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Progress in the Development and Application of Small Molecule Inhibitors of Bromodomain−Acetyl-lysine Interactions

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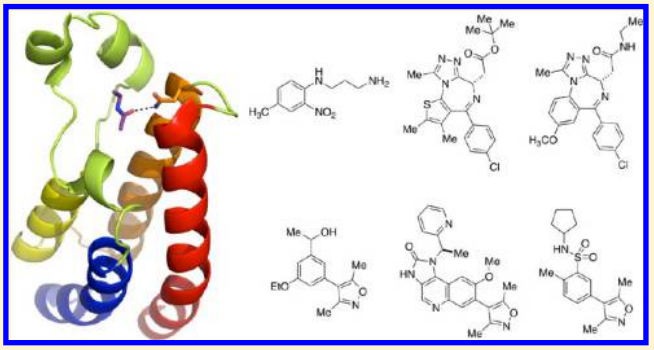
ABSTRACT: Bromodomains, protein modules that recognize and bind to acetylated lysine, are emerging as important components of cellular machinery. These acetyl-lysine (KAc) "reader" domains are part of the write−read−erase concept that has been linked with the transfer of epigenetic information. By reading KAc marks on histones, bromodomains mediate protein−protein interactions between a diverse array of partners. There has been intense activity in developing potent and selective small molecule probes that disrupt the interaction between a given bromodomain and KAc. Rapid success has been achieved with the BET family of bromodomains, and a number of potent and selective probes have been reported. These compounds have enabled linking of the BET bromodomains with diseases, including cancer and inflammation, suggesting that bromodomains are druggable targets. Herein, we review the biology of the bromodomains and discuss the SAR for the existing small molecule probes. The biology that has been enabled by these compounds is summarized.



INTRODUCTION

Acetylation of lysine residues is a widespread protein post-translational modification (PTM) that regulates a diverse array of cellular processes.[1−3] Histones, the core proteins around which nuclear DNA is packaged, are subjected to a range of PTMs, including lysine acetylation (Figure 1A). Acetylation of histone lysine residues was historically proposed to be a hallmark of transcriptionally active genes. Acetylation neutralizes the lysine positive charge, resulting in reduced affinity of histones for negatively charged DNA and an open, accessible chromatin structure.[1,4] This view is now accepted as an oversimplification, and it is thought that multiple other factors involving histones also contribute to transcriptional regulation. The plethora of PTMs to histones has led to the proposal that these marks regulate gene expression as part of a combinatorial code, leading to specific downstream effects.[5−10] There is accumulating evidence that, along with modifications to DNA, some histone PTMs can be maintained through multiple cell cycles. Thus, these PTMs potentially represent a method for epigenetic inheritance, a process that can be defined as the transmission of heritable changes in phenotype that are not encoded in the underlying DNA base sequence.[11−15] The concept of a histone code has led to the proposal that specific protein families exist to add the PTM marks ("writers"), recognize the marks ("readers"), and remove the marks ("erasers"). All three types of protein have been identified for lysine acetylation: histone acetyltransferases (HATs) effect lysine acetylation; histone deacetylases (HDACs) remove acetyl groups; bromodomains bind to acetylated lysine (KAc) and hence act as readers of lysine acetylation state (Figure 1).[16−18] Although the write−read−erase concept has proved to be inspirational, it is probably an oversimplification of the complex underlying chemistry that regulates gene expression in a context-dependent manner. One of the major challenges in biological science is deciphering the molecular details of how histones, and the potentially linked modifications to nucleic acids,[19] regulate genetics.

The possibility of treating disease by modifying cellular lysine acetylation is demonstrated by the HDAC inhibitors vorinostat[20] and romidepsin,[21] which have been approved for the treatment of cutaneous T-cell lymphoma. However, it is only recently that a number of landmark reports have revealed that the "reader" bromodomains are also potential therapeutic targets.[22−29] Excitingly, GlaxoSmithKline's Web site[30] gives details of a phase I/II clinical trial for the triazolobenzodiazepine-based BET bromodomain inhibitor **3** (I-BET/I-BET762, Figure 5)[25] to treat NUT midline carcinoma. Consequently, there is intense interest in bromodomain biology and the development of small molecule probes that disrupt their interactions with KAc-containing binding partners.[31−35] Herein, we introduce the biology associated with the bromodomains and review the small molecule bromodomain inhibitors that have been reported to date. Uses of these compounds in

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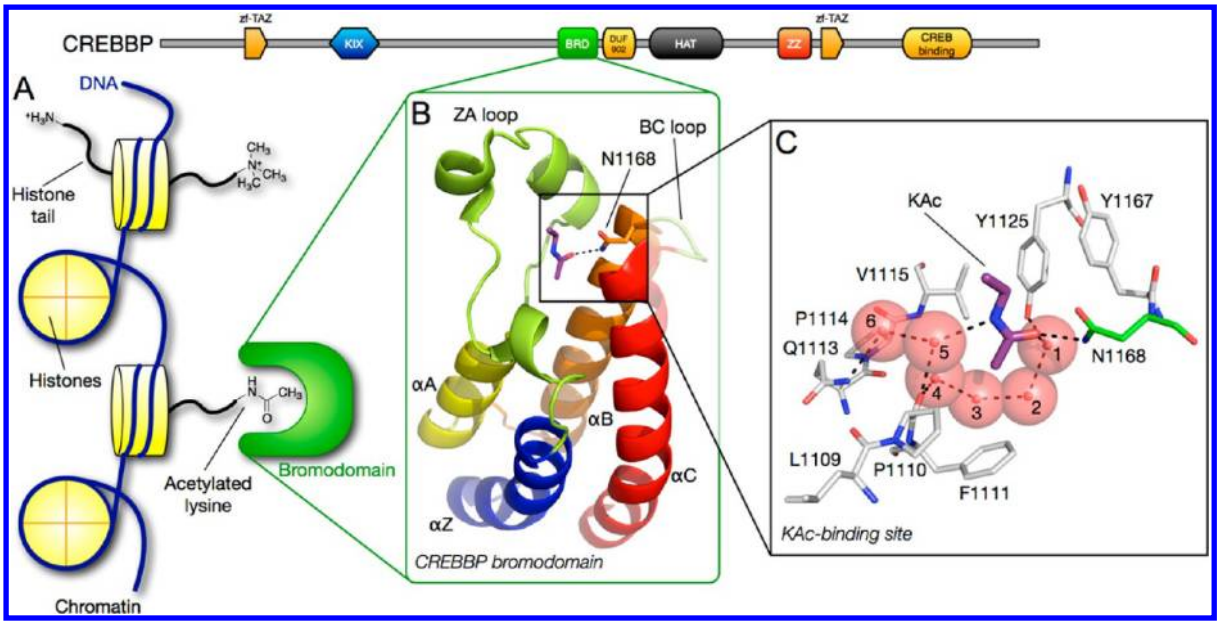

Figure 1. (A) Histone tails are subjected to multiple PTMs, including lysine acetylation. Lysine acetylation state is "read" by bromodomains, protein modules that invariably exist as part of a more complex protein architecture. The example shown is cAMP response element-binding protein (CREB) binding protein (CREBBP or CBP). (B) X-ray crystal structure of the CREBBP bromodomain in complex with KAc (carbon = purple, PDB code 3P1C). The bromodomain fold comprises four helices αZ (blue), αA (yellow), αB (orange), and αC (red) and two loop regions known as the ZA loop and the BC loop (light green). (C) The KAc residue binds in a well-defined pocket and, in CREBBP, forms interactions with N1168 and a second interaction with Y1125, via structured water molecule 1 (red sphere). The pocket is substantially hydrophobic, but four structured water molecules (red spheres) form its base (waters 1−4). Many bromodomains have been crystallized with one or two additional water molecules bound in the ZA channel. In the CREBBP above, water molecules 5 and 6 are observed in the ZA channel. Water 5 binds to the NH of the acetylated lysine residue, P1110, and water 6. Water 6 binds to Q1113 and water 5.[36]

understanding bromodomain biology and validating these proteins as potential drug targets are highlighted.

Bromodomains are named after the *Drosophilia* gene *brahma* where they were first identified.[37,38] These domains have been observed as part of numerous larger protein architectures, many of which are involved in regulating gene transcription, including HATs, ATP-dependent chromatin-remodeling complexes, methyltransferases, and transcriptional coactivators (Figure 1).[31,39] It is likely that in all these cases their primary role is binding to KAc residues. Bromodomains comprise approximately 110 amino acids that form a characteristic antiparallel four-helix bundle containing helices αZ, αA, αB, and αC (Figure 1B). KAc binds in a well-defined pocket at one end of the helical bundle, which is substantially hydrophobic in nature but contains up to four crystallographically well-ordered water molecules. These water molecules are observed in most bromodomains and act as the base of the pocket. Analysis of multiple crystal structures indicates that KAc recognition is mediated by a direct hydrogen bond between the acetyl carbonyl oxygen atom and the NH_2 group of the asparagine amide [N1168 in the bromodomain of CREBBP, Figure 1B,C]. This asparagine residue is highly conserved through the bromodomains, but there is evidence to suggest that it is replaced in certain bromodomains by other hydrogen bond donors, including threonine or tyrosine residues, with retention of KAc-binding activity.[36] A second interaction occurs between the acetyl carbonyl oxygen atom and the side chain of a conserved tyrosine residue [Y1125 in CREBBP] via one of the structured water molecules (water 1, Figure 1C). The ability of bromodomains to discriminate between different protein binding partners is thought to result predominantly from sequence diversity in the ZA and BC loop regions, which bind to residues neighboring KAc in the target protein or peptide (Figure 1B).[16,40]

There are 61 human bromodomains found within 46 separate proteins in the human proteome, with some proteins containing more than one distinct bromodomain.[15,36] Phylogenetic analysis of a structure-based alignment reveals eight distinct families (Figure 2). The biological role of most bromodomain-containing proteins (BCPs) is still unknown; however, some BCPs have been studied in detail and there is increasing information emerging on the link of BCPs to certain diseases, which has been reviewed elsewhere.[15,31,33,34,42,43]

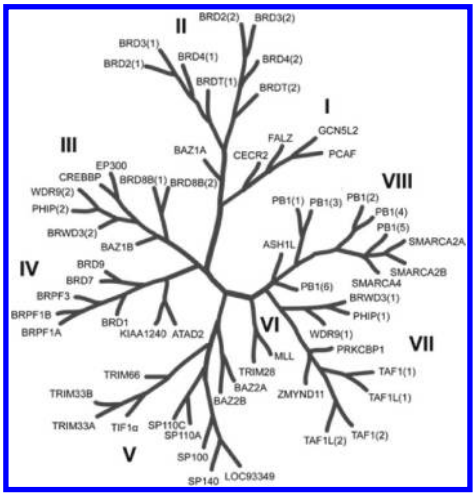

Figure 2. Phylogenetic tree of the human bromodomain family constructed on a structure-based alignment.[41] The eight bromodomain families are highlighted with Roman numerals.

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As bromodomains exist as components of large multidomain proteins, deletion of the whole BCP is a blunt tool to investigate the specific role of the bromodomain within the context of overall protein function. Therefore, an important strategy for the study of bromodomain function is the development of small molecules that selectively prevent the interaction of specific bromodomains with KAc without perturbing the BCP's other cellular roles. These chemical probes will allow a better understanding of the cellular and physiological role of BCPs. This information is essential both from the perspective of fundamental biology and to allow the validation of certain bromodomains as potential therapeutic targets.[44]

To date, small molecule probes have been employed to elucidate the role of the BCP p300/CREB-binding protein-associated factor (PCAF) in HIV transcription, the involvement of bromodomain and extra C-terminal domain (BET) BCPs in cancer and inflammation, and the role of the p53-binding protein CREBBP. The details of these studies are reviewed below.

INHIBITORS OF HIV-1 Tat ASSOCIATION WITH P300/CREB-BINDING PROTEIN ASSOCIATED FACTOR (PCAF)

Pioneering work in the development of small molecule bromodomain ligands was conducted by Zhou and co-workers. This group obtained the first structural information on bromodomains in the form of a solution structure of the PCAF bromodomain and showed that this protein binds specifically to HIV-1 Tat when lysine 50 is acetylated.[45] Further studies showed that Tat coactivator recruitment is mediated by Tat-KAc50 binding to the PCAF bromodomain.[46]

The data obtained by Zhou et al. suggested that the Tat-KAc50−PCAF interaction is important for HIV transcription and therefore is potentially a therapeutic target for anti-HIV therapy.[45−47] To investigate this possibility, Zhou et al. initiated work to identify small molecule bromodomain inhibitors. NMR screening was used to identify small molecules that bound to the PCAF bromodomain. In order to develop compounds that were selective for PCAF over other bromodomains, emphasis was placed on identifying molecules that bound in the peptide-binding groove rather than within the KAc-binding pocket. By use of an ELISA assay, compound **1** (Figure 3) was identified as inhibiting the interaction of biotinylated Tat-KAc50 peptide with an IC_{50} of 1.6 μM.

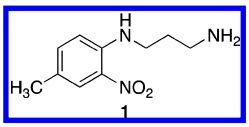

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Figure 3. Structure of the PCAF bromodomain inhibitor (**1**) developed by Zhou et al.[46]

An NMR structure of compound **1** bound to the bromodomain of PCAF (Figure 4A) reveals a binding location between residues E756 in the ZA loop and Y802 on the end of helix αB.[46] The compound forms an electrostatic interaction with E750 in the ZA loop, and a possible hydrogen bond between the nitro group and Y802 is also observed. The methyl group binds in a small hydrophobic pocket in the ZA loop, and this interaction is thought to be partly responsible for the compound's PCAF affinity. An overlay of the above structure with a representative NMR structure of the HIV-1 Tat-KAc50 peptide bound to the PCAF bromodomain demonstrates that compound **1** resides within the peptide-binding groove and not the KAc-binding pocket (Figure 4B).[45] Further studies were undertaken to optimize compound **1**; however, no significant increase in affinity was reported.[48] This work provided the first demonstration that nonpeptidic small molecules can act as bromodomain ligands.

DEVELOPMENT OF LIGANDS FOR THE BET FAMILY OF BROMODOMAINS

The majority of work to date on developing small molecule bromodomain inhibitors has focused on the BET family of bromodomains. The interest in developing BET bromodomain inhibitors results from their potential as anticancer, anti-inflammatory, or antiviral agents.[31,33,34] Four distinct chemotypes have been shown to inhibit the interaction between the BET bromodomains and KAc residues in histones: methyltriazolodiazepines (**2**−**4**) and the related triazepines,[24,25,49−51] 3,5-dimethylisoxazoles (**5**, **6**),[28,52−55] benzimidazoles (**7**)[56] (Figure 5), and 1-acyltetrahydroquinolines.[57] The development of these ligands is described below.

