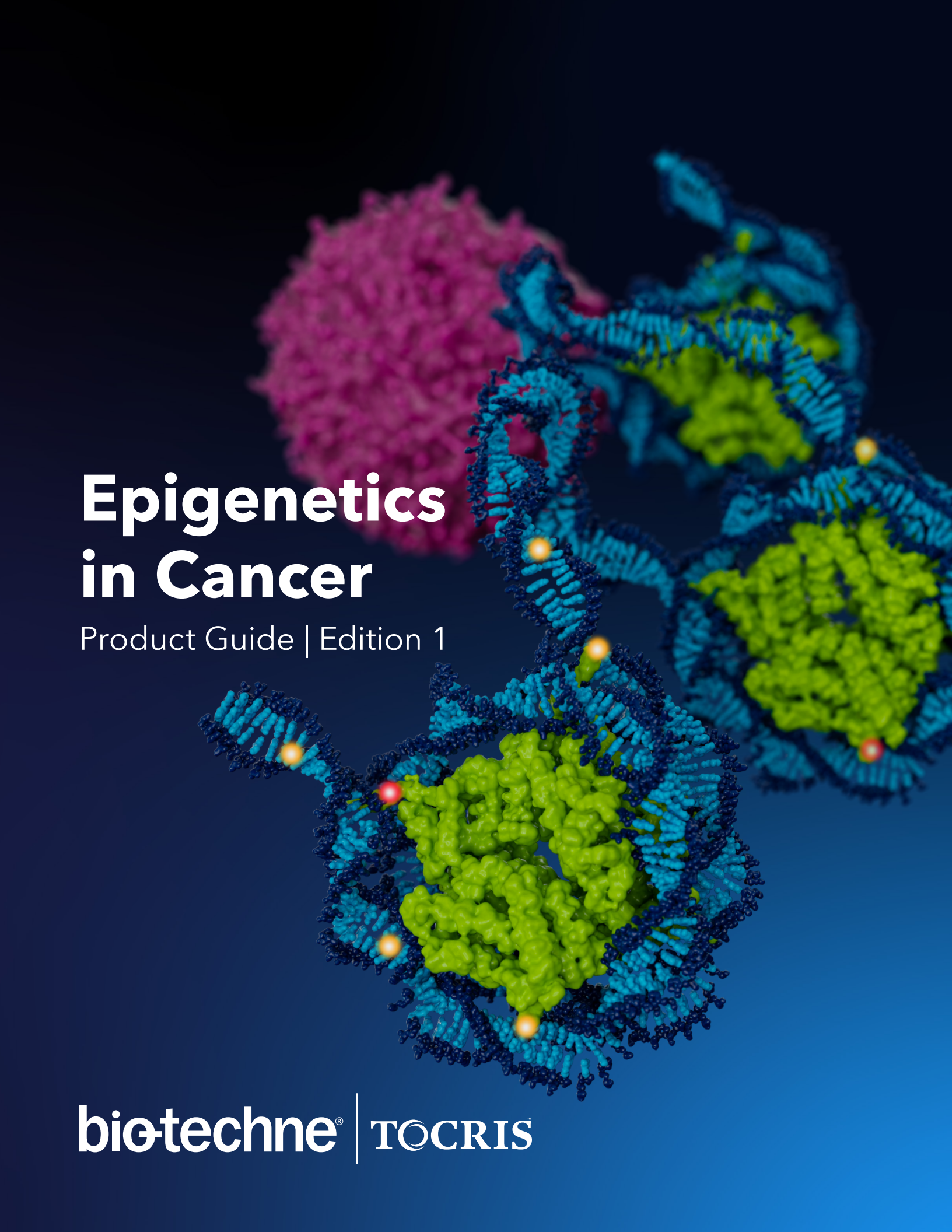


# Epigenetics in Cancer

Product Guide | Edition 1

bio-techne® | TOCRIS™

# Epigenetics in Cancer

Epigenetics can be defined as acquired changes in chromatin structure that arise independently of a change in the underlying DNA sequence. Epigenetic modifications including acetylation and methylation (histone marks) among others, can alter the accessibility of DNA to transcription machinery and therefore influence gene expression (**FIGURE 1**).

The dysregulation of these epigenetic modifications has been shown to result in oncogenesis and cancer progression. For example, the cell cycle, as well as proliferation and metastasis can be regulated by histone modification, DNA methylation and chromatin remodeling. Unlike genetic mutations, epigenetic alterations are reversible and thus make a promising therapeutic target.

Proteins that carry out these epigenetic modifications can be thought of as being either "writers", "readers" or "erasers".

- Epigenetic writers catalyze the addition of epigenetic marks onto either histone tails or the DNA itself.
- Epigenetic reader domains are effector proteins that recognize and are recruited to specific epigenetic marks. "Writer" and "eraser" enzymes may also contain such reader domains, leading to the coordination of "read-write" or "read-erase" mechanisms.
- Epigenetic erasers remove epigenetic marks to alter gene expression.

This guide reviews some of the main areas in cancer epigenetic research, including histone methylation and histone acetylation, ubiquitination, and DNA methylation, among others.

## Histone Methylation

One of the most studied post-translational histone modifications is methylation. Histone methylation is carried out by histone methyltransferases (HMT), which are subdivided according to their target residue: those that methylate the arginine histone tail are known as protein arginine methyltransferases (PRMT), and those that methylate the lysine histone tail are known as lysine methyltransferases (KMT). PRMTs and KMTs regulate both transcriptional activation and repression, as well as DNA repair.

### Histone Methyltransferases (HMT- "writer")

Great efforts have been made to develop inhibitors targeting HMTs including DOT1L, SETD7, EZH2, G9A, and GLP. DOT1L (disruptor of telomeric silencing 1) is an attractive target for drug development. Recent studies have found that aberrant methylation by DOT1L is a fundamental step in the development of mixed-lineage leukemia (MLL)-rearranged leukemia, and preclinical studies have shown that the inhibition of this enzyme increases survival in a mouse model of leukemia. The potent DOT1L inhibitor SGC 0946 (Cat. No. 4541), selectively kills cells transformed with the MLL-AF9 fusion oncogene *in vitro* and lowers levels of MLL target genes HOXA9 and Meis1. The highly potent DOT1L inhibitor EPZ 004777 (Cat. No. 5567) has also been shown to selectively inhibit proliferation and induce apoptosis of MLL-rearranged cells *in vitro*, as well as prolonging survival in a MLL xenograft mouse model. Two other useful research tools for studying MLL are OICR 9429 (Cat. No. 5267) and MM 102 (Cat. No. 5307). OICR 9429 is a high affinity WDR5 antagonist, which disrupts WDR5/MLL interactions and reduces the viability of acute myeloid leukemia cells *in vitro*, as well as disrupting MLL1-RbBP5 interactions. MM 102 is a potent WDR5/MLL interaction inhibitor, which induces HoxA9 and Meis-1 gene expression, two key genes involved in leukemogenesis.

SETD7 (SET domain-containing 7 histone lysine methyltransferases) has a large and diverse number of substrates and has been implicated in multiple cancer pathways. Research suggests that in addition to histone methylation, SETD7 plays an important role in nonhistone methylation of transcription factors and chromatin regulatory complexes, which also leads to changes in gene expression. The exact role of SETD7 is still not fully understood. (R)-PFI 2 (Cat. No. 4892), a potent and selective SETD7 KMT inhibitor, suppresses yes - associated protein (YAP) nuclear translocation and function following activation of the Hippo signaling pathway in breast cancer cells and thus might be a potential therapeutic option for SETD7-mediated cancer development.

