classification of the human BrD sequences can yield slightly different groupings. However, most recent publications agree on a classification of the human BrD family into 8 subgroups [26, 32] (Figure 3). The current structural coverage of the human BrD family, while not exhaustive, is relatively high with 42 out 61 BrD with at least one structure available (mouse orthologs of BRD3 and BRDT are counted). In terms of structural description of ligand binding the coverage is lower, with 17 BrDs having at least one structure in complex with a peptide, and 10 BrDs having at least one structure in complex with a small-molecule ligand (Figure 3). While there is at least one known structure for each of the eight subgroups; groups I, II, IV, and VIII have complete or almost complete coverage, in contrast to groups III, V, VI, VII, which have 50% or less coverage. Due to extensive inhibitor development efforts group II, containing the BET (bromodomain and extraterminal domain) proteins is the most extensively covered group, with every member having at least one structure available in complex with a peptide or small-molecule (Figure 3). The heavily studied domains BRD4-BrD1 and BRD2-BrD1 have 54 and 18 structures, respectively, accounting for 39% of all available BrD structures [47].

The large amount of structural information available for the human BrD family has been leveraged for the structure-guided development or optimization of bromodomain inhibitors, especially for the BET group (Figure 3).

Small-molecule inhibitors of Bromodomains

While multiple studies had shown that BrD-containing proteins participate in disease processes and that BrDs are *bona fide* drug targets [16–20, 46] it was initially thought that BrDs, as mediators of protein-protein interactions, did not belong to the druggable universe

of proteins. The first attempt to develop small-molecule inhibitors of BrDs was conducted by the Zhou group using NMR-based screens to identify compounds that inhibit the PCAF BrD [47]. This study validated the Kac binding pocket as amenable to small-molecule binding and identified a lead compound with potency in the low μM range. While not highly potent, the lead compound was selective for PCAF BrD (vs CBP and TRIM28) showing that it was possible to achieve selectivity within the BrD family. This pioneering work was followed by the structure-guided discovery of CBP BrD inhibitors [48, 49], one of which (ischemin/MS120), was later shown to prevent apoptosis in ischemic cardiomyocytes [49]. Ischemin binds to the CBP BrD by hydrogen bonding to the conserved Asn1168, which also mediates Kac binding (Figure 4A). These initial studies showed that it was possible to develop selective small-molecule inhibitors of BrDs that target the Kac binding site, and which had relevant biological activity in spite of their relatively low potency (K_d of ischemin is 19 μM).

More recently two groups, one at the Structural Genomic Consortium (SGC) and one at GlaxoSmithKline (GSK) independently developed selective nanomolar inhibitors of BrDs in the BET family (Figure 3, Group II), showing for the first time that BrDs might be druggable [50, 51]. The SGC group developed the JQ1 inhibitor using as a starting point thienodiazepine compounds initially reported by Mitsubishi Pharmaceuticals as BET protein ligands [50]. The GSK group developed I-BET using a phenotypic assay to identify apolipoprotein A-1 up-regulators, followed by additional studies [51, 52]. Both I-BET and JQ1 bind to the Kac pocket directly blocking the recognition of Kac by the BrD (Figure 4) by mimicking the hydrogen-bonding and hydrophobic interactions of the native peptide ligand. Selectivity for the BET family is achieved by making additional contacts outside the Kac cavity (Figure 4) [50, 51]. While the compounds are selective for BET BrDs, they do not show marked preference for any particular member of the BET family, and do not distinguish between BrD1 and BrD2 of the BET proteins. Both BET inhibitors have shown cell permeability and *in vivo* efficacy. In the case of I-BET series it was shown that I-BET762 provides protection against LPS-induced endotoxic shock and bacteria-induced sepsis [51]; while JQ1 was shown to promote tumor cell differentiation, decrease tumor size, and enhance survival in a mouse xenograft model of the nuclear protein in testis midline carcinoma (NMC) [50].

The efficacy of these first-generation BET inhibitors has prompted the search for new chemical scaffolds that may lead to the development of inhibitors of non-BET BrDs, as well as inhibitors that may distinguish within the BET family and between BrD1 and BrD2 of BET proteins (Figure 5).

