Recent Updates on Small Molecule Inhibitors of Bromo and Extra C-Terminal Domain (BET) Family of Bromodomains

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Abstract: The bromo and extra C-terminal domain (BET) family of bromodomains (BRDs) are involved in binding epigenetic marks on histone proteins, more specifically ε-N-acetylated lysine residues. Inhibition of these targets leads to profound effects in relevant models of disease. BET BRDs inhibitors reported to date include benzodiazepines, benzotriazepines, 3,5-dimethylisoxoazole, dihydroquinazolinone, tetrahydroquinoline, thiazol-2-one, 4-acylpyrroles, diazobenzene, naphthyridines, and benzimidazole scaffold/moiety in their structure. Some potent inhibitors of BRD4, one of the bromodomain members, bind to asparagine140 residue of the acetylated-lysine site of BETs through triazole or isoxazole moieties. Some BET BRDs inhibitors also act as kinase inhibitors. Small molecules BET BRDs inhibitors have potential as anti-inflammatory, antiviral, and anticancer agents. Actions to produce a contraceptive for male rely on targeting BET family by a potent and selective bromodomain inhibitor. Several inhibitors targeting BRD4 are in preclinical/clinical trials as anticancer drugs.

Keywords: Inhibitors, Bromodomains and extra-terminal (BET) proteins, Benzodiazepines, Benzotriazepines, Tetrahydroquinoline, Dihydroquinazolinone.

INTRODUCTION

In biology/genetics, epigenetics deals with the heritable changes in gene expression or cellular epigenetic phenotype caused by mechanisms other than changes in the underlying DNA sequence. There are two important types of epigenetic modifications on gene regulation involment in chromatin by nature. On the one hand, methylation of DNA occurs, on the other hand, various changes on amino acid side chains of histones take place. It is known that post-translational modifications are regulated by three groups of proteins: writers (e.g. histone acetyltransferases), erasers (e.g. histone deacetylases) and readers (e.g. bromodomains (BRDs) [1-2]. Besides epigenetic readers and writers, which have already yielded promising targets for drug development [3], the epigenetic readers (bromodomains (BRDs) are also gaining attention in drug discovery over the last couple of years, and are now in preclinical and clinical development.

Bromodomains

A bromodomain is an approximately 110 amino acid protein domain. They are the main protein interaction modules known for a specifically recognition of ε-N-acetylated lysines of histones. There are six families of bromodomain-containing proteins. Research over the last decade has shown that members of one such family-the BET (bromodomain and extra-terminal) family (BRD2, BRD3, BRD4, and testis-specific BRDT proteins) collectively called epigenetic “readers” bind to acetyl-lysine residues on the tails of histones H3 and H4 [4, 5]. Bromodomain proteins recruit chromatin-regulating enzymes, including “writers” and “erasers” of histone modification, to target promoters, and to regulate gene expression [6]. The chance, that BETs can be addressed selectively by drug-like small molecules, without interfering with other BRDs, raised research efforts and led to promising inhibitors [7-9]. One of the most interesting facts deals with BRDT, which can only be found in testis and is strictly required for spermatogenesis [10]. Actions to produce a contraceptive for male rely on targeting this protein by a potent and selective bromodomain inhibitor [11].

Bromodomains are highly sequence diverse but they share a conserved fold that comprises a left-handed bundle of four alpha helices (αZ, αA, αB, αC) linked by diverse loop regions (ZA and BC loops) that flank the substrate binding site. The helical bromodomain bundle creates a deep central hydrophobic cavity that specifically recognizes sequences that contain ε-N-acetylated lysine residues. The acetyl-lysine side chain is typically anchored by a hydrogen bond to a conserved asparagines residue and water-mediated interactions with a conserved tyrosine. Crystal structures of bromo and extra terminal (BET) complexes with di-acetylated histone 4 tail peptides showed that the first BRDs of BRD4 and BRDT may accommodate 2 acetyl-lysines in a single binding site [12]. However, the substrates (e.g. the acetylated sequences that are specifically recognized) of most BRDs are not certain [13].

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Dysfunction of BRD-containing proteins has been linked to the development of diverse diseases and BRD inhibition has revealed exciting opportunities for treating a variety of maladies such as cancer, inflammation, obesity, cardiovascular disease, and neurological disorders [14-16].

The acetyl-lysine binding pocket in bromodomains represents an active site for the development of inhibitors. The acetylated-lysine is not charged, thus, binding pockets that specifically recognize pre-transmembrane contain mainly neutral, hydrophobic and aromatic residues allowing the development of cell-permeable acetyl-lysine competitive drug-like molecules. The publication of two potent ligands for the BET bromodomains in 2010 demonstrated that small molecules can inhibit the bromodomain–acetyl-lysine protein–protein interaction [17, 18]. These molecules display strong phenotypic effects in a number of cell lines and affect a range of cancers *in vivo*. These studies stimulated deep interest in developing further ligands for the BET bromodomains [19].

The MYC family of proteins is a group of basic-helix-loop-helix-leucine zipper transcription factors that feature prominently in cancer. Overexpression of MYC is observed in the vast majority of human malignancies. MYC transcription can be suppressed using small molecule inhibitors of BET protein bromodomains [20-22]. Researchers highlighted the potential of BET inhibitors as a novel therapeutic approach to treat prostate tumors driven by MYC over-expression [23].

Researchers have demonstrated that small molecules inhibited the bromodomain–acetyl-lysine protein–protein interaction [24]. A bromodomain inhibitor is a compound being a small molecule, in particular having a molecular weight of less 750, more particularly less than 500. Publicly available bromodomain inhibitors led to discoveries of key functions of BET-proteins in disease and development of new therapeutic strategies [25].

Inhibitors lack selectivity for individual BET family members. In addition, inhibitors development efforts have focused on few BRDs so far. Small molecule inhibition of BET protein bromodomains also selectively suppresses other genes, such as Bcl-2, that have important roles in cancer, as well as some NF-kB-dependent genes that have roles in both cancer and inflammation. The molecular mechanisms behind the promising therapeutic effects of BET bromodomain inhibition have been proposed [26]. Fluorescence recovery after photobleaching assays offer a potentially pan-bromodomain method for generating cell-based assays, allowing the testing of compounds with respect to cell permeability, on-target efficacy and selectivity [27]. This review article provides recent progress in the development of BET bromodomain inhibitors for potential applications in drug discovery.

BET BROMODOMAINS INHIBITORS

Most BET inhibitors described to date related to structural classes such as isoxazoles, amides/ureas and 1,2,4-triazoles [28-39] are described under following heads:

i) Triazolobenzodiazepine and Triazolobenzotria-zepine derivatives

ii) 3,5-Dimethylisoxoazole moiety

iii) Dihydroquinazolinone scaffold

iv) 1–Acetyl-tetrahydroquinoline moiety

v) Thiazol-2-one moiety

vi) 4-Acylpyrroles

vii) Diazobenzene moiety

viii) Naphthyridines

ix) Benziimidazole derivatives

x) Others

xi) Dual inhibitors

The general structures belonging to above classes are shown in Figures **1A-1J**.

ia) Benzodiazepines and Triazolobenzodiazepine–Based BET/BRDs Inhibitors

Researchers have reported benzodiazepines as potent protein interaction inhibitors that selectively bind to acetylated-lysine recognition modules of the BET (bromodomain and extra-terminal) family of transcriptional co-regulators [17, 18]. Drugs such as alprazolam (Figure **2A**) and midazolam (Figure **2B**) have been developed as protein interaction inhibitors that target bromodomains of the BET family [9].