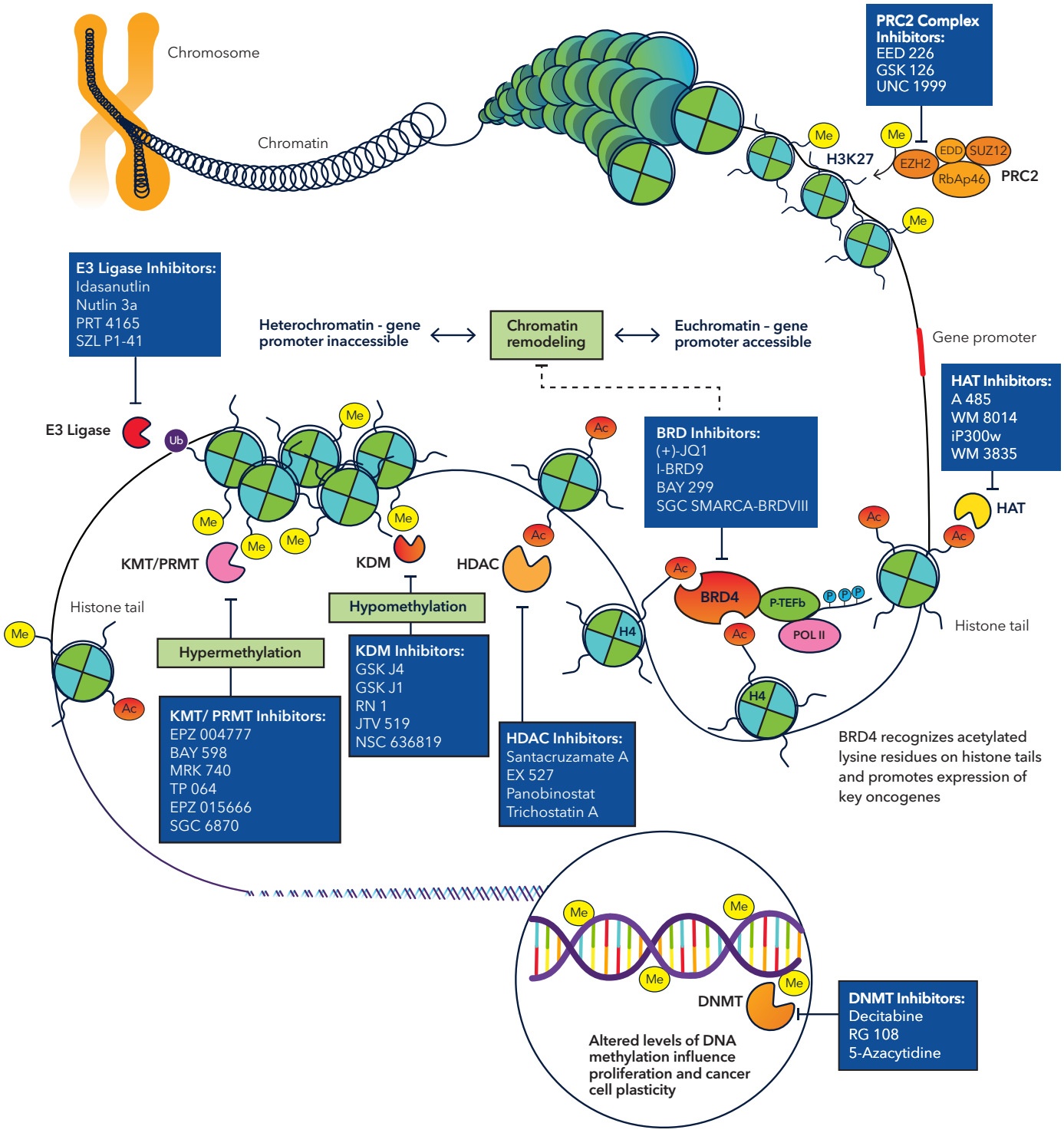


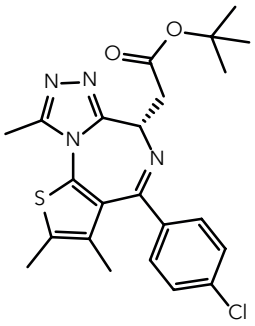

**FIGURE 1: Epigenetic alterations in cancer.** The fundamental unit of chromatin is the nucleosome, which consists of an octamer of the histone proteins H2A, H2B, H3 and H4 (two of each) tightly bound by DNA. Alterations in chromatin structure by post-translational modifications can regulate gene expression 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. Abbreviations: Ac: acetylation, BRD: bromodomains, DNMT: DNA methyltransferases, HAT: histone acetyltransferases, HDAC: histone deacetylases, KDM: histone demethylases, KMT: lysine methyltransferase, Me: methylation, Pol II: RNA polymerase II, PRC2: polycomb repressive complex 2, PRMT: protein arginine methyltransferases, P-TEFb: positive transcription elongation factor b, Ub: ubiquitin.

Enhancer of zeste homolog 2 (EZH2) is an enzymatic catalytic subunit of polycomb repressive complex 2 (PRC2) that can alter downstream target genes expression by trimethylation of Lys-27 in histone 3 (H3K27me3). PRC2 is composed of a core consisting of two methyltransferases EZH1 and EZH2 (enhancer of zeste homolog 1 and 2), the proteins SUZ12, EED and either RBBP4 or RBBP7. Dysregulation of methyltransferase EZH2 is associated with tumor aggressiveness and this protein is upregulated in breast and prostate cancer, as well as lymphoma and glioblastomas. Research into EZH2 as a target for cancer therapy has led to the development of different types of EZH2 inhibitors. The EZH2/EZH1 lysine methyltransferase inhibitor UNC 1999 (Cat. No. 4904), has been shown to inhibit the growth of MLL–rearranged leukemia cells and prolongs survival in a mouse model of leukemia, while GSK 126 (Cat. No. 6790; **BOX 1**) selectively inhibits EZH2 histone methyltransferase activity and inhibits proliferation of B-cell lymphoma (BCL) cell lines *in vitro* and tumor growth in mice bearing BCL xenografts *in vivo*. A selective EDD inhibitor, EDD 226 (Cat. No. 7762; **BOX 1**), regulates histone H3K27 methylation and PRC2 target gene expression *in vitro* and induces tumor regression in a mouse xenograft model.

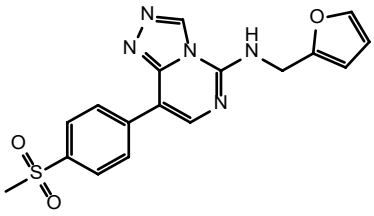
Overexpression of two other methyltransferases G9a and GLP has been found in many different types of cancer and the proteins are involved in the dimethylation and consequent inactivation of the tumor suppressor p53. Several G9a/GLP histone lysine methyltransferase inhibitors, including UNC 0638 (Cat. No. 4343), A 366 (Cat. No. 5163), and UNC 0642 (Cat. No. 5132) have been shown to attenuate dimethylation of histones in cancer cells *in vitro*. UNC 0642 can be used *in vivo* and displays modest brain penetration in mice.