Targeting non-BET BrDs is expected to be challenging given the predicted low druggability of many BRDs [20, 53]. While no nanomolar inhibitors of non-BET BrDs have been reported, some encouraging results suggest that it might be possible to develop drug-like compounds for some of them. Hewings et al. [54] identified 3,5-dimethyloxazoles as a new scaffold that competes for the Kac binding pocket. The compounds showed IC_{50} values below 5 μM for BRD2-BrD1 and BRD4-BrD1, but also moderate activity against CBP BrD. Further structure-guided optimization of this scaffold resulted in a more potent inhibitor with selectivity for BET and CBP BrDs with anti-proliferative effects in acute myeloid

leukemia cells [55]. Chung et al. [56] used a structure-guided fragment-based approach to identify new BrD chemotypes. A phenyl dimethyl isoxazole chemotype was optimized through structure-based design, leading to a sulfonamide series showing anti-inflammatory activity in cellular assays [57]. This study validated the use of fragment-based approaches as an alternative strategy for BrD inhibitor discovery. Some of the fragments identified in this study bound with a consistent mode to different BrDs, including CBP, suggesting their potential as generic templates for BrD inhibitors. Gerona-Navarro et al. developed cyclic-peptide inhibitors of CBP BrD with affinities similar to those of small-molecule CBP BrD inhibitors [58]. Based on a [1,2,4]triazolo[4,3-a]phthalazin scaffold Fedorov et al. [59] developed the first sub-micromolar inhibitors of BrDs outside the BET family. The compounds showed preference for the BrDs of CECR2, BRD4-1, CBP, BRD9, and TAF1L-2. In another study that suggests that it may be possible to target less druggable BrDs Ferguson et al. [60] used a fragment-based approach to develop low-micromolar pyridoindole inhibitors of BAZ2B BrD.

In the area of identifying new scaffolds for BET BrD inhibitors Seal et al. [61] described a new quinolone isoxazole scaffold that produced an inhibitor (I-BET151) with good bioavailability and efficient suppression of bacterial induced inflammation and sepsis *in vivo*. Fish et al. [62] used a fragment-based approach to identify novel BET family inhibitors, which, like in the case of Chung et al. [56], lead to the development of a sulfonamide series of dihydroquinazolinone. One compound, named PFI-1, was selected for profiling and was shown to be selective for BET BrDs with potent cell-based activity. Further studies with PFI-1 showed that it has anti-proliferative effects on leukemic cells [63]. More recently, Mirguet et al. [64] reported the identification of 1,5-naphtyridine derivatives as potent BET BrD inhibitors with good cell activity and pharmacokinetic properties. In another fragment-based study Zhao et al. [65] found that 2-thiazolidinones derived compounds also served as inhibitors of BRD4 BrD. Using a structure-based guided inhibitor design, Gehling et al. [66] designed novel BRD4 BrD inhibitors based on the isoxazole azepine scaffold that have good oral activity and *in vivo* BET inhibition. Vilder et al. [67] used a virtual screening approach, first by using similarity search methods to identify commercial compounds that mimic Kac, followed by molecular docking and visual inspection, to identify four novel BRD4 BrD inhibitor scaffolds. Another new BET modulator fragment was introduced by Lucas et al [68]. The compound XD14 having the 4-acyl pyrolles moiety was discovered by high throughput screening against BRD4-BrD1. This compound shows a K_d in the nM range for the BrDs of BRD2, BRD3 and BRD4, but a low μM affinity for BRDT BrDs.

In a surprising finding, Martin et al. [69] showed that the cyclin-dependent kinase inhibitor Dinaciclib also interacts with the Kac binding site in BRDT (a member of the BET family). Suggesting a new scaffold for the design of BrD inhibitors. In addition, recently Dittmann et al. [70] determined from a quantitative chemoproteomic profiling that two small molecules known as inhibitors of the phosphoinositide 3-kinase (PI3K) and commonly used as probes in cell signaling, are also inhibitors of BET BrDs. These chromen-4-one compounds named LY29002 and LY303511 represent a new pharmacophore for BET BrDs. Their selectivity is higher for BrD1 of the BET family and they exhibit the same anti-inflammatory effects as

the previous established BET inhibitors. This finding was followed by the more recent discovery of three other high potent dual kinase/BET inhibitors [71, 72]. BI-2536 known inhibitor of PLK1 kinase, TG-101209 and TG-101348 inhibitors of JAK2 kinases, show a sub-micromolar affinity for the BET BrDs and specially a nanomolar affinity for BRD4-BrD1. In another recent study, Atkinson et al. [73] designed the first dual epigenome eraser and reader inhibitors. These inhibitors modulate the activity of histone deacetytilase HDAC as well as the BET BrDs. They were designed by combining the structural features of HDAC and BET inhibitors. Among the designed series, a promising compound named DUAL946 shows sub-micromolar inhibition for HDAC class I and IIb, as well as BET BrDs. These dual inhibitors will help gain insights into possible coordinated cellular activities and polypharmacology of HDACs and BET BrDs in regulation of gene expression in human biology of health and diseases.