The first potent and selective bromodomain ligands were published simultaneously by Filippakopoulos et al.[24] and Nicodeme et al.[25] The studies of Nicodeme et al.[25] and Chung et al.[49] that eventually led to the discovery of **3** (Figure 5) were based on a phenotype screening approach to discovering small molecule up-regulators of apolipoprotein A1 (ApoA1). It was not initially known that these compounds functioned by binding to the BET bromodomains. ApoA1 up-regulation is involved in protection from atherosclerosis progression and with anti-inflammatory effects; however, when these studies commenced, no method existed to achieve this up-regulation in a therapeutic manner. A luciferase reporter-based screen was used to identify small-molecule enhancers of ApoA1 expression, which included the triazolobenzodiazepine **4** (GW841819X, Figure 5).[49] This compound has an EC_{50} of 440 nM for the induction of the ApoA1 reporter gene. A program of medicinal chemistry was undertaken to optimize the potency of **4**, in which it was found that the benzodiazepine core was essential for activity, as was the aryl group extending from the 6-position. These studies identified **3** (Figure 5),[25] which had a higher EC_{50} of 700 nM in the reporter gene assay but improved properties for in vivo experiments.

It was noted that the C4 stereochemistry of the molecule had a large influence on its potency, with only the (*S*)-enantiomer showing activity. The dependence of the compound's activity on stereochemistry was interpreted as evidence that its biological effects resulted from defined interactions with specific, but unknown, molecular targets. This observation highlights a significant challenge with phenotypic screens: although this approach is a powerful strategy for the identification of cell-permeant small molecules that effect the desired phenotype, the cellular target or targets that evoke the phenotype must be subsequently identified.[58,59] Since screening against a panel of known drug targets failed to identify a possible mode of action for **3**, a chemoproteomic approach[60,61] was employed to identify the target. An affinity matrix of the active compound was generated and exposed to a lysate of HepG2 cells. This technique identified the bromodomains of BRD2, BRD3, and BRD4, members of the BET family, as potential cellular targets of **3**; and the proposed interaction was

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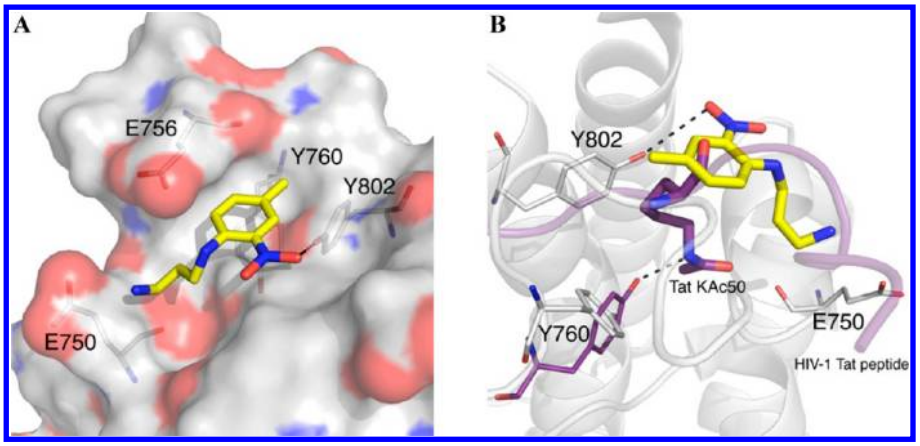

Figure 4. (A) View of an NMR structure of the PCAF bromodomain inhibitor (carbon = yellow, PDB code 1WUG) bound to the bromodomain of human PCAF.[46] Compound **1** binds between residues E756 and Y802. It is positioned to form an electrostatic interaction with E750 and a possible hydrogen bond between the nitro group and Y802. The methyl group binds in a small hydrophobic pocket and is thought to contribute to affinity for PCAF. (B) An overlay of representative NMR structures of the HIV-1 Tat-KAc50 peptide (carbon = purple, PDB code 1WUG) and compound **1** (carbon = yellow, PDB code 1JM4) bound to the PCAF bromodomain. The overlaid structures reveal that compound **1** resides in the peptide-binding groove but not the conserved KAc-binding pocket.[45,46] Structures were aligned using the "align" command in PyMOL.

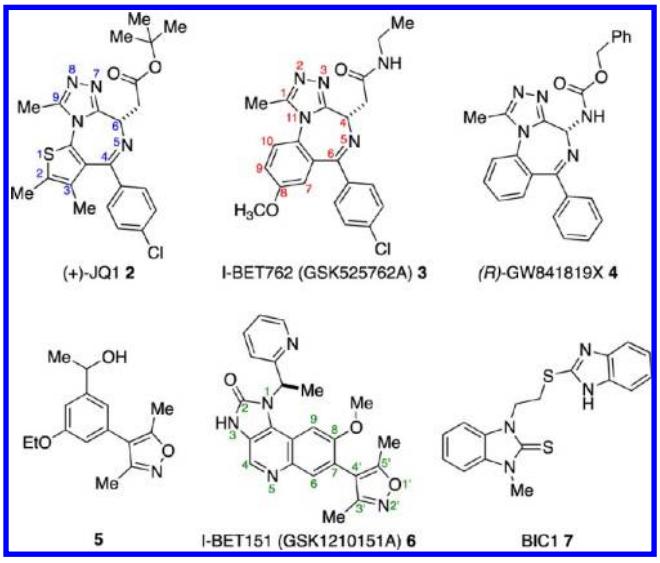

Figure 5. Structures of the BET bromodomain-selective ligands that have been reported to date. The different systems for numbering (+)-**2** [(+)-JQ1],[24] **3**, and **6** (I-BET151)[28] are shown.

confirmed by in vitro ITC and SPR experiments. Furthermore, compound **4** inhibited the binding of a tetra-acetylated histone H4 peptide ($H4_{1-18}$KAc5,8,12,16) to the tandem bromodomains of BRD2, BRD3, and BRD4 in a FRET assay.[49] Similarly, **3** bound to the tandem bromodomains of BRD2−4 [K_D(BRD4) = 55 nM by ITC] and displaced a tetra-acetylated histone H4 peptide [IC_{50}(BRD4) = 36 nM by FRET] while showing no affinity for bromodomains outside the BET family.[25,49] Crystal structures of **4** with BRD2(1) and BRD4(1)/(2) in addition to **3** with BRD2(1) and BRD4(1) reveal essentially identical binding interactions (vide infra).

A reported synthesis of racemic **3** is shown in Scheme 1. The benzodiazepine core (**10**) was formed by the condensation of a 2-aminobenzophenone (**9**) and a methyl aspartate derived acid chloride (**8**). The amide **10** was converted to the thioamide **11** by treatment with Lawesson's reagent. The methyltriazole **12** was afforded via the initial reaction of a thioamide and hydrazine to form a hydrazonamide, which reacted in situ with acetyl chloride to yield the corresponding acetylhydrazone. Cyclization in acetic acid furnished the tricyclic triazole **12**, which was converted to **3** by hydrolysis of the methyl ester **12** to afford the acid **13** and subsequent *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) mediated coupling with ethylamine. The active (*S*)- and inactive (*R*)-enantiomers were separated by preparative chiral HPLC.

A second triazolobenzodiazepine-based BET ligand, **2**, was independently developed by Filippakopoulos et al.[24] based on compounds disclosed in a patent from Mitsubishi Pharmaceuticals.[23] Previous work from this company indicates that related benzodiazepine compounds were identified from phenotypic studies investigating potential therapies for transplant rejection or autoimmune diseases.[22] Filippakopoulos et al. were encouraged by the synthetic availability of the lead compounds and the fact that they each contain a "privileged" structural core, found in drug molecules such as alprazolam and triazolam. Using docking studies based on the X-ray crystal structure of apo-BRD4(1), **2** was designed as a putative ligand. Importantly, a ^{t}Bu ester group was attached at the C6 position to reduce the likelihood of the compound interacting with other known benzodiazepine-binding proteins. Like **3**, only a single enantiomer was active, with (+)-**2** binding to BRD4(1) with a K_D of 49 nM (ITC) and showing excellent selectivity for the BET family of bromodomains. (+)-**2** displaced a tetra-acetylated histone H4 peptide ($H4_{1-21}$KAc5,8,12,16) from BRD4(1) with an IC_{50} of 77 nM, as determined by a bead-based amplified luminescent proximity homogeneous assay (AlphaScreen).[62] Furthermore, (+)-**2** inhibited the interaction of BRD4 with nuclear chromatin in human cells, as demonstrated by fluorescence recovery after photobleaching (FRAP) experiments, which measure the mobility of GFP-labeled BRD4.[24]

Like **3**, compound **2** was initially prepared as a racemate (Scheme 2). Formation of the 2-aminothiophene (**16**) was achieved by reaction of 4-chlorobenzylacetonitrile (**15**), 2-butanone (**14**), and elemental sulfur. Coupling of amine **16** to the α-carboxylate of L-aspartic acid afforded **17**, and nitrogen deprotection gave the amine **18** with 75% ee. Cyclization under acidic conditions afforded the benzodiazepine core (**19**) with

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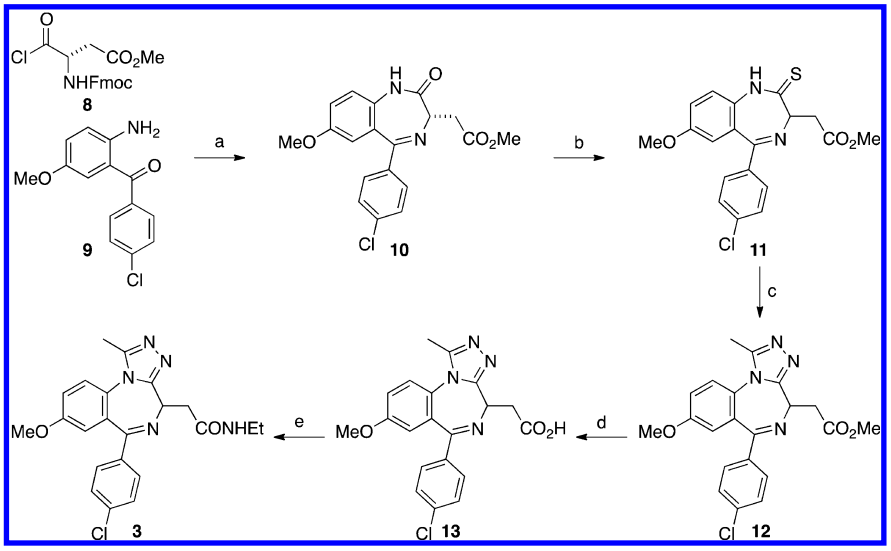
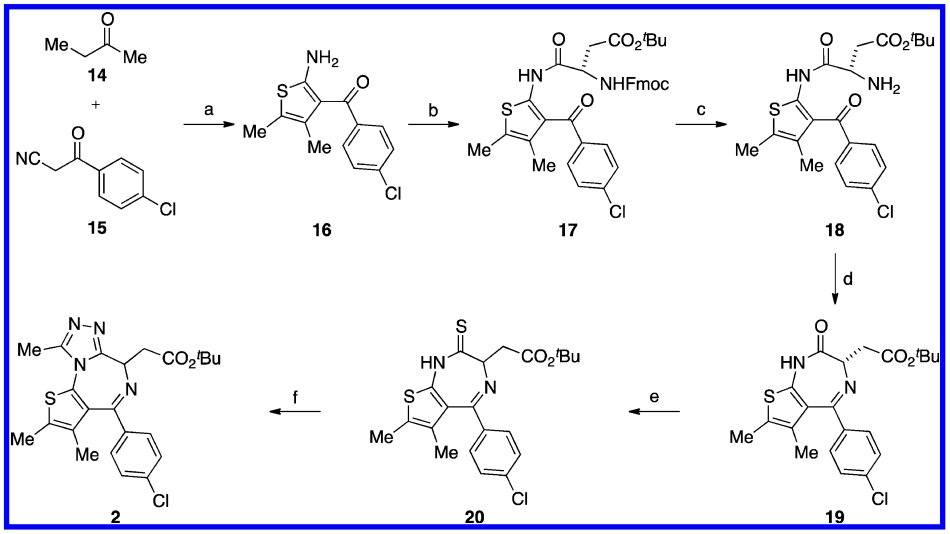
Scheme 1. Synthesis of 3[a]



[a]Conditions: (a) (i) $CHCl_3$, 60 °C; (ii) Et_3N, CH_2Cl_2, reflux; (iii) AcOH, 1,2-dichloroethane, 60 °C, 30%; (b) Lawesson's reagent, toluene, reflux, 81%; (c) (i) H_2N-NH_2·H_2O, THF, 0 °C; (ii) Et_3N, AcCl, 0 °C → rt; (iii) AcOH, THF, rt, 91%; (d) $NaOH_{(aq)}$, THF, rt, 98%; (e) $EtNH_2$, HBTU, $^iPr_2NEt$, THF, rt, 85%.[25,49]

Scheme 2. Synthesis of Racemic 2[a]



[a]Conditions: (a) sulfur, morpholine, EtOH, 70 °C, 70%; (b) Fmoc-Asp(O^tBu)-OH, *O*-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU), $^iPr_2NEt$, DMF, rt, 90%; (c) piperidine, DMF, rt, 90%; (d) AcOH, EtOH, 80 °C, 95%; (e) P_2S_5, $NaHCO_3$, diglyme, 85 °C, 65%; (f) (i) H_2N-NH_2, THF, 0 °C → rt; (ii) $CH_3C(OCH_3)_3$, toluene, 120 °C, 85%.[24]

67% ee. The methyltriazole (**2**) was formed by activation of the amide as a thioamide (**20**), followed by reaction with hydrazine and then trimethyl orthoacetate to form (±)-**2** with complete racemization.

A route to synthesize enantiomerically enriched (+)-**2** and (−)-**2** was also developed (Scheme 3). Again, a single enantiomer of protected aspartic acid was used to introduce the desired stereochemistry, but the route was optimized to reduce racemization. In particular, formation of the triazole **2** in the final step resulted in complete racemization, so milder conditions were used, employing an intermediate phosphorylimidate to activate the amide to nucleophilic attack by acetic hydrazide. The desired (+)-**2** was obtained in 90% ee, with preparative chiral HPLC increasing the ee to >99%.