**BOX 1** Epigenetics Products

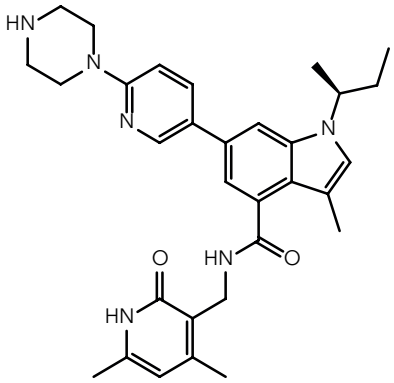
A full list of targets and related products is available on pages 11-15



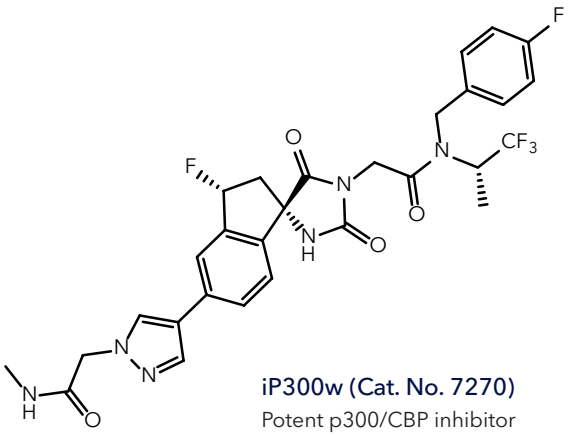
**(+)-JQ1 (Cat. No. 4499)**
Potent and selective BET bromodomain inhibitor; cell permeable



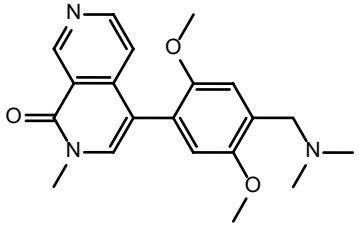
**EED 226 (Cat. No. 7762)**
Potent and selective EED inhibitor



**GSK 126 (Cat. No. 6790)**
Very high affinity and selective EZH2 inhibitor



**iP300w (Cat. No. 7270)**
Potent p300/CBP inhibitor



**BI 9564 (Cat. No. 5590)**
Potent and selective BRD9 and BRD7 inhibitor; orally bioavailable

### Histone Demethylases (HDM- "eraser")

Histone demethylases (HDMs) catalyze the removal of methyl groups from histones and are involved in transcriptional regulation and DNA repair. Recently, the family of lysine (K)-specific demethylase (KDM) proteins, has been shown to be involved in various pathways related to cancer development and a majority of KDMs have been indicated to be oncogenes in both leukemia and solid tumors. Jumonji domain-containing histone-lysine demethylases (Jmj-KDMs) have been found to be upregulated in breast, prostate and colon cancer leading to the interest and development of new inhibitors able to block KDM activity and reduce tumor progression. JIB 04 (Cat. No. 4972) and IOX 1 (Cat. No. 4464) are pan-Jumonji histone demethylase inhibitors. JIB 04 diminishes tumor growth in mouse lung cancer xenograft models and prolongs survival in a mouse model of breast cancer, whereas IOX 1 inhibits JMJD2A-mediated demethylation in cervical cancer cells.

More selective histone demethylase inhibitors include the potent HDM inhibitor GSK J1 (Cat. No. 4593); this compound inhibits the H3K27 histone demethylases JMJD3 (KDM6B) and UTX (KDM6A), as well as KDM5B, KDM5C and KDM5A. GSK J4 (Cat. No. 4594) is the cell permeable ethyl ester derivative of GSK J1. Other histone demethylase inhibitors are: NSC 636819 (Cat. No. 5287) and JQKD 82 (Cat. No. 7676; **BOX 2**). NSC 636819, a KDM4A/KDM4B inhibitor, inhibits histone demethylase activity on H3K9me3 and induces apoptosis in LNCaP prostate cancer cells *in vitro* whereas JQKD 82, a KDM5 selective inhibitor, suppresses multiple myeloma cell growth *in vitro* and in a disseminated tumor mice model.

## Histone Acetylation

Histone acetylation occurs on lysine residues and is predominantly associated with transcriptional activation. Acetylation increases transcription by neutralizing the histone's positive charge, reducing the attraction to the negatively charged DNA, and thus exposing gene promoters on the DNA for transcription. Chromatin conformation is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), which catalyze the addition and removal of acetyl groups, respectively.

### Histone Acetyltransferases (HAT- "writer")

There are three HAT families: Gen5, p300/CREB-binding protein (CBP) and MYST. Studies have found that 51% of cancer cells lines tested have a mutation at p300 and 35% show a mutation in CBP, suggesting that these two genes are important tumor-suppressors. Among selective p300/CBP inhibitors; C 646 (Cat. No. 4200), suppresses histone H3 and H4 acetylation in fibroblast cell lines; A485 (Cat. No. 6387) suppresses proliferation in several hematological malignancies and inhibits tumor growth in a castration-resistant prostate cancer xenograft model; and iP300w (Cat. No. 7270; **BOX 1**) suppresses tumor growth in a mouse CIC-DUX4 sarcoma xenograft model.

Acetyl transferase KAT5 (Tip60) is an interesting target because it plays a key role in chromatin remodeling, which regulates multiple levels of gene transcription and DNA repair. Furthermore, KAT5 acetylation is crucial for the p53-dependent apoptotic pathway. Selective KAT5 inhibitor NU 9056 (Cat. No. 4903), inhibits protein acetylation in prostate cancer cell lines and blocks the DNA damage response. The compound decreases proliferation of LNCaP cells and induces apoptosis via caspase activation.

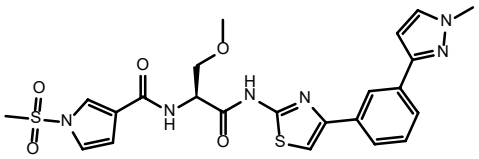
An alternative strategy to reduce histone acetyltransferase is to use the innovative method of selective target protein degradation with small molecule protein Degraders called PROTAC®s. JQAD1 (Cat. No. 7682; **BOX 3**), is a potent and selective histone acetyltransferase EP300 Degrader in neuroblastoma cell lines and causes growth delay of neuroblastoma tumor xenografts.

### Histone Deacetylases (HDAC- "eraser")

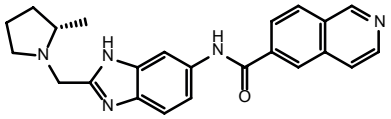
HDACs catalyze acetyl group removal from lysine residues on histones and oncoproteins including p53, YY1 and STAT3. Many cancer cell lines and primary tumors have shown hypoacetylation profiles, in comparison with normal cells. This combined with the observation that HDACs are upregulated in many types of cancers, including prostate colorectal and breast cancer, make HDACs an attractive target. Several HDAC inhibitors have shown promising results, including MS 275 (Cat. No. 6208), FK 228 (Cat. No. 3515), Valproic acid (Cat. No. 2815) and Trichostatin A (Cat. No. 1406), which have shown antiproliferative and antitumor activity *in vivo*. Furthermore, the class I and II HDAC inhibitor SAHA (Cat. No. 4652), induces an accumulation of acetylated histones H2A, H2B, H3 and H4 and suppresses cell growth in a range of cancer cell lines, while inducing apoptosis in cutaneous T cell lymphoma cells *in vitro*. Additional selective inhibitors allow targeting of specific classes of HDACs, including Santacruzamate A (Cat. No. 7191) against HDAC2, Tubastatin A (Cat. No. 6270) and BRD 9757 (Cat. No. 6040) against HDAC6, and RGFP 966 (Cat. No. 6728) against HDAC3.