One of the more difficult tasks in the area of BrD inhibitor selectivity is probably the development of compounds that can distinguish between BrD1 and BrD2 within the BET family. Zhang et al. [74] reported a novel series of BET inhibitors based on a diazobenzene scaffold. A lead compound derived from this scaffold (MS436) shows 10-fold selectivity for BrD1 of BRD4 over BrD2; suggesting that it might be possible to selectively target individual BrDs within the BET family. In another example of selectivity of BrD1 vs BrD2 within a BET protein, Picaud et al. [75] report that RVX-208 (a compound in phase II clinical trials) is a selective inhibitor for the second BrD of BET proteins. A possible explanation for the selectivity of RVX-208 is given by the co-crystal structure of the compound with BrD1 and BrD2 domains. In the complex with BrD2 of BRD2 the BrD2 unique residue His433 flips into the Kac binding site and packs against the phenyl ring of RVX-208.

While the BrD inhibitor development field is extremely active, the coverage of the human BrD family is far from complete, with only the BET family having highly effective inhibitors available (Figure 3). In the process of addressing the challenges described above, a large number of different compounds have been and are being developed. In an attempt to organize this information in a user-friendly resource Meslamami et al. [76] have developed ChEpiMod, a knowledgebase of chemical modulators for epigenome reader domains. A query of ChEpiMod (version 2014.02.03) shows that the BrDs with the most compounds developed (with potency better than 10 μM) are BRD4-BrD1 (196 compounds), BRD4-BrD2 (119), CBP BrD (97 compounds), PCAF/KAT2B BrD (60 compounds), EP300 BrD (26 compounds), and BRD2-BrD1 (12 compounds). ChromoHub [77, 78] is another related database for epigenome reader, writer and eraser domains. Although it is not focused on chemical modulators or their molecular interactions with these domains, ChromoHub is a valuable resource for exploring epigenetic mechanisms concerning cancer genomics.

Applications of bromodomain inhibitors

There are multiple disease areas in which BrD inhibitors have been used [19, 46]; mainly cancer [17], HIV/AIDS [18], cardiovascular disease [49, 79], and inflammation [51]. Additionally applications in the area of male contraception have also been reported [80, 81]. Some common themes emerge from the multitude of studies using bromodomain inhibitors.

In a number of tumor types BET inhibitors (JQ1, I-BET151 and I-BET762) have been shown to have antiproliferative effects via downregulation of *MYC* transcription [82–90], although effects independent of MYC downregulation have also been observed [91]. In the HIV/AIDS field multiple studies have shown that BET inhibitors (JQ1, I-BET, MS417) can reactivate HIV from latency [92–95]. The same effect has been observed with other viruses [96–99]. Table 2 summarizes the currently published applications of BrD inhibitors. Bromodomain inhibitors have proven to be effective tools to investigate the function of BrD-containing proteins, in particular their BrD-associated functions, in a number of systems. The number of new functions discovered for BET proteins in the last three years, since potent inhibitors became available, exemplifies the power of small-molecule modulators as tools in biology. It is expected that as potent inhibitors are developed for other BrDs, outside the BET family, new biology associated with these proteins will be discovered.

Conclusions

The last few years have witnessed enormous activity in the field of bromodomain biology. The structural coverage of the human BrD family is almost complete and focus has shifted towards solving higher order structures (tandem PHD/BrD and BrD/BrD) and the development and application of BrD inhibitors. The development of selective and potent small-molecule inhibitors has not only brought bromodomains into the realm of druggable targets, but has also shown the power of small-molecule modulators as tools to discover and dissect the functions of these epigenome reader domains in human biology. This effect is apparent when seeing the amount of functional information being generated about BRD4 (and BET proteins in general) through the use of selective nanomolar inhibitors. The challenges for the future are many, including: (1) the development of selective and potent inhibitors for non-BET bromodomains; (2) the development of inhibitors that are selective within the BET family; (3) better understanding of the functional differences and specificities of bromodomains in transcription or chromatin-associated proteins in gene transcriptional regulation; and (4) the translation of these inhibitors into new drugs for the treatment of multiple disease in which BrD containing proteins play a crucial role. Recent developments suggest that these challenges, while difficult, can be overcome; and that the field of bromodomain biology is rapidly becoming a focal point in our continued pursuit in better mechanistic understanding of the complex and dynamic nature of gene transcription in chromatin, as well as in the emerging arena of epigenetic targeted drug discovery for a wide array of human diseases that is expected to be even more active in the next few years.

