Epigenetic Probes: Open Access Drug Discovery

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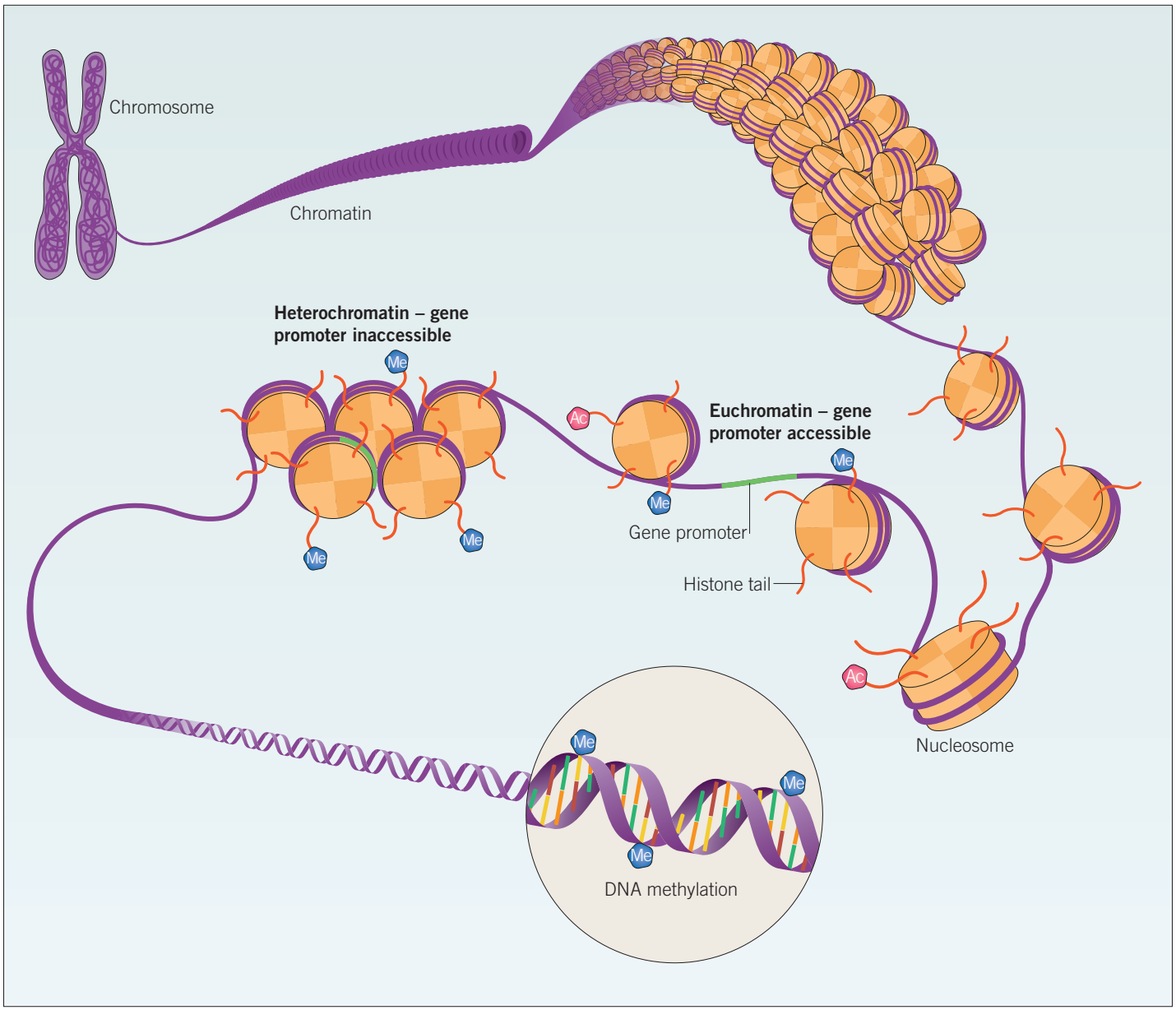
Introduction

Epigenetics is the study of heritable changes which, unlike the genome itself, are cell specific, plastic and responsive to environmental influences. In contrast to genetics, which is based on changes to the DNA sequence, epigenetic changes in gene expression and the resulting cellular phenotype are regulated by interrelated mechanisms. These include DNA modifications like methylation, (which are well-studied due to the availability of established assays); regulator RNAs; and post-translational modifications of the histone tails (Figure 1). Proteins which modify histone tails are generally referred to as readers, writers and erasers, depending on their function of interpreting, adding or removing histone modifications respectively (Figure 2). A variety of modifications have been identified and studied, most notably acetylation and methylation of lysine residues, as well as ubiquitination.[1] The study of these epigenetic modifications has been aided greatly by the development of chemical probes.

Chemical Probes as Useful Tools in Biology and Target Validation

Chemical probes are well-characterized, potent (IC_{50}/K_d < 100 nM), selective (> 30-fold) and cell-active (IC_{50} < 1 µM) tool compounds, that are useful for exploring the physiological function of a protein in cells. They offer several benefits over traditional approaches such as small interfering RNA (siRNA), small hairpin RNA (shRNA) and related methods. Although these methods are well established, they do not provide information about the role of specific domains of a protein or catalytic versus scaffolding functions of an enzyme. To study these aspects, more intricate and complicated genetic and biochemical experiments need to be conducted, unless a specific inhibitor is available to modulate the function of the protein domain of interest. Chemical probes are therefore valuable tools in the study of human biology and have the further advantage of being useful in the validation of the target protein as a potential drug target. Unfortunately, chemical probes for novel targets and emerging research areas are not often available, and those which are accessible are often inadequately characterized, making the interpretation of the biological results difficult. A notable exception is the relatively new field of epigenetics, where well-characterized chemical probes have been made available since the beginning.[2,3] This has had a major impact on the speed with which the field has developed. The first clinical trials based on these inhibitors are already