**BOX 2** Novel Epigenetics Products

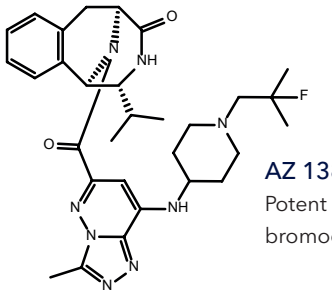
A full list of targets and related products is available on pages 11-15



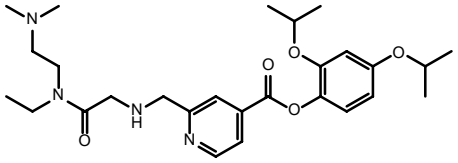
FHT 2344 (Cat. No. 7645)
Potent SMARCA2/4 inhibitor



NVS MLLT-1 (Cat. No. 7482)
Selective MLLT1/3 inhibitor



AZ 13824374 (Cat. No. 7757)
Potent and selective ATAD2 bromodomain inhibitor



JQKD 82 (Cat. No. 7676)
Selective inhibitor of lysine demethylase 5

## Bromodomains (BRDs- "readers")

Bromodomains selectively recognize acetylated lysine residues on histone protein tails. Of particular interest is the BET (bromodomain and extra-terminal) bromodomain family, which comprises the ubiquitously expressed proteins BRD2, BRD3, BRD4; and the testis-specific protein, BRDT. Each of these BET proteins contains two bromodomains, known as bromodomain 1 (BD1) and bromodomain 2 (BD2). BET proteins play a key role at the interface between chromatin remodeling and transcriptional regulation and are integral in the regulation of transcriptional elongation and the cell cycle.

Inhibition of BET downregulates Myc in many malignant hematopoietic cell lines and exhibits therapeutic effects in mouse models of myeloid leukemia. CPI 203 (Cat. No. 5331) is a BET bromodomain inhibitor that downregulates Myc expression, causing G1 cell cycle arrest and attenuating cell proliferation in pancreatic neuroendocrine tumors. It has also been shown to arrest the growth of T cell acute lymphoblastic leukemia cells *in vitro*. Furthermore, CPI 203 enhances the antitumor effect of Rapamycin (Cat. No. 1292). Another BET bromodomain inhibitor, I-BET 762 (Cat. No. 6521), attenuates transcription of oncogenic MYC, suppresses key inflammatory genes, and displays antiproliferative effects *in vitro* and *in vivo*. Some studies have shown that iBET-BD2 (Cat. No. 7458) selectively inhibits the second bromodomains (BD2) of the BET proteins *in vitro* and *in vivo*.

BRD4 influences mitotic progression and is a critical mediator of transcriptional elongation because it binds to transcriptional sites of genes expressed during the M/G1 cell cycle transition. BRD4 increases expression of genes that promote growth by recruiting P-TEFb (positive transcription elongation factor b) to mitotic chromosomes. Furthermore, it has been observed that BRD4 is significantly upregulated in both primary and metastatic melanomas. *In vivo* studies have shown that inhibition of BRD4 impairs tumor growth and metastasis. Key BRD4 inhibitors include the potent, high affinity and selective, archetypical BET bromodomain inhibitor (+)-JQ1 (Cat. No. 4499; **BOX 1**), which induces squamous cell differentiation and arrests tumor growth in BRD4-dependent carcinomas, including tumor growth in midline carcinoma cell xenograft models. CeMMEC1 (Cat. No. 7139), a BRD4 and high affinity TAF1 inhibitor, induces apoptosis and in combination with (+)-JQ1 inhibits proliferation of lung adenocarcinoma cells. I-BET 151 (Cat. No. 4650) potently blocks recruitment of BRD3/4 to chromatin, inducing apoptosis and cell cycle arrest in MLL-fusion leukemia cell lines and improves survival in rodent models of MLL fusion leukemia. Potent and selective BRD4 bromodomain inhibitor, MS 436 (Cat. No. 5173), causes fast acting cytostatic effects and attenuates the proliferation of three melanoma cell lines *in vitro*.

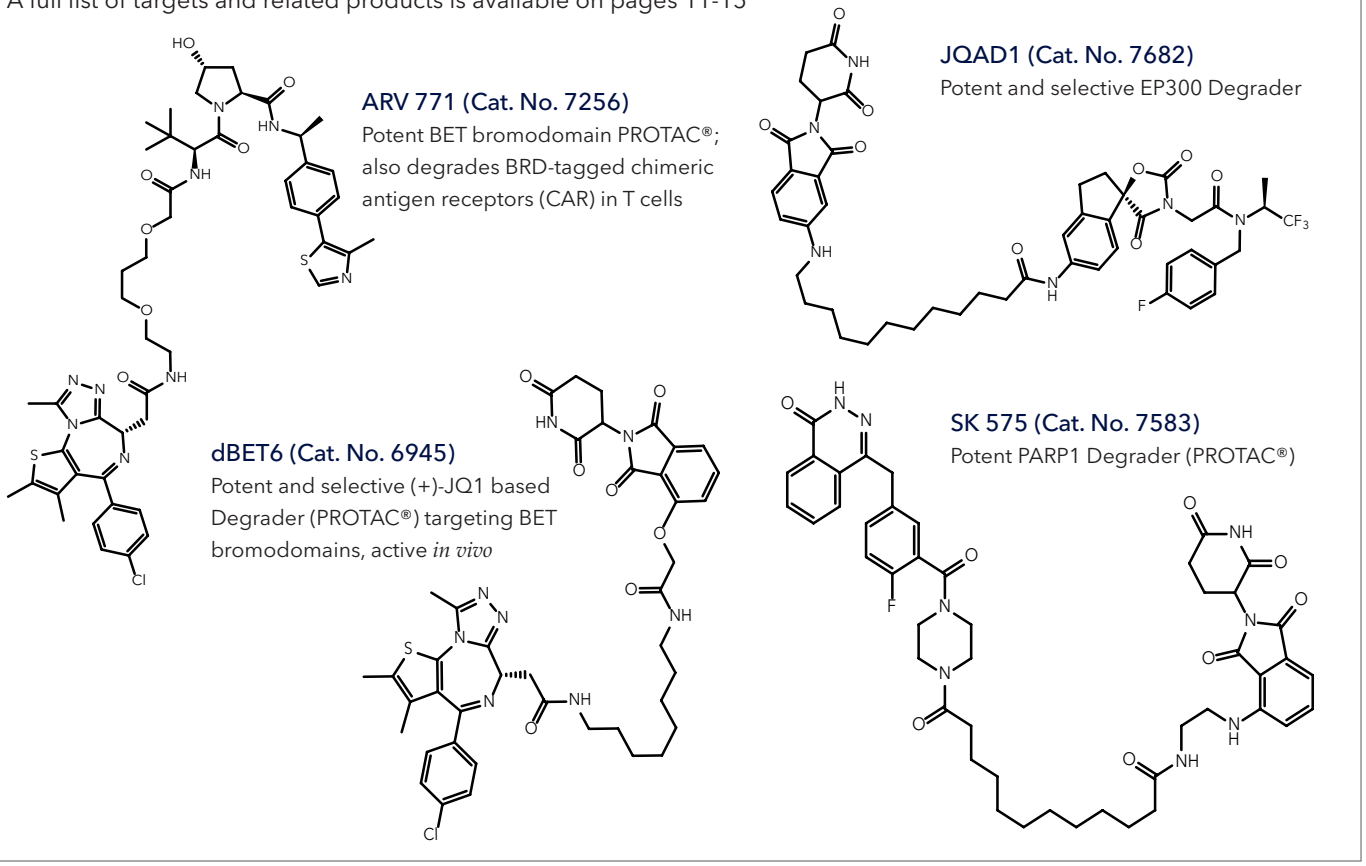
While most BRD research has historically focused on BET domains, the roles of other BRD-containing proteins are starting to generate intense interest. The bromodomain adjacent to zinc finger domain (BAZ) family includes BAZ1A, BAZ1B, BAZ2A, and BAZ2B. BAZ2A, and BAZ2B are involved in chromatin remodeling and the regulation of non-coding RNA. BAZ domains form an interesting research target because BAZ2A has been shown to be involved in prostate cancer growth, and high expression levels of BAZ2B are correlated with B cell acute lymphoblastic leukemia. The selective BAZ2 bromodomain inhibitor BAZ2-ICR (Cat. No. 5266), demonstrates a 15-fold selectivity for the BAZ2 bromodomain over the CERC2 bromodomain and over 100-fold selectivity over a range of other bromodomains. Furthermore, BAZ2-ICR demonstrated a good *in vivo* profile and was suitable for oral administration, making it an ideal tool for investigating the BAZ2 bromodomain.