The core structures of some reported BET BRDs are similar to clinical benzodiazepines such as diazepam (Figure **3**), with a number of unique

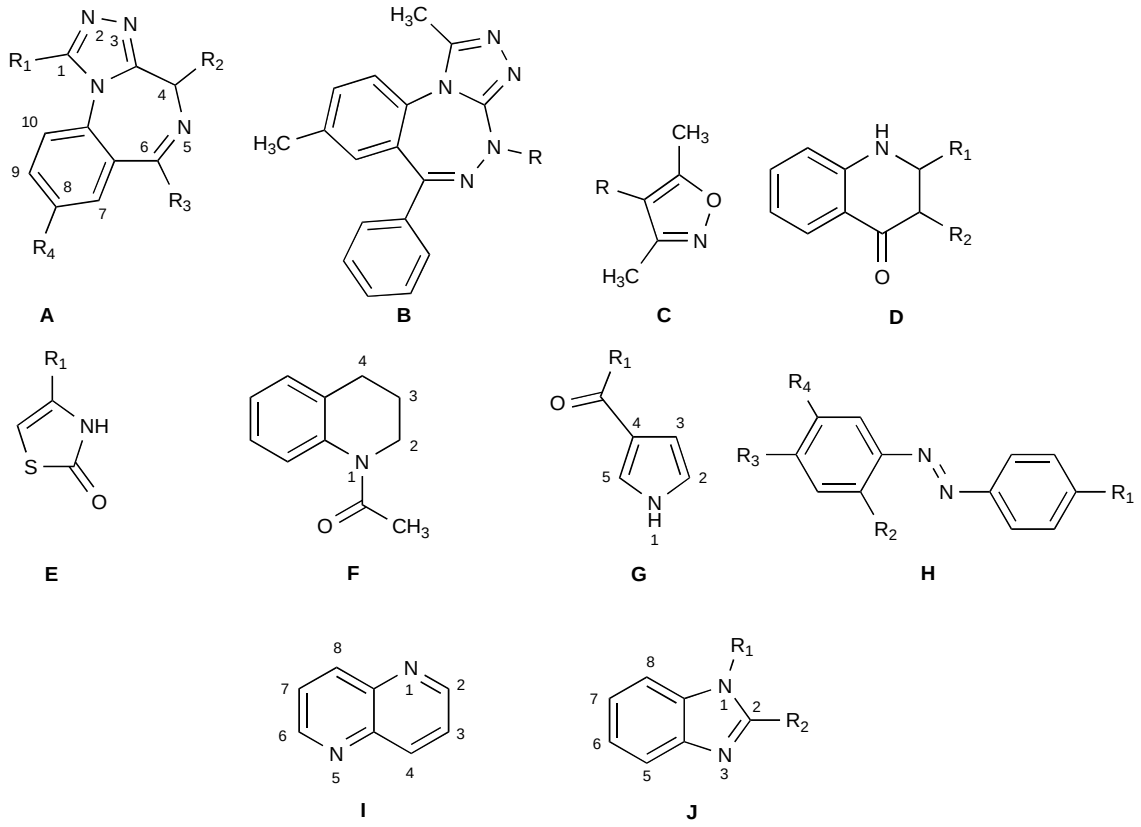


Figure 1: **A**: General structure for triazolobenzodiazepine derivatives. **B**: General structure for triazolobenzotriazepine derivatives. **C**: General structure of 3,5-dimethylisoxoazole moiety. **D**: General structure of dihydroquinazolinone moiety. **E**: Structure of thiazol-2-one moiety. **F**: Structure of 1-acetyl-tetrahydroquinoline moiety. **G**: Structure of 4-acylpyrroles moiety. **H**: General structure of diazobenzene derivative. **I**: Structure of 1,5-naphthyridine moiety. **J**: Structure of benzimidazole moieties.

substitutions conferring binding selectivity for BET bromodomains.

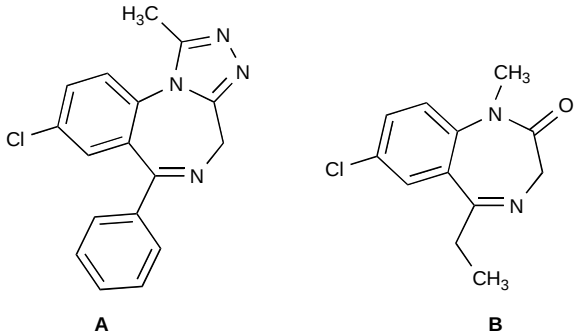


Figure 2: **A**: Structure of Alprazolam. **B**: Structure of Midazolam.

Chemical structures of BET bromodomain inhibitors such as (+)- JQ1, I-BET, GW841819X, OTX015, MS417 and CPI 203 share a common triazolodiazepine scaffold.

(+)-JQ1

(+)-JQ1{(S)-tert-butyl-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl)acetate[Formula:$C_{23}H_{25}ClN_4O_2S$] [M.Wt: 456.99]} (Figure **4**) is an inhibitor of BET bromodomain with IC_{50} of 77 nM/33 nM for BRD4(1/2). (+)-JQ1 binds to BRD4 bromodomains 1 and 2 with K_d values of ~ 50 and 90 nM, respectively and the binding is competitive with acetyl lysine. A close look at (+)- JQ1 bound BRDT confirmed that the acetyl-lysine recognition site of BRDT was blocked [11].

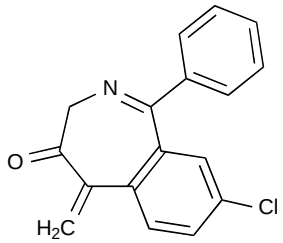


Figure 3: Structure of Diazepam.

(+)- JQ1, a potent and selective inhibitor of the bromodomain testis-specific protein BRDT is essential for chromatin remodeling during spermatogenesis. By blocking BRDT, (+)-JQ1 effectively blocks the production of sperm in the testes and consequently produces effective contraception, without the negative

side effects associated with previously researched hormonal contraceptives for men [17]. Notably, inhibition of BETs by (+)- JQ1 resulted in the down-regulation of oncogenes belonging to the MYC family of transcription factors, including c-Myc, in several cancer cell lines [40].

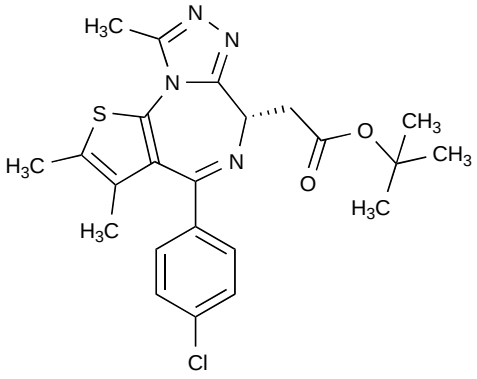


Figure 4: Structure of (+)-JQ1.