Figure 1 | Post-translational modifications of histones and DNA



The fundamental unit of chromatin is the nucleosome, which consists of an octamer of the histone proteins H2A, H2B, H3 and H4 (two of each) tightly bound by DNA. Alterations in chromatin structure by post-translational modifications can regulate gene expression through the formation of heterochromatin or euchromatin, which usually repress or activate gene transcription respectively. Post-translational modifications include DNA methylation and the covalent methylation (Me) and acetylation (Ac) of histone tails. DNA methylation represses transcription by blocking the binding of transcription complexes to the gene promoter. The acetylation of histone tails usually loosens the DNA from around the nucleosomes, increasing the accessibility of gene promoters to transcription complexes, therefore promoting transcription. Alternatively histone tail methylation can repress or promote gene expression, depending on the site and extent of methylation, as well as the presence of other histone modifications in the vicinity. The pattern of these post-translational modifications on a nucleosome determines the transcriptional profile of nearby genes.

ongoing (http://www.cancer.gov/drugdictionary). Here we give a short overview of these epigenetic chemical probes and the emerging biology based on their use by the scientific community.

Impact of Epigenetic Probes

While epigenetic changes include both DNA and histone modifications, in this review we will concentrate on proteins that read, write or erase the histone code (Figure 2). More than 400 domains of these three classes of proteins have been identified and more may yet be discovered (http://apps.thesgc.org/resources/phylogenetic_trees/). While histone deacetylase (HDAC) inhibitors were discovered as early as 1978,[4] other epigenetic proteins and their inhibitors have only recently become the focus of intensive research (Figure 3). Unrestricted access to well-characterized chemical probes has now opened up new areas of biological research and therapeutic possibilities.

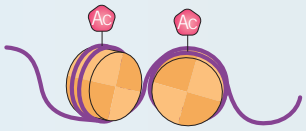
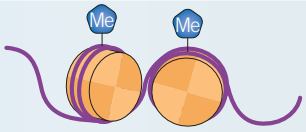
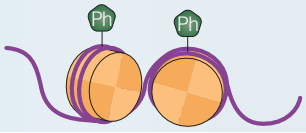
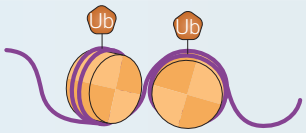
BET Inhibition

The bromo and extra terminal (BET) family belongs to the family of bromodomain (BRD) containing proteins, which read ε-N-acetylated lysine residues on histone as well as non-histone proteins. In humans 61 BRDs, comprising 42 proteins, have been identified. A variety of different proteins contain one or more BRD, including transcriptional co-activators, chromatin modifying enzymes (i.e. histone acetyltransferases and lysine methyltransferases), ATP-dependent chromatin remodeling complexes and adapter proteins.[5] The four BET proteins (BRD2, BRD3, BRD4 and BRDT) are highly conserved, with each containing two conserved N-terminal BRDs and an extra terminal protein interaction motif. Their BRDs recognize acetylated lysine residues in histones H3 and H4. BET proteins regulate the expression of antiapoptotic and growth-related genes, as well as those important for cellular inflammation.[6,7]

Two selective BET inhibitors, the benzo-triazolo-diazepine I-BET and the related thieno-triazolo-diazepine, JQ1 (Figure 4) were simultaneously published in 2010,[8,9] providing promising starting points for the treatment of inflammation and nuclear protein in testis (NUT) midline carcinoma, respectively. NUT midline carcinoma is an aggressive, currently incurable, form of squamous carcinoma, where in-frame chimeric proteins of the tandem N-terminal BRDs of BRD4 or BRD3 are fused with the protein NUT. Treatment of established cell lines with JQ1 led to cell cycle arrest, terminal differentiation and apoptosis. Moreover, using the chemical probe in a patient-derived xenograft model resulted in significant reduction of tumor growth.[9] The I-BET molecule has been tested in a model system of bone marrow-derived macrophages stimulated with bacterial lipopolysaccharide (LPS), where it has been shown to suppress the expression of several pro-inflammatory cytokines and chemokines. Furthermore, I-BET protected treated mice from bacterial LPS-induced endotoxic shock and bacteria-induced sepsis.[8]

More recently, additional BET inhibitors representing alternative chemical scaffolds have become available. PFI 1 (Figure 4) is a dihydroquinazoline-2-one that has been used to study the role of BET proteins in leukemia.[10,11] It has been shown to have antiproliferative effects on leukemic cell lines, efficiently abrogating their clonogenic growth and inducing differentiation of primary leukemic blasts.[10] Studies with PFI 1 also revealed a link between BET proteins and the well-established oncology target Aurora B kinase, downregulating its expression and thereby decreasing phosphorylation of the Aurora substrate histone H3 Ser10.[10] The isoxazole, I-BET 151 (Figure 4), has also

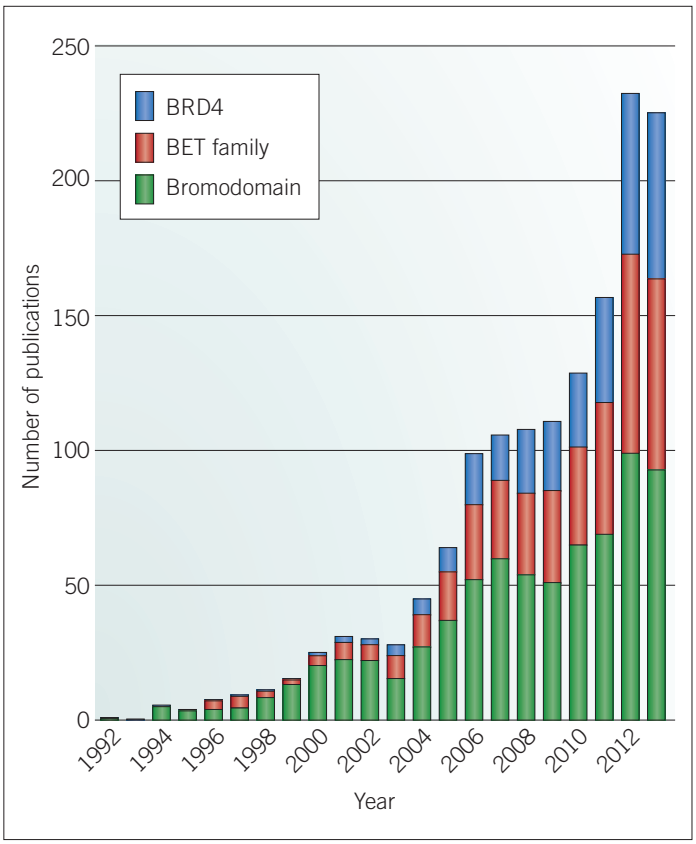
Figure 2 | Readers, writers and erasers of histone modifications