BRDs are also a key component of mammalian chromatin-remodeling complexes, multiprotein complexes that are fundamental in modulating gene expression. They alter chromatin access by acting on nucleosomes in an ATP-dependent manner. The SWI/SNF complex is particularly relevant in cancer and, depending on the complex, comprises multiple BRD-containing subunits including BRD7, PBRM1 and BRD9. All these targets are amenable to inhibition by recognized chemical probes such as BI 9564 (Cat. No. 5590; **BOX 1**) or TP 472 (Cat. No. 6000) against BRD7/9 and the selective BRD9 inhibitor I-BRD9 (Cat. No. 5591). The presence of SMARCA2/4 is ubiquitous across all forms of the SWI/SNF complex and both subunits show noted mutational rates in many solid tumors including breast, lung and colon cancers. This creates an exploitable vulnerability that is being explored within multiple ongoing clinical trials, with several relevant chemical probes available against SMARCA2/4. For example, BRD can be targeted by SGC SMARCA-BRDVIII (Cat. No. 7460) or PFI 3 (Cat. No. 5072). Newer chemical probes like FHT 1015 (Cat. No. 7644) and FHT 2344 (Cat. No. 7645; **BOX 2**) are potent and highly selective inhibitors of the ATP-domains of SMARCA2/4. They alter key cancer-associated transcriptional programs by directly affecting chromatin accessibility.

Other known "reader" protein domains recognize and bind to acetylated lysine residues, such as plant homeodomain (PHD) fingers that recruit transcription factors and chromatin modification complexes. Proteins that contain bromodomains and PHD fingers (BRPF) form scaffold complexes in the HAT MYST family. The BRPF family includes BRPF1, BRPF 2 and BRPF3. MYST complexes are involved in

**BOX 3** Epigenetics PROTAC® Products

A full list of targets and related products is available on pages 11-15



ARV 771 (Cat. No. 7256)
Potent BET bromodomain PROTAC®; also degrades BRD-tagged chimeric antigen receptors (CAR) in T cells

JQAD1 (Cat. No. 7682)
Potent and selective EP300 Degrader

dBET6 (Cat. No. 6945)
Potent and selective (+)-JQ1 based Degrader (PROTAC®) targeting BET bromodomains, active *in vivo*

SK 575 (Cat. No. 7583)
Potent PARP1 Degrader (PROTAC®)

transcription activation and DNA repair and are often translocated in acute myeloid leukemia (AML). OF 1 (Cat. No. 5289) is a selective BRPF1B/2 inhibitor, which exhibits 39-fold selectivity for BRPF1B and BRPF2 over BRD4. Other key BRPF compounds are the potent and selective BRPF1 inhibitors GSK 6853 (Cat. No. 6198) and GSK 5959 (Cat. No. 5385). GSK 5959 is a cell permeable compound that inhibits BRPF1 interaction with histone H3.3 (a histone associated with reshaping the epigenome and several pediatric cancers). Recently discovered, TP 238 (Cat. No. 6670), a high affinity and selective bromodomain PHD finger transcription factor (BPTF) inhibitor, could be an important therapeutic target in cancer treatment.

MLLT1 is another key player in epigenetics signaling, acting as an acetyl/acyl-lysine dependent epigenetic "reader" domain, and its function occurs via its YEATS (Yaf9, ENL, AF9, Taf14, and Sas5) domain. Recently, mutations in the YEATS domain have been implicated in the development of aggressive cancers. Thus, targeting YEATS protein modules with small molecules has been proposed as a potential chemotherapeutic strategy. NVS MLLT-1 (Cat. No. 7482; **BOX 2**), has been identified as a selective inhibitor of YEATS domain of MLLT1 and MLLT3.

In addition to functional inhibition using small molecules, BRD domains have been successfully targeted for protein degradation using Degrader (PROTAC®) compounds. One of them, MZ 1 (Cat. No. 6154), a cell penetrant Degrader based on (+)-JQ1 (Cat. No. 4499; **BOX 1**), exhibits potent cytotoxicity and antiproliferative effects in acute myeloid leukemia (AML) cell lines. Other Degraders (PROTAC®) VZ 185 (Cat. No. 6936) and dBRD9-A (Cat. No. 6943) show selectivity for BRD7/9 and BRD9 respectively. VZ 185 exhibits cytotoxicity *in vitro* towards cancer cell lines whereas dBRD9-A inhibits growth of synovial sarcoma cells *in vitro* and tumor progression in a synovial sarcoma xenograft mouse model.

## Ubiquitination

Compared to other histone modifications, the functions of histone ubiquitination are less well understood. However, increasing evidence points to an important role for this epigenetic modification in the DNA damage response.

### Histone Ubiquitinating Enzymes

One ubiquitin E3 ligase currently under investigation is Skp2, which promotes ubiquitination and degradation of p27, as well as triggering the ubiquitination of Akt. Skp2 is upregulated in many types of cancer, playing an integral role in apoptosis, cell cycle control, cancer progression and metastasis. In addition, Skp2 has been shown to regulate the self-renewal capability of cancer stem cells. SZL P1-41 (Cat. No. 5076) selectively suppresses Skp2-Skp1, Cullin, F-box (SCF) containing complex E3 ligase activity but exhibits no effect on the activity of other SCF complexes. Furthermore, it inhibits Skp2-mediated p27 and Akt ubiquitination *in vitro* and *in vivo*. This compound suppresses the survival of cancer cells and cancer stem cells by triggering cell senescence and inhibiting glycolysis. It also exhibits antitumor effects in multiple animal models and cancer cell lines.

Another important E3 ubiquitin ligase is MDM2, a negative regulator of the p53 tumor suppressor. MDM2 protein functions both as an E3 ubiquitin ligase that binds p53 and as an inhibitor of p53 transcriptional activation. Increased levels of MDM2 have been found in several human tumor types, including soft tissue sarcomas and osteosarcomas as well as

breast tumors. Nutlin-3 (Cat. No. 3984) and Nutlin 3a (Cat. No. 6075) are MDM2 antagonists, that inhibit the MDM2-p53 interaction and activates p53. Nutlin-3 induces apoptosis in cancer cells whereas Nutlin 3a suppresses tumor growth *in vivo* in prostate cancer and osteosarcoma cancer models.

Polycomb-repressive complex 1 (PRC1)-mediated histone ubiquitination plays an important role in aberrant gene silencing in human cancers and is a potential target for cancer therapy. PRT 4165 (Cat. No. 5047) is a potent inhibitor of PRC1-mediated H2A ubiquitylation *in vivo* and *in vitro*.

