RESEARCH BULLETIN

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Epigenetics

Introduction

Epigenetics is the study of acquired changes in chromatin structure that arise independently of a change in the underlying DNA nucleotide sequence. Epigenetic modifications – including acetylation, methylation, phosphorylation and ubiquitination, amongst others – alter the accessibility of DNA to transcription machinery and therefore influence gene expression. Epigenetic mechanisms integrate environmental changes at the cellular level, thereby enabling cellular plasticity. As a result, they have been implicated in a number of diseases including cancer, inflammation and metabolic disorders. Proteins that carry out and interpret these epigenetic modifications can be classified as either writers, readers or erasers (Table 1).

Epigenetic modifications occur most commonly on either the DNA itself, or on the histone octamer around which DNA is coiled. The complex of DNA and the histone octamer is known as 'chromatin', and the basic repeating unit of chromatin is termed the 'nucleosome'. Although DNA must be tightly compacted in order to fit into the nucleus, it must also be temporarily accessible during DNA transcription. Therefore, mechanisms of chromatin uncoiling and recoiling must exist to facilitate this. The addition and removal of specific chemical groups, which are known as 'epigenetic marks', is thought to regulate chromatin architecture and therefore influence gene expression.

Tocris provides a wide range of pharmacological tools for studying such epigenetic targets, a selection of which are highlighted within each section. For a full product listing please visit www.tocris.com/epigenetics.

Table 1 | Epigenetic machinery

Epigenetic Mechanism	Epigenetic Writers	Epigenetic Readers	Epigenetic Erasers
DNA Methylation	DNA Methyltransferases	Methyl-CpG Binding Domains	Active DNA Demethylation (TETs, TDG) Passive DNA Demethylation
Histone Acetylation	Histone Acetyltransferases	Bromodomains Tandem PHD Fingers Pleckstrin Homology Domains	Histone Deacetylases
Histone Arginine Methylation	Protein Arginine Methyltransferases (PRMTs)	Tudor Domains WD40 Domains	Histone Arginine Demethylases (JMJD6) Peptidyl Arginine Deiminases (putative)
Histone Lysine Methylation	Histone Lysine Methyltransferases	Chromodomains MBT Domains PHD Fingers Tudor Domains WD40 Domains	Histone Lysine Demethylases
Histone Phosphorylation	Kinases (JAK2, ATM/ATR, PKC, PKA Haspin, Aurora B kinase, RSK2, AMPK, MSK, MEK)	14.3.3 Proteins BIR Domains BRCT Proteins Chromoshadow Domains	Protein Serine/Threonine Phosphatases Protein Tyrosine Phosphatases
Histone Ubiquitination	Ubiquitin E2 Conjugases Ubiquitin E3 Ligases	Unknown	Deubiquitinating Enzymes

Epigenetic Writers

Tocris Product Areas: Epigenetic Writers

DNA Methyltransferases
Histone Acetyltransferases
Histone Methyltransferases
JAK Kinase
Pim Kinase
Poly(ADP-ribose) Polymerase (PARP)

Epigenetic writers catalyze the addition of chemical groups onto either histone tails or the DNA itself. These modifications are known as epigenetic marks, and are integral to gene expression and silencing.

One such group of epigenetic writers is histone methyltransferases; these enzymes catalyze the transfer of a methyl group onto a lysine or arginine residue on histone tails.

In addition to methyl marks, histone lysine residues may also undergo acetylation through the activity of histone acetyltransferases. The transfer of an acetyl group from the cofactor acetyl-CoA to lysine residues on histone tails neutralizes the positive charge of lysine, thereby weakening the affinity of the histone tail for the DNA and reducing chromatin condensation. Since a more open chromatin architecture enables the recruitment of transcription factors and polymerases, histone acetylation results in the promotion of gene expression.

