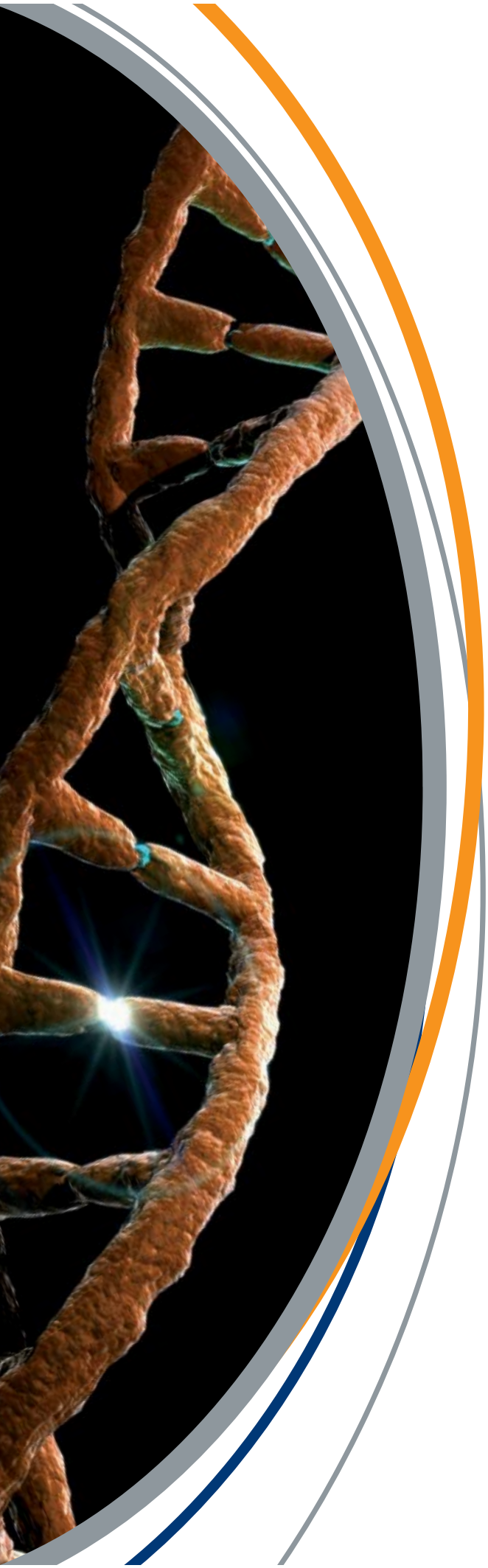

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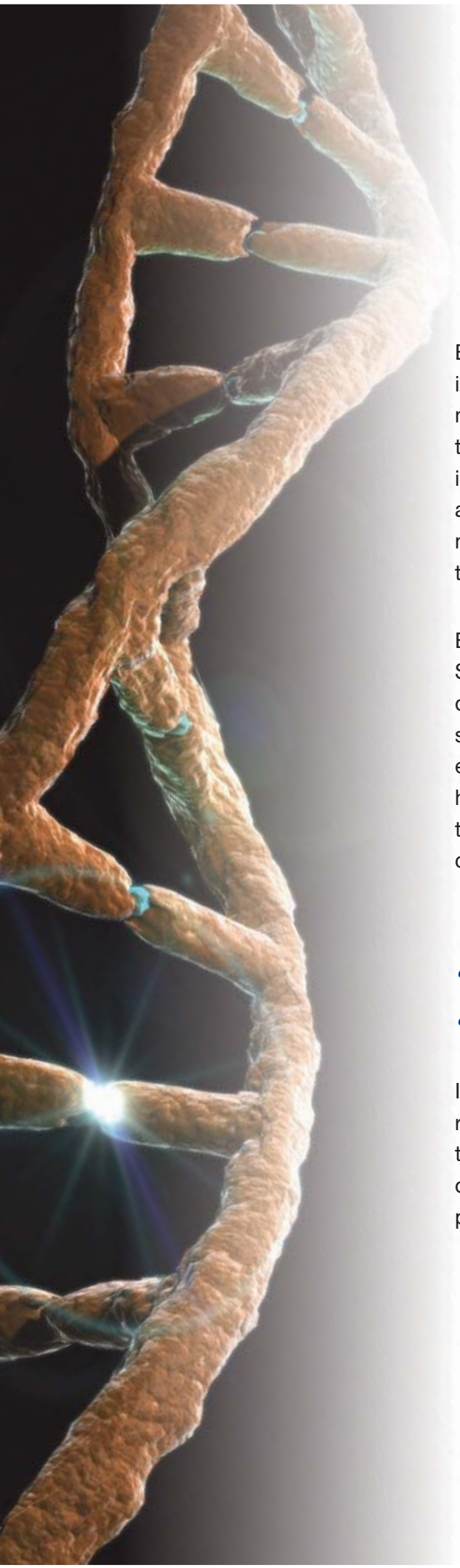

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Enzo Life Sciences, Leader in Epigenetics.

ELS has made many contributions to the field of epigenetics, both commercial and scientific.
For details see page 27.

Introduction

Epigenetics has been defined as the study of heritable changes in gene expression that occur without a change in DNA sequence. However, recent changes in the usage of the term have led to the suggestion that the requirement of heritability be dropped and that epigenetic events might better be defined as "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" [1]. Epigenetic changes in gene expression originate from modified accessibility of the eukaryotic transcription machinery to specific genes, mostly because of alterations in chromatin structure or RNA interference (RNAi). Key components in the processes of epigenetic transcription regulation are DNA methylation, histone modifications and variants, non-histone chromatin proteins, small interfering RNA (siRNA) and micro RNA (miRNA) [2]. DNA methylation takes place by transferring a methyl moiety from S-adenosylmethionine to the 5´ position of the cytidine ring. In mammals, normally cytidines followed by a guanosine, termed CpGs, are methylated. Cytosines followed by a base other than guanine (especially CpA) can also be methylated. CpGs are locally enriched in short stretches of DNA called CpG islands, found near approximately 40% of mammalian promoters.

DNA methylation is established and maintained by enzymes of the DNA methyltransferase (DNMT) family. The effects of DNA methylation on gene expression are exerted by promiscuous mechanisms and generally lead to the reduction of gene expression, so called gene silencing. This can be triggered either by a reduced affinity or complete abolition of transcription factor binding capacity to methylated DNA or by direct interaction of DNMTs with histone deacetylases, methyltransferases or other transcription co-repressors.

Chromatin consists of histone and non-histone proteins bound to genomic DNA. The nucleosome is formed by the wrapping of DNA around histone octamer composed of two H2A–H2B dimers and an H3–H4 tetramer. H1 linker histones outside the nucleosomes promote the formation of zigzag arrays of nucleosomes to form the chromatin fiber, which can be further compacted to chromatin. Two distinct chromatin states can be distinguished: condensed "closed" heterochromatin and de-condensed "open" euchromatin.

The change from transcriptionally silent heterochromatin to gene expressing euchromatin is mediated by posttranslational modifications of histones and use of distinct histone variants. Currently the known covalent modifications of histones (called the "histone code") include acetylation of lysines, methylation of lysines and arginines, phosphorylation of serines and threonines, ADP-ribosylation of glutamic acids, and ubiquitinylation and SUMOylation of lysine residues. These modifications are performed primarily on the N-terminal tails of histone proteins that protrude from the nucleosome.

Epigenetics, through the modulation of genetic information, plays roles in fundamental life processes, such as cell proliferation, cell development and the decision between cell survival and cell death. As a consequence, chromatin modification enzymes are increasingly being considered as targets for therapeutics for human disease with compounds targeting HDACs and DNMTs currently approved and in clinical trials.

LIT: [1] Perceptions of epigenetics: A. Bird; Nature **447,** 396 (2007) ▪ **[2]** Structure of histone acetyltransferases: R. Marmorstein; J. Mol. Biol. **311,** 433 (2001)

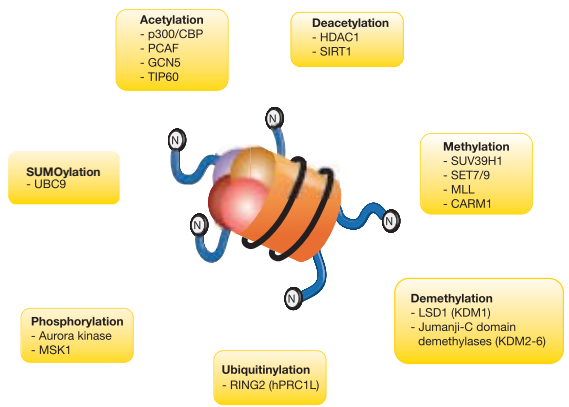


FIGURE 1: Histone N-terminal tails are substrates for a number of chemical modifications including acetylation/deacetylation, methylation/demethylation, ubiquitinylation, SUMOylation and phosphorylation. A sampling of enzymes that catalyse these modifications are indicated.

Adapted from: Chromatin control and cancer-drug discovery: realizing the promise. A.G. Inche & N.B. La Thangue, et al.; Drug Discov. Today ***11,*** *97 (2007)*

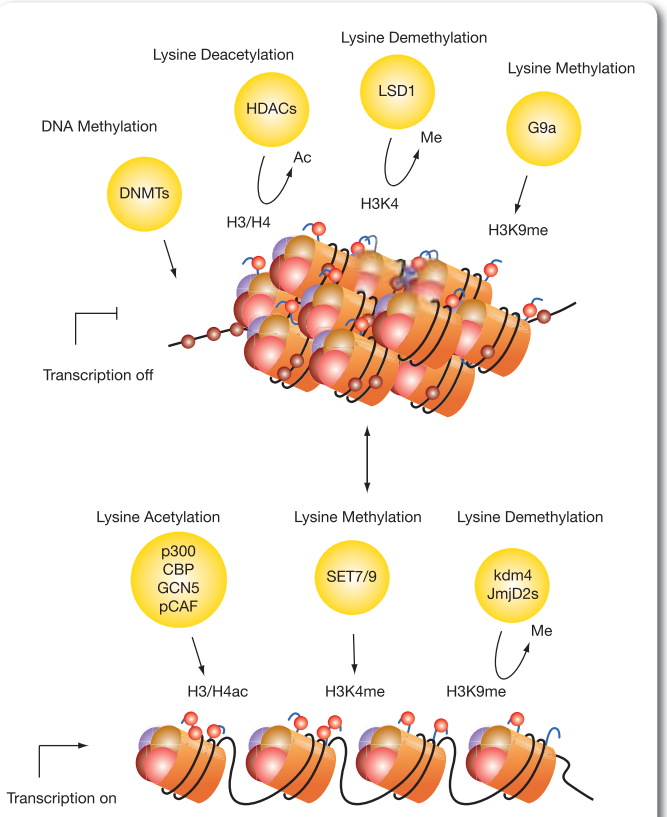


FIGURE 2: The change of chromatin from a condensed and transcriptionally repressed state (top) to an open and transcriptionally activated state (bottom) is regulated in part by specific patterns of DNA methylation and lysine modifications of histone N-terminal tails. These post-translational modifications, along with others not shown, have been proposed to constitute a "histone code".