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Highlights

- Structure and function of bromodomains as the acetyl-lysine binding domains
- Small molecule inhibitors of bromodomains
- Bromodomain inhibitors as epigenetic chemical modulators to control gene transcriptional activation

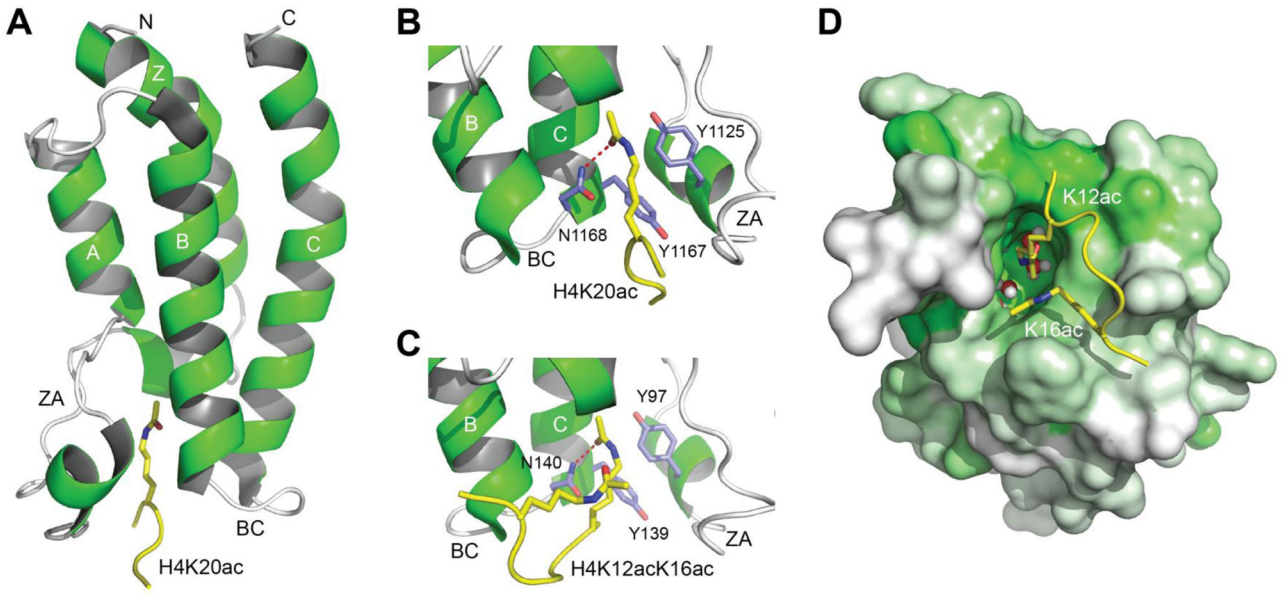

Figure 1. The structural features of the bromodomain as the acetyl-lysine binding domain

(A) The BrD fold. Structure of the CBP BrD in complex with H4K20ac peptide (PDB 4n3w).

(B) Close-up of the acetyl-lysine binding site in the CBP BrD. Key conserved interaction residues are shown. The hydrogen bond between Asn1168 and K20ac is shown in red.

(C) Close-up of the di-acetylated lysine-binding site in the BRD4-BrD1. Key conserved interaction residues are shown. The hydrogen bond between Asn140 and K12ac is shown in red (PDB 3uvx).

(D) Conservation of the acetyl-lysine binding site. The BRD4-BrD1 structure is shown with sequence conservation mapped on the surface. Green represents more conserved position, and white less conserved position. The conservation was computed from a multiple sequence alignment of all human BrDs. Conserved water molecules at the bottom of the K12ac binding pockets are shown as ball-and-stick models.