Crystal structures of **4** (GW8411819X, not shown), **3**, and (+)-**2** in complex with a variety of BET bromodomains have been solved, revealing excellent shape complementarity between ligand and the KAc-binding site (Figure 6A). Analysis of the X-ray crystal structures of (+)-**2** and **3** (Figure 6) in complex with BRD4(1) reveals that the ligand−protein interactions are almost identical for these two molecules. The key interactions of (+)-**2** and BRD4(1) are shown in Figure 6A−C. The 3-methyl-1,2,4-triazole of (+)-**2** acts as a KAc mimic, with the methyl group of (+)-**2** occupying the hydrophobic pocket that recognizes the methyl group of KAc. N(2) forms a water-mediated hydrogen bond to a conserved Tyr residue [Y97 in BRD4(1)], and N(1) accepts a hydrogen bond from the conserved Asn NH [N140 in BRD4(1)], analogous to the interaction between the carbonyl

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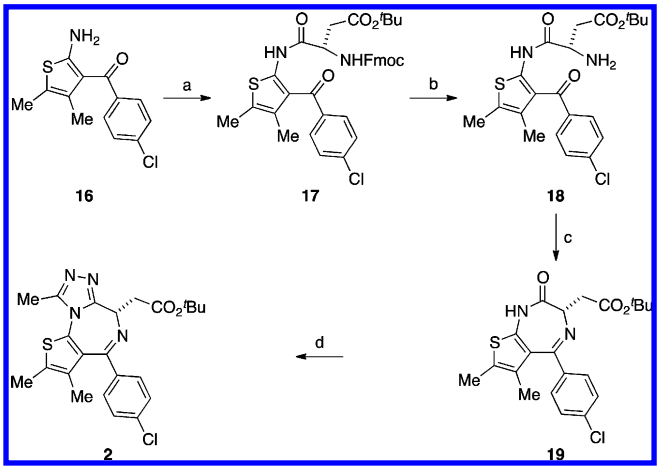
Scheme 3. Synthesis of Enantiomerically Enriched (+)-2[a]



[a]Conditions: (a) Fmoc-Asp(O^{t}Bu)-OH, (benzotriazol-1-yloxy)-tripyrrolidinophosphonium hexafluorophosphate (PyBOP), ^{i}Pr$_2$NEt, DMF, rt, 72%; (b) piperidine, DMF, rt, 90%; (c) SiO_2, toluene, 90 °C, 95%; (d) (i) KO^{t}Bu, THF, −78 °C → rt; (ii) POCl(OEt)$_2$, THF, −78 °C → −10 °C; (iii) AcNHNH$_2$, ^{n}BuOH, 90 °C, 92%, 90% ee.[24]

group of KAc and N140 (Figure 6B). Compounds **4** and **3** form an additional hydrogen bond between the carbamate or amide NH and the carbonyl oxygen atom of N140 (Figure 6D).

The 4-chlorophenyl moieties of (+)-**2** and **3** occupy a hydrophobic part of the peptide-binding groove termed the WPF shelf,[25,49] formed by residues in the BC loop and the ZA loop at the end of helix Z. The WPF motif found at the entrance to this region [W97, P98, F99 in BRD4(1)] is conserved in the BET family of bromodomains, and occupancy of this site is believed to contribute to the selectivity of the above compounds for BET bromodomains.[49] The fused dimethylthiophene of (+)-**2** and the methoxybenzo moiety of **3** are directed into the pocket formed in part by the ZA loop, which is termed the ZA channel.

A further study by Filippakopoulos et al. has provided additional insight into the structure−activity relationships (SARs) in this important class of BET bromodomain inhibitors and has demonstrated that a number of clinically approved benzodiazepines (Figure 7) bind to BET bromodomains with low micromolar affinities.[50] Notably, the widely prescribed anxiolytic alprazolam (**21**) bound to BRD4(1) with a K_D of 2.5 μM [cf. 0.05 μM for (+)-**2**] (Figure 8A,B); however, the authors note that this affinity means that it is unlikely that alprazolam has any effect on bromodomains at clinically relevant doses.[50] Unlike (+)-**2** and **3**, these benzodiazepines are achiral, as they are not substituted at the 4-position of the diazepine ring (Figure 7), simplifying their synthesis. The sedative midazolam **22** (Figure 8C) contains an imidazole rather than a triazole ring and therefore cannot form a hydrogen bond to the KAc-binding Asn residue (Figure 8D). Nonetheless, midazolam still interacts with the BET bromodomains, albeit more weakly than the triazoles. Crystallographic evidence shows that despite this compound binding further out of the KAc-binding pocket, two of the four water molecules found in most apo and ligand-bound BET bromodomain structures are not observed (Figure 8D). Although an imidazole ring is tolerated, a methyl substituent in the KAc-binding site is required for binding affinity, as replacement of the methyl group with H, Et, Ph, NH_2, or carbonyl resulted in a loss of BET bromodomain affinity.[50] This observation is consistent with the work of Vollmuth and Geyer, who showed that BRD2(1) and BRD4(1) bind *N*-propionyl-lysine but to a lesser extent *N*-butyryl-lysine.[63] Interestingly, these larger lysine modifications did not perturb or displace the KAc-binding pocket water molecules.

Recent work by Hewings et al., Dawson et al., and Bamborough et al. has identified the 3,5-dimethylisoxazole moiety as a new class of KAc mimic.[28,52,53] Following the observation that 3,5-dimethylisoxazole-containing compounds (**23**, Figure 10) could occupy the KAc-binding site of BRD4(1), Hewings et al. used a structure-guided design approach to develop low molecular weight 4-phenyl-3,5-dimethylisoxazoles including compound **5**.[52] This compound displayed good selectivity for BET bromodomains and displaced a tetra-acetylated histone ($H4_{1-21}$KAc5,8,12,16) peptide from BRD4(1) with an IC_{50} of 5 μM. An X-ray crystal structure of **5** in complex with BRD4(1) reveals that this compound binds in the KAc-binding pocket and peptide-binding groove in a similar manner to (+)-**2** and **3** and forms many of the same interactions as these compounds (Figure 9). The 3,5-dimethylisoxazole moiety binds in the KAc-binding pocket with the oxygen atom receiving a hydrogen bond from the NH_2 of N140. The nitrogen atom receives a hydrogen bond from the conserved water molecule that also interacts with Y97 (Figure 9B). The ethoxy substituent is directed into the ZA channel but does not appear to hydrogen-bond with the conserved water molecules that also bind here. The methyl substituent of the secondary alcohol binds in the hydrophobic WPF shelf region (Figure 9A). One of the molecules in this series of compounds showed selectivity for the bromodomain of CREBBP over the BET BCPs, further details of which are described below.

3,5-Dimethylisoxazoles were independently identified as bromodomain ligands by a fragment screen of potential KAc mimics against bromodomains by Dawson et al. and Bamborough et al.[28,53] 4-Phenyl-3,5-dimethylisoxazole (not shown) was observed to inhibit the interaction of a **3**-based fluorogenic ligand with bromodomains of BRD2, BRD3, and BRD4. An X-ray crystal structure of the 3,5-dimethylisoxazole in complex with BRD2(1) confirmed this interaction. Synthesis of the 5-ethyl-3-methylisoxazole derivative (**25**, Figure 11B) provided strong evidence that the isoxazole bound with the nitrogen atom forming a water-mediated hydrogen bond to a conserved Tyr residue [Y113 in BRD2(1), equivalent to Y97 in BRD4(1)] while the oxygen atom forms a direct hydrogen bond to the NH_2 of the Asn amide [N156 in BRD2(1)] (Figure 11A). These hydrogen bonds are also consistent with the predicted angles of the nitrogen and oxygen atom lone pairs forming strong hydrogen bonds. The opposite arrangement of the nitrogen and oxygen atoms would be expected to form weaker hydrogen bonds with the water molecule and Asn residue.

These key hydrogen-bonding interactions with the Asn and Tyr residues were the same as those observed by Hewings et al.[53] However, as noted,[53] the phenyl ring of compound **5** prepared by Hewings is shifted out of the KAc-binding pocket toward the WPF shelf relative to 4-phenyl-3,5-dimethylisoxazole. This shift might be due to the methyl group being insufficiently large to form the optimal interactions with the WPF shelf.

To increase the affinity of the fragment hit, Bamborough et al. constructed a 3D pharmacophore model encapsulating the crystallographically observed hydrogen-bonding and hydrophobic interactions. A search of commercially available

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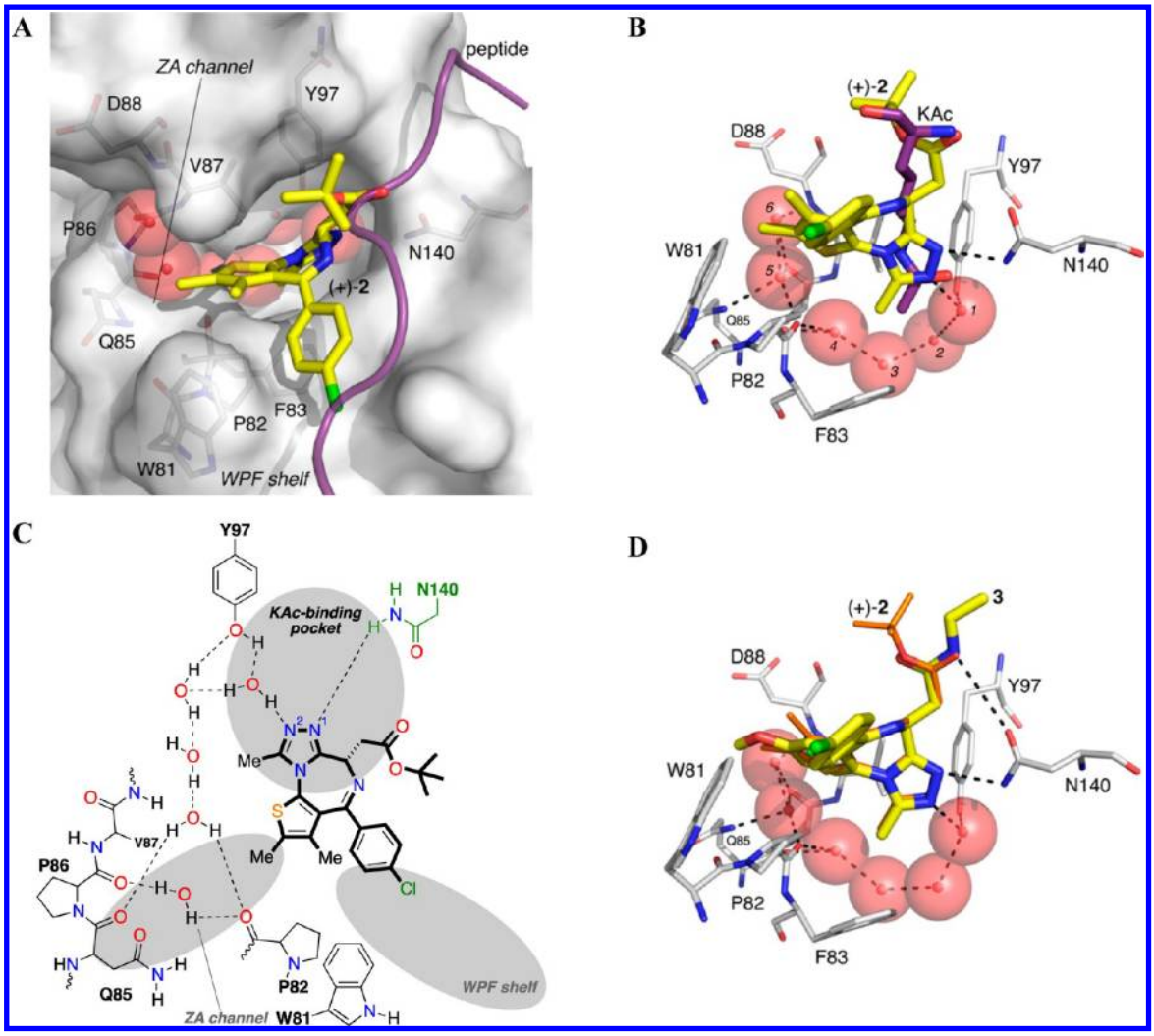

Figure 6. (A) X-ray crystal structure of (+)-**2** bound to human BRD4(1) (PDB code 3MXF, carbon = yellow) showing the excellent shape complementarity between (+)-**2** and the KAc-binding pocket. The structure is overlaid with an X-ray crystal structure of human BRD4(1) in complex with the diacetylated histone peptide $H4_{1-12}$KAc5KAc8 (PDB code 3UVW, purple), demonstrating that (+)-**2** occupies part of the peptide-binding groove. (B) The 3-methyl-1,2,4-triazole of (+)-**2** acts as an effective KAc mimic. N(2) is positioned to form a water-mediated hydrogen bond to Y97, and N(1) accepts a hydrogen bond from N140. (C) Schematic representation of the binding interactions between (+)-**2** and BRD4(1). (D) Overlay of an X-ray crystal structure of (+)-**2** bound to human BRD4(1) (PDB code 3MXF, carbon = yellow) and an X-ray crystal structure of **3** bound to human BRD4(1) (PDB code 3P5O, carbon = orange). (+)-**2** and **3** form similar interactions with the bromodomain, but **3** forms an additional hydrogen bond between its amide NH and the carbonyl oxygen atom of N140.[24] Structures were aligned using the "cealign" command in PyMOL.

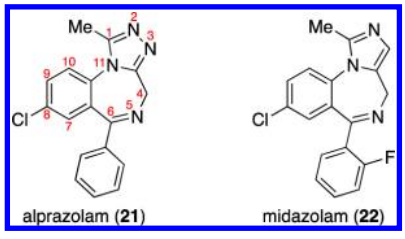

Figure 7. Structures of alprazolam (**21**) and midazolam (**22**).

compounds using the criteria of this pharmacophore identified a series of 4-phenyl-3,5-dimethylisoxazoles with a sulfonamide appended to the phenyl ring meta to the isoxazole (**26**–**31**, Figure 12A). The meta substituents are directed toward the WPF shelf in a similar manner compared to the series prepared by Hewings et al., while the sulfonamide moiety itself forms a number of water-mediated hydrogen-bonding interactions. Exploration of the sulfonamide substituent SAR revealed that small, hydrophobic substituents displayed the highest affinity, consistent with the hydrophobic nature of the WPF shelf. The cyclopropyl-containing compound **29** displayed the greatest activity and showed selectivity for the BET bromodomains in a thermal shift (ΔT_m) binding assay. Furthermore, compound **29** was active in a cellular assay, inhibiting the release of the proinflammatory cytokine IL-6 from lipopolysaccharide (LPS) stimulated peripheral blood mononuclear cells (PBMCs), with an IC_{50} of 3.1 μM. In addition, the compound reduced TNFα release from LPS-stimulated PBMCs. However, this series of compounds suffered from limited aqueous solubility; therefore, analogues possessing solubilizing groups attached to the phenyl ring para to the isoxazole moiety were investigated. This modification was well tolerated and did result in improved aqueous solubility, albeit with reduced ligand efficiency. Compound **30** had IC_{50} of 1–3 μM in a time-resolved FRET (TR-FRET) assay using tandem bromodomain constructs.