I-BET-762

I-BET-762, also known as (GSK525762A),{2-[(4S)-6-(4-chlorophenyl)-8-methoxy-1-methyl-4H-[1,2,4]tri-azolo[4,3-a][1,4]benzodiazepin-4-yl]-N-ethylacetamide [Formula:$C_{22}H_{22}ClN_5O_2$] [M.Wt:423.9]} (Figure **5**) is a highly potent inhibitor of BET family with IC_{50} values of 32.5–42.5 nM. I-BET762 is found to have high affinity with BET (K_d of 50.5–61.3 nM) and it binds to the acetyl-lysine-binding pocket of BET and competes with acetyl-lysine. I-BET762 also has high selectivity, it has no interaction with other bromodomain-containing proteins. I-BET762 is reported to downregulate the expression of genes induced by LPS, thus causing decreased expression of LPS-inducible cytokines and chemokines. *In vivo* assay showed that I-BET762 has an anti-inflammatory potential. Treatment of I-BET762 can cure mice that have started to develop symptoms of an inflammatory disease [41, 42].

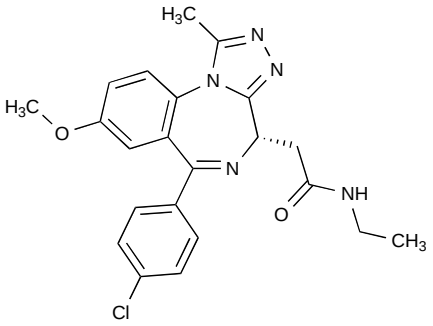


Figure 5: Structure of I-BET762 (GSK525762A).

Further, preclinical studies showed that I-BET762 has a favorable pharmacologic profile as an oral agent and that it inhibits myeloma cell proliferation, resulting in survival advantage in a systemic myeloma xenograft model. These data provided a strong rationale for extending the clinical testing of the novel antimyeloma agent I-BET762 and reveal insights into biologic pathways required for myeloma cell proliferation [43, 44]. In I-BET762, a triazole ring interacts with the critical asparagine residue of the acetylated-lysine site of BETs.

In another study, I-BET762 inhibited the proliferation of RS4;11 cells and promoted cells apoptosis. The mechanisms underlying this phenomenon might be achieved *via* downregulation of Bcl-2 protein thereby inducing apoptosis of leukemia cells [45]. Furthermore, I-BET 762 was under evaluation in a phase I/II clinical trial for nuclear protein in testis (NUT) midline carcinoma and other cancers [46]. The above-mentioned structurally related thieno- and benzo-diazepine triazoles (+)-JQ1 and I-BET762 (Figures **4** and **5**) have potential for use in inflammatory disease [50], atherosclerosis [47], NUT-midline carcinoma [19], acute leukemia [48], lymphoma [49], and HIV infection [50]. An ethyl derivative of an existing small-molecule inhibitor, I-BET762/JQ1 has been identified that binds leucine/alanine mutant bromodomains with nanomolar affinity and achieved up to 540-fold selectivity relative to wild-type bromodomains. Cell culture studies showed that blockade of the first bromodomain alone was found sufficient to displace a specific BET protein, BRD4, from chromatin [51].

GW841819X

GW841819X {(R)-benzyl (6-(4-chlorophenyl)-8-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo [4,3-a][1,4] diazepin-4-yl)carbamate[Formula: $C_{26}H_{22}ClN_5O_3$][M. Wt: 487.94]} (Figure **6**) is an analogue of (+)-JQ1 and a novel inhibitor of BET bromodomains.

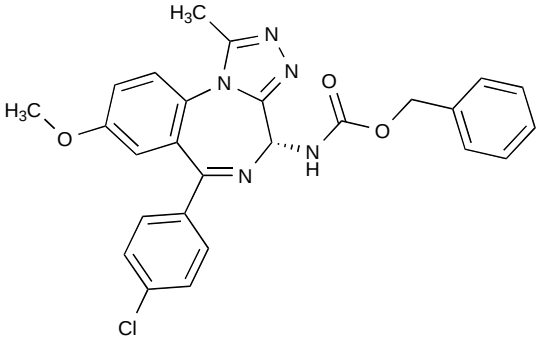


Figure 6: Structure of GW841819X.

GW841819X bind to the acetyl-lysine binding pocket of BET bromodomains with K_d ranges from 50

to 370 nM. GW841819X displayed activity *in vivo* against NUT-midline carcinoma, multiple myeloma, mixed-lineage leukemia, and acute myeloid leukemia [52]. GW841819X bind to both the individual BD1 and BD2 domains with affinities of 46 and 52.5 nM, respectively. GW841819X-BRD3 interaction was estimated to be around 70 nM. GW841819X competed directly with GATA1 site for BD1 binding and also specifically blocked the interaction between BRD3 and acetylated GATA1 [53]. It also potently induced the ApoA1 gene with an EC_{50} of 440 nM and had very little effect on LDL-R expression at concentrations that induced ApoA1 expression. These experiments were performed using reporter gene constructs and suggest that the effect is indeed specific [7]. Recent findings reported that GW841819X compound can be further developed into potential drugs against diseases including cancer, HIV infection and heart disease [54].

OTX015

OTX015 {(6S)-4-(4-chlorophenyl)-N-(4-hydroxy-phenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4] triazolo[4,3-a][1,4]diazepine-6-acetamide}[Formula:$C_{25}H_{22}ClN_5O_2S$] [M.Wt:491.99] (Figure **7**) is an orally bioavailable and small molecule inhibitor of bromodomain and BET family proteins with EC_{50} values of 10-19 nM for BRD2, BRD3, and BRD4. OTX015 has shown preclinical activity in hematologic and solid tumor models as well as promising early results in an ongoing phase I study. OTX015 showed preclinical activity as a single agent in Mantle cell lymphoma (MCL) cells. OTX015 had additive or synergistic activity with several targeted compounds (everolimus, pomalidomide, dexamethasone, and ibrutinib) in multiple MCL cell lines, identifying combinations that may merit further investigation in the preclinical and clinical settings. *In vitro*, OTX015 results in rapid down-regulation of c-MYC expression, and showed the synergistic anti-proliferative effects in combination with ALK inhibitors in ALKpos ALCL cell lines [55-58].

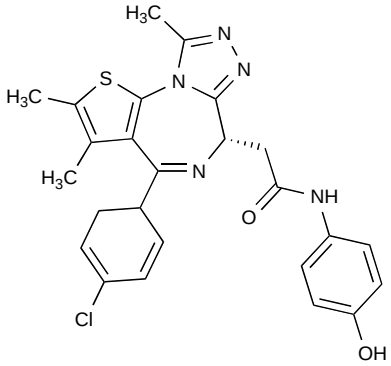


Figure 7: Structure of OTX015.