Enzymes that catalyze the phosphorylation of histone tails are also important epigenetic writers. For example, phosphorylation of histone H3 (H3Y41) by JAK2 disrupts binding of the heterochromatin protein HP1α to chromatin, leading to increased DNA accessibility and the transcription of the oncogene lmo2. Other kinases including Haspin, Pim-1, PKC, Aurora B Kinase and ATM/ATR kinases have also been implicated in the phosphorylation of histone proteins and subsequent modification of gene expression.

Further epigenetic marks that alter gene expression include ubiquitination and ADP-ribosyltation. Lysine residues on histone proteins H2A and H2B can undergo monoubiquitination through the concerted actions of E2 ubiquitin conjugases and E3 ubiquitin ligases, whilst activated PARP interacts with, and ADP-ribosylates, certain histones to destabilize chromatin structure and induce transcription.

DNA can also undergo methylation through different mechanisms. The addition of a methyl group to a nucleotide by DNA methyltransferases (DNMTs) occurs at the major groove of the DNA double helix, and prevents transcription by blocking the binding of transcription factors and polymerases. There are two known types of DNA methylation – *de novo* and maintenance methylation. *De novo* methylation, predominantly carried out by DNA methyltransferases DNMT3A and DNMT3B, catalyzes the addition of methyl groups onto cytosine nucleotides. Since cell replication does not preserve such methylation, maintenance methylation copies these marks from the parent DNA onto the daughter DNA strands. The high affinity of DNMT1 for hemimethylated DNA *in vitro* suggests that this enzyme is primarily responsible for the maintenance of DNA methylation *in vivo*.

Further Reading

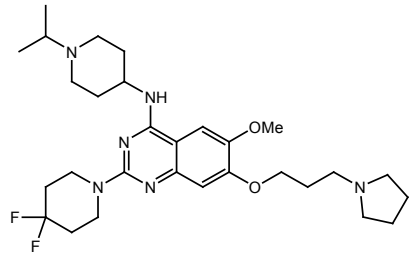
Cole (2008) Chemical probes for histone-modifying enzymes. *Nat.Chem.Biol.* **4** 590

Spannhoff *et al* (2009) The emerging therapeutic potential of histone methyltransferase and demethylase inhibitors. *Chem.Med.Chem.* **4** 1568

Featured Products for Epigenetic Writers

UNC 0642 *Cat. No. 5132*

Histone Methyltransferase Inhibitor

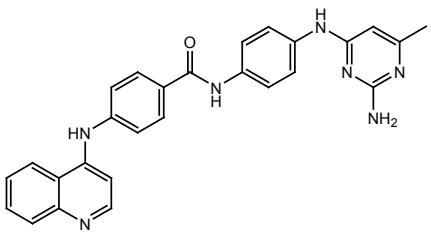


UNC 0642 is a potent and selective G9a and GLP histone lysine methyltransferase inhibitor (IC_{50} < 2.5 nM). The compound exhibits >2,000-fold selectivity for G9a and GLP over PRC2-EZH2 and >20,000-fold selectivity over other methyltransferases. UNC 0642 reduces H3K9 dimethylation levels in MDA-MB-231 cells (IC_{50} = 110 nM) and displays modest brain penetration *in vivo*.

(Supplied in conjunction with the Structural Genomics Consortium)

SGI 1027 *Cat. No. 5155*

DNA Methyltransferase Inhibitor



SGI 1027 is a DNMT3B, DNMT3A and DNMT1 DNA methyltransferase inhibitor (IC_{50} values are 7.5, 8 and 12.5 µM respectively with Poly(dI-dC) as the substrate). The compound reactivates silenced tumor suppressor genes by reducing CpG island hypermethylation.

Epigenetic Readers

Tocris Product Areas: Epigenetic Readers

14.3.3 Proteins
Bromodomains
MBT Domains

Epigenetic reader domains can be thought of as effector proteins that recognize, and are recruited to, specific marks on histones or nucleotides. Enzymes which write or erase epigenetic marks may also contain such reader domains, leading to the coordination of 'read-write' or 'read-erase' epigenetic processes. The structure of reader domains typically provides a cavity or surface groove in which to accommodate a specific epigenetic mark.