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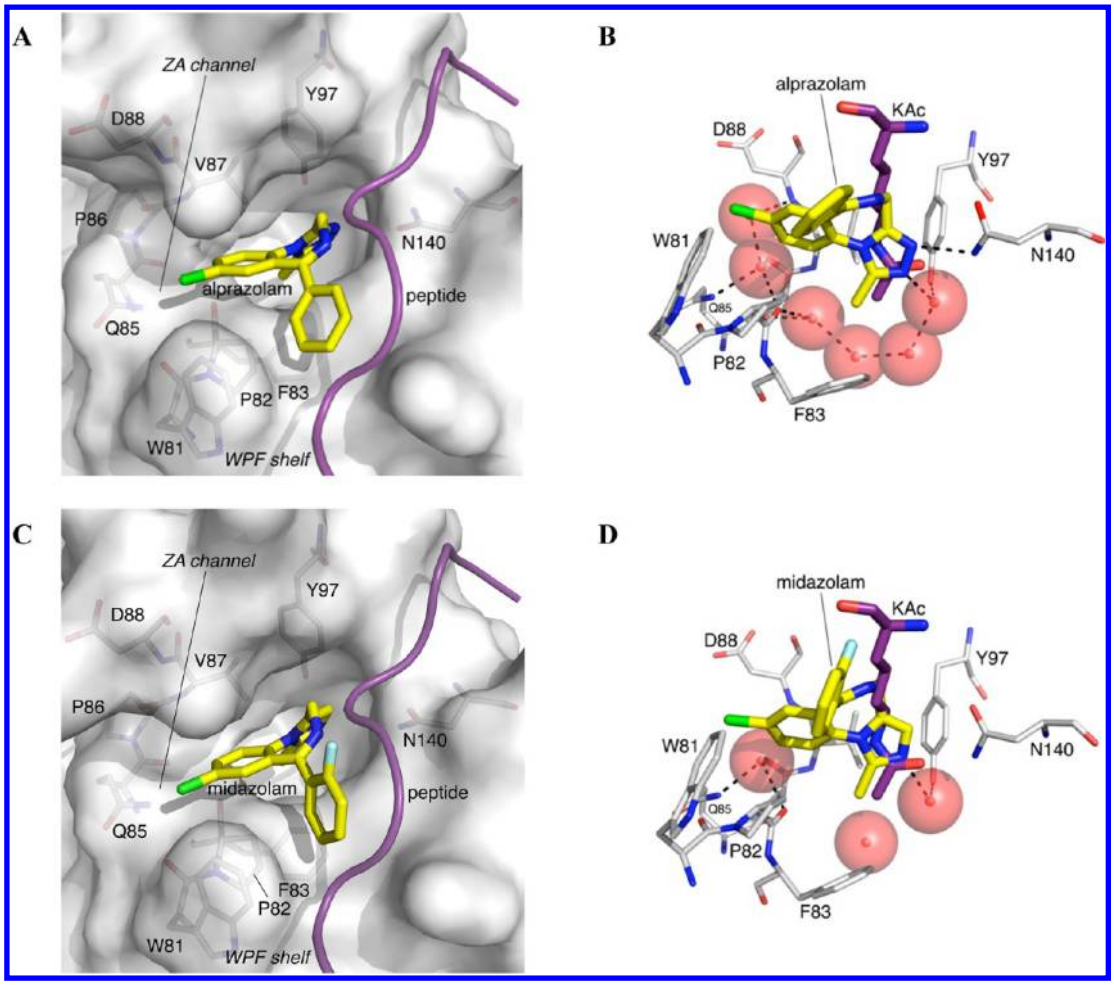

Figure 8. (A) X-ray crystal structure of alprazolam (**21**) bound to human BRD4(1) (PDB code 3U5J, carbon = yellow). The structure is overlaid with the X-ray crystal structure of human BRD4(1) in complex with the diacetylated histone peptide $H4_{1-12}$KAc5KAc8 (PDB code 3UVW, purple), demonstrating that alprazolam occupies part of the peptide-binding groove. (B) The triazole moiety of alprazolam resides in the KAc-binding pocket and forms a hydrogen bond with N140 and a water-mediated hydrogen bond with Y97. (C) X-ray crystal structure of midazolam (**22**) bound to human BRD4(1) (PDB code 3U5K, carbon = yellow). The structure is overlaid with the X-ray crystal structure of human BRD4(1) in complex with the diacetylated histone peptide $H4_{1-12}$KAc5KAc8 (PDB code 3UVW, purple), demonstrating that alprazolam occupies part of the peptide-binding groove. (D) Midazolam **22** contains an imidazole rather than a triazole ring and thus does not hydrogen bond to N140. Despite **22** binding further out of the KAc-binding pocket, two of the four water molecules found in most apo and ligand-bound BET bromodomain structures are not observed.[50] Structures were aligned using the "cealign" command in PyMOL.

Compound **30** gave similar K_D in an SPR assay while showing a 1000-fold increase in solubility over **29**. The cellular activity of compound **30** was maintained at 3.0 μM in the IL-6 assay.

A further 4-aryl-3,5-dimethylisoxazole, **6** (Figure 13), has also been reported as a BET bromodomain inhibitor.[28] Like the triazolobenzodiazepine series that gave rise to **3**, this series arose from a phenotypic HTS campaign, which identified a 7-isoxazoloquinoline (**24**, Figure 10) as a potent up-regulator of ApoA1.[54] SAR studies revealed that a variety of hydrophobic substituents on the amine at position 4 were well tolerated. Cyclization of the adjacent carboxamide onto this amine to form the urea was also tolerated, but the compounds displayed undesirable CYP inhibition. Appropriate choice of amine substituent and the addition of a methoxy substituent at position 6 abolished the CYP liability of these ureas. The resultant compound **6** showed similar activity compared to **3** in ligand displacement, binding, and cellular assays (inhibition of cytokine release) but with markedly improved pharmacokinetic properties over both **3** and (+)-**2**.[28,55] Pharmacokinetic evaluation in mice revealed that **6** had a half-life of 7.2 h and AUC_∞ (area under the curve) of 680 μM·h, compared to a half-life of 1.6 h and AUC_∞ of 40 μM·h for **3**, with (+)-**2** showing similar properties compared to **3**.

The 4-aryl-3,5-dimethylisoxazole core has, in most instances, been synthesized by Suzuki coupling between an aryl halide and a 3,5-dimethylisoxazole-4-boronic acid **37** or derivative. Hewings et al. employed the potassium aryltrifluoroborate salt as shown in Scheme 4.[64] An alternative CH-activation procedure employing 3,5-dimethylisoxazole itself gave lower yields of the desired product.[65]

Compound **6** was synthesized using 3,5-dimethylisoxazole-4-boronic acid (**37**, Scheme 5),[28] and the sulfonamides reported by Bamborough et al. were afforded by coupling the corresponding pinacol ester to the appropriate aryl bromides.[53] These authors also reported the reverse procedure involving the coupling of 4-iodo-3,5-dimethylisoxazole and arylboronic acids, illustrating that the isoxazole moiety can be introduced as either the organoboron or organohalide species.

A benzimidazole-based BET bromodomain inhibitor, **7** (BIC1, Figure 5), was identified by Ito et al.[56] This compound

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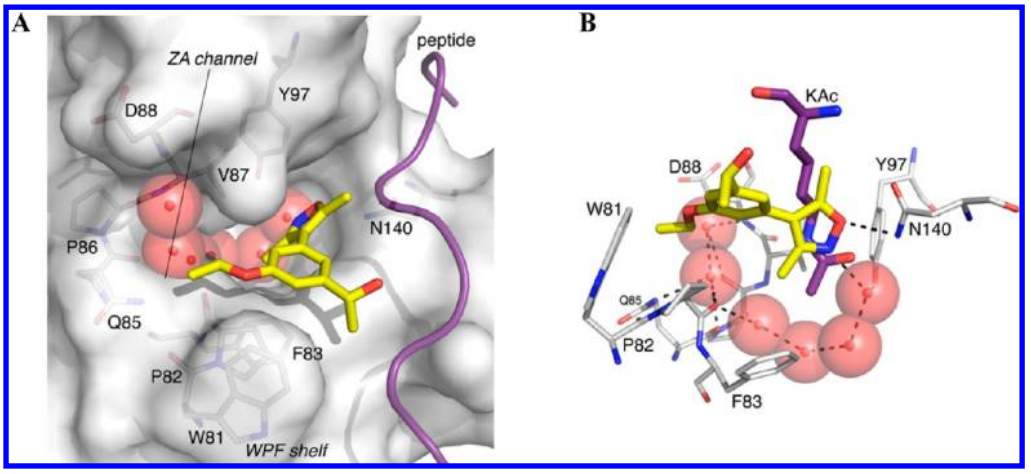

Figure 9. (A) X-ray crystal structure of 3,5-dimethylisoxazole **5** bound to human BRD4(1) (PDB code 3SVG, carbon = yellow). The structure is overlaid with the X-ray crystal structure of human BRD4(1) in complex with the diacetylated histone peptide $H4_{1-12}$KAc5KAc8 (PDB code 3UVW, purple), demonstrating that the isoxazole moiety resides in the KAc-binding pocket, the methyl group binds to the WPF shelf, and the ethoxy substituent is oriented toward the ZA channel. (B) The isoxazole resides in the KAc-binding pocket and acts as a KAc mimic, with the oxygen atom accepting a hydrogen bond from N140. KAc from the diacetylated histone peptide $H4_{1-12}$KAc5KAc8 (PDB code 3UVW, carbon = purple) is shown for comparison. The nitrogen atom forms a water-mediated hydrogen bond with Y97.[52] Structures were aligned using the "cealign" command in PyMOL.

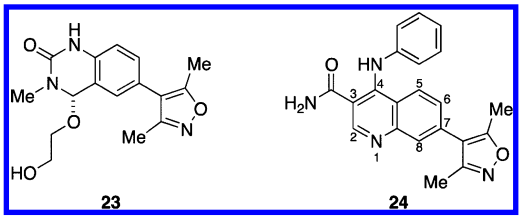

Figure 10. Structures of the lead compounds reported by Hewings et al.[52] (**23**) and Mirguet et al.[28] (**24**).

was shown to block the interaction of the BRD2 bromodomains to a H4KAc12-mimicking peptide in a novel cell-based assay for histone acetylation. The interaction of **7** with BRD2(1) was confirmed by SPR, which gave a K_D of 28 μM. X-ray crystallography reveals that the benzene ring of the benzimidazole moiety occupied the KAc-binding pocket (Figure 14A) while the benzimidazole-2-thione was directed toward the WPF shelf (Figure 14B). The compound does not form hydrogen bonds to the conserved Asn [N156 in BRD2(1)] and Tyr residues [Y113 in BRD2(1)], perhaps explaining its lower affinity for BRD2 compared to other reported ligands. No data were presented on the selectivity of this compound for BRD2(1) over other bromodomains.

A number of patents have been filed in the area of bromodomain ligands, and although many of the compounds found in these patents have also been reported in the academic literature, some have not. These ligands include compounds based on the 1-acyl THQ moiety, which is a distinct KAc mimic that is disclosed in a GlaxoSmithKline patent (Figure 15).[57] A number of compounds were potent in whole blood assays, which measure their effect on levels of inflammatory mediators TNFα and IL-6. The THQ amine is normally capped with an acetyl group, although some variations are claimed (**50**). The 4-amino group is derivatized as a carbamate, aniline, or amino heterocycle. The isopropyl carbamate found in compounds **46** and **47** seems to be preferred, but a methyl carbamate is also reported (**48**). The scope in the patent demonstrates a considerable effort to explore SAR on the exocyclic aryl group with heteroaromatic, basic, neutral, and acidic function-

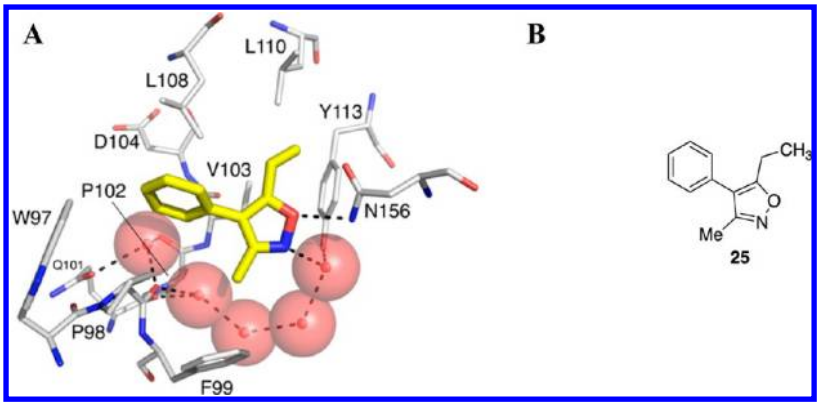

Figure 11. (A) X-ray crystal structure of the 5-ethyl-3-methylisoxazole derivative (**25**) bound to human BRD2(1) (PDB code 4A9O, carbon = yellow) showing the isoxazole oxygen atom forming a hydrogen bond with N156. The isoxazole nitrogen atom forms a water-mediated hydrogen bond with Y113. The presence of the ethyl groups makes it easy to distinguish between the two isoxazole heteroatoms.[53] (B) Structure of the 5-ethyl-3-methylisoxazole derivative (**25**).

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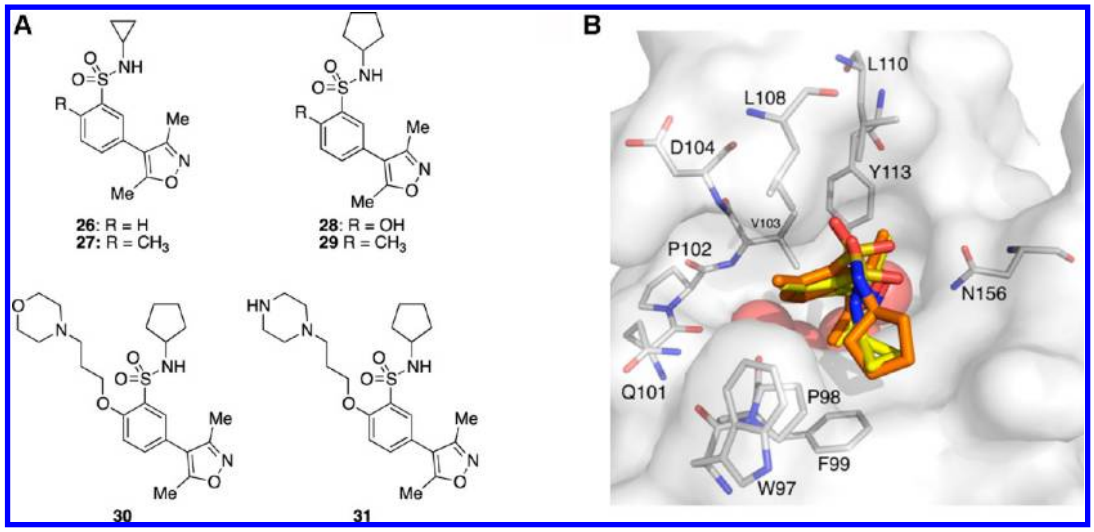

Figure 12. (A) Structures of a selection of the isoxazole-based BET bromodomains inhibitors reported by Bamborough et al. (B) X-ray crystal structure of isoxazole **27** (PDB code 4A9N, carbon = yellow) overlaid with the X-ray crystal structure isoxazole **29** (PDB code 4A9M, carbon = orange), bound to human BRD2(1), showing that the cyclopropyl and cyclopentyl rings bind in the WPF shelf.[53] Structures were aligned using the "cealign" command in PyMOL.