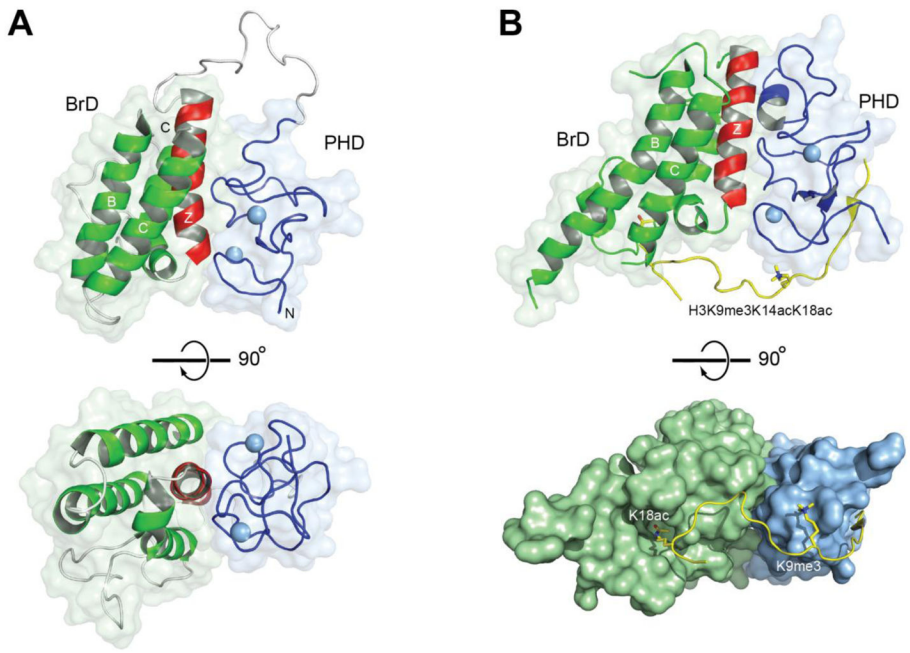

Figure 2. Structures of tandem modules of epigenome reader domains

(A) Tandem PHD/BrD module of TRIM28. The PHD finger is shown in blue with Zn atoms shown as spheres, and the BrD is shown in green with the Z helix in red (PDB 2ro1).

(B) Tandem PHD/BrD of TRIM33 in complex with H3K9me3K14acK18ac peptide. The PHD finger is shown in blue with Zn atoms shown as spheres, the BrD is shown in green with the Z helix in red, and the H3K9me3K14acK18ac peptide is shown in yellow (PDB 3u5o).