Modification	Reader	Writer	Eraser
	Bromodomains; Tandem PHD domains	Histone acetyltransferases	Histone deacetylases
	Chromodomains; Tudor domains; MBT domains; ZF-CW proteins; Ankyrin repeats; WD40; PWWP	Histone methyltransferases	Lysine demethylases (KDMs); JMJD6 (arginine demethylase)
	14.3.3 proteins; BRCT proteins; Chromoshadow domains	Kinases (RSK2; AMPK; ATM/ATR; Aurora B kinase; JAK2; PKC)	Protein Tyr phosphatases; Protein Ser/Thr phosphatases
	Unknown	Ubiquitin E2 conjugases; Ubiquitin E3 ligases	Deubiquitinating enzymes

Enzymes adding a post-translational modification, such as an acetyl or methyl group, to lysine or arginine residues in the histone tail are referred to as writers; those that remove these epigenetic marks are called erasers. Domains that recognize these modifications and interpret them are called readers. Examples of each class of protein are shown.

Abbreviations: Ac, acetyl; AMPK, adenosine monophosphate-activated kinase; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and RAD3 related; BRCT, breast cancer type 1 susceptibility protein C terminus; JAK2, Janus kinase 2; JMJD6, Jumonji domain-containing 6 protein; MBT, malignant brain tumor; Me, methyl; PHD, plant homeo domain; Ph, phosphate; PKC, protein kinase C; PWWP, Proline-Tryptophan-Tryptophan-Proline; RSK2, ribosomal S6 kinase; Ub, ubiquitin; ZF-CW, zinc finger CW.

Figure 3 | Bromodomain publications



The number of publications per year regarding BRD4, BET family members and all BRD-containing proteins since 1992. Data shown are from manual Google Scholar searches for the keywords, 'BRD4', 'BET family' and 'Bromodomain'. Several BET family inhibitors became available in late 2010, most notably for BRD4; this resulted in a significant increase in the number of publications.

shown significant efficacy against human leukemia cell lines *in vitro* and in a mouse model transplanted with MLL-AF9 leukemia cells, as well as a xenotransplant model of human MLL-AF4 leukemia.[12] Other promising starting points for BET inhibition, such as 3,5-dimethylisoxazole derivatives, 2-thiazolidinones or fragment hits, have also been reported.[13-16]

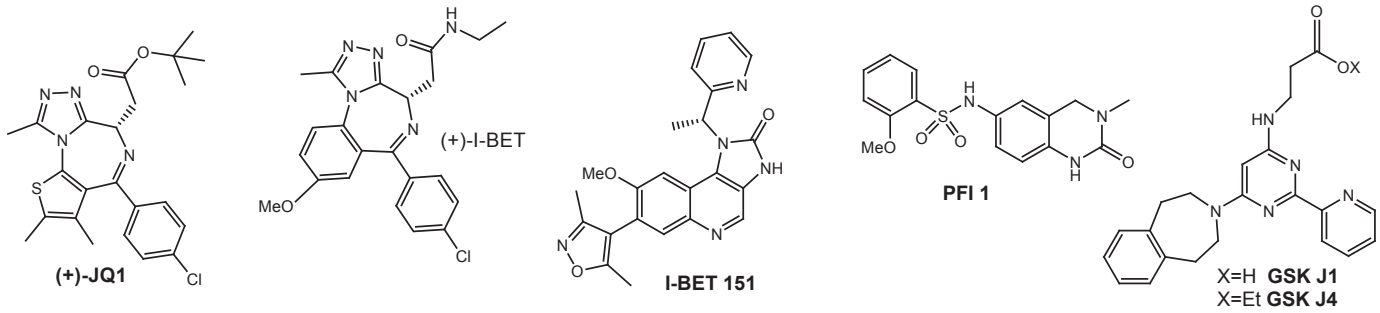
The accessibility of several of these well-characterized chemical probes to the scientific community has led to a plethora of new information on BET family members, and opened up new therapeutic avenues. Besides the already described role of BRD4 in leukemia,[17-19] BET inhibition has also been found to be effective in several solid tumor models including non-small cell lung cancer,[20] prostate cancer[21] and glioblastoma.[22] Although the precise mechanism of inhibitor action has not yet been elucidated in every model, it is clear that the effect of JQ1 and other BET inhibitors on tumor growth can be mediated by c-Myc-dependent as well as c-Myc-independent mechanisms.[22-25] Contrary to studies in several types of c-Myc–driven hematopoietic cancers, where downregulation of c-Myc by JQ1 is the central mechanism mediating transcriptional changes and attenuating cell-cycle progression, additional targets like Bcl-xL and p21$^{CIP1/WAF1}$ are also affected by BET inhibition in the glioblastoma model.[22] In addition, JQ1 also repressed the N-Myc isoform as shown in a model of neuroblastoma.[26]