Proteins that contain reader domains can be broadly classified into four groups: chromatin architectural proteins; chromatin remodeling enzymes; chromatin modifiers; and adaptor proteins that recruit other machinery involved in gene expression. The first group of these, chromatin architectural proteins, binds to nucleosomes and can either directly induce chromatin compaction or alternatively act as a shield to prevent the binding of proteins involved in RNA transcription.

In contrast to chromatin architectural proteins, chromatin remodeling enzymes prompt a more open chromatin architecture. The increased accessibility of chromatin facilitates DNA transcription, promoting gene expression.

Many other proteins that contain reader domains cannot directly influence chromatin architecture, but instead serve to recruit secondary chromatin modifiers to further modify chromatin or to reverse an existing chromatin modification. One such example is the yeast chromatin remodeling enzyme complex, RSC, which contains a tandem bromodomain within its Rsc4 subunit that recruits the complex to acetylated lysine residues on histone H3 (H3K14). RSC is involved in a number of cellular processes including nucleosome remodeling, the consequence of which is the promotion of RNA polymerase II recruitment to the underlying DNA, prompting gene transcription.

The final class of reader domain-containing proteins is adaptor proteins: the principal function of these domains is to recruit factors that are linked to DNA metabolism processes including transcription, DNA damage repair, DNA recombination, DNA replication and RNA processing.

Further Reading

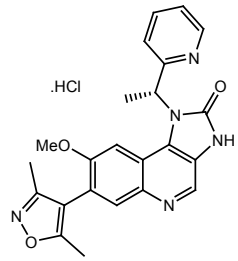
Belkina and Davis (2012) BET domain co-regulators in obesity, inflammation and cancer. *Nat.Rev.Cancer* **12** 465

Musselman *et al* (2012) Perceiving the epigenetic landscape through histone readers. *Nat.Struct.Mol.Biol.* **19** 1218

Featured Products for Epigenetic Readers

I-BET 151 hydrochloride — *Cat. No. 4650*

BET Bromodomain Inhibitor

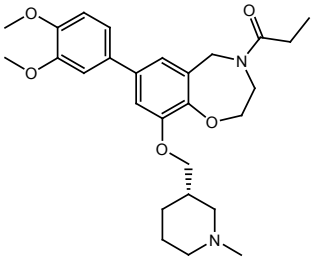


I-BET 151 is a BET bromodomain inhibitor that blocks the recruitment of BET to chromatin. The compound induces apoptosis and G_0/G_1 cell cycle arrest in MLL-fusion leukemic cell lines *in vitro* (IC_{50} values are 15, 26, 120 and 192 nM for NOMO1, MV4;11, MOLM13 and RS4;11 cell lines respectively). I-BET 151 hydrochloride reduces BCL2 expression in NOMO1 cells and improves survival in two rodent models of MLL-fusion leukemia *in vivo*.

(Sold for research purposes under agreement from GlaxoSmithKline)

I-CBP 112 — *Cat. No. 4891*

CBP/p300 Bromodomain Inhibitor

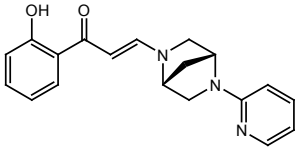


I-CBP 112 is a selective CBP/p300 bromodomain inhibitor (IC_{50} values are 142-170 and 625 nM respectively). The compound is selective for CBP/p300 over ATAD2, BAZ2B, BRD2(2), BRD4(1), PB1(5), PCAF, PHIP(2) and TIF1α bromodomains.

(Supplied in conjunction with the Structural Genomics Consortium)

PFI 3 — *Cat. No. 5072*

SMARCA2/4 and Polybromo 1 Inhibitor



PFI 3 is a potent and selective polybromo 1 (PB1) and SMARCA4 inhibitor (K_d values are 48 and 89 nM respectively). The compound also inhibits SMARCA2. PFI 3 displays selectivity for PB1 and SMARCA2/4 over other bromodomains.