Histone Acetyltransferases

Histone acetyltransferases (HATs), a group of transcription regulators, covalently modify the epsilon amino group of lysines found in the N-terminal tails of histones, or other proteins, by the addition of an acetyl group from acetyl coenzyme A. Acetylation of lysines inside histone tails generally leads to transcriptional activation and deacetylation results in silencing. In addition, the stability and function of many non-histone proteins are regulated by the acetylation state of specific lysine residues [1, 2]. Known protein families with histone acetyl transferase activity include GNAT (GCN5, PCAF), CBP/p300, TAFII250 (TFIID), SRC-1, ACTR and MYST [3-8]. HAT complexes are involved in such diverse processes as transcriptional activation, gene silencing, DNA repair and cell-cycle progression. Dysfunction of these enzymes is often associated with diseases, ranging from neurodegenerative disorders to cancer [9].

LIT: [1] Structure of histone acetyltransferases: R. Marmorstein; J. Mol. Biol. **311,** 433 (2001) ▪ **[2]** Regulating histone acetyltransferases and deacetylases: G. Legube & D. Trouche; EMBO Rep. **4,** 944 (2003) ▪ **[3]** Functions of myst family histone acetyltransferases and their link to disease: N. Avvakumov & J. Cote; Subcell Biochem. **41,** 295 (2007) ▪ **[4]** Structure and functions of the GNAT superfamily of acetyltransferases: M. W. Vetting, et al.; Arch Biochem Biophys **433,** 212 (2005) ▪ **[5]** Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300: H. Chen, et al.; Cell **90,** 569 (1997) ▪ **[6]** Steroid receptor coactivator-1 is a histone acetyltransferase: T. E. Spencer, et al.; Nature **389,** 194 (1997) ▪ **[7]** Requirement for TAF(II)250 acetyltransferase activity in cell cycle progression: E. L. Dunphy, et al.; Mol. Cell Biol. **20,** 1134 (2000) ▪ **[8]** CBP/p300 histone acetyl-transferase activity is important for the G1/S transition: S. Ait-Si-Ali, et al.; Oncogene **19,** 2430 (2000) ▪ **[9]** The diverse functions of histone acetyltransferase complexes: M.J. Carrozza, et al.; Trends Genet. **19,** 321 (2003)

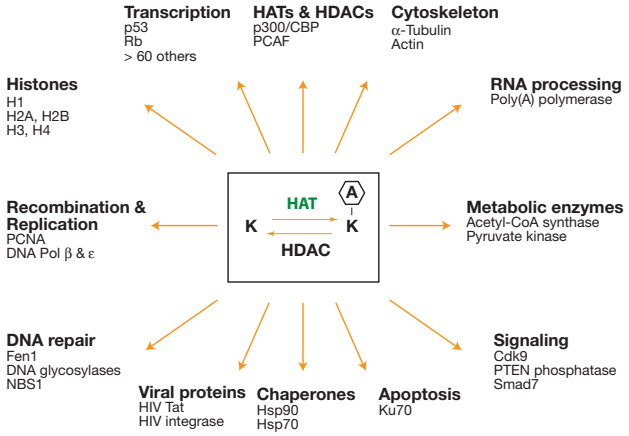


FIGURE 3: Schematic illustration of the prevalence of reversible lysine (K) acetylation in diverse cellular processes. The hexagon with the letter A refers to acetylation. For each process, only representative proteins are listed. Cdk9, cyclin-dependent kinase 9; Fen1, Flap endonuclease 1; NBS1, Nijmegen breakage syndrome protein 1; PCNA, proliferating cell nuclear antigen; PTEN, phosphatase and tensin homolog; Rb, retinoblastoma suppressor protein.

Latest Insight

A recent proteomic study characterized the "acetylome", identifying over 3600 acetylation sites on 1750 proteins. Acetylated proteins were identified with known functions in chromatin remodeling, cell cycle, splicing, nuclear transport and actin nucleation.

LIT: Lysine acetylation targets protein complexes and co-regulates major cellular functions: C. Choudhary, et al.; Science **325,** 834 (2009)

Enzymes

p300 (human), (rec)
BML-SE451-0100 100 µg

Produced in *E. coli.* Catalytic domain (aa 1284-1673) of human p300.

PCAF (human), (rec.)
[P300/CBP-associated factor]

BML-SE271-0050 50 µg

Produced in *E. coli.* Catalytic domain (aa 492-658) of human pCAF.

PCAF (mouse), (rec.) (His-tag)
[P300/CBP-associated factor]

ALX-201-281-C020 20 µg

Produced in SF21 cells. Fused to a His-tag.

CBP (human), (rec.)
BML-SE452-0100 100 µg

Produced in *E. coli.* Catalytic domain (aa 1319-1710) of human CBP.

CBP (mouse), (rec.) (His-tag)
ALX-201-279-C002 2 µg

Produced in SF21 cells. Fused to a His-tag.

GCN5 (human), (rec.)
[GCN5L2]

BML-SE272-0050 50 µg

Produced in *E. coli.* Catalytic domain (aa 497-663) of human GCN5.

GCN5 (human), (rec.) (His-tag)
[GCN5L2]

ALX-201-280-C002 2 µg

Produced in SF21 cells. Fused to a His-tag.

Substrates

p53 peptide (368-386)
[Ac-His-Leu-Lys-Ser-Lys-Lys-Gly-Gln-Ser-Thr-Ser-Arg-His-Lys-Lys-Leu-Met-Phe-Lys-NH_2]

BML-P198-0025 2.5 mg

Derived from the C-terminal regulatory domain of p53, this peptide comprises five lysines, including three preferentially acetylated by p300 (373, 381, 382). May be used as a substrate for assay of p300 and CBP HATs (BML-SE451 and BML-SE452).

Histone H3 peptide
[Ac-Gln-Thr-Ala-Arg-Lys-Ser-Thr-Gly-Gly-Lys-Ala-Pro-Arg-Lys-Gln-Leu-Ala-Thr-Lys-NH_2]

BML-P271-0500 0.5 mg

Comprising residues 5-23 of the human histone H3 N-terminal tail, this peptide is centered on Lys^{14}, preferred acetylation site for the GCN5/pCAF family of histone acetyltransferases (HATs). May be used as a substrate for assay of PCAF and GCN5 HATs.

Histone Acetyltransferases

Inhibitors

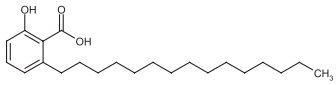
Anacardic acid

ALX-270-381-M005 5 mg

ALX-270-381-M025 25 mg

Synthetic. Cell permeable salicylic acid analog that acts as a potent, non-competitive inhibitor of p300 and PCAF (p300/CBP-associated factor) histone acetyltransferase (HAT) activities (IC_{50}~8.5μM and ~5μM, respectively).

LIT: Anacardic Acid: molluscicide in cashew nut shell liquid: J.T. Sullivan, et al.; Planta Med. **44,** 175 (1982) ▪ Inhibition of lipoxygenase and prostaglandin endoperoxide synthase by anacardic acids: R. Grazzini, et al.; BBRC **176,** 775 (1991) ▪ Antibacterial activity of anacardic acid and totarol, alone and in combination with methicillin, against methicillin-resistant Staphylococcus aureus: H. Muroi & I. Kubo; J. Appl. Bacteriol. **80,** 387 (1996) ▪ Small molecule modulators of histone acetyltransferase p300: K. Balasubramanyam, et al.; J. Biol. Chem. **278,** 19134 (2003) ▪ Inhibition of histone acetyltransferase activity by anacardic acid sensitizes tumor cells to ionizing radiation: Y. Sun, et al.; FEBS Lett. **580,** 4353 (2006)



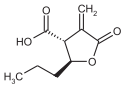
Butyrolactone 3

[Gcn5 Inhibitor 1; MB-3]

ALX-270-411-M005 5 mg

Synthetic. Racemic. Inhibitor of human histone acetyltransferase Gcn5.

LIT: Design, synthesis, and biological evaluation of a small-molecule inhibitor of the histone acetyltransferase Gcn5: M. Biel, et al.; Angew. Chem. Int. Ed. Engl. **43,** 3974 (2004)



Curcumin

BML-EI135-0001 1 g

BML-EI135-0005 5 g

Curcumin (high purity)

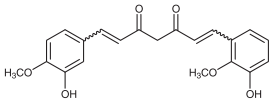
ALX-350-028-M010 10 mg

ALX-350-028-M050 50 mg

ALX-350-028-M250 250 mg

Isolated from turmeric (*Curcuma longa*). Dual inhibitor of 5-lipoxygenase (IC_{50}=8μM) and cyclooxygenase (IC_{50}=52μM). Antioxidant. Curcumin has been shown to inhibit NF-κB, possibly thru inhibition of JNK (IC_{50}=10μM), the COP9 signalosome kinase (IC_{50}=10μM) and p300/CBP.

LIT: Curcumin, an atoxic antioxidant and natural NFkappaB, cyclooxygenase-2, lipooxygenase, and inducible nitric oxide synthase inhibitor: a shield against acute and chronic diseases: S. Bengmark; JPEN J. Parenter. Enteral. Nutr. **30,** 45 (2006) ▪ Curcumin is an inhibitor of p300 histone acetylatransferase: M.G. Marcu, et al.; Med. Chem. **2,** 169 (2006) ▪ Multiple biological activities of curcumin: a short review: R.K. Maheshwari, et al.; Life Sci. **78,** 2081 (2006) ▪ The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats: T. Morimoto, et al.; J. Clin. Invest. **118,** 868 (2008) ▪ **For a comprehensive bibliography please visit our website.**



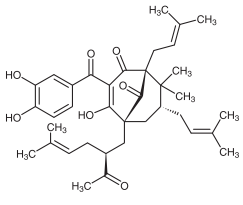
Garcinol

BML-GR343-0010 10 mg

BML-GR343-0050 50 mg

Garcinol is a polyisoprenylated benzophenone derivative isolated from *Garcinia indica*. It is a potent inhibitor of histone acetyltransferases (HATs) p300 (IC_{50}=7μM) and PCAF (IC_{50}=5μM) both *in vitro* and *in vivo*. HAT activity-dependent chromatin transcription was inhibited by garcinol, but it had no effect on naked DNA transcription. It is a potent inducer of apoptosis in HeLa cells where it down-regulates global gene expression.

LIT: Cytotoxic benzophenone derivatives from Garcinia species display a strong apoptosis-inducing effect against human leukemia cell lines: K. Matsumoto, et al.; Biol. Pharm. Bull. **26,** 569 (2003) ▪ Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression: K. Balasubramanyam, et al.; J. Biol. Chem. **279,** 33716 (2004)



Activator

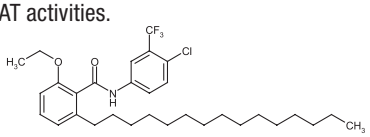
CTPB

ALX-420-033-M001 1 mg

ALX-420-033-M005 5 mg

Potent activator of p300 HAT (histone acetyltransferase), but not of PCAF (p300/CBP-associated factor) HAT activities.