MS417

MS417 {methyl [(6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl]acetate}[Formula:$C_{20}H_{19}ClN_4O_2S$] [M.Wt: 414.91] (Figure **8**) is a novel BRD inhibitor with high affinity and specificity for the BRDs of BET proteins with IC_{50} of 30 nM.

MS417 inhibition of BRD4 binding to NF-κB blocks target gene activation and represented a new therapeutic approach for treating NF-κB-mediated inflammation and kidney injury in HIV-associated nephropathy [59].

Researchers have reported that BET inhibitor compounds ((+)-JQ1, I-Bet, and MS417) reactivate HIV from latency. This is evident in polyclonal Jurkat cell populations containing latent infectious HIV, as well as in a primary T-cell model of HIV latency. They showed that this activation was found dependent on the positive transcription elongation factor p-TEFb but independent from the viral Tat protein, arguing against the possibility that removal of the BET protein BRD4, which functions as a cellular competitor for Tat, serves as a primary mechanism for BET inhibitor action. Instead, they found that the related BET protein, BRD2, enforced HIV latency in the absence of Tat, pointing to a new target for BET inhibitor treatment in HIV infection. In shRNA-mediated knockdown experiments, knockdown of BRD2 activated HIV transcription to the same extent as JQ1 treatment, while a lesser effect was observed with BRD4. In single-cell time-lapse fluorescence microscopy, quantitative analyses across ~2,000 viral integration sites confirmed the Tat-independent effect of (+)-JQ1 and pointed to positive effects of (+)-JQ1 on transcription elongation, while delaying re-initiation of the polymerase complex at the viral promoter. Together, these results identify BRD2 as a new Tat-independent suppressor of HIV transcription in latently infected cells and underscore the therapeutic potential of BET inhibitors in the reversal of HIV latency [60].

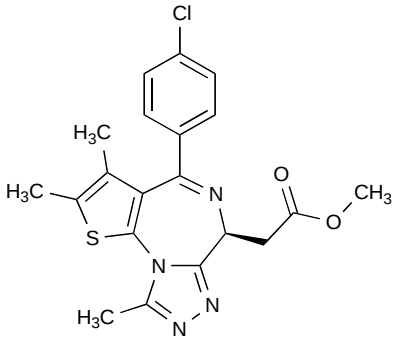


Figure 8: Structure of MS 417.

CPI-203

CPI-203{(6S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a] [1,4]diazepine-6-acetamide}{(S)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepin-6-yl) acetamide} [Formula:$C_{19}H_{18}ClN_5OS$] [M.Wt: 399.9] (Figure **9**) is a potent BET bromodomain inhibitor with IC_{50} value of 37 nM for BRD4. CPI-203 is a primary amide analog of (+)-JQ1 which has shown superior bioavailability with oral or i.p. administration [70]. It is comparable or superior to (+)-JQ1 in inhibiting BRD4 binding and action *in vitro* or in cells [70]. CPI-203 arrests the growth of leukemic T cells *in vitro* (EC_{50}= 91nM) and rapidly suppresses leukemia burden in mice [61].

CPI-203 is a potent, selective and competitive small molecule inhibitor of BET bromodomain with a mean GI50 value of 0.23μM in MCL cell lines [62]. As an inhibitor of BET proteins, CPI-203 inhibits BRD4 *in vitro* and in cells. It inhibits the specific Ser2 phosphorylation of both endogenous BRD4 and exogenous mutant BRD4 (BRD4 FEE-AAA) *in vivo*, thus, blocking the recruitment of BRD4 to chromatin. The CPI-203-lenalidomide combination is reported to be a promising strategy in MCL cases refractory to proteasome inhibition [62].

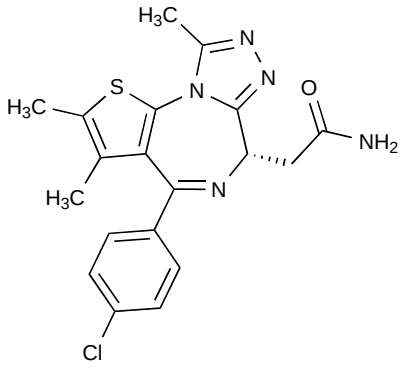


Figure 9: Structure of CPI 203.

Fused Triazoles

Structurally related fused triazoles (compound I) (Figure **10**) have been described in patents from GSK [63] and compound II (Figure **11**) from constellation [64] as potent BET inhibitors.

ib) Triazolobenzotriazepine Derivatives (BzTs)

Recently, triazolobenzotriazepines were discovered as a new versatile scaffold with strong BRD4 binding affinity. In this context, researchers have described the structural requirements for high affinity interactions of

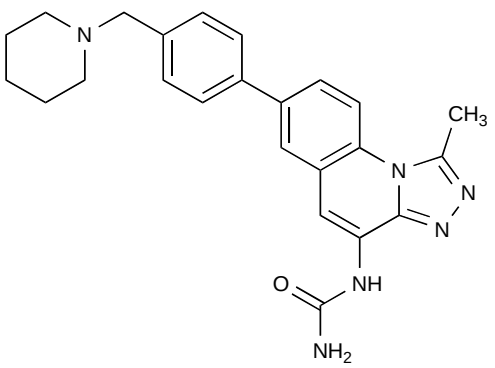


Figure 10: Structure of Compound I.

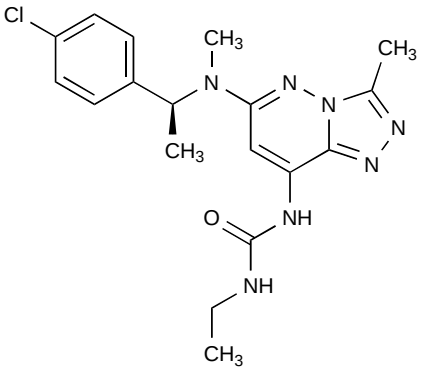


Figure 11: Structure of Compound II.

BzTs with BET bromodomains and discussed an initial structure–activity relationship for these BzTs [65]. Researchers have reported compounds of the triazolobenzo-triazepine class as submicromolar inhibitors of BET bromodomains with similar binding mode as benzodiazepines (i.e. BRD4 (1) acetyl lysine binding site) and excellent ligand efficiency. The co-crystal structure with BRD4(1) showed that the triazolo ring acts as an acetyl lysine mimetic scaffold forming a hydrogen bond with the conserved residue N140 that acts as a hydrogen bond anchor point for acetylated substrates in most bromodomains. Results revealed the importance of the 1-methyl triazolo ring system for BET binding and suggested modifications for the development of further high affinity bromodomain inhibitors. The binding mode of benzotriazepines (BzT-7) (Figure **12**) was found to be reminiscent of the related benzodiazepines alprazolam with identical conservation of the four water molecules [9].

ii) 3,5-dimethylisoxoazole Moiety

The 3,5-dimethylisoxazole moiety has been demonstrated as an effective acetylated-lysine mimic[33-36, 66]. Compound III (Figure **13**) binds to the BET bromodomains, having IC_{50}= 4.8 μM against BRD4(1) [33].