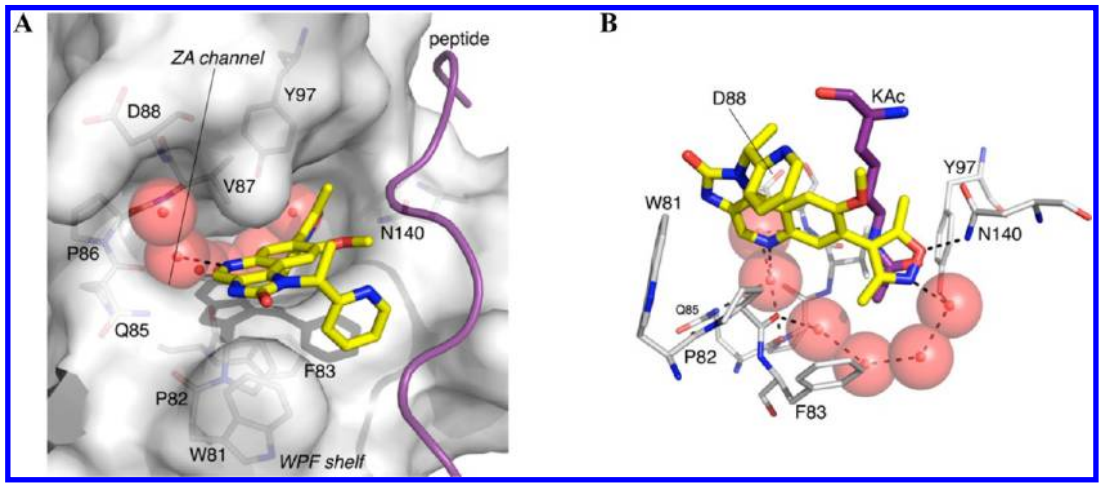

Figure 13. (A) X-ray crystal structure of **6** bound to human BRD4(1) (PDB code 3ZYU, carbon = yellow). The structure is overlaid with the X-ray crystal structure of human BRD4(1) in complex with the diacetylated histone peptide H4KAc5KAc8 (PDB code 3UVW, purple), demonstrating that the isoxazole moiety resides in the KAc-binding pocket, the pyridyl group binds to the WPF shelf, and the quinolone nitrogen atom accepts a hydrogen bond from one of the ZA channel water molecules. (B) The the isoxazole of **6** forms similar interactions with the KAc-binding pocket to KAc and compound **5**.[28] Structures were aligned using the "cealign" command in PyMOL.

ality all exemplified. Additionally, in vivo data are reported for **45**. High doses of endotoxin were administered to mice in order to elicit a shock response, analogous to human sepsis and septic shock. Survival rates of mice administered with 10 mg/kg **45** were compared to controls. The results indicated a significant increase in survival rates for mice treated with **45** (58%) compared to the control (6%). Further **45** was tested in an oncology growth assay, giving an IC_{50} of 35−844 nM for heme cell lines and 40−9200 nM for breast cell lines. It seems, therefore, that these compounds are promising BET ligands, and it is to be hoped that full details of their synthesis and biological activity are disclosed in due course.

■ BET BROMODOMAINS SAR

With an increasing number of ligands for BET bromodomains being reported, the structure−activity requirements for potent and selective BET ligands are beginning to emerge. The three key areas of interaction with the BET bromodomains are the KAc-binding pocket, the WPF shelf, and the ZA channel. The most potent compounds described to date, namely, (+)-**2**,[24] **3**,[25] and **6**,[28] in addition to the compounds described by Hewings et al.[52] and Bamborough et al.,[53] use methyl-substituted five-membered heterocycles to mimic the KAc residue.

There are four main classes of KAc mimic that have been employed in the BET bromodomain inhibitors reported to date, and the binding modes of these compounds are outlined in Figure 17. Both (+)-**2** and **3** possess the triazolodiazepine moiety in which the triazole unit acts as the KAc mimic (Figure 16B). In (+)-**2** (Figure 16A, partial structure of (+)-**2** shown), one of the triazole nitrogen atoms forms a hydrogen bond with the highly conserved Asn residue [N140 in BRD4(1)]. One of the other triazole nitrogen atoms forms a hydrogen bond with the conserved water molecule that is also hydrogen bonded to the conserved Tyr residue [Y97 in BRD4(1)]. The methyl

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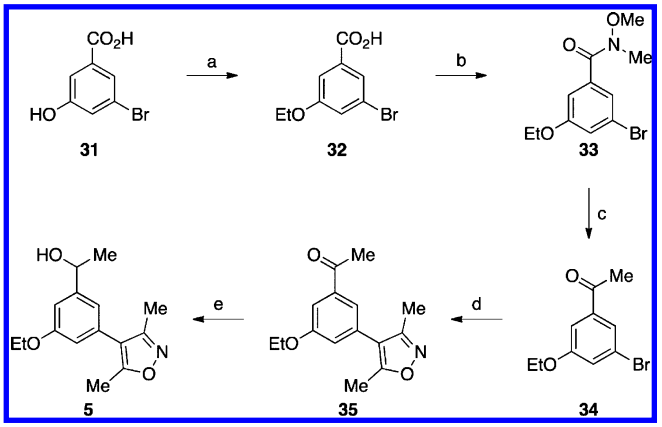
Scheme 4. Synthesis of Compound 5[a]



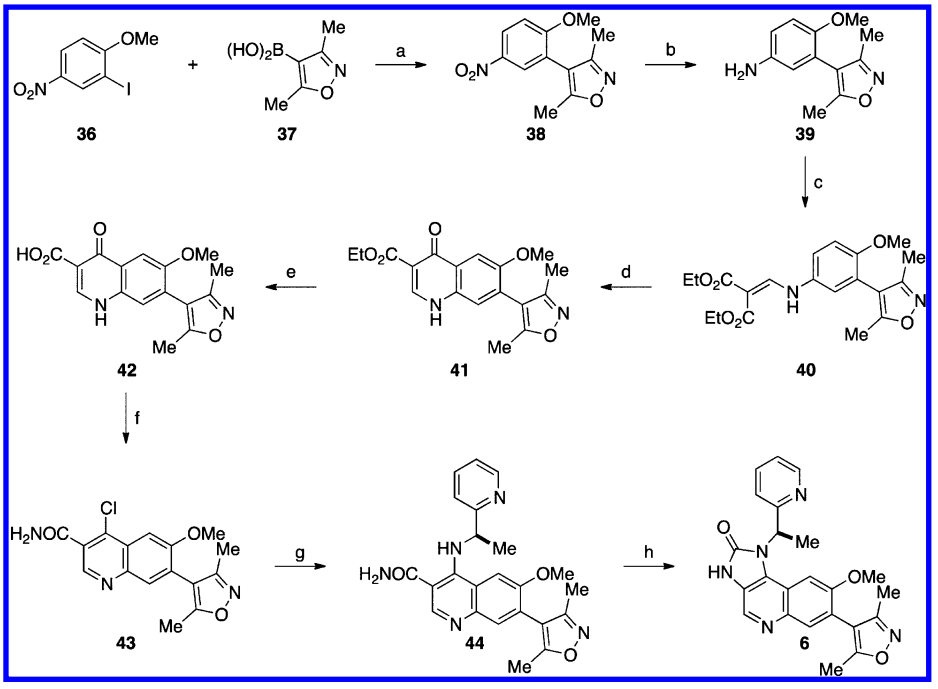
[a]Conditions: (a) (i) EtBr, K_2CO_3, DMF, 100 °C (microwave), 15 min, 96%; (ii) LiOH, THF, H_2O, rt, 23 h, 95%; (b) NHMe(OMe)·HCl, HBTU, $^iPr_2NEt$, DMF, rt, 14 h, 80%; (c) MeMgBr, THF, rt, 15 h, 86%; (d) potassium (3,5-dimethylisoxazol-4-yl)-trifluoroborate, Na_2CO_3, $Pd(OAc)_2$ RuPhos, EtOH, 85 °C 64 h, 67%; (e) $NaBH_4$, MeOH, rt, 2 h, 81%.[52]

group is directed into a small hydrophobic pocket formed by a valine and a phenylalanine residue [F83 and V87 in BRD4(1)]. The 3,5-dimethylisoxazole moiety forms similar interactions compared to the triazole ring (Figure 16A). The isoxazole oxygen atom forms a hydrogen bond with the Asn residue [N140 in BRD4(1)], and the nitrogen atom forms a water-mediated hydrogen bond with the conserved Tyr residue [Y97 in BRD4(1)]. The methyl group proximal to the nitrogen atom is directed into the bottom of the KAc-binding pocket. The carbonyl oxygen atom of the 1-acyl THQ moiety found in the compounds shown in Figure 15 also forms a hydrogen bond with the conserved Asn residue.[57] These interactions closely mimic those of the natural KAc ligand. It is also possible for the Asn amide to accept an extra hydrogen bond via its carbonyl oxygen atom, such as the additional hydrogen bond formed by **3** (Figure 6D) or the reciprocal hydrogen bonds formed by the dihydroquinazolinone fragment (Figure 17C).[66] Given that the nature of the KAc-binding pocket is highly conserved between bromodomains, it currently seems unlikely that it will be possible to obtain interbromodomain selectivity from the KAc bioisostere. However, these interactions are important for bromodomain recognition and orientation of the ligand.

A key feature of potent and selective BET bromodomain ligands is the presence of a lipophilic substituent that binds to the WPF shelf (Figure 16B). This region also includes a Met and a residue that has been defined by some as a "gatekeeper",[33,42,49,53,66] which is Ile in the first BET bromodomains [BRDX(1)] and Val in the second BET bromodomains [BRDX(2)]. The 4-chlorophenyl moieties of both **3** and (+)-**2** (Figure 16B) occupy the WPF shelf, while the 2-pyridyl group of **6** binds to a similar region (Figure 16B). The methyl group of compound **5**, reported by Hewings et al., is also observed to bind to the WPF shelf. Bamborough et al. explored a variety of substituents directed into this channel and found a cyclopentyl group to be optimal.[24,25,49−51,53]

The third region of the BET bromodomains that can potentially influence ligand binding is the ZA channel (Figure 16C). This channel initially appears to form a second pocket that can be exploited to gain further ligand potency and selectivity. However, this pocket is invariably occupied by a crystallographically conserved water molecule, which forms interactions with the backbone carbonyl groups of P82 and

Scheme 5. Synthesis of 6[a]



[a]Conditions: (a) Cs_2CO_3, PEPPSI, dimethoxyethane (DME), H_2O, 90 °C, 83%; (b) (i) 5% aq Na_2SO_3, EtOAc, rt; (ii) H_2, Pd/C, EtOH, rt, 100%; (c) diethyl [(ethyloxy)methylidene]propanedioate, 150 °C, 100%; (d) (i) Ph_2O, 255 °C; (ii) EtOAc, reflux, 64%; (e) 2 M aq NaOH, EtOH, reflux, 85%; (f) (i) $POCl_3$, 120 °C; (ii) NH_4OH, THF, 0 °C, 89%; (g) [(1*R*)-1-(2-pyridinyl)ethyl]amine, $^iPr_2NEt$, NMP, 120 °C, 77%; (h) (i) KOH, MeOH; (ii) $PhI(OAc)_2$, 0 °C, 77%.[28,45]

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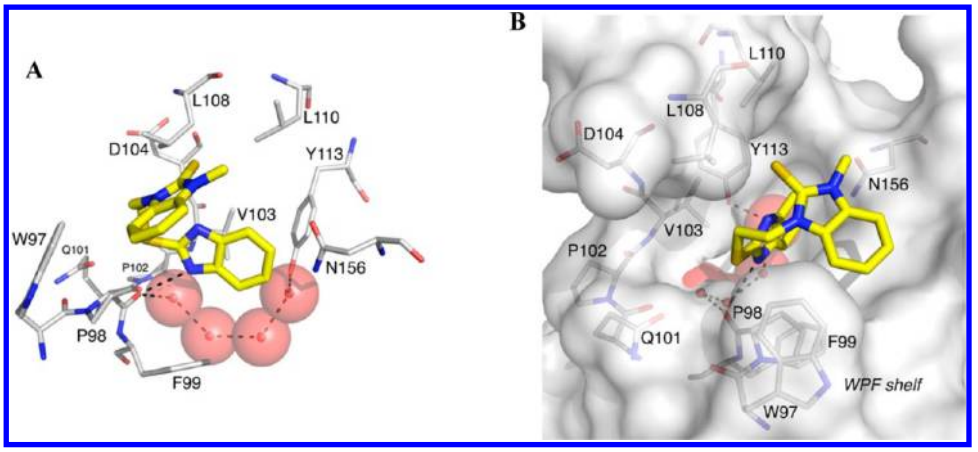

Figure 14. (A) X-ray crystal structure of **7** bound to human BRD2(1) (PDB code 3AQA, carbon = yellow) showing that the benzimidazole moiety resides in the KAc-binding pocket but does not form a hydrogen bond with N156 or Y113. One of the benzimidazole nitrogen atoms forms a hydrogen bond with the oxygen atom of the P98 carbonyl group. (B) The benzimidazole-2-thione group of **7** occupies the WPF shelf.[46,56]