(Supplied in conjunction with the Structural Genomics Consortium)

Epigenetic Erasers

Tocris Product Areas: Epigenetic Erasers

Histone Deacetylases
Histone Demethylases
Protein Serine/Threonine Phosphatases
Protein Tyrosine Phosphatases

Epigenetic marks are not necessarily permanent modifications; instead, they can be removed by a group of enzymes known as epigenetic 'erasers' in order to alter gene expression. There are multiple categories of epigenetic erasers that target histones; these include histone deacetylases, histone serine/threonine/tyrosine phosphatases, histone deubiquitinases and histone lysine/arginine demethylases.

The removal of acetyl groups through the actions of histone deacetylases (HDACs) is an important mechanism for increasing chromatin condensation and therefore repressing gene transcription. HDACs can be divided into class I and class II HDACs.

Protein phosphatases can target either phosphorylated serine, threonine or tyrosine residues on histone proteins. PP1, PP2A and PP4, amongst others, have been reported to dephosphorylate histone proteins. For example, the catalytic subunit of PP2A colocalizes with phosphorylated H2AX, a phosphorylated sequence variant of histone protein H2A that is rapidly concentrated within chromatin domains flanking DNA double-strand breaks. Phosphorylated H2AX acts as a docking site for DNA repair proteins, and is released from chromatin once double-strand breaks have been rejoined; this mechanism is thought to involve PP2A.

The removal of ubiquitin groups from histone lysine residues is catalyzed by proteases known as deubiquitinating enzymes (DUBs). These proteins target histones H2A and H2B, where they regulate transcription, DNA repair, gene expression and cell cycle progression. Compared to other histone modifications, the functions of histone ubiquitination are less well understood, yet increasing evidence points to an important role for this epigenetic modification in the DNA damage response.

The first histone lysine demethylase to be discovered was lysine-specific demethylase 1 (LSD1), also known as KDM1A. LSD1 contains an amino oxidase domain that binds the cofactor, flavin adenine dinucleotide (FAD), crucial for demethylation. A further family of lysine demethylases have since been identified; these are termed Jumonji C domain-containing demethylases (JMJD). JMJD do not require FAD as a cofactor but instead are dependent on Fe^{2+}/2-oxoglutarate (2-OG) for catalysis. To date, two enzymes that can remove methyl groups from arginine residues have been identified: peptidyl arginine deiminases and the histone demethylase JMJD6.

The process of DNA demethylation can be either passive or active. Passive demethylation involves the loss of methyl groups from DNA during DNA replication. This requires the absence of DNA methylation machinery that would otherwise maintain DNA methylation. The mechanism of active demethylation is less well-understood. One proposed mechanism involves the ten-eleven translocation (TET) enzyme family and thymine DNA glycosylase (TDG), which are involved in DNA base excision repair. TET enzymes can oxidize methylated cytosine residues, producing intermediates which can be subsequently excised from DNA by TDG, resulting in DNA demethylation. Further study is needed to identify other DNA modifying enzymes involved in the physiological regulation of the DNA demethylation pathway.

Further Reading

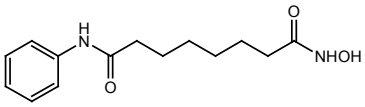
Arrowsmith *et al* (2012) Epigenetic protein families: a new frontier for drug discovery. *Nat.Rev.Drug Discov.* **11** 384

Kooistra and Helin (2012) Molecular mechanisms and potential functions of histone demethylases. *Nat.Rev.Mol.Cell.Biol.* **13** 297

Featured Products for Epigenetic Erasers

SAHA — *Cat. No. 4652*

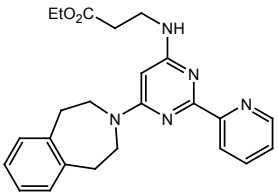
Histone Deacetylase Inhibitor



SAHA is a Class I and II histone deacetylase (HDAC) inhibitor that induces accumulation of acetylated histones H2A, H2B, H3 and H4 in transformed cultured cells. The compound suppresses cell growth in a range of cancer cell lines and induces apoptosis in cutaneous T-cell lymphoma cells *in vitro*.