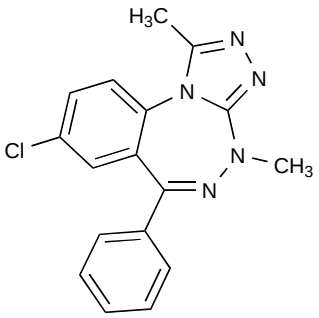


Figure 12: Structure of benzotriazepines (BzT-7).

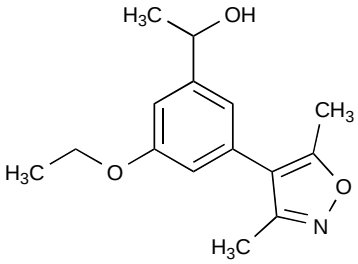


Figure 13: Structure of Compound III.

I-BET151 showed low nanomolar potency *in vitro* and in cell based assays. This quinoline based inhibitor I-BET-151 carries an isoxazole group that interacts with the critical asparagines residue [67]. Although less potent than JQ1 or I-BET-762, I-BET-151 showed marked acceleration of apoptosis and perturbation of growth in primary cells obtained from patients with mixed lineage leukemia [52].

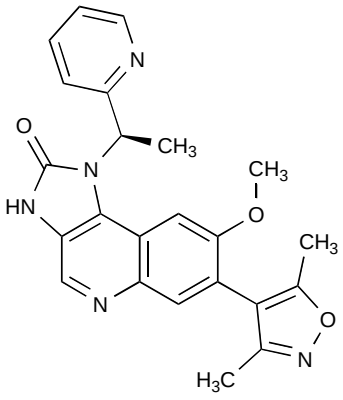


Figure 14: Structure of I-BET151.

Researchers have reported the structure-guided optimization of 3,5-dimethylisoxazole derivatives to develop potent inhibitors of the BET bromodomain family with good ligand efficiency. X-ray crystal structures of the most potent compounds revealed key interactions required for high affinity at BRD4(1). Cellular studies demonstrated that the phenol and acetate derivatives of the lead compounds showed strong antiproliferative effects on MV4; 11 acute myeloid leukemia cells, as shown for other BET bromodomain inhibitors and genetic BRD4 knockdown [37].

iii) Dihydroquinazolinone

Researchers have reported a highly potent dihydroquinazoline-2-one inhibitor; PFI-1 (Figure **15**) resulting from optimization of a fragment-derived hit. PFI-1 blocked the interaction of BET BRDs with acetylated histone tails. Moreover, PFI-1 binds to BET BRDs with low nanomole potency. PFI-1 showed antiproliferative activity in leukemia cell lines arising from the induction of G1 arrest, MYC down-regulation, and apoptosis. Exposure of leukemia cells to PFI-1 resulted in induction of caspase-dependent apoptosis, differentiation, and in down regulation of the Aurora B kinase [69].

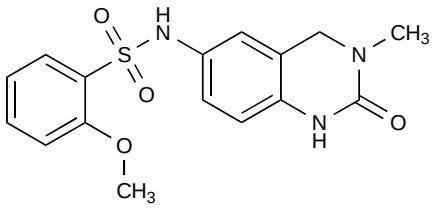


Figure 15: Structure of PFI-1.

iv) Tetrahydroquinoline

Researchers have reported the identification of a novel tetrahydroquinoline series through up-regulation of apolipoprotein A1 and the optimization into potent compounds active in murine models of septic shock and neuroblastoma. At the molecular level, these effects are produced by inhibition of BET bromodomains. Based on X-ray crystallography results, I-BET726 (GSK1324726A) (Figure **16**) represents a new, potent, and selective class of tetrahydroquinoline-based BET inhibitors [70].

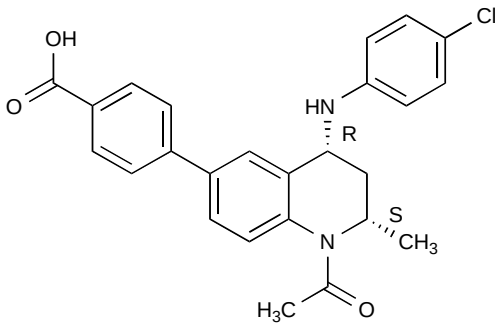
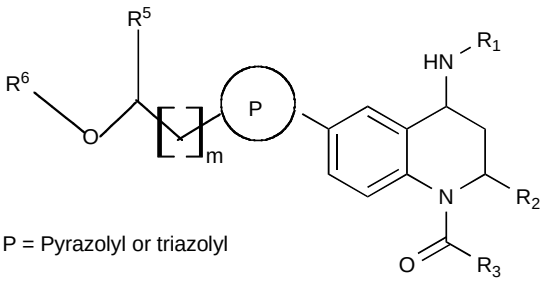


Figure 16: Structure of I-BET-726 (GSK1324726A).

Tetrahydroquinoline compounds (Figure **17**) pharmaceutical compositions containing such compounds and their use in therapy was reported [71].



P = Pyrazolyl or triazolyl

R^1 = C(O)OR^4; R^4 = C_{1-3} alkyl or C_{3-7} cycloalkyl or

R^1 = phenyl, pyridyl, pyrazinyl and pyrimidinyl

R^2 = C_{1-4} alkyl; R^3 = C_{1-4} alkyl

R^5 = H or C_{1-4} alkyl; R^6 = C_{1-4} alkyl

Figure 17: General structure of tetrahydroquinoline compounds.

v) Thiazol-2-One Moiety

To contribute novel scaffolds for developing into bromodomain inhibitors, researchers have utilized a fragment-based drug discovery approach. By successively applying docking and X-ray crystallography, they were able to identify nine fragment hits from diffracting more than sixty crystals. Further, they described four of them and carried out the integrated lead optimization for fragment IV (Figure **18**) which bears a 2-thiazolidinone core.

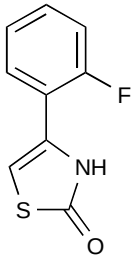


Figure 18: Structure of fragment IV.

Exploring the structure-activity relationship of 2-thiazolidinones resulted in a novel and potent BRD4 inhibitor with IC_{50} ~0.3 µM. The PK study *in vitro* and cellular activity assays further demonstrated that the 2-thiazolidinone is an interesting scaffold, which is both druggable and has potential to be developed into inhibitors that antagonize acetyllysine-bromodomains interactions [72].

In the optimization of another series of 2-thiazolidinones as BRD4 inhibitors, researchers reversed the sulfonamide group and identified a new binding mode. A structure-activity relationship study on series led to several potent BRD4 inhibitors (Figure **19**) with IC_{50} of about 0.05-0.1 µM in FP binding assay and GI_{50} of 0.1-0.3 µM in cell based assay. Further investigations on its effects on BRD4 downstream protein c-Myc, its selectivity amoung five different bromodomain proteins, as well as the metabolic stability test, reinforced the utility of 2-thiazolidinone scaffold as BET bromodomain inhibitors in novel anticancer drug development [73].