LIT: Small molecule modulators of histone acetyltransferase p300: K. Balasubramanyam, et al.; J. Biol. Chem. **278,** 19134 (2003)



Antibodies

p300 (human), pAb

ALX-210-228-C100 100 μg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 139-151 (G^{139}TSGPNQGPTQST^{151}C) in the nuclear factor binding domain of human p300 (E1A binding protein p300). **SPECIFICITY:** Recognizes human p300. Detects a band of ~265kDa by Western blot. **APPLICATION:** WB. **BP:** ALX-165-038.

CBP, pAb

ALX-210-229-C100 100 μg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 162-176 (A^{162}TSSPATSQTGPGIC^{176}) in the nuclear factor binding domain of human CBP (CREB binding protein). **SPECIFICITY:** Recognizes human and mouse CBP. Detects a band of ~265kDa by Western blot. **APPLICATION:** WB. **BP:** ALX-153-023.

SRC-1, mAb (1135/H4)

ALX-804-191-R100 100 μl

CLONE: 1135/H4. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Synthetic peptide corresponding to aa 477-947 of human SRC-1 (steroid receptor coactivator-1). **SPECIFICITY:** Recognizes human, mouse and monkey SRC-1. Detects a band of ~165kDa (and an unspecific band of 30-40kDa) by Western blot. **APPLICATION:** IHC (PS), IP, WB.

SRC-1, pAb

ALX-210-283-C100 100 μg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 8-24 (S^{8}SDPANPDSHKRKGSPC^{24}) of human SRC-1 (steroid receptor coactivator-1). **SPECIFICITY:** Recognizes human, mouse and japaneese quail SRC-1. Detects a band of ~165kDa and an unspecific band of ~40kDa by Western blot. **APPLICATION:** IHC (FS), ICC, WB. **BP:** ALX-167-020.

AIB1 (human), mAb (AX15.3)

ALX-804-283-C100 100 μg

CLONE: AX15.3. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human AIB1 (amplified in breast cancer 1) (aa 605-1294). **SPECIFICITY:** Recognizes human AIB1. Detects a band of ~155kDa by Western blot. **APPLICATION:** IP, WB.

LIT: Production and characterization of monoclonal antibodies to the steroid receptor coactivator AIB1: D.O. Azorsa, et al.; Hybridoma **18,** 28 (1999)

AIB1 (human), pAb

ALX-210-255-C100 100 μg

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to aa 3-15 (G^{3}LGENLDPLASDS^{15}C) of human AIB1 (amplified in breast cancer 1). **SPECIFICITY:** Recognizes human AIB1. Detects a band of ~155kDa by Western blot. **APPLICATION:** WB. **BP:** ALX-151-034.

Histone Deacetylases (HDACs) & Sirtuins

Histone deacetylases (HDACs), more properly termed lysine deacetylases, reverse the action of histone acetyltransferases (HATs) by catalysing the removal of acetyl groups from the lysine ε-amino group of histones and other non-histone substrates. HDACs can be divided into four classes: class I, including HDAC1, 2, 3 and 8; class II, including HDAC4, 5, 6, 7, 9 and 10; class IV (HDAC11) and class III, the NAD^+-dependent Sir2-like deacetylases or sirtuins. Class I, II and IV HDACs are zinc-dependent hydrolases. The sirtuins (class III HDACs), which are phylogenetically unrelated to the hydrolase HDACs (classes I, II and IV), catalyse a reaction that couples lysine deacetylation to the consumption of NAD^+, forming nicotinamide and O-acetyl-ADP-ribose [1-7]. With the exception of HDAC8, functional HDACs exist in high molecular weight multi-protein complexes in which different HDAC subtypes are often associated with specific co-regulators and chromatin-modifying enzymes. HDAC activity is tightly controlled through targeted recruitment, protein-protein interactions and posttranslational modifications. HDACs play roles in the regulation of gene transcription, cell growth, survival and proliferation. Dysfunction of these enzymes is often associated with disease, ranging from neurodegenerative disorders to cancer [7-9]. Sirtuins (SIRTs 1-7) target a wide range of cellular proteins in the nucleus, cytoplasm, and mitochondria for deacetylation of acetyllysine (SIRT1, -2, -3, -6 and -7) or mono-ADP-ribosylation (SIRT4) [9-19]. Sirtuins are involved in important biological processes including apoptosis, cancer prevention, DNA damage repair, regulation of energy expenditure, stress resistance, and aging.

LIT: **[1]** Molecular evolution of the histone deacetylase family: Functional implications of phylogenetic analysis: I.V. Gregoretti, et al.; J. Mol. Biol. **338,** 17 (2004) ▪ **[2]** The role of NAD+ dependent histone deacetylases (sirtuins) in ageing: J. Trapp & M. Jung; Curr. Drug Targets **7,** 1553 (2006) ▪ **[3]** HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics: P. Gallinari, et al.; Cell Res. **17,** 195 (2007) ▪ **[4]** Silent information regulator 2 family of NAD- dependent histone/protein deacetylases generates a unique product, 1-O-acetyl-ADP-ribose: K.G. Tanner et al.; PNAS **97,** 14178 (2000) ▪ **[5]** Coupling of histone deacetylation to NAD breakdown by the yeast silencing protein Sir2: Evidence for acetyl transfer from substrate to an NAD breakdown product: J.C. Tanny & D. Moazed; PNAS **98,** 415 (2000) ▪ **[6]** Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase: S. Imai et al.; Nature **403,** 795 (2000) ▪ **[7]** Genome-wide analysis of HDAC function: K. Ekwall; Trends Genet. **21,** 608 (2005) ▪ **[8]** The molecular mechanism of HDAC inhibitors in anticancer effects: G. Bi and G. Jiang; Cell. Mol. Immunol. **3,** 285 (2006) ▪ **[9]** HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis: R.N. Saha & K. Pahan; Cell Death Differ. **13,** 539 (2006) ▪ **[10]** Sirtuins (histone deacetylases III) in the cellular response to DNA damage--facts and hypotheses: M. Kruszewski & I. Szumiel; DNA Repair (Amst) **4,** 1306 (2005) ▪ **[11]** NAD metabolism and sirtuins: metabolic regulation of protein deacetylation in stress and toxicity: T. Yang & A.A. Sauve; AAPS J. **8,** E632 (2006) ▪ **[12]** Sirtuins: critical regulators at the crossroads between cancer and aging: L.R. Saunders & E. Verdin; Oncogene **26,** 5489 (2007) ▪ **[13]** NAD+-dependent deacetylation of H4 lysine 16 by class III HDACs: A. Vaquero, et al.; Oncogene **26,** 5505 (2007) ▪ **[14]** Sirtuins: The ‚magnificent seven', function, metabolism and longevity: N. Dali-Youcef, et al.; Ann. Med. **39,** 335 (2007) ▪ **[15]** Sirtuins in mammals: insights into their biological function: S. Michan & D. Sinclair; Biochem. J. **404,** 1 (2007) ▪ **[16]** SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic ß cells: M.C. Haigis et al.; Cell **126,** 941 (2006) ▪ **[17]** SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin: E. Michishita et al.; Nature **452,** 492 (2008) ▪ **[18]** Substrates and regulation mechanisms for the human mitochondrial sirtuins Sirt3 and Sirt5: C. Schlicker et al.; J. Mol. Biol. **382,** 790 (2008) ▪ **[19]** Sirt7 Increases Stress Resistance of Cardiomyocytes and Prevents Apoptosis and Inflammatory Cardiomyopathy in Mice: O. Vakhrusheva, et al.; Circ Res. **102,** 703 (2008)

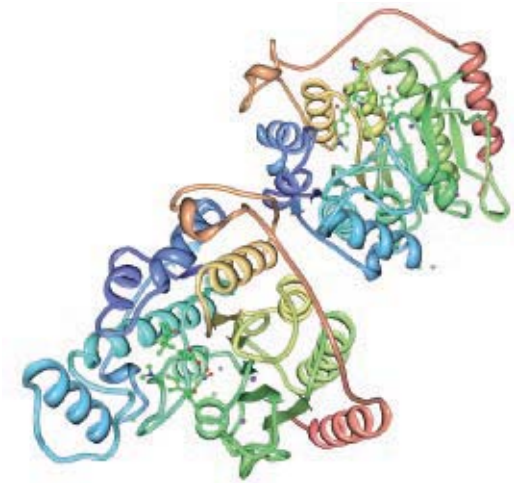


FIGURE 4: Crystal structure of human HDAC8 complexed with Trichostatin A.
Open source picture from RCSB (protein data bank)

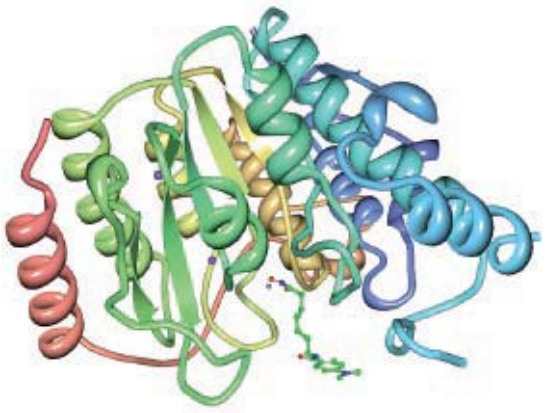


FIGURE 5: Crystal structure of human HDAC8 complexed with M344.
Open source picture from RCSB (protein data bank)

Histone Deacetylases (HDACs) & Sirtuins

Drug Discovery / Cell-free Assay Systems

The *Fluor de Lys*™ (Fluorescent deacetylation of lysine) Assay System was developed for simple, nonradioactive measurement of deacetylase activity. Deacetylation of a *Fluor de Lys*™ Substrate by a source of deacetylase activity, including purified enzyme or enzyme complex, cell lysate or whole cells, sensitizes the substrate so that, in a second step, treatment with the appropriate *Fluor de Lys*™ Developer produces a fluorophore (see Figure 6). The assay is compatible with class I and IIb HDACs and sirtuins and a variety of substrates are available based on acetylated sites found in p53 and histones (see Table 1, page 11). *Fluor de Lys*™ Substrate (Prod. No. BML-KI104) is cell-permeable and allows cell-based determination of HDAC activity (see page 9). This system is the basis of several activity assays/drug discovery kits for HDACs (Prod. No. BML-AK500, BML-AK511, BML-AK518) and sirtuins (Prod. No. BML-AK555, BML-AK556 and BML-AK557).

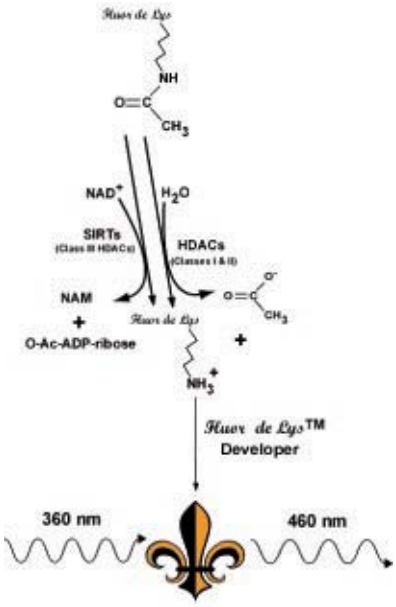


FIGURE 6: Mechanism of the *Fluor de Lys*™ HDAC assay. Deacetylation of the substrate sensitizes it to the developer, which then generates a fluorophore (symbol). The fluorophore is excited with 360nm light and the emitted light (460nm) is detected on a fluorometric plate reader.