The SCF family of ubiquitin ligases is involved in transcription and cell cycle control (specifically the control of G1/G2 transition). They catalyze the ubiquitination of proteins which then undergo proteasomal degradation. SMER 3 (Cat. No. 4375) is a selective inhibitor of a yeast SCF family E3 ubiquitin ligase (SCFMet30), and studies have demonstrated its ability to block cell proliferation *in vitro* and *in vivo*, as well as enhancing the inhibition of mTOR by Rapamycin (Cat. No. 1292).

### Histone Deubiquitinating Enzymes

The removal of ubiquitin groups from histone lysine residues is catalyzed by proteases known as deubiquitinating enzymes (DUBs). Ubiquitin-specific protease (USP) and JAMM (JAB1/MPN/MOV34) family members have been shown to regulate transcription, DNA repair, gene expression and cell cycle progression. USP7 is a master regulator of p53-mediated apoptosis, modulating the effects of KAT5, Mdm2 and p53. USP7 deubiquitinates and stabilizes KAT5 and Mdm2; Mdm2 then ubiquitinates p53, which leads to its destruction, therefore maintaining normal p53 levels. The USP7 inhibitor P 22077 (Cat. No. 4485) destabilizes KAT5 and suppresses the p53-dependent apoptotic pathway. It also inhibits USP47 and HDM2.

Beclin 1 and p53 are two important tumor suppressors, which are frequently mutated in cancer. Beclin 1 regulates the activities of USP10 and USP13, which in turn regulate p53 levels. The autophagy inhibitor Spautin 1 (Cat. No. 5197), inhibits USP10 and USP13 activity and selectively promotes apoptosis of cancer cells under starvation conditions. This compound also promotes the degradation of the Beclin 1/Vps34 complex.

## Other Histone Modifications

Other enzymes involved in histone posttranslational modification include kinases, phosphatases, and proteases, see the products relating to these categories in the Tocris products list.

## DNA Methylation

DNA methyltransferases (DNMTs) are a family of enzymes that catalyze the transfer of a methyl group from S-adenosyl methionine (SAM) to the target DNA. DNA methylation usually occurs on the 5′ position of the cytosine (5mC) ring within CpG dinucleotides. Widespread DNA hypomethylation and hypermethylation have been observed at CpG islands and short CpG-rich DNA regions in gene promoters and are thought to promote tumorigenesis. Key research compounds for studying DNA methylation include a synthetic analog of cytidine, Zebularine (Cat. No. 2293), an orally active DNA methyltransferase inhibitor, which attenuates tumor cell proliferation and reactivates silenced genes in bladder carcinoma cells. Decitabine (Cat. No. 2624) is a cytosine analog that is incorporated into DNA and acts as a suicide substrate for DNA methyltransferase,

resulting in DNA hypomethylation and activation of silent genes. This compound is a widely used chemotherapeutic agent that suppresses growth of human tumor cell lines and has received the US Food and Drug Administration (FDA) approval for the treatment of acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), and myelodysplastic syndrome (MDS). Another commonly used anticancer and immunosuppressive agent is 6-Thioguanine (Cat. No. 4061); this compound disrupts cytosine methylation by DNA methyltransferases after incorporation into DNA and displays cytotoxic and antineoplastic properties.

## Poly (ADP-ribose) Polymerase (PARP)

Poly (ADP-ribose) polymerases (PARPs) are important components of DNA damage/repair pathways, playing key roles in genomic stability and tumor cell survival. The PARP family consists of seventeen enzyme categories able to catalyze the post-translational modification of proteins by the addition of multiple ADP-ribose moieties. PARP transfers ADP-ribose from nicotinamide dinucleotide (NAD) to Glu/Asp residues on the substrate protein. Poly-ADP-ribosylation of histone proteins is emerging as an important epigenetic regulatory mechanism and as a result, PARPs have been an attractive target for anti-cancer therapies resulting in the successful development of several PARP inhibitors for various cancers.

Many inhibitors in preclinical and clinical trials target PARP1 and PARP2. Among them, Olaparib (Cat. No. 7579) and Rucaparib (Cat. No. 6230) have been approved by the US food and drug administration (FDA) for the treatment of breast cancer and ovarian cancer. PARP inhibitor AZD 2461 (Cat. No. 6060), a novel small molecule structurally analogous to Olaparib, exhibits anticancer effects in BRCA1 mutant, but not wild-type breast cancer cell lines *in vitro*. In addition, another member of PARP family, PARP14, has been gradually emerging as a promising anti-cancer drug target. PARP14 selective inhibitors, including GeA-69 (Cat. No. 6795) and H10 (Cat. No. 6228), have shown apoptosis activity in cancer cells *in vitro*.

An alternative strategy to target specific degradation of PARP is to use PROTAC® molecules, such as SK 575 (Cat. No. 7583; **BOX 3**), a potent PARP1 Degrader (PROTAC®). It comprises the PARP1/2 inhibitor Olaparib (Cat. No. 7579), joined by a linker to cereblon/cullin 4A ligand Thalidomide (Cat. No. 0652). This enables SK 575 to drive the selective ubiquitination of PARP1, leading to its subsequent degradation by the endogenous proteasome system. Using this method, SK 575 effectively reduces PARP1 protein levels in mouse SW620 tumor xenograft models.

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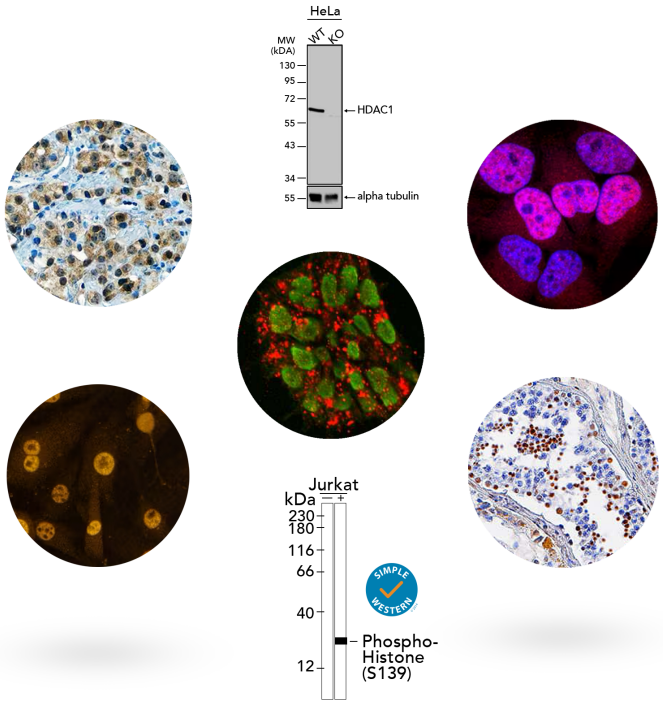