A protein containing two bromodomains has emerged as an attractive therapeutic target for several types of cancer as well as inflammatory diseases. Using a fragment-based in silico screening approach, researcher have identified two small molecules (Figure **20**) that bind to the first bromodomain of BRD4 with low-micromolar affinity and favourable ligand efficiency (0.37 kcal/mol per non-hydrogen atom), selectively over other families of bromodomains. Notably, the hit rate of the fragment-based in silico approach is about 10% as only 24 putative inhibitors, from an initial library of about 9 million molecules, were tested *in vitro* [74].

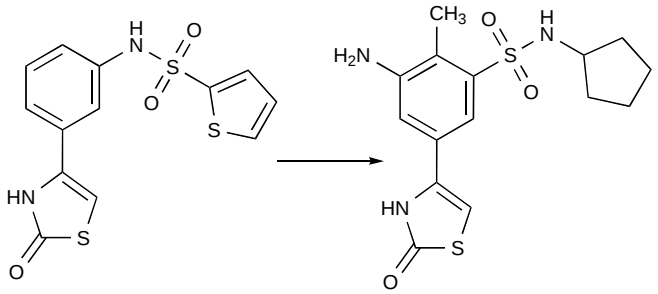


Figure 19: Structure of potent BRDs inhibitors generated due to reversal of the sulfonamide group.

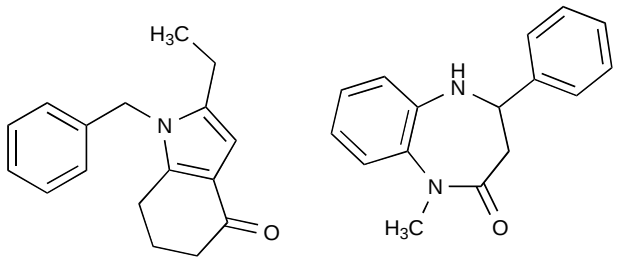


Figure 20: Structure of BRD4 inhibitors with low-micromolar affinity.

vi) 4-Acylpyrroles

XD46 (Figure **21**), a starting template for the development of potent inhibitors of other bromodomain families have exhibited significant inhibitory activity against the BET bromodomain BRD4 [94]. Since XD46 is a new chemical entity, it could serve as a novel drug platform in the epigenetics field. A virtual screening approach, followed by X-ray crystallography and ITC measurements has identified a 4-acyl pyrrole derivative

XD14 (an optimized derivative of XD46) (Figure **22**), which potently inhibits BET BRDs and has a K_D of 237 nM against BRD4(1) by ITC and a K_D value of 160 nM in the BROMO scan ligand displacement assay. X-ray structure revealed that the ketone carbonyl group mimics the carbonyl of acetylated-lysine in its hydrogen-bonding interactions, while a methyl group at C(5) occupies a similar position as the terminal methyl group of acetylated-lysine [75]. Xd14 potently inhibits specific bromodomains and exhibits antiproliferative activity against leukemia cell lines and it is not acute toxic in mice [76].

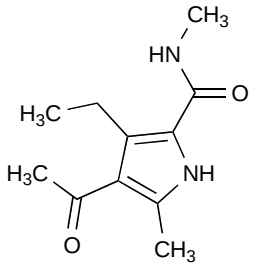


Figure 21: Structure of XD46.

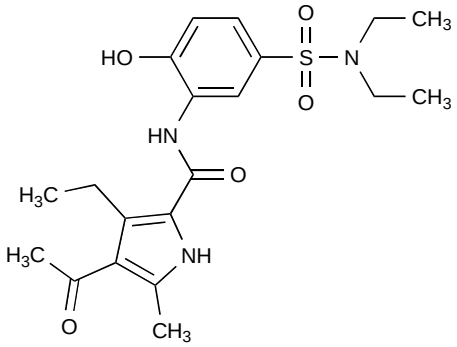


Figure 22: Structure of XD14.

vii) Diazobenzene Compound

MS436 (Figure **23**) is a potent and selective small-molecule inhibitor of BRD4 with Ki values of ＜0.085 μM and 0.34 μM, respectively for BRD1 and BRD2. *In vitro* fluorescent anisotropy assay showed that MS436 has about 10-fold higher affinity of BRD1 over BRD2. MS436 binds to BRD4 through a set of water-mediated interaction and this is the molecular basis for the binding affinity. MS436 also has activity to CBP BRD [77].

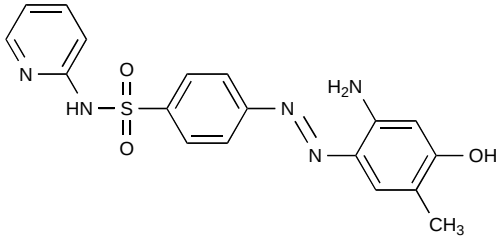


Figure 23: Structure of MS 436.

viii) Naphthyridines

Researchers have reported the discovery of new naphthyridine analogues as potent BET bromodomain family inhibitors. To expand the chemical diversity of BET inhibitors and enhance solubility of the parent chemical series, namely the isoxazoloquinoline derivatives, one extra nitrogen atom was added to the quinoline template. The higher affinity of the 1,5-naphthyridine derivatives was observed over the others isomers. The most promising compound (Figure **24**) were progressed in a mouse model of inflammation and exhibited dose-dependent anti-inflammatory pharmacology and showed efficacy [78].

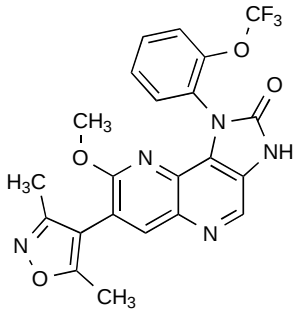


Figure 24: Structure of potent 1,5-naphthyridine derivative.

ix) Benzimidazole Derivatives

Based on a validated virtual screening approach, novel acetylated-lysine mimetics including benzimidazole derivative (Figure **25**) has been reported as potent inhibitors of BRD4 [79].

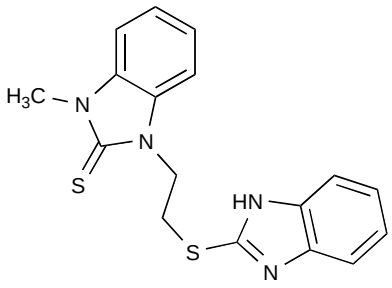


Figure 25: Structure of benzimidazole derivative.

Simple 1-substituted 5- and 6-isoxazolyl-benzimidazoles (Figure **26**) have been shown to be potent inhibitors of the BET bromodomains BRD4(1) (pIC50 = 6.7) with selectivity over the related bromodomain of CBP (pIC50 < 4).

Benzimidazole derivatives with general formula (Figure **27**) which may act as inhibitors of, or which

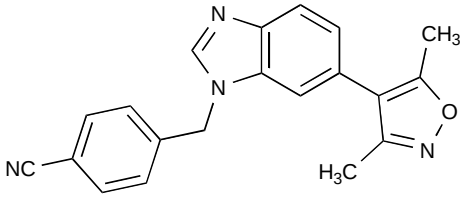


Figure 26: Structure of 1-substituted 5-isoxazolyl-benzimidazoles derivative.

may otherwise modulate the activity of, a bromodomain-containing protein, including BRD4, and their compositions and formulations containing such compounds, and methods of preparations were reported [80].