General *Fluor de Lys*™ Activity Assays

- Useful for assaying lysates, immunoprecipitates or inhibitor screening using the nuclear extract provided.
- Includes HeLa nuclear extract, a rich source of HDACs 1 & 2 for use as a positive control or as a source of HDAC activity for screening.
- Compatible with class I & IIb HDAC and sirtuins (with addition of NAD^+).
- Includes enough reagent for 100-200 assays.

Product	Prod. No.	Size
HDAC fluorometric activity assay kit	BML-AK500-0001	1 Kit
HDAC colorimetric activity assay kit	BML-AK501-0001	1 Kit

New & Improved HDAC Assay!

Fluor de Lys™-Green HDAC assay

BML-AK530-0001 — 1 Kit

An improved *Fluor de Lys*™ HDAC assay with *Fluor de Lys*™-Green, a new substrate offering higher sensitivity and an excitation and emission (485/530nm) that avoids quenching and fluorescent interference from compounds absorbing in the near UV and blue range.

Fluor de Lys™ Drug Discovery Assays for Specific Deacetylases

- Useful for inhibitor screening or characterising enzyme kinetics.
- Includes optimal substrate selected from a panel of acetylated peptides derived from acetylated sites in p53 and histones, plus everything needed to perform the assay.
- Supplied with enough recombinant enzyme for 96 assays (1 x 96-well plate).

Product	Prod. No.	Size
HDAC1 fluorometric drug discovery kit	BML-AK511-0001	1 Kit
HDAC8 fluorometric drug discovery kit	BML-AK518-0001	1 Kit
SIRT1 fluorometric drug discovery kit	BML-AK555-0001	1 Kit
SIRT2 fluorometric drug discovery kit	BML-AK556-0001	1 Kit
SIRT3 fluorometric drug discovery kit	BML-AK557-0001	1 Kit

Fluor de Lys™ Cellular HDAC Assay

HDACs are typically found in large multiprotein complexes and are tightly regulated by subcellular localisation, phosphorylation, and other mechanisms. A cellular HDAC assay, which allows the determination of deacetylase activity within an undisturbed cellular environment, is likely to provide more accurate activity information than a cell-free assay. In addition, a cellular assay allows the study of the effects of upstream regulators on deacetylase activity and the detection of inhibitors or activators that act indirectly to affect deacetylase activity. The HDAC Cellular Assay (Prod. No. BML-AK503) utilises the cell-permeable *Fluor de Lys*™ Substrate (Prod. No. BML-KI104), to provide a straightforward approach to measuring deacetylase activity in cultured cells. The substrate can be deacetylated by a broad range of deacetylases, and unlike histone-based assays, deacetylation of the *Fluor de Lys*™ Substrate can reveal activity of enzymes that act on non-histone substrates as well.

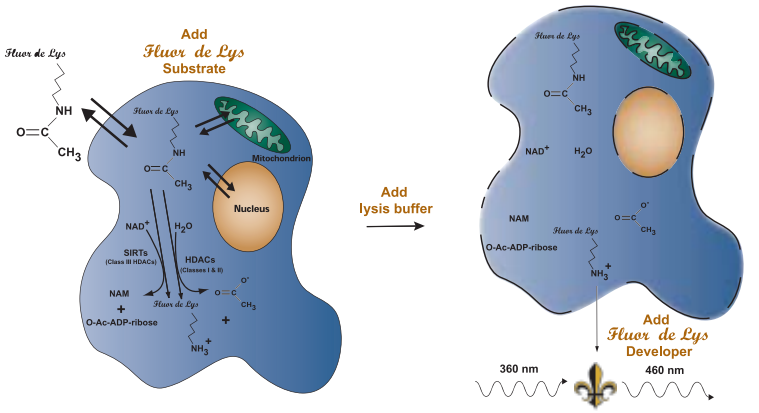


FIGURE 7: Mechanism of the *Fluor de Lys*™ HDAC Fluorometric Cellular Activity Assay. The *Fluor de Lys*™ Substrate (Prod. No. BML-KI104) is cell permeable and is deacetylated by cellular HDACs. The deacetylated substrate accumulates inside cells and deacetylation can be quantitated by addition of developer to lysed cells and measurement of fluorescence.

Product	Prod. No.	Size
HDAC fluorometric cellular activity assay kit	BML-AK503-0001	1 Kit

Selected citations using the *Fluor de Lys*™ activity assays:

X. Zhou et al.; PNAS **98,** 10572 (2001) ▪ K.J. Bitterman et al.; J. Biol. Chem. **277,** 45099 (2002) ▪ B. Heltweg and Jung, M.; Anal. Biochem. **302,** 175 (2002) ▪ K. Ito et al.; PNAS **99,** 8921 (2002) ▪ S. Milutinovic et al.; J. Biol. Chem. **277,** 20974 (2002) ▪ R.M. Anderson et al.; Science **302,** 2124 (2003) ▪ K.T. Howitz et al.; Nature **425,** 191 (2003) ▪ G.V. Kapustin et al.; Org. Lett. **5,** 3053 (2003) ▪ D.K. Kim et al.; J. Med. Chem. **46,** 5745 (2003) ▪ C.G. Kleer et al.; PNAS **100,** 11606 (2003) ▪ K. Zhao et al.; Nat. Struct. Biol. **10,** 864 (2003) ▪ T. Suzuki et al.; Bioorg. Med. Chem. Lett. **13,** 4321 (2003) ▪ B.G. Cosio et al.; Am. J. Respir. Crit Care Med. **170,** 141 (2004) ▪ C.M. Gallo et al.; Mol. Cell Biol. **24,** 1301 (2004) ▪ N. Gurvich et al.; Cancer Res. **64,** 1079 (2004) ▪ L.H. Wang et al.; Nat. Med. **10,** 40 (2004) ▪ J.G. Wood et al.; Nature **430,** 686 (2004) ▪ F. Yeung et al.; EMBO J. **23,** 2369 (2004) ▪ J.L. Avalos et al.; Mol. Cell **17,** 855 (2005) ▪ K. Ito et al.; N. Engl. J. Med. **352,** 1967 (2005) ▪ A. Mai et al.; J. Med. Chem. **48,** 7789 (2005) ▪ E. Michishita et al.; Mol. Biol. Cell **16,** 4623 (2005) ▪ A.D. Napper et al.; J. Med. Chem. **48,** 8045 (2005) ▪ T. Suzuki et al.; J. Med. Chem. **48,** 1019 (2005) ▪ P. Aksoy et al.; BBRC **349,** 353 (2006) ▪ V.C. de Boer et al.; Mech. Ageing Dev. **127,** 618 (2006) ▪ S.L. Gantt et al.; Biochemistry **45,** 6170 (2006) ▪ W. Gu et al.; Bioorg. Med. Chem. **14,** 3320 (2006) ▪ D. Herman et al.; Nat. Chem. Biol. **2,** 551 (2006) ▪ X. Li et al.; Cancer Res. **66,** 9323 (2006) ▪ V.M. Nayagam et al.; J. Biomol. Screen. **11,** 959 (2006) ▪ J.M. Solomon et al.; Mol. Cell Biol. **26,** 28 (2006) ▪ P.H. Kiviranta et al.; Bioorg. Med. Chem. Lett. **17,** 2448 (2007) ▪ T.F. Outeiro et al.; Science **317,** 516 (2007) ▪ S. Lain et al.; Cancer Cell **13,** 454 (2008) ▪ B. Jung-Hynes et al.; J. Biol. Chem. **284,** 3823 (2009)

Histone Deacetylases (HDACs) & Sirtuins

HDAC & Sirtuin Enzymes

HDAC (rat)
ALX-202-052-L002 2 ml

Isolated from rat liver.

LIT: Improvement and validation of the fluorescence-based histone deacetylase assay using an internal standard: K. Hoffmann, et al.; Arch. Pharm. **334,** 248 (2001)

HDAC1 (human), (rec.)
BML-SE456-0050 50 µg

Produced in insect cells.

HDAC2 (human), (rec.)
BML-SE500-0050 50 µg

Produced in insect cells.

HDAC2 (full-length) (human), (rec.)
BML-SE533-0050 50 µg

Produced in insect cells.

HDAC3 (human), (rec.)
BML-SE507-0050 50 µg

Produced in insect cells.

HDAC3/NCOR1 complex (human), (rec.)
BML-SE515-0050 50 µg

Produced in insect cells. Approximately 1:1 stoichiometric complex of recombinant human HDAC3 (histone deacetylase 3) with a GST fusion protein comprising the Deacetylase Activation Domain (DAD) of human NCOR1 (amino acids 397-503; SANT1 domain plus amino flanking residues). Produced by coexpression of HDAC3 with a GST-NCOR1(DAD) construct in an insect cell system, the complex displays ~100-fold higher deacetylase activity than isolated HDAC3 (based on equal weights HDAC3 assayed at a saturating concentration (100µM) of a fluorogenic peptide substrate p53 379-382, K(Ac)382; BML-KI177).This HDAC3 preparation is active with various fluorogenic peptide substrates, including *Fluor de Lys*™ Substrate (BML-KI104) and *Fluor de Lys*™-SIRT1 (p53 379-382, K(Ac)382; BML-KI177).

HDAC6 (human), (rec.)
BML-SE508-0050 50 µg

Produced in insect cells.

HDAC8 (human), (rec.)
BML-SE145-0100 100 U

Produced in *E. coli*. **BIOLOGICAL ACTIVITY:** Like other class I HDACs, HDAC8 exhibits trichostatin A-inhibitable histone deacetylase activity and can mediate transcription repression.

HDAC10 (human), (rec) (His-tag) NEW
BML-SE559-0050 50 µg

Expressed with a C-terminal His-tag in an insect cell system.

HDAC11 (human), (rec) (His-tag) NEW
BML-SE560-0050 50 µg

Expressed with a N-terminal His-tag in an insect cell system.

SIRT1 (human), (rec.) (His-tag)
BML-SE239-0100 100 U

Produced in *E. coli*. Has a N-terminal His-tag.

SIRT2 (human), (rec.) (His-tag)
BML-SE251-0500 500 U

Produced in *E. coli*. Contains a N-terminal His-tag.

SIRT3 (human), (rec.) (His-tag)
BML-SE270-0500 500 U

Produced in *E. coli*. Active SIRT3 (aa 102-199). Contains a N-terminal His-tag.

SIRT5 (human), (rec.) (His-tag)
BML-SE555-0500 500 µg

Produced in *E. coli*. Comprises residues 37-310 of SIRT5 isoform 1, with an N-terminal His-tag. Recombinant human mitochondrial processing protease cleaves full-length SIRT5 after residue thirty-six, suggesting that the mature, *in vivo* form may begin with residue 37. Consistent with this, N-terminal sequence of mouse SIRT5 processed in 293T cells also commences at residue 37.

SIRT5 (full-length) (human), (rec.) (His-tag)
ALX-201-448-C010 10 µg

ALX-201-448-C050 50 µg

Produced in *E. coli*. The full-length human sirtuin 5 (aa 1-310) is fused at the N-terminus to a His-tag.