Following the original discovery of I-BET and the involvement of BET family members in inflammatory diseases, several groups have extended these studies using BET inhibitors. Nicodeme *et al* reported that BET inhibition by I-BET strongly diminished expression of specific pro-inflammatory LPS-inducible genes without affecting general gene transcription.[8] Further studies demonstrated that these effects are at least in part mediated by the direct association of both BRD4 and BRD2 with the promoters of inflammatory cytokine genes in macrophages, which can be abolished by treatment with BET inhibitors.[27] Moreover, data from Belkina *et al* suggested that both BRD2 and BRD4 regulate pro-inflammatory cytokine expression in a chromatin-independent mechanism by directly binding to and activating the NF-κB protein, RelA, through association with acetylated RelA K310.[27] Inhibition of this interaction by a small molecule BET inhibitor prevented association of the BET BRD to RelA and tethering to NF-κB-responsive genes, providing a dual mechanism of negatively regulating inflammatory responses. Furthermore, T cell-mediated inflammation and autoimmunity was also regulated by BET proteins. The BET inhibitor I-BET 762 upregulated expression of anti-inflammatory genes and downregulated several pro-inflammatory cytokines in naïve CD4$^+$ T cells, modulating early T cell differentiation. It also had a positive effect in a mouse model of experimental autoimmune encephalomyelitis, suppressing T cell inflammatory function.[29]

BET inhibition has also paved the way for new therapeutic strategies in other disease states. Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease with a poorly understood pathobiological mechanism. Lung fibroblasts from IPF patients show increased histone H4 lysine 5 acetylation (Ac-H4K5) and an aberrantly high level of BRD4 bound to the gene of the pro-inflammatory cytokine, IL-6. Treatment of cells with JQ1 reverted this phenotype and decreased IL-6 expression. The therapeutic effect of JQ1 in this disease has also been shown in a mouse model of bleomycin-induced lung fibrosis, suggesting that BRD4 inhibitors have the potential as novel therapeutics for IPF and other fibrotic indications.[30]

Overexpression of BRD4 in cardiac hypertrophy has led to a new field of BET research in cardiovascular diseases. Cardiac hypertrophy refers to a thickening of the heart muscle in response to cardiac stress. In a recent study, BRD4 was identified as one of the proteins whose expression is increased during cardiac hypertrophy. In response to hypertrophic signals, BRD4 is recruited to the transcriptional start site of the gene encoding for atrial natriuretic factor (ANF). However, the precise significance of this is still unknown as ANF itself does not promote hypertrophy. Additional target genes need to be identified to establish a more direct link between BRD4 and cardiac hypertrophy.[31]

Figure 4 | Chemical structures of bromodomain and demethylase inhibitors



(Bold text denotes compounds available from Tocris at time of publication)

BRD4 regulation of transcription occurs at the level of transcriptional elongation and is mediated by its interaction with the positive transcription elongation complex (P-TEFb) (Figure 5). Recruitment of P-TEFb, which comprises cyclin dependent kinase 9 (CDK9) and its activator cyclin T, to promoter regions results in phosphorylation of the C-terminal heptad repeat domain of RNA polymerase II and transcriptional elongation.[32] This cellular transcription machinery is also used by viruses, which usurp the proteins necessary for cellular maintenance for their own needs. Epstein-Barr virus (EBV) is a key etiologic agent in the development of numerous cancers. Immortalization of cells is thereby dependent on transcription of the viral C promoter (Cp), to which pTEFb is recruited by BRD4. JQ1 significantly reduced Cp transcript production, indicating inhibition of BRD4 as a potential mechanism for anti-EBV treatment.[33]

In addition to the transcription of viral genes, BRD4 has been implicated in viral replication and integration (Figure 5). In Merkel cell polyomavirus (MCV), BRD4 directly interacts with the large T antigen, a protein critical for viral DNA replication. Treatment of virus-infected cells with JQ1 resulted in an increase in viral replication, presumed to result from an increased availability of BRD4 for assembly of MCV replication complexes, through its release from bound chromatin.[34] The herpes virus (HPV) replication machinery also makes use of BRD4, with BRD4 interacting with the viral factor E2, which is crucial for HPV replication and viral transcription. BRD4, together with other cellular proteins, is recruited to actively replicating HPV16. Like in the case for MCV replication, displacement of BRD4 from acetylated chromatin by JQ1 leads to an increase of freely available BRD4, which enhances HPV16 DNA replication.[35] BRD4 also interacts with retroviral integrases, proteins that catalyze integration of proviral genomes into host DNA. Murine leukemia virus (MLV) frequently integrates near transcription start sites where the integrase directly interacts with BET proteins, stimulating the catalytic activity of the enzyme. Displacement of BRD4 from chromatin by treatment of cells with JQ1 and I-BET released BET proteins from the transcriptional start site, thus decreasing MLV integration.[36]

The crucial role of BRD4 in HIV replication has been known for many years (for review see Zhou *et al*).[37] The subsequent availability of specific BET inhibitors has opened up new avenues for potential HIV treatment. The more recent finding that BRD2 also regulates HIV latency via association with the E2F1 transcription factor, which together with NF-κB, binds to the HIV enhancer and represses HIV transcription, offers the possibility of a dual target in the therapy of HIV.[38]