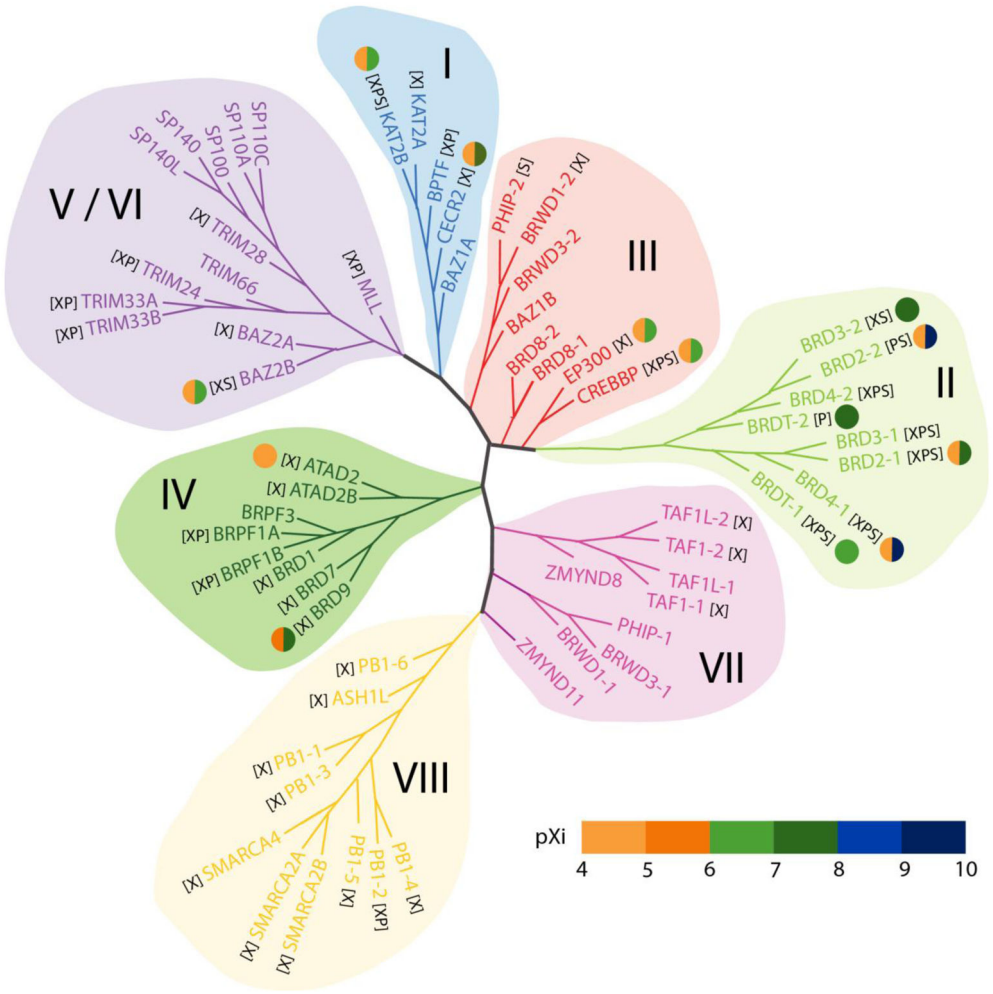

Figure 3. Sequence-based classification of the human BrD family

For consistency the numbering of the subgroups has been adjusted to correspond to those of Filippakopoulos et al. [23]. Meaning of symbols: [X] experimental ligand-free structure available, [P] structure in complex with peptide available, [S] structure in complex with small-molecule available. The colored circles illustrate the range of affinities of ligands tested against a particular BrD. Affinities expressed as K_i, K_d, or IC_{50} where converted to pX_i, where $pX_i = -\log_{10}(X_i)$, with X_i expressed in M. The left side of the circle is the minimum value of pX_i and the right side the maximum value, which are color-coded according to the pXi scale shown at the right lower corner. This figure was produced with data extracted from the ChEpiMod knowledgebase [76] as of February 2014.

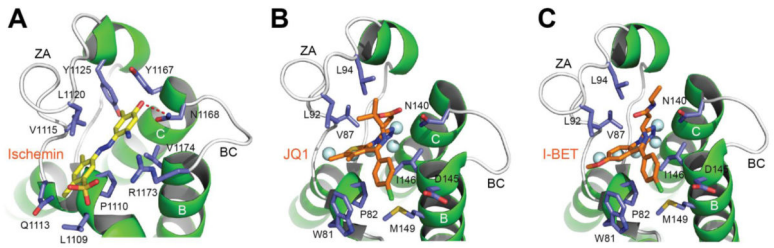

Figure 4. Structures of BrD-inhibitor complexes

(A) Ischemin bound to CBP BrD. Binding site residues are shown as sticks, with ischemin in yellow, and the hydrogen bond with the conserves Asn (1168 in CBP) in red (PDB 2l84).

(B) JQ1 bound to BRD4-BrD1. Binding site residues are shown as sticks, with JQ1 in orange, and conserved water molecules in light blue (PDB 3mxf).

(C) I-BET bound to BRD4-BrD1. Binding site residues are shown as sticks, with JQ1 in orange, and conserved water molecules in light blue (PDB 3p5o).

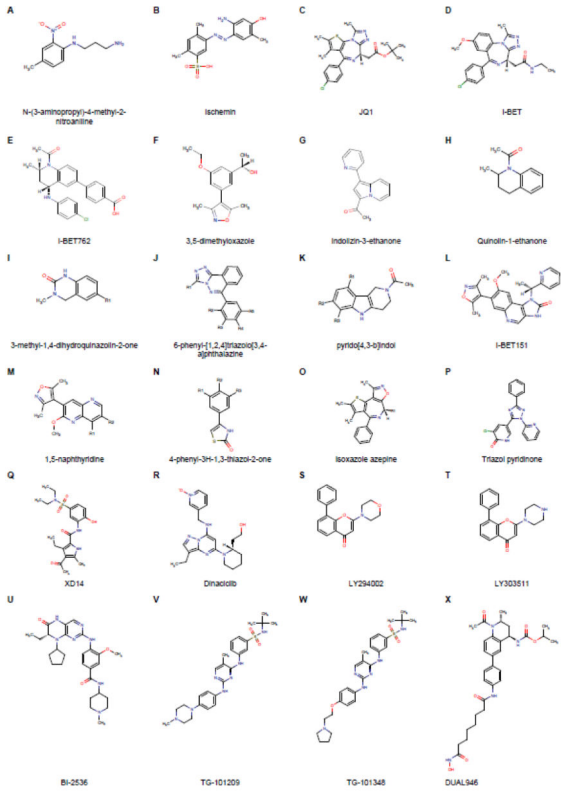

Figure 5. Representative BrD inhibitors

(A) Zeng et al. [47]. **(B)** Borah et al. [49]. **(C)** Filippakopoulos et al. [50]. **(D, E)** Nicodeme et al. [51]. **(F)** Hewings et al. [54, 55]. **(G, H, I)** Chung et al. [56]. **(J)** Fedorov et al. [59]. **(K)** Fergusson et al. [60]. **(L)** Seal et al. [61]. **(M)** Mirguet et al. [64]. **(N)** Zhao et al. [65]. **(O)** Gehling et al. [66]. **(P)** Vilder et al. [67]. **(Q)** Lucas et al. [68]. **(R)** Martin et al. [69]. **(S, T)** Dittmann et al. [70]. **(U, V)** Ember et al. [72]. **(W)** Ciceri et al. [71]. **(X)** Atkinson et al. [73].

Table 1

Human BrD-containing Proteins [100]

Isoforms of BRPF1, SMARCA2, SP110, and TRIM33 are not listed separately.