GSK J4 — *Cat. No. 4594*

Histone Demethylase Inhibitor



GSK J4 is a histone lysine demethylase (KDM) inhibitor that blocks GSK demethylation of histone H3K27. The compound attenuates lipopolysaccharide-induced proinflammatory cytokine production in primary human macrophages (IC_{50} = 9 µM for the inhibition of TNF-α release). GSK J4 is cell permeable, and is an ethyl ester derivative of GSK J1 (Cat. No. 4593).

(Supplied in conjunction with the Structural Genomics Consortium)

Epigenetics Compounds Available from Tocris

Category	Product Name	Cat. No.	Primary Action
Bromodomains			
	BAZ2-ICR	5266	Selective BAZ2 bromodomain inhibitor
	Bromosporine	4758	Broad spectrum bromodomain inhibitor
	I-BET 151 hydrochloride	4650	BET bromodomain inhibitor
	I-CBP 112	4891	Selective CBP/p300 bromodomain inhibitor
	(+)-JQ1	4499	Potent and selective BET bromodomain inhibitor; cell permeable
	MS 436	5173	Potent and selective BRD4(1) bromodomain inhibitor
	OF 1	5289	Selective BRPF1B and BRPF2 bromodomain inhibitor
	OXF BD 02	4928	Selective BRD4(1) inhibitor
	PFI 1	4445	BET bromodomain inhibitor
	PFI 3	5072	Potent and selective SMARCA4 and polybromo 1 inhibitor; also inhibits SMARCA2
	SGC-CBP30	4889	Potent CBP/p300 bromodomain inhibitor
DNA Methyltransferases			
	Decitabine	2624	DNA methyltransferase inhibitor
	RG 108	3295	Non-nucleoside DNA methyltransferase inhibitor
	SGI 1027	5155	DNA methyltransferase inhibitor
	Zebularine	2293	DNA methyltransferase and cytidine deaminase inhibitor
Histone Acetyltransferases			
	Anacardic acid	3084	Noncompetitive PCAF/p300 HAT inhibitor
	C 646	4200	Selective p300/CBP HAT inhibitor
	L002	5045	p300 inhibitor
	NU 9056	4903	Inhibitor of KAT5 (Tip60) histone acetyltransferase
Histone Deacetylases			
	Apicidin	4846	Potent histone deacetylase inhibitor
	CI 994	2952	Class 1 histone deacetylase inhibitor; orally active
	FK 228	3515	Potent and selective class I histone deacetylase inhibitor
	LMK 235	4830	Selective HDAC4/HDAC5 inhibitor
	MC 1568	4077	Selectively inhibits HDAC class II (IIa)
	NCH 51	3747	Histone deacetylase inhibitor
	PCI 34051	4643	Potent and selective histone deacetylase 8 (HDAC8) inhibitor
	Pyroxamide	4403	Histone deacetylase inhibitor
	SAHA	4652	Class I and II HDAC inhibitor
	Sodium 4-Phenylbutyrate	2682	Histone deacetylase inhibitor
	Tubacin	3402	HDAC6 inhibitor; inhibits α-tubulin deacetylation
Histone Demethylases			
	GSK J1	4593	Potent histone demethylase JMJD3/UTX inhibitor
	GSK J2	4688	Inactive isomer of GSK J1 (Cat. No. 4593)
	GSK J4	4594	Histone lysine demethylase (KDM) inhibitor; cell permeable
	GSK J5	4689	Inactive isomer of GSK J4 (Cat. No. 4594); cell permeable
	IOX 1	4464	Histone demethylase inhibitor; cell permeable
	JIB 04	4972	Pan Jumonji histone demethylase inhibitor; active *in vivo*
	RN 1 dihydrochloride	4977	Lysine-specific demethylase 1 (LSD1) inhibitor
	TC-E 5002	5089	Selective histone lysine demethylase KDM2/7 subfamily inhibitor
	Tranylcypromine hydrochloride	3852	Irreversible inhibitor of lysine-specific demethylase 1 (LSD1); also inhibits MAO