SIRT6 (human), (rec.) (His-tag)
ALX-201-449-C010 10 µg

ALX-201-449-C050 50 µg

Produced in *E. coli*. The mature human sirtuin 6 (aa 1-355) is fused at the N-terminus to a His-tag.

Nuclear extract from HeLa cells
BML-KI140-0100 100 µl

Prepared by high salt extraction of HeLa nuclei (human cervical cancer cell line), this extract is a rich source of HDAC activity, useful for inhibitor screening or immunoprecipitation of HDAC containing complexes. Typically, 0.5µl or less per well is sufficient for assay with the HDAC *Fluor de Lys* ™ Fluorogenic Activity Assay (BML-AK500).

See page 11 for the substrate preferences of these enzymes.

HDAC & Sirtuin Substrates

The *Fluor de Lys*™ substrates form the core of the *Fluor de Lys*™ Substrate/Developer system for measuring HDAC activity. These include the original *Fluor de Lys*™ Substrate (Prod. No. BML-KI104), which incorporates a single acetylated lysine and is the most versatile of the series. It is acted upon by class I, class II and sirtuin deacetylases, and is suitable for measuring activity from extracts, immunoprecipitated or recombinant protein, and intact cells (see page 9). Further substrates have been developed based on short peptide sequences (4-6 amino acids) from lysine acetylation sites in histones H3 and H4, and p53. Interestingly, we observe selectivity of various enzymes toward these substrates (see Table 1) and other proteins. Therefore, optimal substrates can be selected for any given enzyme. The *Fluor de Lys*™-SIRT1 (Prod. No. BML-KI177), *Fluor de Lys*™-SIRT2 (Prod. No. BML-KI179), and *Fluor de Lys*™-HDAC8 (Prod. No. BML-KI178) substrates are examples, which are deacetylated at a rate 8-, 50- and 10-fold faster than the original Substrate by SIRT1, SIRT2 and HDAC8, respectively. The *Fluor de Lys*™-H4-AcK16 Substrate (Prod. No. BML-KI174) is based on lysine-16 of histone H4, thought to be critical in determining regions of chromatin silencing, and is the preferred substrate for both SIRT1 and SIRT2 within a group patterned on acetylation sites in histone H3 and H4 N-terminal tails. While no substrate is absolutely specific for a given enzyme, the following tables provide our recommendations for the optimal substrates to use with a given enzyme:

Product	Sequence source (a.a.)	Sequence	Prod. No.	Size
Fluor-de-Lys™ Deacetylase Substrate*	ε-acetyl-lysine	K(Ac)	BML-KI104-0050	50 µl
Fluor-de-Lys™-H4-AcK16*	Histone H4 (12-16)	KGGAK(Ac)	BML-KI174-0005	0.5 µmol
Fluor-de-Lys™-HDAC8**	p53 (379-382)	RHK(Ac)K(Ac)	BML-KI178-0005	0.5 µmol
Fluor-de-Lys™-SIRT1**	p53 (379-382)	RHKK(Ac)	BML-KI177-0005	0.5 µmol
Fluor-de-Lys™-SIRT2**	p53 (317-320)	QPKK(Ac)	BML-KI179-0005	0.5 µmol
Fluor-de-Lys™ Developer concentrate			BML-KI105-0300	300 µl
Fluor-de-Lys™ Developer II			BML-KI176-1250	1.25 ml

Other substrates based on acetylated sites can be produced on a custom basis. Contact customquote-usa@enzolifesciences.com.
*Must be used in conjunction with Fluor de Lys™ Developer concentrate (Cat. # BML-KI105)
**Must be used in conjunction with Fluor de Lys™ Developer II (Cat. # BML-KI176)

Substrate Preferences for HDAC & Sirtuin Enzymes

Substrate	HDAC								Sirtuin			
	1	2	3	6	8	10	11	HeLa nuclear extract	1	2	3	5
Fluor de Lys™	+++	++	++++	++	+	++++	++	++	+	+	+	+
Fluor de Lys™-H4-AcK16	+++	+++	++++	+++++	+++	n.d.	+++	+++	++++	+++	+	+++
Fluor de Lys™ HDAC8	++++	+++++	++++	+++++	+++++	n.d.	+++++	+++++	+++	++++	+++	+++++
Fluor de Lys™-SIRT1	+++++	+++++	+++++	+++++	+	+++++	+++++	+++++	+++++	+++++	+++	+++++
Fluor de Lys™-SIRT2	+++	++++	++++	++++	++	+	+	++++	++	+++++	+++++	++++
Fluor de Lys™ Substrate concentration	5µM	5µM	50µM	50µM	25µM	25µM	5µM	25µM	25/500µM	25µM	10µM	500µM

TABLE 1: Substrate preferences for a number of HDACs and sirtuins. The table indicates the substrate preferences for the indicated deacetylase among a panel of *Fluor de Lys*™ substrates based on short stretches of human histones H3, H4 and p53 sequence (see product listing above). The concentration of each substrate is indicated in the last row. For sirtuins the concentration of the co-substrate NAD^+ was 500µM. Class IIa enzymes (HDACs 4, 5, 7 and 9) were not included because they exhibit very little activity with standard peptide substrates (see Unraveling the hidden catalytic activity of vertebrate class IIa histone deacetylases: Lahm et al.; PNAS **104,** 17335 (2007)). n.d. indicates not determined.

Histone Deacetylases (HDACs) & Sirtuins

HDAC Inhibitors

Apicidin

BML-GR340-0001 1 mg

BML-GR340-0005 5 mg

Cyclopeptide class I & II HDAC inhibitor. Apicidin is a novel cyclic tetrapeptide HDAC inhibitor. It inhibits proliferation of human endometrial and ovarian cancer cells but not normal cells. Antiproliferative activity on HeLa cells is accompanied by morphological changes, cell cycle arrest at the G1 phase and induction of $p21^{WAF1/Cip1}$ and gelsolin.

LIT: Apicidin, a novel histone deacetylase inhibitor, has profound anti-growth activity in human endometrial and ovarian cancer cells: T. Ueda, et al.; Int. J. Mol. Med. **19,** 301 (2007) ▪ Apicidin, a histone deacetylase inhibitor, inhibits proliferation of tumor cells via induction of p21WAF1/Cip1 and gelsolin: J.W. Han, et al.; Cancer Res. **60,** 6068 (2000) ▪ Apicidin, a histone deacetylase inhibitor, induces differentiation of HL-60 cells: J. Hong, et al.; Cancer Lett. **189,** 197 (2003) ▪ Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells: S.H. Kwon, et al.; J. Biol. Chem. **277,** 2073 (2002) ▪ Regulation of the HIF-1alpha stability by histone deacetylases: S.H. Kim, et al.; Oncol. Rep. **17,** 647 (2007)

BML-210

BML-GR330-0001 1 mg

BML-GR330-0005 5 mg

Novel HDAC inhibitor developed at Enzo Life Sciences. Inhibits HDAC activity in HeLa nuclear extracts (IC_{50}= 5-10µM).

BML-266

BML-GR346-0010 10 mg

BML-GR346-0050 50 mg

BML-266 is a structurally novel SIRT2 inhibitor (IC_{50}= 56.7 µM).

LIT: N,N'-Bisbenzylidenebenzene-1,4-diamines and N,N'-Bisbenzylidenenaphthalene-1,4-diamines as Sirtuin Type 2 (SIRT2) Inhibitors: P.H. Kiviranta et al.; J Med. Chem. **49,** 7907 (2006)

Depudecin

BML-EI319-0100 100 µg

Depudecin is a fungal metabolite that reverts ras- and src-transformed NIH3T3 cells to a flat phenotype (1µg/ml). It is a potent HDAC inhibitor (IC_{50}=4.7µM for HDAC1) and displays anti-angiogenic activity.

LIT: Depudecin, a microbial metabolite containing two epoxide groups, exhibits anti-angiogenic activity in vivo: T. Oikawa et al.; Biol. Pharm. Bull. **18,** 1305 (1995) ▪ Depudecin induces morphological reversion of transformed fibroblasts via the inhibition of histone deacetylase: H.J. Kwon et al.; PNAS **95,** 3356 (1998) ▪ Synthesis and cellular characterization of the detransformation agent, (-)-depudecin: J. Shimada et al.; Chem. Biol. **2,** 517 (1995) ▪ Depudecin: a novel compound inducing the flat phenotype of NIH3T3 cells doubly transformed by ras- and src-oncogene, produced by Alternaria brassicicola: M. Matsumoto et al.; J Antibiot. (Tokyo) **45,** 879 (1992)

HC Toxin

BML-GR320-0001 1 mg

A potent, cell-permeable histone deacetylase (HDAC) inhibitor. Inhibits HDACs in maize, *Physarum* and chicken. IC_{50}=30nM for *E. tenella* HDAC. Displays antiprotozoal and antineoplastic activity. Induces expression of the γ-globin gene in erythroid cells.

LIT: Inhibition of maize histone deacetylases by HC toxin, the host-selective toxin of Cochliobolus carbonum: G. Brosch et al.; Plant Cell **7,** 1941 (1995) ▪ Apicidin: a novel antiprotozoal agent that inhibits parasite histone deacetylase: S.J. Darkin-Rattray et al.; PNAS 93, 13143 (1996) ▪ Induction of gamma-globin by histone deacetylase inhibitors: P.G. McCaffrey et al.; Blood **90,** 2075 (1997)

ITSA-1

BML-GR350-0025 25 mg

BML-GR350-0100 100 mg

ITSA-1 counteracts trichostatin A-induced cell cycle arrest, histone acetylation and transcriptional activation but not the activity of other HDAC inhibitors.

LIT: Chemical genetic modifier screens: small molecule trichostatin suppressors as probes of intracellular histone and tubulin acetylation: K.M. Koeller et al.; Chem. Biol. **10,** 397 (2003)

M344

ALX-270-297-M001 1 mg

ALX-270-297-M005 5 mg

Potent inhibitor of histone deacetylases (HDACs) (IC_{50}≤1µM).

LIT: Amide analogues of trichostatin A as inhibitors of histone deacetylase and inducers of terminal cell differentiation: M. Jung, et al.; J. Med. Chem. **42,** 4669 (1999)

MC 1293

ALX-270-344-M005 5 mg

Inhibitor of histone deacetylase 1 (HDAC1) (IC_{50} = 4.5µM) and maize histone deacetylase HD2 (IC_{50}=1.9µM).