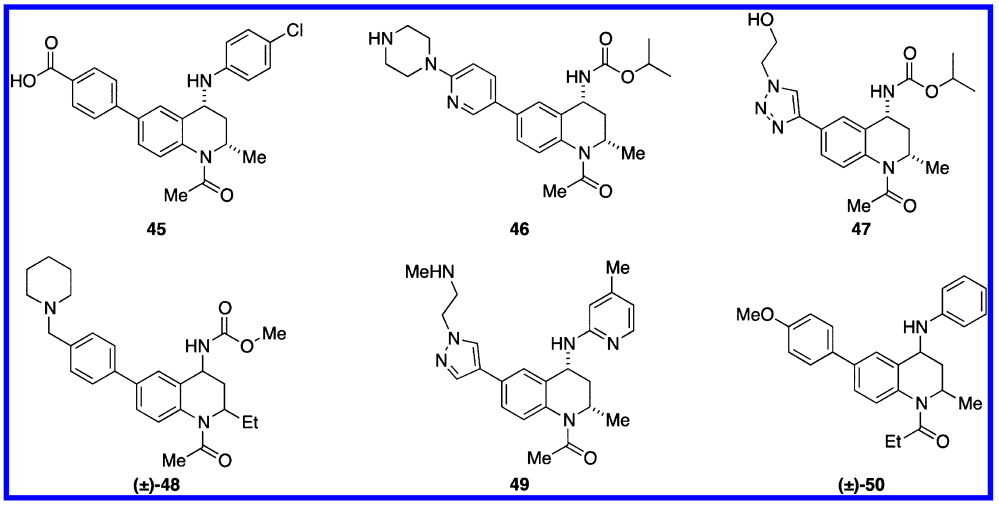

Figure 15. Structures of putative BET ligands (**45**–**50**) containing the 1-acyltetrahydroquinoline (THQ) motif reported in a GlaxoSmithKline patent.[57]

Q85 and the side chain amide of Q85 in BRD4(1). Comparison of eight ligand-bound BRD4(1) structures shows that this water molecule is usually present and that its position is constant (Figure 16D). Interestingly, this water molecule is in contact with the fourth KAc-binding pocket water molecule via their P82 interactions. We therefore suggest that this water can be viewed as the fifth conserved water molecule, all of which play an important role in defining the shape and size of the KAc- and peptide-binding regions of the BET bromodomains. This observation is supported by the fact that no reported BET bromodomain ligands displace this water molecule; indeed, only **6** forms a hydrogen bond with this water molecule, via its quinoline nitrogen atom (Figure 16C). An additional, and less-conserved, water molecule is also observed binding in the ZA channel, forming interactions with the backbone NH of D88 and the other water molecule (Figure 16D). Any docking studies aimed at designing BET bromodomain ligands should take these water molecules into account.

The potent BET bromodomain ligands reported to date show good selectivity for the BET family over other classes of BCPs, at least in in vitro assays. It is not possible to draw general conclusions about the structural reasons for this selectivity. However, all potent BET bromodomain ligands contain substituents that bind to the WPF shelf. This region of the protein varies significantly between bromodomain classes, and it seems that large substituents in this position are not universally tolerated among bromodomains.

In summary, good progress has been made toward the design, synthesis, and biological characterization of potent and selective BET bromodomain ligands. Two main chemotypes, the triazolobenzodiazepines and the 3,5-dimethylisoxazoles, have emerged as the basis of potent and selective BET bromodomain inhibitors. The development of ligands that show selectivity between bromodomains within the BET family remains a key challenge in this area, due to the high sequence homology between the first BET bromodomains [BRDX(1)] and between the second BET bromodomains [BRDX(2)].

APPLICATION OF BET LIGANDS TO THE STUDY OF BIOLOGICAL SYSTEMS

An important endorsement of the utility of the BET bromodomain ligands, described above, is their application in exploring the roles of these proteins in vivo. Work by Filippakopoulos et al. investigated the effect of (+)-**2** on BRD4-dependent NUT midline carcinoma (NMC), which is a gene fusion between BRD4 and the usually testis specific NUT

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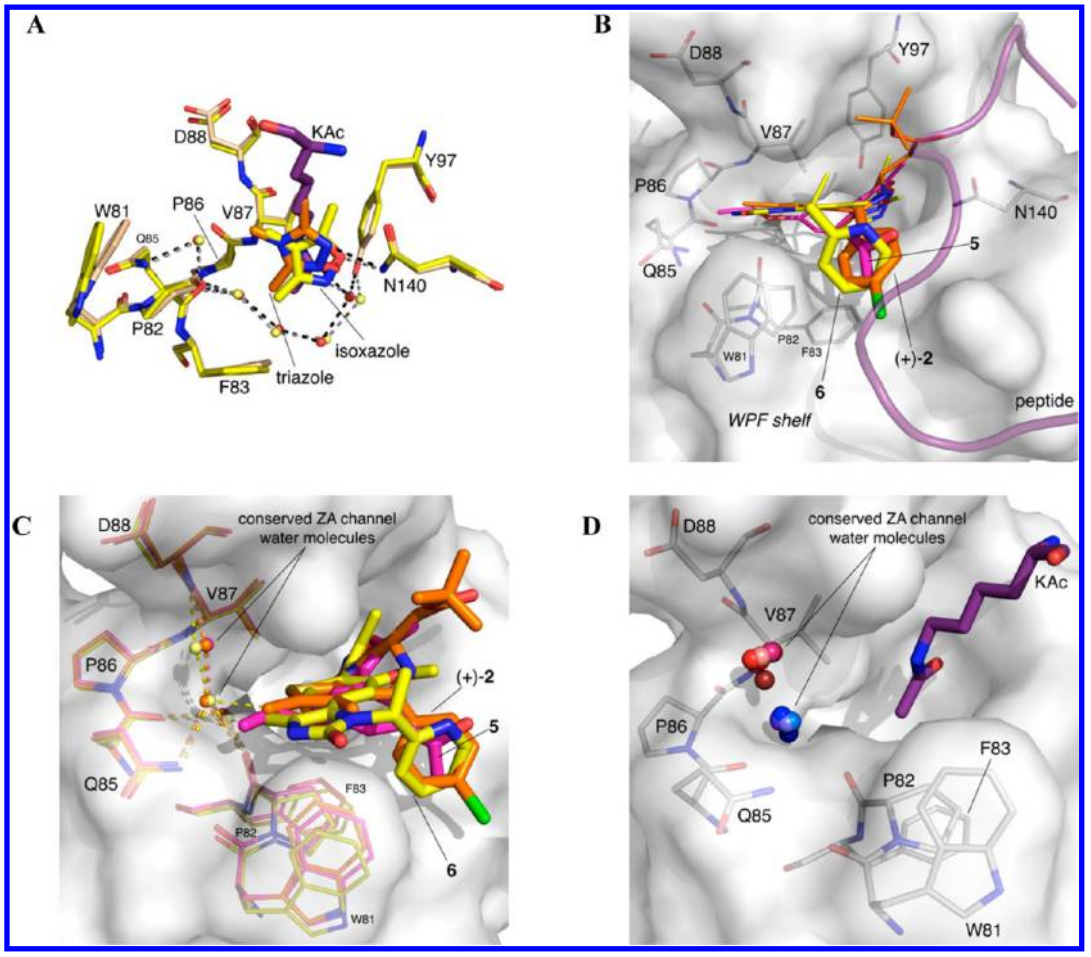

Figure 16. (A) Overlaid X-ray crystal structures of KAc from the diacetylated histone peptide $H4_{1-12}$KAc5KAc8 (PDB code 3UVW, carbon = purple), the isoxazole moiety of **6** (PBD code 3ZYU, carbon = yellow), and the triazole unit of (+)-**2** (PDB code 3MXF, carbon = orange) all bound to human BRD4(1) (the remainder of (+)-**2** and **6** is omitted for clarity). The key KAc-binding bromodomain residues of the **6** (yellow) and (+)-**2** (light orange) structures are shown. The four water molecules found at the base of the KAc-binding pocket are shown (**6** structure = yellow, (+)-**2** = orange). The conserved water molecule bound in the ZA channel is also shown. (B) Overlaid X-ray crystal structures of **6** (PBD code 3ZYU, carbon = yellow), (+)-**2** (PDB code 3MXF, carbon = orange), and compound **5** (PBD code 3SVG, carbon = magenta) all bound to human BRD4(1). The WFP-binding component of each ligand is highlighted as a stick structure. (C) Overlaid X-ray crystal structures of **6** (PBD code 3ZYU, carbon = yellow), (+)-**2** (PDB code 3MXF, carbon = orange), and compound **5** (PBD code 3SVG, carbon = magenta) all bound to human BRD4(1), focusing on the ZA channel region. The two ZA channel conserved water molecules are shown as spheres for each structure. (D) Overlaid ligand-bound X-ray crystal structure of BRD4(1) 3P5O, 3SVG, 3U5J, 3U5K, 3U5L, 3UVW, 3ZYU, and 3MXF. The more conserved P82-bound water molecule is shown for each structure in shades of blue. The less well-conserved D88-bound water molecule is shown for each structure in shades of red. Structures were aligned using the "cealign" command in PyMOL.

protein of unknown function. They observed that (+)-**2** led to differentiation, growth arrest, and apoptosis in patient-derived NMC cell lines, phenocopying the effect of BRD4-NUT siRNA knock-down. (+)-**2** treatment also led to the down-regulation of BRD4-dependent genes. In patient-derived NMC mouse xenografts, (+)-**2** led to tumor shrinkage and increased overall survival rate.[24] (+)-**2** was also used to corroborate the findings of an RNAi screen that identified BRD4 as a therapeutic target for acute myeloid leukemia (AML).[26] In addition to reproducing the antiproliferative and differentation-inducing effects of BRD4 shRNA in AML cells, (+)-**2** showed single-agent antileukemic effects in an AML mouse model. Intriguingly, the effect of (+)-**2** on leukemia cells was due, at least in part, to down-regulation of the *Myc* oncogene, an attractive but challenging therapeutic target. A reduction in *Myc* expression was also shown to be partly responsible for the activity of (+)-**2** in multiple myeloma (MM).[29] Down-regulation of *Myc* and its target genes was accompanied by reduced proliferation and cell cycle arrest in cell lines and primary samples. Similar findings have been reported by Mertz et al. who independently demonstrated the *Myc*-dependent antiproliferative effects of (+)-**2** in leukemia and MM cell lines and showed activity in xenograft models of AML and Burkitt's lymphoma.[67] Encouragingly, (+)-**2** showed selectivity for malignant cells in all studies; however, the reason underpinning the observed selectivity for transformed cells remains to be fully elucidated.

The isoxazole-containing BET probe **6** showed efficacy against leukemia cell lines and xenograft leukemia models containing mixed lineage leukemia (MLL) gene fusions. The authors suggest that BET bromodomain-containing proteins recruit members of the superelongation complex (SEC) or polymerase-associated factor complex (PAFc). SEC and PAFc are regulators of transcriptional elongation, and since components of these complexes form fusions with MLL proteins in leukemias, they are crucial to the transforming

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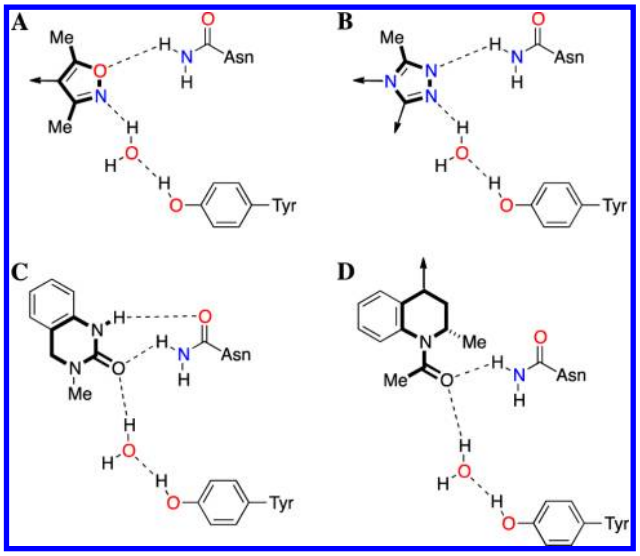

Figure 17. Schematic representations of the bromodomain-binding interactions of the (A) 3,5-dimethylisoxazole-, (B) triazole-, (C) dihydroquinazolinone-, and (D) 1-acyl THQ-based KAc mimics.

effects of the translocations. Known MLL target genes, including *Bcl2*, *Cdk6*, and *Myc*, were down-regulated by **6** treatment, consistent with the observed phenotype of the compound. These findings strengthen the association between inhibition of BET bromodomains and *Myc* down-regulation, suggesting that the inhibition of BET bromodomains is a promising therapeutic strategy for treating MLL-fusion leukemias.[26,28] Because of the potential for a *Myc*-targeted agent to act as a cancer therapy,[29] there is considerable interest in establishing the effects of a *Myc*-down-regulating compound, such as the BET bromodomain inhibitors, in other tumor types.[68]

Inhibitors of BET bromodomains have shown potential as anti-inflammatory agents. The BET bromodomains inhibitors **3** and **4** were initially identified as up-regulators of *ApoA1*.[25,49] ApoA1 is the major component of high-density lipoprotein and is associated with anti-inflammatory effects. Nicodeme et al. investigated the effects of **3** on the inflammatory response in LPS-stimulated macrophages.[25] **3** down-regulated the expression of LPS-inducible cytokines in stimulated macrophages but had little effect on unstimulated cells. Anti-inflammatory activity was also demonstrated in vivo, with **3** improving survival and down-regulating proinflammatory genes in mouse models of endotoxic shock and bacterial sepsis. Compound **6** also showed activity as both a prophylactic and a therapeutic agent in the same mouse model of sepsis.[55] A **2** analogue reported by Zhang et al. suppressed NF-κB-dependent transcription of proinflammatory genes in kidney cells and reduced inflammatory kidney damage in a mouse model of HIV-associated kidney disease.[51] The anti-inflammatory activity is likely due, at least in part, to inhibition of the interaction between BRD4 and a subunit of NF-κB, acetylation of which is important for transcriptional activation.[25,69] BET inhibitors have also been suggested as antiviral agents targeting the Epstein−Barr virus (EBV), as BRD4-dependent transcription of EBV nuclear antigens is inhibited by (+)-**2** treatment.[70] Together, these studies demonstrate that the disruption of bromodomain−histone interactions represent a promising therapeutic strategy for multiple disease states and that developing small molecule probes of these interactions is a valuable method of target validation.[44]

BET probes have been used to elucidate the role of BET bromodomain-containing proteins in nonpathogenic states.[71,72] Zhao et al. showed that treatment of cells with (+)-**2** slowed postmitotic mRNA synthesis, implying an important role for BET bromodomain-containing proteins (notably BRD4) in transcriptional activation after cell division. This is likely due in part to a role for BRD4 in chromatin decompaction, which is as yet poorly understood. The authors suggest that histone lysine acetylation plays a key role in "bookmarking" genes for rapid postmitotic transcriptional reactivation and that the association of BRD4 with KAc is crucial in promoting chromatin decompaction and recruiting important components of the transcriptional machinery.[71] A (+)-**2** analogue, bearing a primary amide in place of a *tert*-butyl ester, was used in a recent study by Devaiah et al. They showed that the N-terminal bromodomain-containing region of BRD4 possesses atypical kinase activity and directly phosphorylates the C-terminal domain of RNA polymerase II (Pol II), promoting transcriptional elongation. Treatment with a (+)-**2** analogue does not affect BRD4 kinase activity in vitro but suppresses BRD4-dependent Pol II phosphorylation in cells, suggesting that phosphorylation by BRD4 is dependent on its recruitment to acetylated chromatin.[73]

Lysine acetylation has also been observed in a diverse range of non-histone proteins, with roles in the regulation of both nuclear and cytoplasmic function.[2] **4** was used to probe the function of GATA1, an acetylated transcription factor involved in erythroid maturation.[72] Lamonica et al. identified BRD3 as a GATA1-interacting partner and used mutagenesis to demonstrate that the interaction is dependent on GATA1 acetylation. However, determining the effect of the GATA1−BRD3 interaction on GATA1 function proved to be challenging, as BRD3 knock-down affects cell viability and growth. Consequently, **4** was used to inhibit the association of BRD3 with GATA1, showing that disruption of this interaction reduced the transcription of GATA1 gene targets involved in erythroid maturation. Therefore, the authors caution that treatments using BET bromodomain inhibitors might lead to side effects arising from interference with hematopoiesis. This work does, however, demonstrate the utility of bromodomain ligands in elucidating the function of bromodomain-interacting proteins.