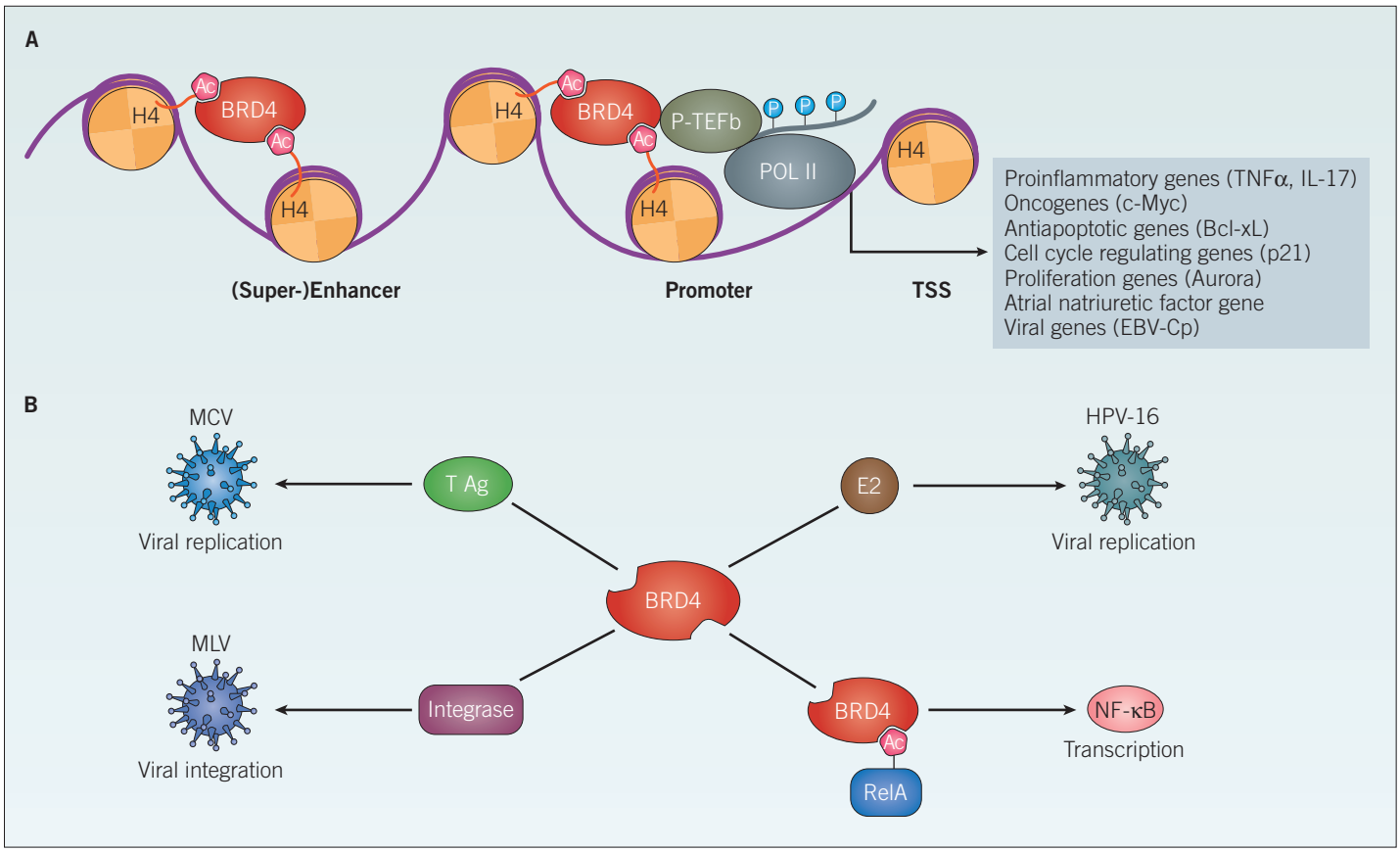
Histone Demethylase Inhibitors

JMJD3

The histone lysine demethylase (KDM) 6B, also called Jumonji Domain Containing 3 (JMJD3), catalyzes the removal of methyl groups from lysine 27 of histone H3 (H3K27me3). JMJD3 plays a crucial role in development, but has also been implicated in several diseases, notably cancer and inflammation.[40] JMJD3 expression is regulated through the NF-κB signaling pathway in LPS-stimulated macrophages,[41] with JMJD3 being associated with more than 7% of LPS-induced gene expression, influencing transcription in both a methylation-dependent and independent manner.[42]

This role of JMJD3 in inflammation was interrogated further by the small molecule inhibitor GSK J1 (Figure 4), which targets both JMJD3 and the related H3K27 demethylase, ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX).[43] This first selective inhibitor of the 2-oxoglutarate dependent histone KDMs contains a pyridyl-pyrimidine biaryl group, which chelates the central metal ion necessary for catalysis. Due to the presence of a highly polar carboxyl group, a prodrug is necessary to use GSK J1 in a cellular setting. Treatment of primary human macrophages with this prodrug, GSK J4, significantly reduced the expression of the majority of LPS-induced cytokines.[43] Furthermore, treatment of macrophages derived from patients with rheumatoid arthritis with GSK J4 resulted in significant reduction of the key pro-inflammatory cytokine TNF-α, opening an interesting avenue of therapeutic intervention by regulation of these epigenetic targets. Given the wide disease implication of JMJD3 in the T cell immune response and systemic lupus erythematosus,[44]

Figure 5 | BRD4 regulation of gene transcription and viral DNA replication



A. BRD4 is recruited to enhancer/super-enhancer regions, as well as the promoter regions of a variety of genes. This regulates the expression of genes involved in a range of biological and disease functions. Figure adapted from Müller *et al.*[39] **B**. BRD4 can also regulate biological function by binding to acetylated RelA to activate NF-κB transcriptional activity, or interacting with factors that are crucial for viral integration and replication. These include murine leukemia virus retroviral integrase, Merkel cell polyomavirus large T antigen and viral factor E2 (which is important for HPV16 replication).

Abbreviations: Ac, acetyl; Bcl-xL, B-cell lymphoma-extra large; BRD4, bromodomain-containing protein 4; E2, viral factor E2; EBV-Cp, Epstein-Barr virus C promoter; H4, histone H4; HPV-16, herpes virus 16; integrase, retroviral integrase; IL-17, interleukin 17; MCV, Merkel cell polyomavirus; MLV, murine leukemia virus; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; P, phosphorylation; P-TEFb, positive transcription elongation factor b; POL II, RNA polymerase II; T Ag, large T antigen; TNF-α, tumor necrosis factor alpha.

as well as its overexpression in breast carcinoma,[45] prostate cancer[46] and renal cell carcinoma,[47] further use of GSK J1 and GSK J4 in these disease areas will result in interesting new applications.