LIT: 3-(4-aroyl-1H-pyrrol-2-yl)-N-hydroxy-2-propenamides, a new class of synthetic histone deacetylase inhibitors: S. Massa, et al.; J. Med. Chem. **44,** 2069 (2001) ▪ Binding mode analysis of 3-(4-benzoyl-1-methyl-1H-2-pyrrolyl)-N-hydroxy-2-propenamide: a new synthetic histone deacetylase inhibitor inducing histone hyperacetylation, growth inhibition, and terminal cell differentiation: A. Mai, et al.; J. Med. Chem. **45,** 1778 (2002)

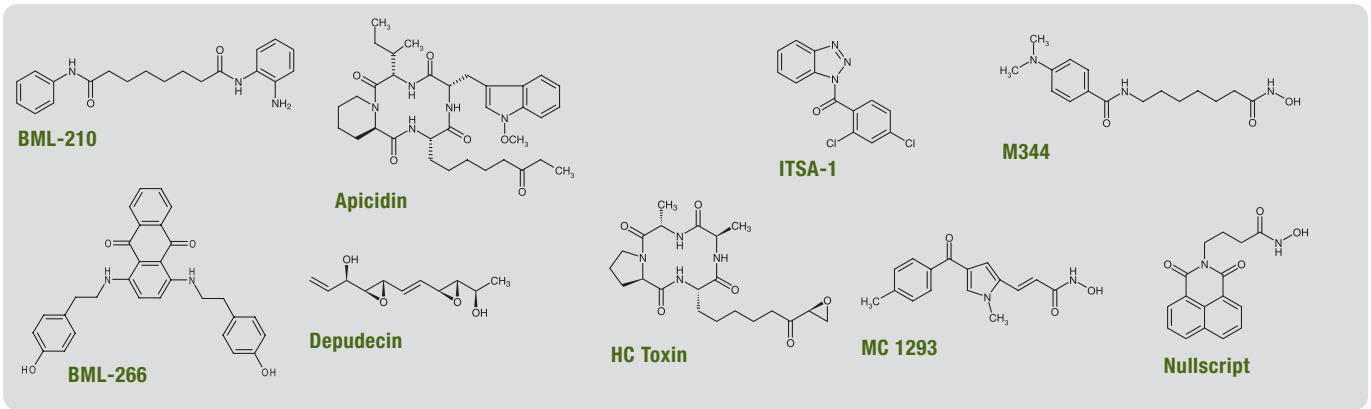
Nullscript

BML-GR327-0001 1 mg

BML-GR327-0005 5 mg

A negative control compound for the histone deacetylase inhibitor scriptaid.

LIT: A novel histone deacetylase inhibitor identified by high-throughput transcriptional screening of a compound library: G.H. Su et al.; Cancer Res. **60,** 3137 (2000)



Oxamflatin

ALX-270-379-M001 1 mg

ALX-270-379-M005 5 mg

Potent inhibitor of mammalian HDACs (IC_{50}=15.7nM). Acts as a ligand for the enzyme active site metal ion. Elevates the expression of extracellular matrix proteins fibronectin and gelsolin. Induces apoptosis in P-glyoprotein (Pgp) positive and Pgp negative multidrug resistant cells.

LIT: Oxamflatin: a novel compound which reverses malignant phenotype to normal one via induction of JunD: H. Sonoda, et al.; Oncogene **13,** 143 (1996) ▪ Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase: Y.B. Kim, et al.; Oncogene **18,** 2461 (1999) ▪The novel anti-tumour agent oxamflatin differentially regulates urokinase and plasminogen activator inhibitor type 2 expression and inhibits urokinase-mediated proteolytic activity: A.E. Dear & R.L. Medcalf; Biochim. Biophys. Acta **1492,** 15 (2000) ▪ Novel mechanisms of apoptosis induced by histone deacetylase inhibitors: M.J. Peart, et al.; Cancer Res. **63,** 4460 (2003)

Phenylbutyrate sodium

BML-EI320-0001 1 g

Weak HDAC inhibitor. Induces differentiation, growth arrest and apoptosis in a number of cell lines. Protects against cerebral ischemic injury and displays neuroprotective effect in a mouse model of Huntington`s disease.

LIT: Neuroprotective effects of phenylbutyrate in the N171-82Q transgenic mouse model of Huntington's disease: G. Gardian et al.; J Biol. Chem. **280,** 556 (2005) ▪ Sodium 4-phenylbutyrate protects against cerebral ischemic injury: X. Qi et al.; Mol Pharmacol. **66,** 899 (2004) ▪ Histone deacetylase inhibitor 4-phenylbutyrate suppresses GAPDH mRNA expression in glioma cells: I.B. Appelskog et al.; Int. J Oncol. **24,** 1419 (2004) ▪ Sodium 4-phenylbutyrate induces apoptosis of human lung carcinoma cells through activating JNK pathway: X. Zhang et al.; J Cell Biochem. **93,** 819 (2004)

Scriptaid

BML-GR326-0001 1 mg

BML-GR326-0005 5 mg

A novel histone deacetylase inhibitor with lower toxicity compared to Trichostatin A. Optimal concentration is 2-2.5µg/ml (6-8µM). A negative control compound, Nullscript, is also available.

LIT: A novel histone deacetylase inhibitor identified by high-throughput transcriptional screening of a compound library: G.H. Su et al.; Cancer Res. **60,** 3137 (2000)

Sodium butyrate

[Butyric acid . sodium salt]

ALX-270-301-G001 1 g

Short-chain fatty acid shown to be an inhibitor of histone deacetylases (HDACs). Induces apoptosis.

LIT: Sodium butyrate inhibits histone deacetylation in cultured cells: E.P. Candido, et al.; Cell **14,** 105 (1978) ▪ Reduction of telomerase activity in human liver cancer cells by a histone deacetylase inhibitor: M. Nakamura, et al.; J. Cell Physiol. **187,** 392 (2001) ▪ Effects of retinoic acid and sodium butyrate on gene expression, histone acetylation and inhibition of proliferation of melanoma cells: K. Demary, et al.; Cancer Lett. **163,** 103 (2001) ▪ **For a comprehensive bibliography please visit our website.**

Splitomicin

BML-GR331-0005 5 mg

BML-GR331-0025 25 mg

Splitomicin is a potent and selective inhibitor of Sir2 HDAC activity (IC_{50}=60 µM).

LIT: Identification of a small molecule inhibitor of Sir2p: A. Bedalov et al.; PNAS **98,** 15113 (2001)

Suberoyl bis-hydroxamic acid

BML-GR323-0100 100 mg

BML-GR323-0500 500 mg

Induces cellular differentiation. Inhibits histone deacetylases.

LIT: Cytodifferentiating agents affect the replication of herpes simplex virus type 1 in the absence of functional VP16: C.M. Preston & McFarlane, M.; Virology **249,** 418 (1998)

Trichostatin A

BML-GR309-0001 1 mg

BML-GR309-0005 5 mg

Trichostatin A (TSA) is a potent and reversible inhibitor of histone deacetylases. In HeLa cells, TSA blocked cell cycle progression at G1 and induced a 12-fold increase in intracellular levels of gelsolin. In cells latently infected with HIV-1, TSA induced the transcriptional activation of the HIV-1 promoter, which resulted in a marked increase in virus production. In NIH 3T3 cells, TSA induced reversion of oncogenic ras-transformed cells to a normal morphology. In Jurkat cells, TSA inhibited IL-2 gene expression (IC_{50}=73nM) and displayed immunosuppressive activity in a mouse model. Induces increased acetylation of GATA4, a cardiac-specific transcription factor and increases cardiac muscle cell differentiation. Trichostatin A is a useful tool for induction of hyperacetylation of cellular histones and for further elucidation of their role in gene expression.

LIT: Histone deacetylase: a regulator of transcription: A.P. Wolffe; Science **272,** 371 (1996) ▪ Trichostatin A induces morphological changes and gelsolin expression by inhibiting histone deacetylase in human carcinoma cell lines: Y. Hoshikawa, et al.; Exp. Cell Res. **214,** 189 (1994) ▪ Trichostatin A inhibits both ras-induced neurite outgrowth of PC12 cells and morphological transformation of NIH3T3 cells: M. Futamura, et al.; Oncogene **10,** 1119 (1995) ▪ Selective inhibition of IL-2 gene expression by trichostatin A, a potent inhibitor of mammalian histone deacetylase: I. Takahashi, et al.; J. Antibiot. (Tokyo) **49,** 453 (1996)

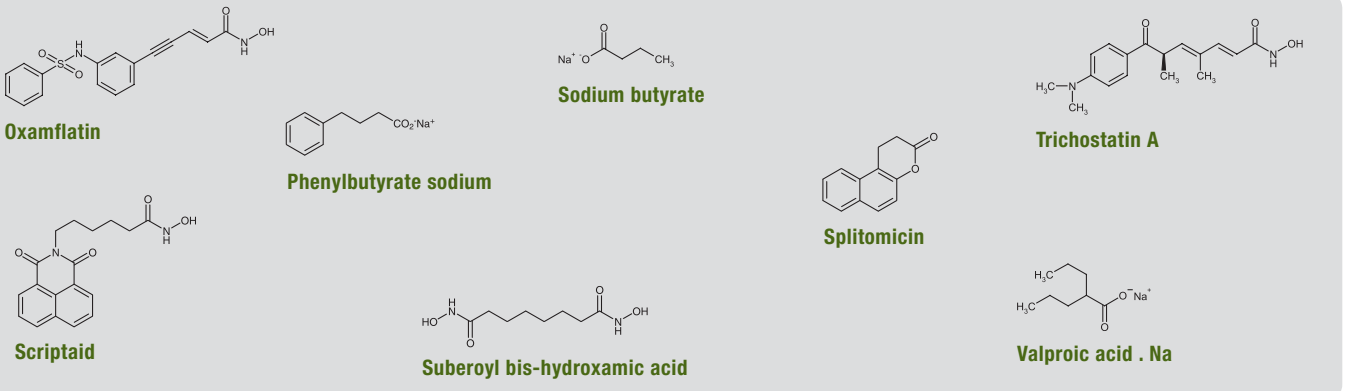
Valproic acid . Na

[2-Propylpentanoic acid . Na; Sodium 2-propylpentanoate]

ALX-550-304-G005 5 g

Anticonvulsant. Anti-depressant. Potent teratogen. Inhibits histone deacetylase 1 (HDAC1) (IC_{50}=0.4mM). Inhibitor of human cytochrome P450 2C9 isoform (K_i=600µM). Inducer of apoptosis in human leukemia cells. In nude mice experiments, inhibits significantly human uterine tumor growth without toxic side effects.

LIT: Sodium valproate: a review of its pharmacological properties and therapeutic efficacy in epilepsy: R.M. Pinder, et al.; Drugs **13,** 81 (1977) ▪ Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen: C.J. Phiel, et al.; J. Biol. Chem. **276,** 36734 (2001) ▪ Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells: M. Gottlicher, et al.; Embo J. **20,** 6969 (2001) ▪ Valproic acid induces apoptosis in human leukemia cells by stimulating both caspase-dependent and -independent apoptotic signaling pathways: R. Kawagoe, et al.; Leuk. Res. **26,** 495 (2002) ▪ Histone deacetylase inhibitors have a profound antigrowth activity in endometrial cancer cells: N. Takai, et al.; Clin. Cancer Res. **10,** 1141 (2004) ▪ Transcription factor NF-kappaB differentially regulates death receptor 5 expression involving histone deacetylase 1: S. Shetty, et al.; Mol. Cell. Biol. **25,** 5404 (2005) ▪ **For a comprehensive bibliography please visit our website.**



Histone Deacetylases (HDACs) & Sirtuins

Sirtuin Activators

In 2003, BIOMOL scientists discovered that resveratrol and other polyphenols activate SIRT1 and, in subsequent collaboration with researchers at Harvard University, Brown University, and the University of Connecticut, found that activators of sirtuins extend lifespan in *S. cerevisiae* [1,2], *C. elegans* and *Drosophila melanogaster* [3]. This has led to investigations into the therapeutic potential of sirtuin activators for a variety of age-related disorders including diabetes, metabolic, neurodegenerative and cardiovascular disease [4-7]. Indeed, SIRT1 activators mimic the beneficial effects of calorie restriction in lower organisms and prolong survival of mice fed a high-fat diet [8-10]. Furthermore, pharmacological activation of SIRT1 with resveratrol or other sirtuin activators attenuates the generation of Aβ peptides *in vitro*, promotes the survival of mouse dopaminergic neurons Parkinson's models, and rescues neurons from polyglutamine-specific cell-death in Huntington's disease models [11-13]. Whether these effects are entirely and directly due to the sirtuin stimulation properties of these molecules requires further investigation, but nevertheless, these findings provide encouraging data for further study and testing of sirtuin activators as potential therapeutics.