Name	Synonyms	Name	Function	# BrDs
ASH1L	ASH1, KMT2H, KIAA1420	absent small and homeotic disks protein 1 homolog	Histone-lysine methyltransferase	1
ATAD2	ANCCA	ATPase family AAA domain-containing protein 2	Transcriptional regulator	1
ATAD2B	KIAA1240	ATPase family AAA domain-containing protein 2B	unknown	1
BAZ1A	ACF1, WCRF180, hWALp1	Bromodomain adjacent to zinc finger domain protein 1A	chromatin-remodeling factor	1
BAZ1B	WBSC10, WBSCR10, WBSCR9, WSTF	Bromodomain adjacent to zinc finger domain protein 1B	tyrosine-protein kinase; transcriptional regulator	1
BAZ2A	KIAA0314, TIP5	Bromodomain adjacent to zinc finger domain protein 2A	Transcriptional repressor	1
BAZ2B	hWALp4, KIAA1476	Bromodomain adjacent to zinc finger domain protein 2B	unknown	1
BPTF	FAC1, FALZ	Bromodomain and PHD finger-containing transcription factor	chromatin-remodeling factor	1
BRD1	BRL, BRPF2	Bromodomain-containing protein 1	Transcriptional regulator	1
BRD2	KIAA9001, RING3	Bromodomain-containing protein 2	Transcriptional regulator	2
BRD3	KIAA0043 RING3L	Bromodomain-containing protein 3	Transcriptional regulator	2
BRD4	HUNK1	Bromodomain-containing protein 4	Transcriptional regulator	2
BRD7	BP75, CELTIX1	Bromodomain-containing protein 7	Transcriptional regulator	1
BRD8	SMAP, SMAP2	Bromodomain-containing protein 8	Transcriptional regulator	2
BRD9		Bromodomain-containing protein 9	unknown	1
BRDT		Bromodomain testis-specific protein	chromatin-remodeling factor	2
BRPF1	BR140, Peregrin	Bromodomain and PHD finger-containing protein 1	Transcriptional activator	1
BRPF3	KIAA1286	Bromodomain and PHD finger-containing protein 3	Transcriptional regulator	1
BRWD1	C21orf107, WDR9	Bromodomain and WD repeat-containing protein 1	Chromatin remodeling factor	2
BRWD3		Bromodomain and WD repeat-containing protein 3	JAK/STAT signalling	2
CECR2	KIAA1740	Cat eye syndrome critical region protein 2	Chromatin remodeling factor	1
CREBBP	CBP, KAT3A	CREB-binding protein	Histone acetyltransferase	1
EP300	P300, KAT3B	E1A-associated protein p300	Histone acetyltransferase	1
KAT2A	GCN5, GCN5L2, HGCN5	General control of amino acid synthesis protein 5-like 2	Histone acetyltransferase	1
KAT2B	PCAF	P300/CBP-associated factor	Histone acetyltransferase	1
MLL	KMT2A, ALL1, CXXC7, HRX, HTRX, MLL1, TRX1	Myeloid/lymphoid or mixed-lineage leukemia	Histone methyltransferase	1
PB1	PBRM1, BAF180	Polybromo-1	Transcriptional regulator	6
PHIP	WDR11	PH-interacting protein	Insulin signaling	2

Name	Synonyms	Name	Function	# BrDs
SMARCA2	BAF190B, BRM, SNF2A, SNF2L2	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 2	Chromatin remodeling factor	1
SMARCA4	BAF190A, BRG1, SNF2B, SNF2L4	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4	Chromatin remodeling factor	1
SP100		Nuclear autoantigen Sp-100	Transcriptional regulator	1
SP110		Sp110 nuclear body protein	Transcriptional regulator	1
SP140	LYSP100	Nuclear body protein SP140	Transcriptional regulator	1
SP140L	LOC93349	Nuclear body protein SP140-like protein	unknown	1
TAF1	BA2R, CCG1, CCGS, TAF2A, TAF(II)250	Transcription initiation factor TFIID subunit 1	Transcription initiation	2
TAF1L	TAF(II)210	Transcription initiation factor TFIID subunit 1-like	Transcription initiation	2
TRIM24	RNF82, TIF1, TIF1α	Transcription intermediary factor 1-alpha	Ubiquitin E3 ligase, Transcriptional regulator	1
TRIM28	KAP1, RNF96, TIF1β	Transcription intermediary factor 1-beta	SUMO E3 ligase, Transcriptional regulator	1
TRIM33	KIAA1113, RFG7, TIF1γ	Transcription intermediary factor 1-gamma	Ubiquitin E3 ligase, Transcriptional regulator	1
TRIM66	C11orf29, KIAA0298	Tripartite motif-containing protein 66	Transcriptional repressor	1
ZMYND8	KIAA1125, PRKCBP1, RACK7	Zinc finger MYND domain-containing protein 8, Protein kinase C-binding protein 1	Transcriptional regulator	1
ZMYND11	BS69	Zinc finger MYND domain-containing protein 11	Transcriptional repressor	1