While the triazolobenzodiazepine BET inhibitors (+)-**2**, **3**, and **4** have already yielded valuble insights into bromodomain biology, these first generation compounds suffer from some drawbacks. The dimethylisoxazole-based **6** has more favorable PK properties, as discussed above. The pan-BET activity of these inhibitors also limits their utility in elucidating the specific functions of individual BET bromodomains. As discussed, development of compounds showing selectivity within the BET family is likely to be challenging because of the highly conserved nature of the KAc-binding sites within the BET family. However, as noted by Filippakopoulos et al., there is diversity between a number of residues around the edge of the binding site, which might allow the development of selective inhibitors for BET bromodomain family members.[24]

■ DEVELOPMENT OF LIGANDS FOR THE CREBBP BROMODOMAIN

The bromodomain-containing protein CREBBP is emerging as another target for small molecule bromodomain inhibitors. CREBBP was first discovered as a nuclear protein that binds to

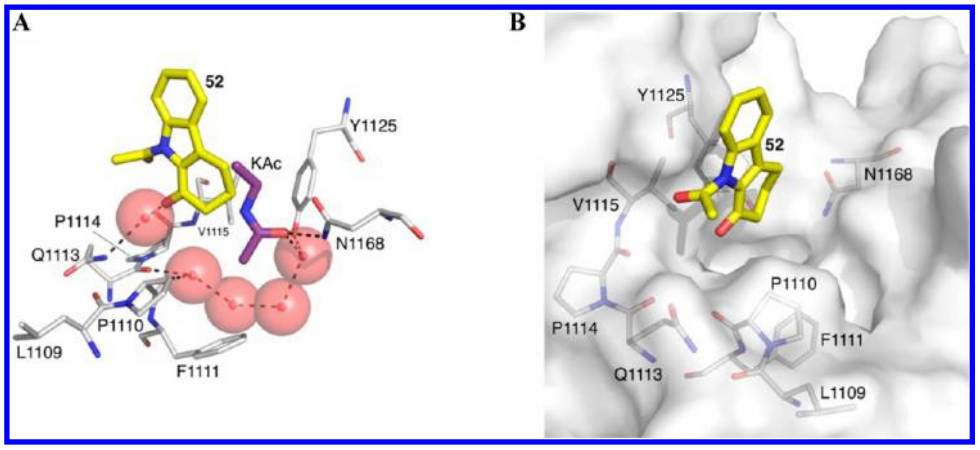

Figure 18. (A) View of an X-ray crystal structure of the CREBBP bromodomain in complex with KAc (carbon = purple, PDB code 3P1C) and the NMR structure of **52** (MS7972)[83] in complex with the CREBBP bromodomain (carbon = yellow, PDB code 2D82). The residues and water molecules shown are from 3P1C. Compound **52** does not reside deep in the KAc-binding pocket, and the intended KAc mimic does not form interactions with N1168 or Y1125. (B) NMR structure of **52** in complex with the CREBBP bromodomain (carbon = yellow, PDB code 2D82). The intended KAc-mimicking moiety in **52** is oriented toward that ZA channel.[83]

phosphorylated CREB and was shown to regulate protein kinase A activity as part of a multiprotein complex.[74] Its paralogue, p300 (also known as EP300), was identified as a binding partner of the adenovirus early region 1A protein (E1A).[75] Both CREBBP and p300 were later revealed to possess HAT catalytic activity.[76,77] The two proteins are now known to have at least 400 interacting protein partners making them key nodes in the known mammalian protein−protein interactome.[78] One such interacting partner, p53, is acetylated on multiple lysine residues at its C-terminus in response to extracellular stress or DNA damage.[79] CREBBP and p300 have been shown to acetylate several sites on p53, including K373 and K382.[80,81] In addition to effecting this acetylation via their HAT domains, CREBBP and p300 have been shown to recognize and bind specifically to KAc382 via their bromodomains.[82] The specific recognition of KAc382 by the CREBBP/p300 bromodomain is required for acetylation-dependent coactivator recruitment after DNA damage.

Zhou et al. were the first to investigate small molecule ligands for the CREBBP bromodomain,[83] based on their NMR structure of CREBBP bromodomain in complex with a K382 p53-mimicking peptide.[82] In order to identify CREBBP bromodomain ligands, a focused library of 200 compounds from a ChemBridge Corporation collection of ∼14 000 small molecules were selected to include a KAc mimic to interact with the KAc-binding pocket. In addition, electron-rich functional groups were favored, as the entrance to the CREBBP bromodomain is positively charged. The library of compounds was screened using $^{1}H-^{15}N$ HSQC spectra acquired in the presence and absence of a mixture of eight fragments. Differences in the chemical shifts of protein amide backbone resonances between the spectra were deconvoluted to determine which of the eight fragments in each screening mixture were binding to CREBBP. Fourteen CREBBP bromodomain-binding compounds were identified from the library. Of these, all but one bound selectively to the CREBBP bromodomain over the PCAF bromodomain, but wider selectivity data were not presented. Two compounds, **51** (MS2126) and **52** (Figure 19), were found to block the p53 KAc382−CREBBP bromodomain interaction at 100 and 50 μM, respectively. An NMR structure of the most potent compound, **52**, bound to the CREBBP bromodomain reveals

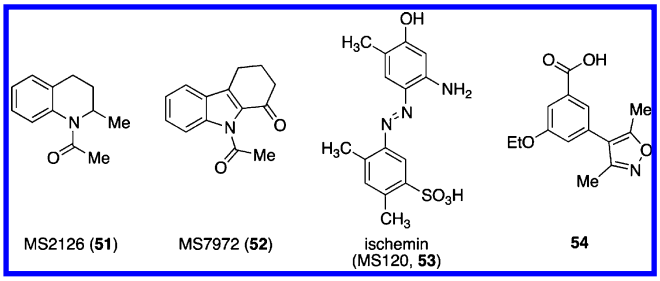

Figure 19. Structures of the CREBBP bromodomain ligands **51**, **52**,[83] ischemin (MS120, **53**),[84] and compound **54**.[52]

that the compound resides partly in the KAc-binding pocket (Figure 18). However, no interactions are formed with N1168 or Y1125, as the cyclohexenone ring is oriented into the KAc-binding pocket (Figure 18A). The intended KAc mimic points toward the ZA channel, and the overlaid structures indicate that the oxygen atoms of both carbonyl groups might interact with one of the ZA channel water molecules, although these water molecules are not observed in the NMR structure (Figure 18B).

The effect of compounds **51** and **52** on p53 translational activation was investigated in human bone osteosarcoma epithelial cells. Following doxorubicin-induced DNA damage, the effect on p53 levels was assessed using Western blot. Treatment with either compound at 200 μM dramatically suppressed the doxorubicin-induced increase in p53 levels. A corresponding decrease in p53-mediated p21 activation was also observed, supporting an inactivation of p53 by these small molecules.[83]

The same group screened a library of 3000 compounds using the $^{1}H-^{15}N$ HSQC-based assay. From this screen, 10 promising compounds were identified, several of which possessed the azobenzene structure.[84] The NMR structure of one compound, 4-hydroxyphenylazobenzenesulfonic acid (MS456), bound to the CREBBP bromodomain was solved, showing that the compound resided at the entrance to the KAc-binding pocket. On the basis of the structure of 4-hydroxyphenylazobenzenesulfonic acid, 27 azobenzene derivatives were synthesized and evaluated for their ability to bind to the CREBBP bromodomain. The ability of these compounds to

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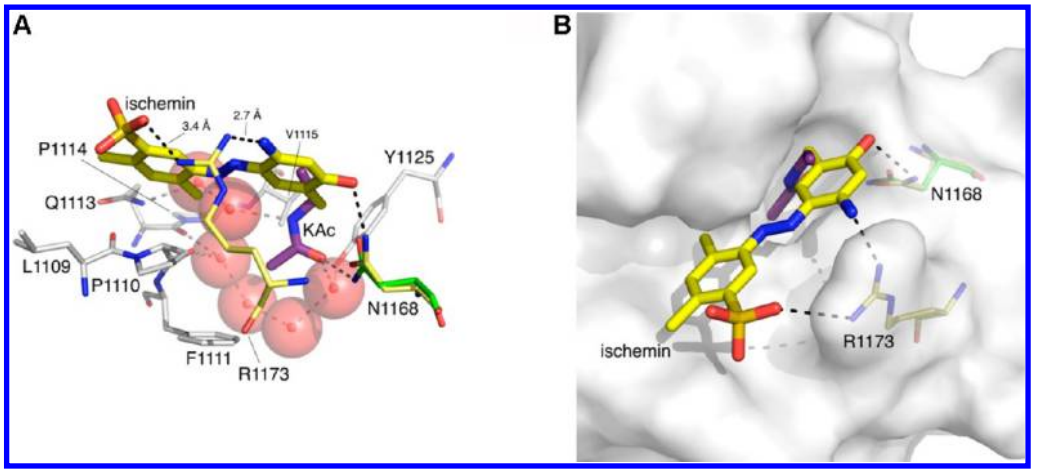

Figure 20. (A) View of an X-ray crystal structure of the CREBBP bromodomain in complex with KAc (carbon = purple, PDB code 3P1C) and the NMR structure of ischemin (**53**) in complex with the CREBBP bromodomain (carbon = yellow, PDB code 2D82). The phenolic hydroxyl group of **53** forms a hydrogen bond with N1168. The sulfonate group forms an electrostatic interaction with R1173, which also interacts with the aniline amine group. (B) As a consequence of the phenol and sulfonate interactions with the CREBBP bromodomain, the entrance to the KAc-binding pocket is blocked.[84] Residues with carbon = white and green are from 3P1C, and those with carbon = light yellow are from 2D82. Structures were aligned using the "cealign" command in PyMOL.

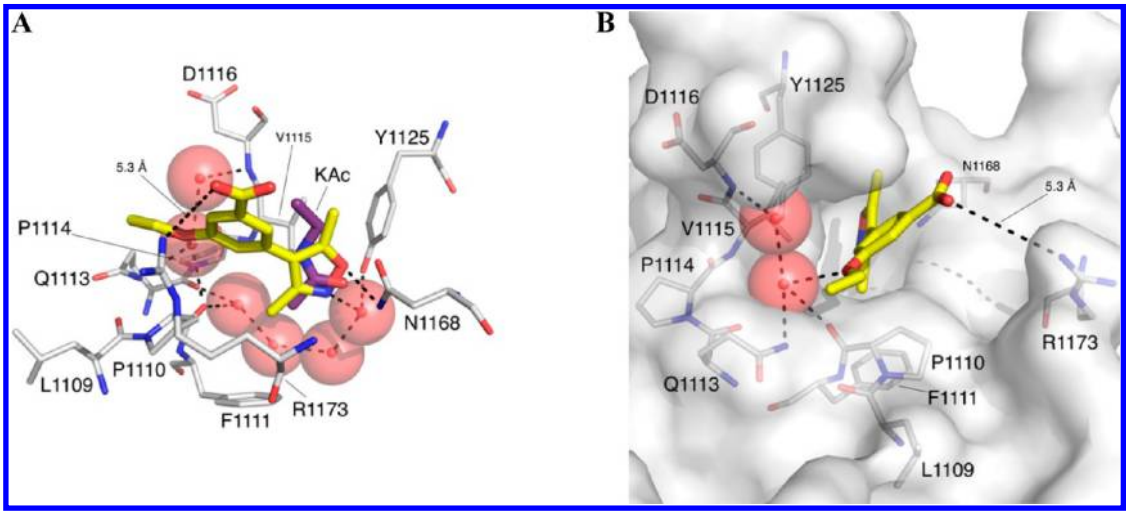

Figure 21. (A) View of an X-ray crystal structure of the CREBBP bromodomain in complex with KAc (carbon = purple, PDB code 3P1C) overlaid with the X-ray crystal structure of **54** bound to the CREBBP bromodomain (carbon = yellow, PDB code 3SVH). The oxygen atom of the 3,5-dimethylisoxazole moiety accepts a hydrogen bond from N1168, and the nitrogen atom forms a water-mediated interaction with Y1125. (B) The oxygen atom of the ethoxy group forms a hydrogen bond with one of the ZA channel water molecules. The carboxylate group might interact with R1173, although at 5.3 Å, the distance between the carboxylate group and R1173 is too long to be a close contact.

modulate DNA-damage-induced p53 activation in cells was measured using a p53-dependent, p21 luciferase reporter gene assay. Although the original hit only gave 4.6% inhibition at 50 μM, all subsequent analogues were more potent. Six of the compounds showed over 80% inhibition, and these were counterscreened in a fluorescence-based assay to determine K_D values. The most potent compound, ischemin (**53**) with a K_D of 19 μM, had an IC_{50} of 5 μM in the cell-based p21-luciferase assay.