Histone Methyltransferase Inhibitors

Histone methyltransferases (HMTs) catalyze the transfer of up to three methyl groups from the cofactor S-adenosylmethionine (SAM) to the ε-amino group of a lysine residue, or up to two methyl groups to the guanidine group of an arginine residue.[1,48] In the case of arginine methylation, dimethylation can occur in a symmetrical or asymmetric manner. The majority of HMTs contain a SET (Suppressor of variegation 3-9 (Su(var)3-9), Enhancer of Zeste (E(z)), and Trithorax (Trx)) domain. HMTs recognize specific peptide sequences, with the size of the binding pocket controlling the maximum number of methyl groups added to each residue.[49]

G9a/GLP (EHMT2/EHMT1)

The closely-related G9a and GLP proteins are responsible for the mono and dimethylation of H3K9 and K373 of the tumor suppressor p53.[50] G9a is overexpressed in various human cancers and it has been shown that knockdown of G9a inhibits prostate, lung, and leukemia cell growth.[50-53] Inhibition of G9a induced reprogramming of cells into stem cells,[54-56] with G9a recently being shown to mediate Sharp-1 inhibition of skeletal muscle differentiation.[57] The first potent inhibitor of G9a and GLP, BIX 01294 (Figure 6), was published in 2007[58] and its crystal structure with GLP was published in 2009.[59] Optimization of this template by Jin and colleagues resulted in UNC 0638 (Figure 6),[60] which exhibited higher cellular potency and lower cell toxicity than BIX 01294. Recently, UNC 0642 (Figure 6) was disclosed as an *in vivo* inhibitor of G9a and GLP, which is also brain penetrant.[61]

EZH2

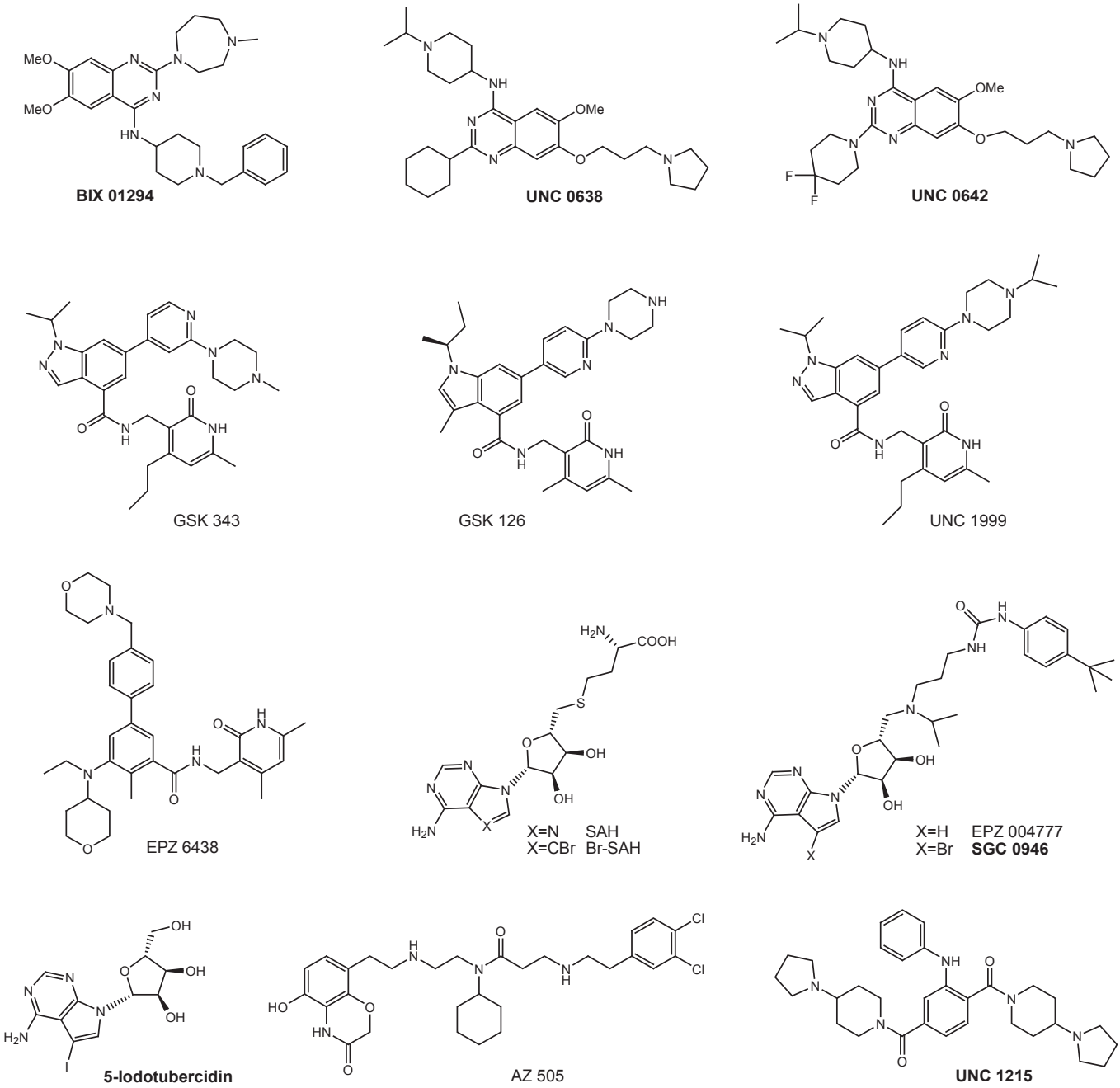
Enhancer of Zeste homolog 2 (EZH2) is a key feature in many oncology programs and is responsible for trimethylation of

H3K27, which is transcriptionally repressive. Mutations of the EZH2 gene at the Tyr641 residue have been identified in several cancers. Mutant EZH2 enzymes exhibit negligible activity in the methylation of unmodified H3K27, whilst being more efficient than wild-type EZH2, at catalyzing di and trimethylation of H3K27me1 and H3K27me2 respectively. Interestingly the expression of the mutant EZH2 gene is heterozygous, leading to an intriguing scenario where the action of both mutant and wild-type proteins gives rise to an oncogenic phenotype.[62] GSK 343, GSK 126, UNC 1999 and EPZ 6438 are potent inhibitors of EZH2 (Figure 6),[63-67] representing a common chemical template with differing selectivities over EZH1.