Table 2

Applications of BrD inhibitors

Inhibitor	Effect	Ref.
Cancer		
JQ1	Rescue erythropoietin differentiation of human erythroleukemia cell line UT7.	[101]
JQ1	Anti-proliferative effect in multiple myeloma mouse models by down-regulation of *MYC* transcription.	[83]
JQ1	Increased sensitivity of human lymphoma B cells to Rituximab.	[102]
JQ1	Prolonged survival of mice harboring medulloblastoma xenografts and reduced tumor burden by down-regulation of *MYC* expression.	[84]
I-BET762	Reduced *MYC* expression in prostate cancer cell lines and patient-derived tumor model with inhibition of cell growth and reduction of tumor burden *in vivo*.	[85]
JQ1	Reduced growth in paediatric B-precursor acute lymphoblastic leukaemia xenograft models, both as a single agent and in combination with dexamethasone, via c-Myc depletion and down-regulation.	[86]
JQ1	Reduces the viability of B-cell acute lymphoblastic leukemia cell lines via down-regulation of *MYC* and *ILR7* transcription.-	[87]
I-BET151 I-BET762	Induce cell cycle arrest and apoptosis in myeloma cells associated with *MYC* down-regulation and HEXIM1 up-regulation.	[88]
JQ1	Impaired growth and induced apoptosis in neuroblastoma via transcriptional regulation of *MYCN*.	[89]
JQ1	Decrease in *MYC*-amplified medulloblastoma cell viability via down-regulation of *MYC* expression.	[90]
JQ1	Induced marked G_1 cell-cycle arrest and apoptosis in glioblastoma xenograft lines.	[103]
JQ1	Significant tumor regression in mice lung cancer models expressing mutant *kras*.	[104]
JQ1	Triggers rapid differentiation and death of IDH2 mutant acute myeloid leukemia via inhibition of BRD4.	[105]
JQ1	Supression of Tax-mediated NF-kappaB activation and tumorigenesis	[99]
I-BET151	Growth inhibition in a human erythroleukemic cell line as well as in erythroid precursors isolated from polycythemia vera patients.	[106]
I-Bet151 JQ1	Blocked cell proliferation and cell-cycle progression in primary effusion lymphoma (PEL) cell lines, and significantly reduced tumor growth and improved survival in a xenograft model of PEL.	[107]
JQ1	G_1 arrest and extensive apoptosis in a variety of leukemia and lymphoma cell lines, and significant anti-tumor activity in xenograft models of Burkitt's lymphoma and acute myeloid leukemia, via down-regulation of *MYC* transcription.	[82]
JQ1	Lung adenocarcinoma cells are inhibited by JQ1 through a mechanism independent of c-MYC down-regulation.	[91]
HIV/AIDS		
JQ1	Increased proviral transcriptional elongation and alleviated HIV-1 latency in cell-line models.	[92]
JQ1 I-BET MS417	Reactivate HIV from latency via inhibition of BRD2.	[93]
JQ1	HIV transcription reactivation in models of latent T cell infection and latent monocyte infection	[94]
JQ1	Activates viral latency without inducing global T cell activation.	[95]
Inflammation and autoimmunity		
JQ1	Suppression of interleukin 17–producing T helper cells mediated pathology in mouse models.	[108]
JQ1	Eliminates cytokine production *in vitro* and blunts the "cytokine storm" in endotoxemic mice by reducing levels of IL-6 and TNF-α while rescuing mice from LPS-induced death.	[109]
MS417	Attenuates activation of pro-inflammatory genes in kidney cells treated with TNF-α or infected by HIV, and ameliorates inflammation and kidney injury in HIV-1 transgenic mice via down-regulation of NF-kappaB transcriptional activity.	[110]
I-BET762	Suppresses the inflammatory functions of T cells via reduced expression of c-myc and GM-CSF.	[111]
Cardiovascular disease		

Inhibitor	Effect	Ref.
JQ1	Suppression of cardiomyocyte hypertrophy *in vitro* and pathologic cardiac remodeling *in vivo*.	[79]
I-BET151	Increases ApoA1 expression.	[112]
Others		
JQ1	Reversible inhibition of spermatogenesis in mice.	[81]
JQ1	Inhibits profibrotic effects of idiopathic pulmonary fibrosis lung fribroblasts and attenuates bleomycin-induced lung fibrosis in mice.	[113]