# List of Products

| Target Protein | Product Name | Catalog # | Action |
|---|---|---|---|
| **Bromodomains (BRDs)** | | | |
| Inhibitors | AZ 13824374 | 7757 | Potent and selective ATAD2 bromodomain inhibitor |
| | BAY 299 | 5970 | Potent and selective BRD1 and TAF1 inhibitor |
| | BAZ2-ICR | 5266 | Selective BAZ2 inhibitor |
| | BI 9564 | 5590 | Potent and selective BRD9 and BRD7 inhibitor; orally bioavailable |
| | CeMMEC1 | 7139 | TAF1 inhibitor |
| | CPI 203 | 5331 | BET bromodomain inhibitor; arrests cell cycle at G1 phase |
| | FHT 1015 | 7644 | Potent and selective SMARCA4/2 inhibitor |
| | FHT 2344 | 7645 | Potent SMARCA2/4 inhibitor |
| | GSK 5959 | 5385 | Potent and selective BRPF1 inhibitor |
| | GSK 6853 | 6198 | Potent and selective BRPF1 inhibitor |
| | I-BET 151 | 4650 | BET bromodomain inhibitor; also promotes differentiation of hiPSCs into megakaryocytes |
| | I-BET 762 | 6521 | Potent and high affinity BET bromodomain inhibitor; anti-inflammatory; orally bioavailable |
| | iBET-BD2 | 7458 | Potent and selective pan-BD2 inhibitor; orally bioavailable |
| | I-BRD9 | 5591 | Potent and selective BRD9 inhibitor |
| | (+)-JQ1 | 4499 | Potent and selective BET bromodomain inhibitor; cell permeable |
| | MS 436 | 5173 | Potent and selective BRD4(1) inhibitor |
| | NVS MLLT-1 | 7482 | Selective MLLT1/3 inhibitor |
| | OF 1 | 5289 | Selective BRPF1B and BRPF2 inhibitor |
| | PFI 3 | 5072 | Potent and selective SMARCA2/4 and polybromo 1 inhibitor |
| | Rapamycin | 1292 | mTOR inhibitor; immunosuppressant |
| | SGC SMARCA-BRDVIII | 7460 | Potent and selective SMARCA2/4 and PB1(bromo 5)-selective SWI/SNF bromodomain inhibitor |
| | TP 238 | 6670 | CECR2 and BPTF/FALZ inhibitor |
| | TP 472 | 6000 | Potent BRD9/7 inhibitor |
| **Deubiquitination** | | | |
| Inhibitors | P 22077 | 4485 | USP7 inhibitor |
| | Spautin 1 | 5197 | USP10 and USP13 inhibitor; inhibits autophagy |

| Target Protein | Product Name | Catalog # | Action |
|---|---|---|---|
| **DNA Methyltransferases (DNMTs)** | | | |
| Inhibitors | 5-Azacytidine | 3842 | DNA methyltransferase inhibitor |
| | Decitabine | 2624 | DNA methyltransferase inhibitor |
| | EGCG | 4524 | β-secretase (BACE) inhibitor; inhibits amyloid assembly |
| | Fisetin | 5016 | Naturally occurring flavonoid and antioxidant; neuroprotective |
| | Lomeguatrib | 4359 | MGMT inhibitor |
| | RG 108 | 3295 | Non-nucleoside DNA methyltransferase inhibitor |
| | SGI 1027 | 5155 | DNA methyltransferase inhibitor |
| | Zebularine | 2293 | DNA methyltransferase and cytidine deaminase inhibitor |
| Other | 6-Thioguanine | 4061 | Anticancer and immunosuppressive agent |
| **Histone Acetyltransferases (HATs)** | | | |
| Inhibitors | A485 | 6387 | Potent and selective p300/CBP inhibitor; orally bioavailable |
| | C 646 | 4200 | Selective p300/CBP inhibitor |
| | iP300w | 7270 | Potent p300/CBP inhibitor |
| | NU 9056 | 4903 | Inhibitor of KAT5 (Tip60) |
| | WM 8014 | 6693 | Potent and selective KAT6A and KAT6B inhibitor |
| | WM 3835 | 7366 | Lysine acetyltransferase HBO1 (KAT7) inhibitor |
| **Histone Deacetylases (HDACs)** | | | |
| Inhibitors | BRD 9757 | 6040 | Potent and selective HDAC6 inhibitor |
| | CI 994 | 2952 | Class I histone deacetylase inhibitor; orally bioavailable |
| | EX 527 | 2780 | Selective SIRT1 inhibitor |
| | FK 228 | 3515 | Potent and selective class I histone deacetylase inhibitor; antitumor |
| | MC 1568 | 4077 | Selective HDAC class IIa inhibitor |
| | MS 275 | 6208 | HDAC (Class I) inhibitor |
| | Panobinostat | 7629 | Pan-histone deacetylase inhibitor |
| | PCI 34051 | 4643 | Potent and selective HDAC8 inhibitor |
| | RGFP 966 | 6728 | Potent and selective HDAC3 inhibitor |
| | SAHA | 4652 | Class I and II HDAC inhibitor |
| | Santacruzamate A | 7191 | Highly potent and selective HDAC2 inhibitor |

| Target Protein | Product Name | Catalog # | Action |
|---|---|---|---|
| | Sodium butyrate | 3850 | Histone deacetylase inhibitor |
| | Sodium 4-Phenylbutyr-ate | 2682 | Histone deacetylase inhibitor |
| | Trichostatin A | 1406 | Potent histone deacetylase inhibitor |
| | Tubacin | 3402 | HDAC6 inhibitor; inhibits α-tubulin deacetylation |
| | Tubastatin A | 6270 | Potent HDAC6 inhibitor |
| | Valproic acid | 2815 | Histone deacetylase inhibitor |
| **Histone Demethylases (KDMs)** | | | |
| Inhibitors | GSK J1 | 4593 | Potent JMJD3/UTX inhibitor |
| | GSK J4 | 4594 | Histone KDM inhibitor; cell permeable |
| | IOX 1 | 4464 | Histone demethylase inhibitor; cell permeable |
| | JIB 04 | 4972 | Pan Jumonji inhibitor; active *in vivo* |
| | JQKD 82 | 7676 | Selective inhibitor of lysine demethylase 5 |
| | JTV 519 | 4564 | Ryanodine receptor (RyR) inhibitor |
| | NSC 636819 | 5287 | KDM4A/KDM4B inhibitor |
| | RN 1 | 4977 | LSD1 inhibitor |
| | TC-E 5002 | 5089 | Selective KDM2/7 inhibitor |
| Other | GSK J2 | 4688 | Inactive control of GSK J1 (Cat. No. 4593) |
| **Lysine Methyltransferases (KMTs)** | | | |
| Inhibitors | A 366 | 5163 | Potent and selective G9a/GLP inhibitor |
| | BAY 598 | 5991 | Potent and selective SMYD2 inhibitor |
| | Chaetocin | 4504 | SUV39H1 inhibitor |
| | EPZ 004777 | 5567 | Highly potent DOT1L inhibitor |
| | MM 102 | 5307 | Potent WDR5/MLL interaction inhibitor |
| | MRK 740 | 6803 | Potent PRDM9 inhibitor |
| | (*R*)-PFI 2 | 4892 | Potent and selective SETD7 inhibitor |
| | SGC 0946 | 4541 | Highly potent and selective DOT1L inhibitor; cell permeable |
| | UNC 0224 | 3861 | Potent G9a and GLP inhibitor |