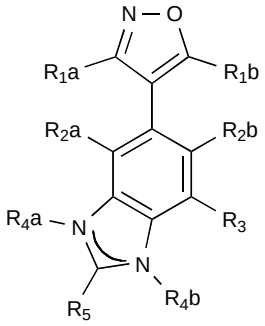


Figure 27: Structure of benzimidazole derivatives.

x) Others

Commercially sourced and de novo synthesized substituted [1,2,4]triazolo[4,3-*a*]phthalazines are potent inhibitors of both the BET bromodomains such as BRD4 as well as bromodomains outside the BET family such as BRD9, CECR2, and CREBBP. This new series of compounds is the first example of submicromolar inhibitors of bromodomains outside the BET subfamily. Representative compounds are active in cells exhibiting potent cellular inhibition activity in a FRAP model of CREBBP and chromatin association. The compounds described are valuable starting points for discovery of selective bromodomain inhibitors and inhibitors with mixed bromodomain pharmacology [41].

Benzodiazepine compounds of formula (Figure **28**), and pharmaceutical compositions containing such compounds and their use in therapy, in particular in the treatment of diseases or conditions for which a bromodomain inhibitor were reported.

Another series of small molecule BET inhibitors was identified. Using crystallographic binding modes of an amino-isoxazole fragment (Figure **29**) and known BET inhibitors, a structure-based drug design effort lead to a isoxazole azepine compound V containing dimethyl

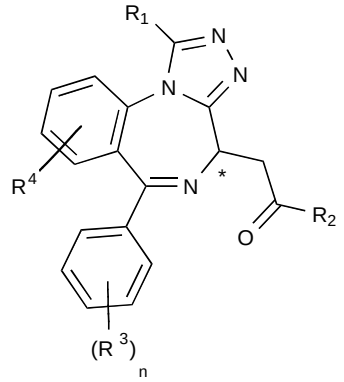


Figure 28: Structure of benzodiazepine compounds.

thiophene isoxazole azepine carboxamide moiety (Figure **30**). This scaffold showed good potency in biochemical and cellular assays and oral activity in an *in vivo* model of BET inhibition. Compound V inhibits MYC mRNA expression *in vivo* after PO dosing in a dose-dependent manner [81].

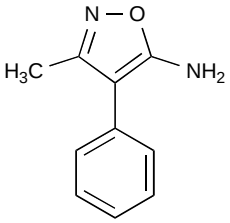


Figure 29: Structure of amino oxazole moiety.

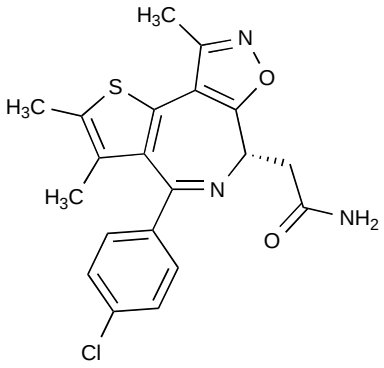


Figure 30: Structure of compound containing isooxazole-azepine scaffold.

CPI-0610

The compound, CP-0610, is chemically similar to that of JQ1, OTX015, and I-BET762, but contains an azepine scaffold instead. CPI-0610 (Figure **31**) inhibits BET proteins with potential antineoplastic activity. Upon administration, the BET inhibitor CPI-0610 binds to the acetylated lysine recognition motifs on the bromodomain of BET proteins, thereby preventing the interaction between the BET proteins and acetylated

histone peptides. This disrupts chromatin remodeling and gene expression. A Phase 1 clinical trial of CPI-0610 as a potential treatment for lymphoma has been initiated. The Phase 1 trial in myeloma and another Phase 1 trial in patients with acute leukemias and myelodysplastic syndromes have been added based on preclinical research showing blood-related cancers may be highly sensitive to BET inhibition [82].

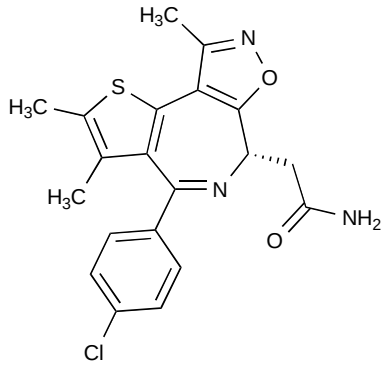


Figure 31: Structure of CPI-0610.

ZEN-3365

ZEN3365 is a novel BET bromodomain inhibitor for the treatment of hematologic malignancies and solid tumors. ZEN-3365 more potently represses superenhancer driven MYC expression in MM.1S than regular enhancer/promoter driven BCL-2 in MM.1S or regular promoter-driven MYC in MV4-11 cells [83].

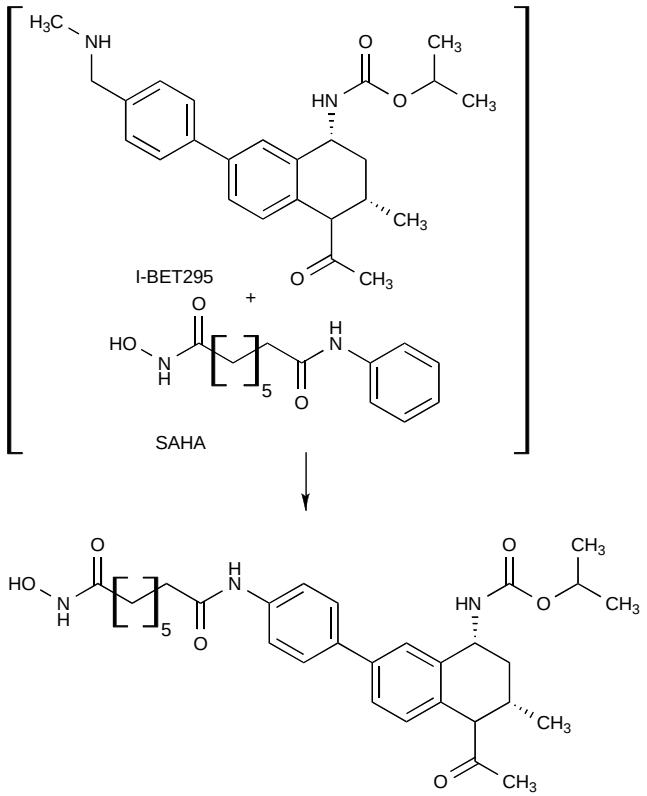


Figure 32: Structure of DUAL946.

xi) Dual Inhibitors

DUAL946

The design and synthesis of a dual active histone deacetylase (HDAC)/BET small molecule tool inhibitor, DUAL946 (Figure **32**) was reported. Researchers have achieved the functionalisation of a BET active tetrahydroquinoline core, with a hydroxamic acid HDAC inhibitor motif. Dual inhibition of BET and HDAC proteins was confirmed by *in vitro* biochemical and biophysical testing and through chemoproteomic competition experiments in cell lysates. Moreover, this activity was translated into potent cellular activity in both immune and cancer cells [84].