LIT: [1] Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan: K.T. Howitz et al.; Nature **425,** 191 (2003) ▪ **[2]** Design and synthesis of compounds that extend yeast replicative lifespan: H. Yang et al.; Aging Cell **6,** 35 (2007) ▪ **[3]** Sirtuin activators mimic caloric restriction and delay ageing in metazoans: J. G. Wood et al.; Nature **430,** 686 (2004) ▪ **[4]** A review of Sirt1 and Sirt1 modulators in cardiovascular and metabolic diseases: S. Pillarisetti; Recent Pathents Cardiovasc. Drug Discov. **3,** 156 (2008) ▪ **[5]** Sirtuins: novel targets for metabolic disease in drug development: W.J. Jiang; BBRC **373,** 341 (2008) ▪ **[6]** Therapeutic role of sirtuins in neurodegenerative disease: T.F. Outeiro et al.; Biochim. Biophys. Acta **1782,** 363 (2008) ▪ **[7]** Therapeutic potential of sirtuin-activating compounds in Alzheimer's disease: L. Gan Drug News Perspect. **20,** 233 (2007) ▪ **[8]** Resveratrol improves health and survival of mice on a high-calorie diet: J.A. Baur JA et al.; Nature **444,** 337 (2006) ▪ **[9]** Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha; M. Lagouge et al.; Cell **127,** 1109 (2006) ▪ **[10]**. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes: J.C. Milne et al.;Nature **450,** 712 (2007) ▪ **[11]** Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer disease amyloid neuropathology by calorie restriction: W. Qin et al.; J. Biol. Chem. **281,** 21745 (2006) ▪ **[12]** Resveratrol protects dopaminergic neurons in midbrain slice culture from multiple insults: M. Okawara et al.; Biochem. Pharmacol. **73,** 550 (2007) ▪ **[13]** Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons: J.A. Parker et al.; Nat. Genet. **37,** 349 (2005)

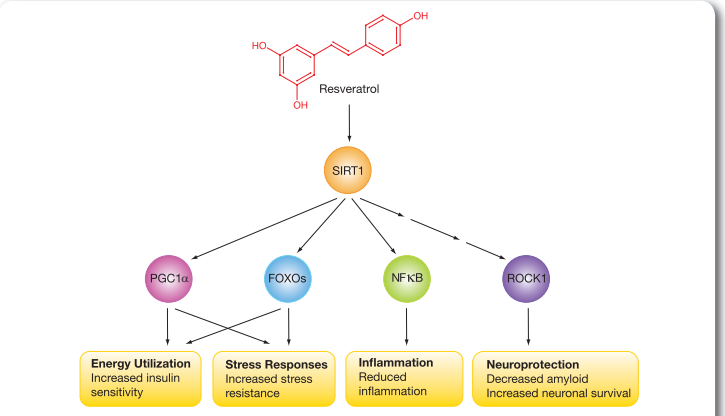


FIGURE 8: Stimulation of SIRT1 and perhaps other sirtuins modulates key signal transduction pathways and transcriptional mechanisms to have beneficial effects on energy utilisation, stress responses, inflammation and neuronal survival.

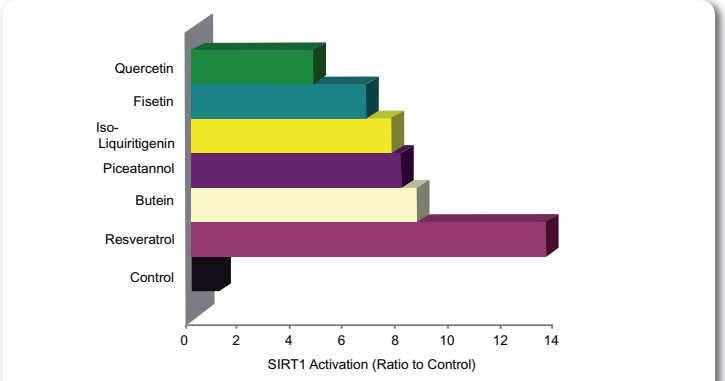


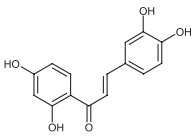
FIGURE 9: SIRT1 Activation by various polyphenols using the SIRT1 Drug Discovery Assay Kit (Prod. No. BML-BML-AK555). Initial deacetylation rates of SIRT1 were determined at 25μM *Fluor de Lys*™–SIRT1 (Prod. No. BML-KI177), 25μM NAD^+ (37°C) in the absence (Control) or presence of 100μM of the indicated compound.

Butein

ALX-350-246-M010 — 10 mg

Plant polyphenol. Activator of human deacetylase SIRT1.

LIT: Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan: K.T. Howitz, et al.; Nature **425,** 191 (2003) ▪ **For a comprehensive bibliography please visit our website.**

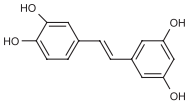


Piceatannol

ALX-270-202-M001 — 1 mg
ALX-270-202-M005 — 5 mg
ALX-270-202-M010 — 10 mg
ALX-270-202-M050 — 50 mg

Synthetic. Originally isolated from *Euphorbia lagascae.* Activator of human deacetylase SIRT1.

LIT: Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan: K.T. Howitz, et al.; Nature **425,** 191 (2003) ▪ **For a comprehensive bibliography please visit our website.**

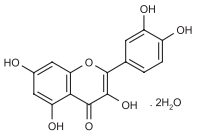


Quercetin . $2H_2O$

ALX-385-001-G005 — 5 g
ALX-385-001-G025 — 25 g

Isolated from *Sophora japonica L.* Antioxidant flavonoid.

LIT: Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan: K.T. Howitz, et al.; Nature **425,** 191 (2003) ▪ **For a comprehensive bibliography please visit our website.**

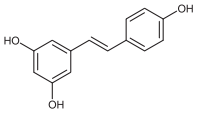


Resveratrol

BML-FR104-0100 — 100 mg
BML-FR104-0500 — 500 mg

A phenolic antioxidant found in grapes and wine. Activates SIRT1.

LIT: Inhibition of protein kinase D by resveratrol: R.S. Haworth & Avkiran, M.; Biochem. Pharmacol. **62,** 1647 (2001) ▪ Resveratrol induces extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in acute lymphoblastic leukemia cells: J. Dorrie et al.; Cancer Res. 61, 4731 (2001) ▪ Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan: K.T. Howitz et al.; Nature **425,** 191 (2003) ▪ **For a comprehensive bibliography please visit our website.**



Sirtuin Inhibitors

6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxamide

[EX-527]

ALX-270-437-M001 1 mg

Potent cell permeable and metabolically stable specific inhibitor of hSIRT1 (IC_{50}=98nM *in vivo* / IC_{50}=38nM *in vitro*; compared to hSIRT2: IC_{50}=19μM and hSIRT3: IC_{50}=48μM) with no effect on human histone deacetylases (HDACs) class I and class II, nor NAD glycohydrolase (IC_{50}>100μg). Inhibits the deacetylation of p53 (IC_{50}=1μM).

LIT: Discovery of indoles as potent and selective inhibitors of the deacetylase SIRT1: A.D. Napper, et al.; J. Med. Chem. **48,** 8045 (2005) ▪ Inhibition of SIRT1 Catalytic Activity Increases p53 Acetylation but Does Not Alter Cell Survival following DNA Damage: J.M. Solomon, et al.; Mol. Cell. Biol. **26,** 28 (2006) ▪ Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes: J.C. Milne, et al.; Nature **450,** 712 (2007)

AGK2

ALX-270-484-M001 1 mg

ALX-270-484-M005 5 mg

Potent, selective and cell permeable inhibitor of sirtuin 2 (SIRT2) (IC_{50}=3.5μM). Rescues α-synuclein-mediated toxicity. Modifies inclusion morphology in a cellular model of Parkinson's disease. Protects against dopaminergic cell death. Leads to an increase in acetylated tubulin.

LIT: Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease: T.F. Outeiro, et al.; Science **317,** 516 (2007)

Aristoforin

ALX-350-129-M001 1 mg

Stable and water-soluble derivative of hyperforin (Prod. No. ALX-350-097) inducing apoptosis. Antitumor agent. Inhibits sirtuins.

LIT: Aristoforin, a novel stable derivative of hyperforin, is a potent anticancer agent: M. Gartner, et al.; Chembiochem. **6,** 171 (2005) ▪ Phloroglucinol Derivatives Guttiferone G, Aristoforin, and Hyperforin: Inhibitors of Human Sirtuins SIRT1 and SIRT2: C. Gey, et al.; Angew. Chem. Int. Ed. Engl. **46,** 5219 (2007)

BML-266

BML-GR346-0010 10 mg

BML-GR346-0050 50 mg

BML-266 is a structurally novel SIRT2 inhibitor (IC_{50}= 56.7μM).

Suramin . 6Na

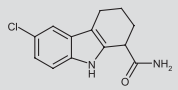
ALX-430-022-M050 50 mg

ALX-430-022-M250 250 mg

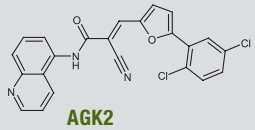
ALX-430-022-G001 1 g

Polysulfonated naphthylurea. P_{2x} and P_{2y} purinergic receptor antagonist. Antitumor, antiangiogenic and antiparasitic compound. Inhibits sirtuin 1, sirtuin 5, topoisomerase II and several growth factors, including FGFa, FGFb and PGDF. Blocks association of G protein α and β/γ-subunits.