An NMR structure of **53** bound to the CREBBP bromodomain (Figure 20) reveals that this compound occupies the peptide-binding groove of the bromodomain, but like the compounds previously reported by Zhou et al., the compound does not protrude far into the KAc-binding pocket. The phenolic hydroxyl group of **53** does form a hydrogen bond with N1168, but this is its only interaction in the KAc-binding pocket (Figure 20A). Interestingly, the sulfonate group forms an electrostatic interaction with R1173, which is located on the BC loop and forms part of the basic rim at the entrance to the CREBBP bromodomain previously identified by Zhou et al.[85] R1173 also interacts with the aniline amine group, which is presumably not protonated at physiological pH (Figure 20A). As a consequence of the phenol and sulfonate anchoring interactions, the entrance to the KAc-binding pocket is blocked. One of the aromatic methyl groups binds in the ZA channel (Figure 20B) and forms a hydrophobic interaction with L1120 (not shown). The bound water molecules are not visible in the NMR structure, but an overlay with the X-ray crystal structure of KAc bound to the CREBBP bromodomain suggests that the aromatic ring binding in the ZA channel displaces one of the water molecules often observed to bind here. The selectivity of ischemin (**53**) for the bromodomain of CREBBP over other bromodomains was investigated using a fluorescence assay. It was shown that **53** was up to 5-fold selective for CREBBP

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bromodomain over PCAF, BRD4(1), and BAZ2B. Zhou et al. have also developed cyclic peptides that bind to the CREBBP bromodomain with a K_D of 8 μM and high selectivity for the CREBBP bromodomain over other BCPs.[86] Fluorescent derivatives of these peptides proved to be useful tools in the identification of ischemin.

A number of the 3,5-dimethylisoxazole derivatives reported by Hewings et al.[52] bind to the CREBBP bromodomain in addition to the BET BCPs. The 3,5-dimethylisoxazole acts as a KAc-mimic residing within the KAc-binding pocket in an orientation similar to those of the 3,5-dimethylisoxazole-based BET bromodomain ligands (Figure 21A). The oxygen atom of the ethoxy group forms a hydrogen bond with one of the ZA channel water moieties (Figure 21B). Interestingly, the compound that showed similar potency for the BET and CREBBP bromodomains possesses a carboxylate group, which seems to interact with R1173 (Figure 21B), the same arginine residue that binds ischemin. Although the distance between the carboxylate group and R1173 is too long to be a close contact at 5.3 Å, it is possible that this interaction contributes to the affinity of this compound for the CREBBP bromodomain. Compound **54** has an IC_{50} of 32 μM against the CREBBP bromodomain and showed modest selectivity for this bromodomain over BRD4(1). This compound did, however, have an IC_{50} of 28 μM against BRD2(1). This work demonstrates that the 3,5-dimethylisoxazole moiety can act as a KAc mimic when binding to the CREBBP bromodomain. Despite this modest potency, compound **54** is an encouraging lead for the development of more potent and selective 3,5-dimethylisoxazole-based CREBBP bromodomain ligands.

In summary, there are presently many fewer small molecule ligands for the CREBBP bromodomain than for the BET bromodomains, and consequently it is not possible to observe significant trends in their structure−activity relationships. It is notable, however, that some of the ligands reported by Zhou et al. and compound **54** reported by Hewings et al. interact with R1173, suggesting that some form of association with this residue might be favorable for CREBBP bromodomain selectivity and affinity. It is encouraging that the 3,5-dimethylisoxazole moiety, which has formed the basis of potent and selective BET bromodomains inhibitors, also acts as a KAc mimic when binding to the CREBBP bromodomain. It is clear that the development of potent and selective CREBBP bromodomain ligands is essential to dissect the complex biology of this pivotal protein.

COMMENT ON ASSAYS USED TO DEVELOP BROMODOMAIN LIGANDS

For a detailed discussion on assay formats used to identify small-molecule and peptide ligands for bromodomains, the reader is directed to a recent review by Chung and Witherington,[41] and only brief coverage will be given here.

Label-free biochemical techniques have been widely used, particularly in earlier studies. These experiments do not require prior knowledge of a suitable peptide or small molecule ligand and so remain the only option for many bromodomains for which no cognate peptide sequence or chemical probe has been identified. The use of NMR was pioneered by Zhou and colleagues, who have identified small molecules (**1**, **51**−**53**) binding to the CREBBP[83,84] and PCAF[46] bromodomains, as well as cyclic peptide inhibitors of the CREBBP bromodomain.[86] Although NMR provides insights into the ligand binding mode, it requires significant quantities of protein and is unlikely to be suitable for large screening campaigns. Surface plasmon resonance (SPR) has the advantage of reduced protein consumption and provides K_D and kinetic data on compound binding. To date, SPR has been used only as a secondary assay to validate hits obtained using other techniques.[28,49,56] Differential scanning fluorimetry (DSF) or thermal shift has proved to be especially useful in evaluating compound selectivity over multiple bromodomains.[24,25,28,49,52,55] Isothermal titration calorimetry (ITC), despite its low throughput and high protein consumption, remains the "gold standard" method for evaluating small-molecule−bromodomain interactions because of its direct measurement of the thermodynamics of binding.[24,25,49]

The first report of a displacement assay to quantify the interaction of small molecules with the bromodomain emerged from the laboratory of M.-M. Zhou. In an ELISA employing immobilized PCAF bromodomain and a GST-tagged KAc-containing peptide, they determined IC_{50} values for a compound series derived from an NMR screening hit.[46] The modest throughput of ELISA limits its use in compound screening, but ELISA and similar formats have been used to identify cognate peptides for bromodomains,[87] most recently in a detailed analysis of the interactions of 33 bromodomains with acetylated and nonacetylated histone peptides.[36] Identification of cognate peptides and small molecule probes for bromodomains by such studies facilitates the development of biochemical assays for bromodomain-binding compounds. Because of the low inherent affinity of bromodomains for acetylated peptides, highly sensitive assay techniques such as AlphaScreen have been employed. Researchers at the Structural Genomics Consortium (SGC) reported the use of AlphaScreen technology to screen 13 bromodomains against a library of modified histone peptides. Strongly binding peptides were then used in an AlphaScreen peptide-displacement assay, which evaluated the effects of various solvents on bromodomain−peptide binding. Interestingly, they demonstrated that several solvents, including DMSO and NMP, inhibited the binding of acetylated peptides to bromodomains, with NMR and X-ray crystallography confirming that these compounds acted as KAc mimics.[62] The AlphaScreen format has also been used by the SGC to characterize inhibitors of the BET family of bromodomains.[24,52] Time-resolved Förster resonance energy transfer (TR-FRET) assays also require a suitable peptide binding partner for the bromodomain and have been used to validate hits against the BET bromodomains.[25,49] The discovery of tight-binding small molecules for bromodomains has allowed the development of sensitive fluorescence anisotropy (FA) assays in which the displacement of a fluorescently labeled probe molecule is detected. A FA assay employing a fluorescent analogue of **3** was developed by Chung and colleagues at GSK[28,49] and subsequently used in a fragment screen to identify compounds that bind to BET bromodomains.[53,66] A fluorescent analogue of ischemin, which binds to the CREBBP bromodomain,[84] was used in a FA assay to evaluate cyclic peptide ligands for CREBBP.[86] Fluorescent analogues of these peptides were then used in FA studies to determine their selectivity across multiple bromodomains.

Cell-based methods have also proved to be important in identifying small-molecule ligands for bromodomains. In particular, the methyltriazolodiazepine series of BET inhibitors was identified in a phenotypic screen for up-regulators of the *ApoA1* gene (vide supra), and bromodomains were only later identified as the molecular target.[25,49] An in-cell FRET assay for

histone acetylation was developed by Yoshida and colleagues,[88] who used a similar technique to identify a small-molecule inhibitor of BRD2−histone H4K12ac interaction (**7**).[56]

As noted by Bamborough et al.,[53] K_D or IC_{50} values obtained using different assay techniques can vary significantly in part because of the differing protein concentrations employed and differing substrate affinities (in the case of ligand displacement assays), among other factors. Similarly, measured peptide affinities are dependent on the assay format. Consequently care must be taken when comparing affinities from different assay methods. There is also a need to develop cell-based assays that are physiologically relevant and suitable for SAR studies to ensure that the action of bromodomain ligands can be readily assessed in a more complex setting.

■ CONCLUSIONS

It can be seen that significant progress has been made in developing small molecules that block the interaction of KAc with several bromodomain classes. In particular, potent and selective inhibitors of the BET bromodomains have been identified. The value of these molecules has been demonstrated by the significant biological studies that have been conducted, which have begun to elucidate the cellular role of the BET bromodomains. Whether the exciting biological data translate into the therapeutic use of BET bromodomain inhibitors remains to be seen; an ongoing clinical trial involving **3** will go some way to answering this question. The success observed in the field of BET bromodomains provides encouragement that it will be possible to develop small molecules that possess high potency and selectivity for other bromodomain classes. It is clear that these small molecule probes will be invaluable tools for dissecting the biological role of bromodomains and also potentially of great value as therapeutic leads. Finally, it is noted that the BET bromodomain story demonstrates how "classical" phenotypic screens, coupled to structure-based design and modern molecular biology, can rapidly elucidate a new set of therapeutic targets involving the tractable and selective disruption of protein−protein interactions.

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Notes

The authors declare no competing financial interest.

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Timothy P. C. Rooney received an M.Chem. at University of Oxford, U.K., in 2009, having carried out his part II project under the supervision of Dr. Angela Russell. He then joined the Systems Approaches to Biomedical Science Industrial Doctoral Centre in Oxford and completed two short epigenetic projects within the Conway group and with Prof. Chris Schofield. Tim is now continuing with work on the development of chemical probes for bromodomains in a collaborative project with the SGC and Pfizer Neusentis.

Laura E. Jennings studied chemistry at the University of Oxford, U.K., working under the supervision of Prof. Véronique Gouverneur for her final year project. She graduated with an M.Chem. in 2010, before enrolling at the Systems Approaches to Biomedical Science Industrial Doctoral Centre in Oxford. Following a short project in stem cell research under the supervision of Prof. Udo Oppermann, she is now working toward a D.Phil. in novel small-molecule approaches to target bromodomains with the cosupervision of Dr. Stuart Conway and Dr. Paul Brennan at Oxford and with Dr. Philip Humphreys at GSK Stevenage.

Duncan Hay received his M.Chem. from the University of Edinburgh, U.K. In 2003, he joined Discovery Chemistry at Pfizer's Sandwich laboratories. During this time, he worked on various preclinical medicinal chemistry projects, including antivirals and allergy and respiratory targets. In 2011, Duncan joined the Structural Genomics Consortium at the University of Oxford, U.K., to work on developing chemical probes for epigenetic targets. His work will form a D.Phil. thesis for which he is cosupervised by Dr. Paul Brennan at the Structural Genomics Consortium and Prof. Chris Schofield at the University of Oxford.

Christopher J. Schofield studied for a First Degree in Chemistry at the University of Manchester, U.K. (1979−1982), before moving to Oxford to study for a D.Phil. with Professor Jack Baldwin. In 1985 he became a Departmental Demonstrator in the Dyson Perrins Laboratory, Oxford University, followed by appointment as Lecturer in Chemistry and Fellow of Hertford College, U.K., in 1990. In 1998 he became Professor of Chemistry and in 2011 was appointed Head of Organic Chemistry. Chris is an international leader in functional, structural, and mechanistic studies on enzymes employing oxygen and 2-oxoglutarate cosubstrates. His work has opened up new fields in antibiotic research, oxygen sensing, and gene regulation. This work has identified new opportunities for medicinal intervention that are being pursued by numerous academic and commercial laboratories.

Paul E. Brennan received his Ph.D. in Organic Chemistry from the University of California, Berkeley, under the mentorship of Paul Bartlett. Following 3 years of postdoctoral research with Steve Ley in Cambridge University, U.K., Paul returned to California to take a position at Amgen. His research focused on designing and synthesizing kinase inhibitors for oncology. After 2 years at Amgen, Paul accepted a position as Medicinal Chemistry Design Lead at Pfizer in Sandwich, U.K. Here Paul designed and synthesized compounds for most major drug classes: GPCRs, CNS targets, ion channels, and metabolic enzymes. In 2011 Paul joined the Structural Genomics Consortium at the University of Oxford, U.K., as the principal investigator for medicinal chemistry. His research is focused on the design and synthesis of chemical probes for epigenetic proteins.

Stefan Knapp studied chemistry at the University of Marburg, Germany, and the University of Illinois. He received his Ph.D. in Protein Crystallography at the Karolinska Institute in Sweden (1996), and he continued his career at the Karolinska Institute as a postdoctoral scientist (1996−1999). He joined the Pharmacia Corporation in 1999 as a principal research scientist in structural biology and biophysics and left the company in 2004 to build up a group at the Structural Genomics Consortium. Since 2008, he has been a Professor at the Nuffield Department of Clinical Medicine at Oxford University, U.K. His research interests are the rational design and development of selective inhibitors that target protein kinases as well as the development of inhibitors for protein interactions mediated by epigenetic reader domains such as bromodomains.

Stuart J. Conway is a Lecturer in Chemistry at the University of Oxford, U.K., and the Fellow in Organic Chemistry at St. Hugh's

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■ ABBREVIATIONS USED

AML, acute myeloid leukemia; ApoA1, apolipoprotein A1; AUC_{∞}, area under the curve from time zero to infinity; BAZ2B, bromodomain adjacent to zinc finger domain 2B; BCP, bromodomain-containing protein; BET, bromodomain and extra C-terminal domain; BRD, bromodomain; BRDX(1)/(2), first/second bromodomain of bromodomain-containing protein X; CREBBP, cyclic adenosine monophosphate response element binding protein binding protein; CYP, cytochrome P450; DSF, differential scanning fluorimetry; EBV, Epstein−Barr virus; ELISA, enzyme-linked immunosorbent assay; FA, fluorescence anisotropy; FRAP, fluorescence recovery after photobleaching; FRET, Förster (fluorescence) resonance energy transfer; GATA1, GATA-binding factor 1; HAT, histone acetyltransferase; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HCTU, *O*-(1*H*-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HDAC, histone deacetylase; HTS, high-throughput screening; IL-6, interleukin 6; ITC, isothermal titration calorimetry; KAc, acetyl-lysine; LPS, lipopolysaccharide; MLL, mixed lineage leukemia; MM, multiple myeloma; NF-κB, nuclear factor κ light-chain enhancer of activated B cell; NMC, NUT midline carcinoma; NUT, nuclear protein in testis; PAFc, polymerase-associated factor complex; PCAF, p300 cyclic adenosine monophosphate response element binding protein binding protein associated factor; PEPPSI, pyridine-enhanced precatalyst preparation stabilization and initiation; PMBC, peripheral blood mononuclear cell; Pol II, RNA polymerase II; PTM, post-translational modification; PyBOP, (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; RuPhos, 2-dicyclohexylphosphino-2′,6′-diisopropoxybiphenyl; SAR, structure−activity relationship; SEC, superelongation complex; SPR, surface plasmon resonance; Tat, transactivator of transcription; THQ, tetrahydroquinoline; TNFα, tumor necrosis factor α; TR-FRET, time-resolved Förster (fluorescence) resonance energy transfer

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