Dual Kinase/Bromodomain Inhibitors

Based on the importance of the conserved asparagine in BRDs for acetylated-lysine binding, it was thought that the most potent BET inhibitors reported to date should also target asparagine for anchoring to BET BRDs [40]. The potent cyclin-dependent kinase inhibitor dinaciclib binds to the acetylated-lysine recognition site of BRDT bromodomain 1 (BRDT-1) [85]. In this context, researchers have demonstrated that the acetylated-lysine site of BRD4-1 interacts with structurally diverse

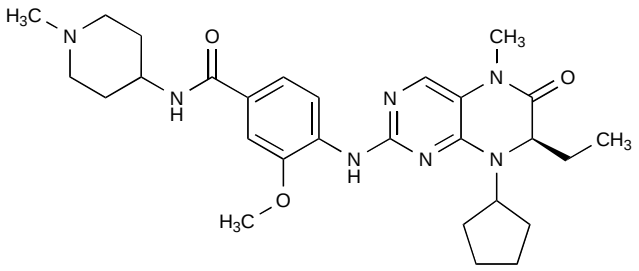


Figure 33: Structure of BI2536.

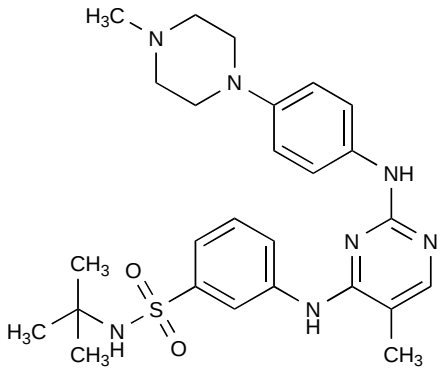


Figure 34: Structure of TG101209.

kinase inhibitors. Among the 14 compounds identified, the PLK1 inhibitor BI2536 (Figure **33**) was the most potent BET inhibitor with IC_{50} values of 25 and 260 nM against BRD4-1 and BRDT-1, respectively. The JAK2 inhibitor TG101209 (Figure **34**) displayed strongest inhibitory potential against BRD4 (IC_{50} values 130 nM). Both inhibitors displayed high selectivity for BET bromodomains; and profiling against 32 human BRDs demonstrated high selectivity of these kinase inhibitors for BET proteins. Comparative structural analysis revealed markedly different binding modes of kinase hinge-binding scaffolds in the acetylated-lysine binding site, suggesting that BET proteins are potential off-targets of diverse kinase inhibitors. Further, the binding modes of BI2536 and TG101209 suggested that the concomitant interaction of kinase inhibitors with both Asn140 and Pro82 provided highest binding potential. Overall, these findings provided a new structural framework for the rational design of next-generation BET-selective and dual-activity BET-kinase inhibitors [86].

According to another study, nanomolar activity on BRD4 by BI-2536 (PLK1kinase inhibitor) (Figure **33**) and TG-101348(JAK2/FLT3 kinase inhibitor) (Figure **35**) of is particularly notable as these combinations of activities on independent oncogenic pathways illustrate a novel strategy for rational single agent polypharmacological targeting. PLK1 inhibitors BI-2536 and JAK inhibitor TG-101348 showed nanomolar affinities (K_d: 37 ±3 nM; K_ds and 164 ±10 nM, respectively) towards BRD4(1). The p38 inhibitor SB-202190 (Figure **36**) and the PI3K inhibitor PP-242 (Figure **37**) showed slightly weaker activity (K_ds: 3.4 ±0.13 μM; 1.7 ±0.076 μM, respectively). Further, structure-activity relationships and co-crystal structures based identification assist design features that enable a general platform for the rational design of dual kinase/bromodomain inhibitors [87]. Dual JAK/BET inhibition exemplified by TG-101348 could also be beneficial in the IL7R-driven subset of acute lymphoblastic leukemia where JAK and BET activities promote the same oncogenic pathway [88]. In addition, dual PLK/BET inhibitors may also be an efficacious combination due to the functions of both targets in mitosis.

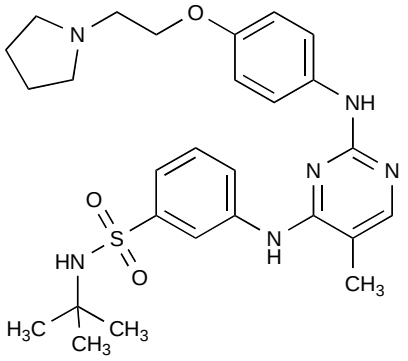


Figure 35: Structure of TG-101348.

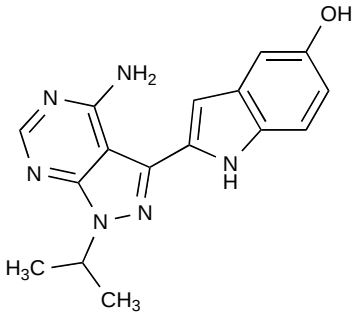


Figure 36: Structure of PP-242.

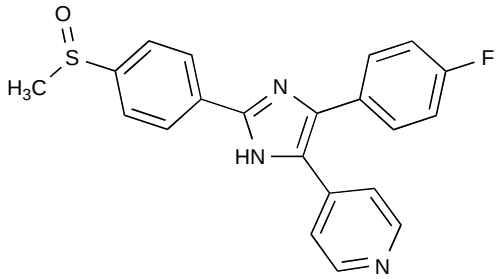


Figure 37: Structure of SB-203580.

CONCLUSION AND PERSPECTIVES

Epigenetic reader domains of the BET bromodomain family have recently emerged as novel targets for many therapies. Many small molecule BET BRDs inhibitors have been exploited as potential drugs for cancer, inflammation, and for research to produce contraceptives. Most BET inhibitors described to date have a methyl group adjacent to a hydrogen bond acceptor which mimics the acetyl group of acetyl lysine. Many research efforts are currently underway to discover new chemical scaffolds for hit-to-lead development campaigns of BET inhibitors as novel therapeutics. In this direction, small molecule containing hydroxamate moiety has not been exploited. Further, there is a strong need to design small molecule dual inhibitors for BET bromodomains/other families. Furthermore, no attempt has been made to involve metal ions in the struggle of BET BRDs inhibition. In this context, the inhibitors chelated with metal ions at non-essential sites of inhibitors should be exploited for better understanding of BET BRDs inhibition mechanism.

NOTE

The author of this article Dr. B.S. Sekhon expired after submitting the article. Keeping in mind the importance of this article I took the initiative to answer some of the querries putforth by the referee. Inspite of my every care to reply correctly to each aspect there may still be somepart remaining unanswered, the readers are requested to go through the references quoted in this paper – Dr. S.K. Munshi, Professor of Biochemistry and Biotechnology, PCTE, Baddowal, Ludhiana, India.

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