DOT1L

DOT1L is the only HMT that does not contain a SET domain and more closely resembles protein arginine methyltransferases (PRMTs) in the phylogenetic tree. Although DOT1L is only weakly active on peptide substrates, it is highly active on nucleosomes. Screening of kinase inhibitors in an attempt to discover starting points for chemical optimization resulted in the weak inhibitor 5-Iodotubercidin (Figure 6) which was crystallized with DOT1L. The iodo atom in 5-Iodotubercidin was found to occupy a cleft in DOT1L that is not present in other HMTs, indicating that this molecule might be selective for DOT1L. This halo-substitution was introduced into

Figure 6 | Chemical structures of histone methyltransferase inhibitors and methyllysine binder antagonists



(Bold text denotes compounds available from Tocris at time of publication)

S-adenosylhomocysteine (SAH; Figure 6) and was found to increase the DOT1L inhibitory potency (IC_{50}) from 600 nM to 77 nM.[68] Shortly after this discovery, the potent inhibitor EPZ 004777 (Figure 6) was published,[69] with the subsequent introduction of a bromine into the 5-position of EPZ 004777 yielding SGC 0946 (Figure 6) which, while only slightly more potent *in vitro*, was 10 times more potent in cellular assays measuring reduction of H3K79me2 levels.[70] This increase in cellular potency was surprising and upon further investigation was explained by an increase in cellular permeability and residence time, relative to EPZ 004777, as measured by surface plasmon resonance (SPR). SGC 0946 has been shown to potently kill cord blood cells containing an MLL-AF9 oncogene whereas cells containing an unrelated oncogene (TLS-ERG) were unaffected.[70]

SMYD2

SET and MYND domain-containing protein 2 (SMYD2) methylates K370 of p53 and K860 of pRb, in addition to H3K36. Methylation of these non-histone targets leads to impairment of their tumor-supressing abilities as SMYD2 is overexpressed in many solid tumors. AZ 505 (Figure 6) was discovered in 2011 as a potent peptide-competitive SMYD2 inhibitor, the crystal structure of which has been solved.[71]

Methyllysine Binder Antagonists

The malignant brain tumor (MBT)[72] family of methyllysine binders, whose members bind mono and dimethylated lysines, is part of the methyllysine binding domain family, which consists of approximately 250 members. The methyllysine binding domain family can be subdivided into MBTs, chromodomains, Plant Homeo Domains (PHD), PWWP domains, WD40 domains, and Tudor domains.[73] To date only a single chemical probe for one member of this large family has been identified, the L3MBTL3 inhibitor, UNC 1215 (Figure 6).

L3MBTL3

The MBT family consists of nine members which contain two, three, or four MBT repeats. Unlike BRDs, which require only acetyllysine binding, isolated MBT domains do not bind methyllysines, since multiple MBT domains are required for this interaction. UNC 1215 was reported as the first chemical probe for this family, and shows potent and selective binding to L3MBTL3.[72] Cellular activity of this probe was demonstrated by using a standard fluorescence recovery after photobleaching (FRAP) assay with GFP-labeled protein; however, the recovery time was significantly faster than those observed with FRAP assays for BRDs. The crystal structure of UNC 1215 bound to L3MBTL3 shows an interesting 2:2 UNC 1215:L3MBTL3 stoichiometry in which one arm of UNC 1215 binds to MBT2 of protein A and the other arm of UNC 1215 binds to MBT1 of protein B (and vice versa for the other UNC 1215 molecule). This result is also corroborated by gel filtration studies which show L3MBTL3 as a dimer in the presence of UNC 1215, but a monomer when unliganded. In addition, isothermal titration calorimetry (ITC) data on mutant L3MBTL3 indicated that binding of UNC 1215 was totally ablated in the case of the D381A mutation (MBT2) and partially ablated in the case of the D274A mutation (MBT1). Proteomic studies indicate that BCLAF-1 is an interaction partner and this protein has been shown to co-localize with L3MBTL3. This interaction is ablated in the presence of UNC 1215.

Conclusion

Well-characterized chemical probes which are available to the scientific community have opened up new areas of biology for specific targets and provided the first step for therapeutic intervention in many disease areas. New tools are particularly important for underexplored epigenetic target areas like methyllysine binders and histone KDMs, where few or no chemical inhibitors are available. While the impact of open access chemical tools has been highlighted here for the BRDs, we expect similar effects from the sharing of other chemical probes. As more well-characterized compounds become available, the impact on target de-orphanization and therapeutic potential will become obvious (http://www.thesgc.org/chemical-probes/epigenetics). A key aspect of this open access strategy is maintaining a strong collaborative community that is supported by the development of high quality chemical probes.