| Target Protein | Product Name | Catalog # | Action |
|---|---|---|---|
| Inhibitors | UNC 0638 | 4343 | Selective G9a and GLP inhibitor |
| | UNC 0642 | 5132 | Potent and selective G9a and GLP inhibitor |
| | UNC 1999 | 4904 | Potent and selective EZH2/EZH1 inhibitor |
| Other | OICR 9429 | 5267 | High affinity and selective WDR5 antagonist |
| | (*S*)-PFI 2 | 5400 | Negative control of (*R*)-PFI 2 hydrochloride (Cat. No. 4892) |
| | WDR5 0103 | 5323 | WDR5 antagonist |
| **Poly (ADP-ribose) Polymerases (PARPs)** | | | |
| Inhibitors | AZD 2461 | 6060 | Potent PARP inhibitor; orally bioavailable |
| | EB 47 | 4140 | Potent PARP-1 inhibitor |
| | GeA-69 | 6795 | Selective allosteric PARP14 inhibitor |
| | H10 | 6228 | PARP14 inhibitor; cell-permeable |
| | JW 55 | 4514 | Tankyrase inhibitor |
| | Nicotinamide | 4106 | PARP-1 inhibitor |
| | Olaparib | 7579 | Potent PARP inhibitor |
| | PJ 34 | 3255 | Potent PARP inhibitor |
| | Rucaparib camsylate | 6230 | PARP inhibitor |
| | Thalidomide | 0652 | Binds cereblon; also TNF-α synthesis inhibitor |
| | XAV 939 | 3748 | Potent tankyrase inhibitor |
| | WIKI4 | 4855 | Potent tankyrase inhibitor |
| **Polycomb Repressor Complex** | | | |
| Inhibitors | A 366 | 5163 | Potent and selective G9a/GLP inhibitor |
| | EED 226 | 7762 | Potent and selective EED inhibitor |
| | GSK 126 | 6790 | Very high affinity and selective EZH2 inhibitor |
| | UNC 0638 | 4343 | Selective G9a and GLP inhibitor |
| | UNC 0642 | 5132 | Potent and selective G9a and GLP inhibitor |
| | UNC 1999 | 4904 | Potent and selective EZH2/EZH1 inhibitor |
| **PROTAC® Degraders** | | | |
| | dBRD9-A | 6943 | Potent BRD9 Degrader (PROTAC®) |
| | JQAD1 | 7682 | Potent and selective EP300 Degrader (PROTAC®) |

| Target Protein | Product Name | Catalog # | Action |
|---|---|---|---|
| **PROTAC® Degraders** | | | |
| | MZ 1 | 6154 | (+)-JQ1 based Degrader (PROTAC®) that selectively degrades BRD4 |
| | SK 575 | 7583 | Potent PARP1 Degrader (PROTAC®) |
| | VZ 185 | 6936 | Potent and selective BRD7/9 Degrader (PROTAC®) |
| **Protein Arginine Methyltransferases (PRMTs)** | | | |
| Inhibitors | C 21 | 5128 | Selective PRMT1 inhibitor |
| | EPZ 015666 | 6516 | Potent and selective PRMT5 inhibitor |
| | SGC 6870 | 7182 | Potent and selective PRMT6 allosteric inhibitor |
| | TP 064 | 6008 | Potent and selective PRMT4 inhibitor |
| **Protein Ser/Thr Phosphatases** | | | |
| Inhibitors | GSK 2830371 | 5140 | Potent and selective allosteric inhibitor of Wip1 phosphatase |
| | Okadaic acid | 1136 | Protein phosphatase 1 and 2A inhibitor |
| | Sanguinarine chloride | 2302 | Inhibitor of protein phosphatase 2C (PP2C) |
| **Protein Tyrosine Phosphatases** | | | |
| Inhibitors | Alexidine dihydrochloride | 3979 | Selective inhibitor of PTPMT1 |
| | Sodium orthovanadate | 2821 | Protein tyrosine phosphatase inhibitor |
| **RNA/DNA Polymerase** | | | |
| Inhibitors | Mithramycin A | 1489 | Inhibitor of DNA and RNA polymerase |
| Other | Triptolide | 3253 | Inhibits RNAPII-mediated transcription; antitumor, anti-inflammatory and immunosuppressive |
| **Ubiquitination** | | | |
| Inhibitors | Idasanutlin | 6904 | Potent MDM2 inhibitor; inhibits MDM2-p53 interaction |
| | PRT 4165 | 5047 | Inhibitor of Bmi1/Ring1A; blocks histone H2A ubiquitination |
| | Rapamycin | 1292 | mTOR inhibitor; immunosuppressant |
| | SMER 3 | 4375 | Selective inhibitor of E3 ubiquitin ligase |
| | SZL P1-41 | 5076 | Selective Skp2 inhibitor; suppresses E3 ligase activity |
| Other | Nutlin-3 | 3984 | MDM2 antagonist; inhibits MDM2-p53 interaction |
| | Nutlin 3a | 6075 | MDM2 antagonist; active enantiomer of Nutlin-3 (Cat. No. 3984) |

# A Selection of Related Products Available from our Sister Brands

| Product Name | Catalog # | Species |
|---|---|---|
| BMI-1 Antibody | MAB33341 | Human |
| CBP Antibody | MAB2676 | Human/Mouse/Rat |
| DNMT1 Antibody | AF6110 | Human |
| DNMT3A Antibody | MAB6315 | Human |
| EED Antibody | AF5827 | Human/Mouse |
| EZH2 Antibody | AF4767 | Human/Mouse |
| Histone Deacetylase 2/ HDAC2 Antibody | MAB7679 | Human/Rat |
| Histone Deacetylase 4/ HDAC4 Antibody | MAB6205 | Human |
| Jumonji/JARID2 Antibody | AF6090 | Human/Mouse |
| G9a/EHMT2 Antibody | PP-A8620A-00 | Human/Mouse |
| Lysine (K)-specific Demethylase 3A/ KDM3A Antibody | AF6746 | Human |
| Lysine (K)-specific Demethylase 4A/ KDM4A Antibody | AF6434 | Human/Mouse |
| Lysine (K)-specific Demethylase 4C/ KDM4C Antibody | AF6430 | Human |
| Lysine (K)-specific Demethylase 6B/ KDM6B Antibody | AF7300 | Human |
| PARP Antibody | MAB8095 | Human |
| PARP Antibody | AF-600-NA | Human/Mouse |
| PRMT1 Antibody | AF6016 | Human/Mouse |
| WDR5 Antibody | AF5810 | Human/Mouse |

## Further Reading


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**Ramaiah** *et al* (2021) Epigenetic modulation and understanding of HDAC inhibitors in cancer therapy. *Life Sci*. **277** 119504

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**Schwalm and Knapp** (2022) BET bromodomain inhibitors. *Curr. Opin.Chem.Biol*. **68** 102148

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**Yu** *et al* (2012) Catalytic site remodelling of the DOT1L methyltransferase by selective inhibitors. *Nat.Commun*. **3** 1288

# List of Acronyms

| Acronym | Definition |
|---|---|
| AML | Acute myeloid leukemia |
| BAZ | Bromodomain adjacent to zinc finger domain |
| BET | Bromodomain and extra-terminal bromodomain |
| BPTF | Bromodomain PHD finger transcription factor |
| BRD | Bromodomains |
| BRPF | Bromodomain and PHD finger containing |
| CBP | CREB-binding protein |
| DOT1L | Disruptor of telomeric silencing 1-like |
| DNMT | DNA methyltransferases |
| DUB | Deubiquitinating enzymes |
| EED | Embryonic ectoderm development |
| EZH | Enhancer of zeste homolog |
| GLP | Glucagon-like peptide |
| HAT | Histone acetyltransferases |
| HDAC | Histone deacetylases |
| HDM | Histone demethylases |
| HMT | Histone methyltransferases |
| KMT | Lysine methyltransferases |
| JAMM | JAB1/MPN/MOV34 |
| MDM2 | Mouse double minute 2 |
| MLL | Mixed-lineage leukemia |
| NAD | Nicotinamide adenine dinucleotide |
| PARP | Poly (ADP-ribose) polymerases |
| PHD | Plant homeodomain |
| PRC | Polycomb repressive complexes |
| PRMT | Protein arginine methyltransferases |
| PROTAC | Proteolysis-targeting chimera |
| SAM | S-adenosyl methionine |
| SETD7 | SET domain containing lysine methyltransferase 7 |
| USP | Ubiquitin-specific protease |
| YAP | Yes associated protein |

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