Category	Product Name	Cat. No.	Primary Action
Histone Methyltransferases			
	A 366	5163	Potent and selective G9a/GLP histone lysine methyltransferase inhibitor
	C 21	5128	Selective PRMT1 arginine methyltransferase inhibitor
	Chaetocin	4504	Histone methyltransferase SUV39H1 inhibitor
	(R)-PFI 2 hydrochloride	4892	Potent and selective SETD7 histone lysine methyltransferase inhibitor
	Ryuvidine	2609	Protein lysine methyltransferase SETD8 inhibitor; also CDK4 inhibitor
	SGC 0946	4541	Highly potent and selective DOT1L inhibitor; cell permeable
	TC-E 5003	5099	Selective PRMT1 arginine methyltransferase inhibitor
	UNC 0224	3861	Potent G9a histone lysine methyltransferase inhibitor
	UNC 0638	4343	Selective G9a/GLP histone lysine methyltransferase inhibitor
	UNC 0642	5132	Potent and selective G9a/GLP inhibitor; active *in vivo*
	UNC 0646	4342	Potent and selective G9a/GLP inhibitor
Histone Phosphorylation			
	H 89 dihydrochloride	2910	Protein kinase A inhibitor
	Kaempferol	3603	RSK2 inhibitor; blocks histone $H3Ser^{10}$ phosphorylation
	NSC 33994	4338	JAK2 inhibitor
	PF 03814735	4821	Aurora kinase A and B inhibitor; inhibits histone H3 phosphorylation
	SB 747651A dihydrochloride	4630	Potent MSK1 inhibitor; also inhibits other AGC group kinases
	SL 327	1969	Selective inhibitor of MEK1 and MEK2; brain penetrant
	SNS 314 mesylate	4584	Potent pan-Aurora kinase inhibitor
	TCS PIM-1 4a	3714	Selective, ATP-competitive Pim kinase inhibitor
	U0126	1144	Potent and selective inhibitor of MEK1 and MEK2
	ZM 447439	2458	Inhibits Aurora kinase B
Histone Ubiquitination			
	PRT 4165	5047	Inhibitor of Bmi1/Ring1A; blocks histone H2A ubiquitination
MBT Domains			
	UNC 1215	4666	Potent inhibitor of L3MBTL3 Kme reader domain; cell permeable
	UNC 926 hydrochloride	4516	L3MBTL1 Kme reader domain inhibitor
Protein Ser/Thr Phosphatases			
	Fostriecin sodium salt	1840	Potent PP2A and PP4 inhibitor
	Okadaic acid	1136	Protein phosphatase 1 and 2A inhibitor
Tocriscreen Collections			
	Tocriscreen Epigenetics Toolbox	5268	80 Epigenetic modulators supplied pre-dissolved in DMSO (250 µL 10 mM solutions)
Transcriptional Modulators			
	α-Amanitin	4025	Inhibitor of RNA polymerase II
	3-Aminobenzamide	0788	PARP inhibitor
	IOX 2	4451	Potent, selective HIF-1α prolyl hydroxylase-2 (PHD2) inhibitor
	PJ 34 hydrochloride	3255	Potent PARP inhibitor
	Retinoic acid	0695	Endogenous retinoic acid receptor agonist
	Triptolide	3253	Inhibits RNA polymerase II-mediated transcription; antitumor, anti-inflammatory and immunosuppressive

For a complete and up-to-date product listing please visit www.tocris.com

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