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Epigenetic Compounds Available from Tocris

Cat. No.	Product Name	Primary Action
Bromodomains		
4758	Bromosporine	Broad spectrum bromodomain inhibitor
4650	I-BET 151 hydrochloride	BET bromodomain inhibitor
4891	I-CBP 112	Selective CBP/p300 bromodomain inhibitor
4499	(+)-JQ1	Potent, selective BET bromodomain inhibitor; cell permeable
5173	MS 436	Potent and selective BRD4 bromodomain inhibitor
4928	OXF BD 02	Selective BRD4(1) inhibitor
4445	PFI 1	BET bromodomain inhibitor
5072	PFI 3	Potent and selective SMARCA4 and polybromo 1 inhibitor; also inhibits SMARCA2
4889	SGC-CBP30	Potent CBP/p300 bromodomain inhibitor
DNA Methyltransferases		
3842	5-Azacytidine	DNA methyltransferase inhibitor
2624	Decitabine	DNA methyltransferase inhibitor
1745	5-Iodotubercidin	Potent adenosine kinase inhibitor; decreases DNA methylation
4359	Lomeguatrib	MGMT inhibitor
3295	RG 108	Non-nucleoside DNA methyltransferase inhibitor
5155	SGI 1027	DNA methyltransferase inhibitor
4061	6-Thioguanine	Anticancer and immunosuppressive agent; disrupts cytosine methylation
2293	Zebularine	DNA methyltransferase and cytidine deaminase inhibitor
Histone Acetyltransferases		
3084	Anacardic acid	Noncompetitive PCAF/p300 histone acetyltransferase inhibitor
4200	C 646	Selective CBP/p300 histone acetyltransferase inhibitor
5045	L002	p300 inhibitor
4903	NU 9056	Inhibitor of KAT5 (Tip60) histone acetyltransferase
Histone Deacetylases		
4846	Apicidin	Potent histone deacetylase inhibitor
2952	CI 994	Class I histone deacetylase inhibitor; orally active
3515	FK 228	Potent and selective class I histone deacetylase inhibitor; antitumor
4001	KD 5170	Class I and II histone deacetylase inhibitor
4830	LMK 235	Selective histone deacetylase 4/5 (HDAC4/HDAC5) inhibitor
2771	M 344	Histone deacetylase inhibitor
4077	MC 1568	Selectively inhibits histone deacetylase class II (IIa)
3747	NCH 51	Histone deacetylase inhibitor
2521	NSC 3852	Histone deacetylase inhibitor
4643	PCI 34051	Potent and selective histone deacetylase 8 (HDAC8) inhibitor
4403	Pyroxamide	Histone deacetylase inhibitor
4652	SAHA	Class I and II histone deacetylase inhibitor
3810	SBHA	Histone deacetylase inhibitor
2421	Scriptaid	Histone deacetylase inhibitor
3850	Sodium butyrate	Histone deacetylase inhibitor
2682	Sodium 4-Phenylbutyrate	Histone deacetylase inhibitor
4270	TC-H 106	Class I histone deacetylase inhibitor
4805	TCS HDAC6 20b	Selective histone deacetylase 6 (HDAC6) inhibitor
1406	Trichostatin A	Potent histone deacetylase inhibitor
3402	Tubacin	Histone deacetylase 6 (HDAC6) inhibitor; inhibits α-tubulin deacetylation
2815	Valproic acid, sodium salt	Histone deacetylase inhibitor

Cat. No.	Product Name	Primary Action
Histone Demethylases		
4684	Daminozide	Selective histone demethylase KDM2/7 subfamily inhibitor
4593	GSK J1	Potent histone demethylase JMJD3/UTX inhibitor
4688	GSK J2	Inactive isomer of GSK J1 (Cat. No. 4593)
4594	GSK J4	Histone demethylase JMJD3/UTX inhibitor; cell permeable
4689	GSK J5	Inactive isomer of GSK J4 (Cat. No. 4594); cell permeable
4464	IOX 1	Histone demethylase inhibitor; cell permeable
4972	JIB 04	Pan Jumonji histone demethylase inhibitor; active *in vivo*
4977	RN 1 dihydrochloride	LSD1 inhibitor
5089	TC-E 5002	Selective histone demethylase KDM2/7 subfamily inhibitor
3852	Tranylcypromine hydrochloride	Irreversible inhibitor of MAO-A, MAO-B and LSD1
Histone Methyltransferases		
5163	A 366	Potent and selective G9a and GLP histone lysine methyltransferase inhibitor
3364	BIX 01294	G9a-like protein and G9a histone lysine methyltransferase inhibitor
5128	C 21	Selective PRMT1 arginine methyltransferase inhibitor
4504	Chaetocin	Histone methyltransferase SUV39H1 inhibitor
4703	3-Deazaneplanocin A hydrochloride	Histone methyltransferase inhibitor
4892	*(R)*-PFI 2 hydrochloride	Potent and selective SETD7 histone lysine methyltransferase inhibitor
4541	SGC 0946	Highly potent and selective DOT1L inhibitor; cell permeable
5099	TC-E-5003	Selective PRMT1 arginine methyltransferase inhibitor
3861	UNC 0224	Potent G9a histone lysine methyltransferase inhibitor
4343	UNC 0638	Selective G9a and GLP histone lysine methyltransferase inhibitor
5132	UNC 0642	Potent and selective G9a and GLP histone lysine methyltransferase inhibitor
4342	UNC 0646	Potent and selective G9a and GLP inhibitor
MBT Domains		
4666	UNC 1215	Potent inhibitor of L3MBTL3 Kme reader domain; cell permeable
4516	UNC 926 hydrochloride	L3MBTL1 domain inhibitor
RNA Polymerase		
1229	Actinomycin D	Inhibits RNA polymerase
4025	α-Amanitin	Inhibitor of RNA polymerase II
1489	Mithramycin A	Inhibitor of DNA and RNA polymerase
1567	Thiolutin	Bacterial RNA polymerase inhibitor
3253	Triptolide	Inhibits RNAPII-mediated transcription; antitumor, anti-inflammatory and immunosuppressive
Screening Libraries		
5268	Tocriscreen Epigenetics Toolbox	80 Epigenetic modulators supplied pre-dissolved in DMSO (250 µl 10 mM solutions)

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