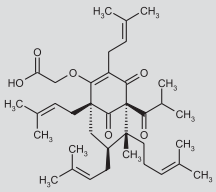
LIT: Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan: K. T. Howitz, et al.; Nature **425,** 191 (2003) ▪ Regulation of SIRT 1 mediated NAD dependent deacetylation: a novel role for the multifunctional enzyme CD38: P. Aksoy, et al.; BBRC **349,** 353 (2006) ▪ Structural basis of inhibition of the human NAD+-dependent deacetylase SIRT5 by suramin: A. Schuetz, et al.; Structure **15,** 377 (2007) ▪ Structure-activity studies on suramin analogues as inhibitors of NAD+-dependent histone deacetylases (sirtuins): J. Trapp, et al.; ChemMedChem **2,** 1419 (2007) ▪ DBC1 is a negative regulator of SIRT1: J.E. Kim, et al.; Nature **451,** 583 (2008) ▪ The Sirtuin family: therapeutic targets to treat diseases of aging: J.C. Milne & J.M. Denu; Curr. Opin. Chem. Biol. **12,** 11 (2008) ▪ **For a comprehensive bibliography please visit our website.**



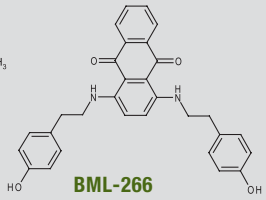
6-Chloro-2,3,4,9-tetrahydro-1H-carbazole-1-carboxamide



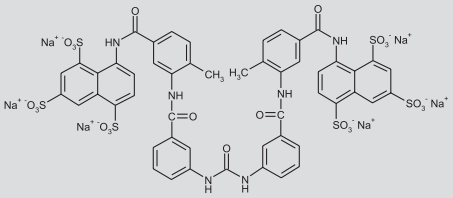
AGK2



Aristoforin



BML-266



Suramin . 6Na

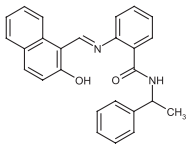
Sirtinol

ALX-270-308-M001 1 mg

ALX-270-308-M005 5 mg

Specific cell permeable inhibitor of the sirtuin family of NAD-dependent deacetylases (ySir2: IC_{50}=48μM; hSIRT1: IC_{50}=131μM; hSIRT2: IC_{50}=58μM) with no effect on human HDAC1. Reported to inhibit Sir2p transcriptional silencing activity *in vivo* (IC_{50}=25μM) and NAD-dependent histone deacetylase activity of purified recombinant yeast Sir2p (IC_{50}=70μM) and hSIRT2 (IC_{50}=40μM) *in vitro*.

LIT: Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening: C.M. Grozinger, et al.; J. Biol. Chem. **276,** 38837 (2001) ▪ Human telomeric position effect is determined by chromosomal context and telomeric chromatin integrity: C.E. Koering, et al.; EMBO Rep. **3,** 1055 (2002) ▪ Design, synthesis, and biological evaluation of sirtinol analogues as class III histone/protein deacetylase (Sirtuin) inhibitors: A. Mai, et al.; J. Med. Chem. **48,** 7789 (2005) ▪ Sirt1 inhibitor, Sirtinol, induces senescence-like growth arrest with attenuated Ras-MAPK signaling in human cancer cells: H. Ota, et al.; Oncogene **25,** 176 (2006)



HIGHLIGHT

Histone Deacetylases (HDACs) & Sirtuins

HDAC & Sirtuin Antibodies

Product	Specificity	Application	Prod. No.	Size
HDAC1, mAb (10E2)	Human and mouse	IHC, ICC, IP, WB	ALX-804-599-C200	200 µg
HDAC1, pAb	Human, mouse, rat, hamster and dog	IHC, ICC, WB	ALX-210-256-C100	100 µg
HDAC1, pAb	Human, rat and mouse	IHC, IP, WB	BML-SA401-0100	100 µg
HDAC2, pAb	Human, rat and mouse	IHC, IP, WB	BML-SA402-0100	100 µg
HDAC2, pAb	human, mouse, rat, hamster and dog	FC, IHC, ICC, IP, WB	ALX-210-257-C100	100 µg
HDAC3, pAb	Human, canine, hamster and rat	IP, WB	BML-SA403-0100	100 µg
HDAC3, pAb	Human, rat, hamster and dog	WB	ALX-210-258-C100	100 µg
HDAC4 (human), pAb	Human	WB	BML-SA404-0100	100 µg
HDAC4 (human) (CT), pAb	Human	WB	ALX-210-338-C100	100 µg
HDAC4 (NT), pAb	Human and mouse	WB	ALX-210-339-C100	100 µg
HDAC5, pAb	Human and mouse	IP, WB	ALX-210-340-C100	100 µg
HDAC6, pAb	Human and mouse	IP, WB	ALX-210-341-C100	100 µg
SIRT1 (human), pAb	Human	WB	BML-SA427-0100	100 µl
SIRT1 (human), pAb	Human	ELISA, WB	ALX-210-486-C100	100 µg
SIRT2 (human), pAb	Human	WB	BML-SA444-0100	100 µl
SIRT2 (human), pAb	Human	ELISA, WB	ALX-210-487-C100	100 µg
SIRT3, pAb	Human, rat	WB	BML-SA463-0100	100 µl
SIRT5, pAb	Human, mouse, rat	WB	BML-SA464-0100	100 µl
AROS (human), pAb (AT135)	Human	WB	ALX-210-961-C100	100 µg
RbAp46 (human), pAb	Human	IHC, WB	ALX-210-287-C100	100 µg
RbAp48 (human), pAb	Human	WB	ALX-210-288-C100	100 µg

Activity Assay of Immunoprecipitated HDACs

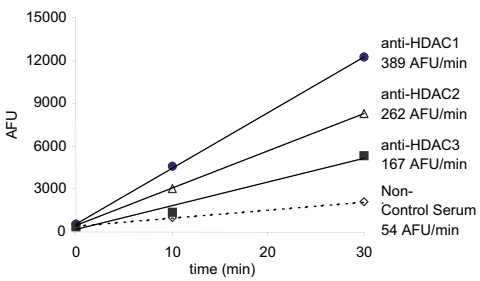


FIGURE 10: Activity assay of immunoprecipitated HDACs using BML-SA401, BML-SA402 and BML-SA403. HDAC's were immunoprecipitated from HeLa Nuclear Extract (BML-KI140) using 10µg of each anti-HDAC antibody (Anti-HDAC's 1-3 are Prod. No. BML-SA401, BML-SA402, BML-SA403) or 10µl of control serum. The washed beads with bound HDAC/Anti-HDAC complexes were incubated with *Fluor de Lys*™ Substrate for indicated time and assayed using the *Fluor de Lys*™ HDAC Assay (BML-AK500).

Arginine and Lysine Methyltransferases

Methylation of proteins on the epsilon-amino group of lysine or the guanidine group of arginine using the S-adenosyl-L-methionine (SAM) as the methyl group donor has been described for some time [1,2]. Lysine can be mono-, di- and trimethylated, while arginine can be both monomethlated symmetrically or asymmetrically dimethylated (see figure 11). Multiple lysine and arginine residues present in histone N-terminal tails, many of which are methylated *in vivo*, and the various degrees of methylation has the potential to encode a great deal of regulatory information into chromatin. Histone lysine methylation is catalysed by a family of proteins that contain a SET domain and by yeast Dot1 and its mammalian homologue, DOT1L, which use a novel enzymatic domain [3,4]. Arginine methylation is performed by the PRMT class of histone methyltransferases [5]. LSD1, a flavin-containing amine oxidase homolog and component of various corepressor complexes, was the first enzyme demonstrated to be capable of lysine demethylation and is elevated in a number of cancers [6,7]. The identification of LSD1 demonstrated that methylation is reversible and opened the door to the identification of a much larger family of demethylase enzymes, namely the Jumonji C (JmjC) domain proteins, a large family comprising 28 enzymes in humans [8]. The recent identification of histone demethylase enzymes, and the characterisation of their role in cancer in other diseases, provides a new avenue for research into epigenetic regulation and a set of novel targets for pharmacological intervention.

LIT: [1] The diverse functions of histone lysine methylation: C. Martin & Y. Zhang; Nat. Rev. Mol. Cell Biol. **6,** 838 (2005) ▪ **[2]** The many faces of histone lysine methylation: M. Lachner & T Jenuwein; Curr. Opin. Cell Biol. **14,** 286 (2002) ▪ **[3]** Histone methyltransferases in tumor suppression: K.C. Kim & S. Huang; Cancer. Biol. Ther. **2,** 491 (2003) ▪ **[4]** Structure and function of histone methyltransferases: R.C. Trievel; Crit Rev Eukaryot Gene Expr **14,** 147 (2004) ▪ **[5]** Genome regulation by polycomb and trithorax proteins: B. Schuettengruber, et al.; Cell **128,** 735 (2007) ▪ **[6]** Histone demethylation mediated by the nuclear amine oxidase homolog LSD1: Y. Shi et al.; Cell **119,** 941 (2004) ▪ **[7]** Histone H3 lysine 4 demethylation is a target of nonselective antidepressive medications: M.G. Lee et al.; Chem. Biol. **13,** 563 (2006) ▪ **[8]** Regulation of histone methylation by demethylimination and demethylation: R. J. Klose & Y. Zhang; Nat. Rev. Mol. Cell Biol. **8,** 307 (2007)

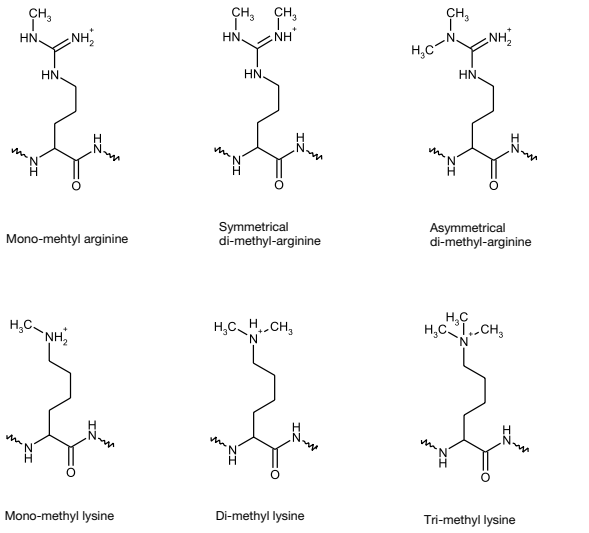


FIGURE 11: Methylation states of arginine and lysine residues in histones. Arginine can be methylated to form mono-methyl, symmetrical di-methyl and asymmetrical di-methylarginine. Lysine can be methylated to form mono-methyl, di-methyl and tri-methyl lysine.

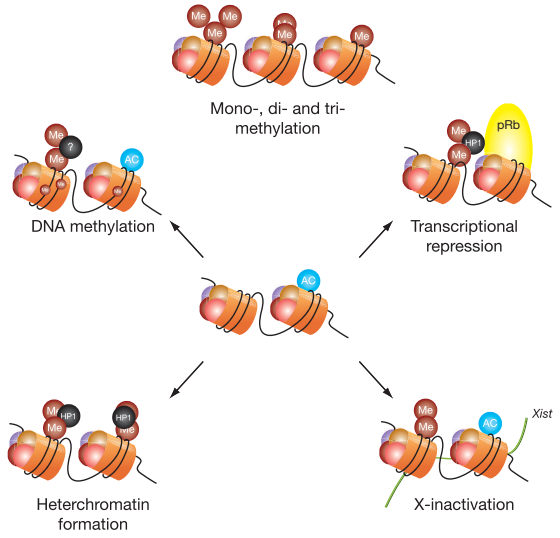


FIGURE 12: Mono-, di- and tri- methylation of histones play a roles in a variety of cellular processes.

*Adapted from: The many faces of histone lysine methylation: M. Lachner and T. Jenuwein; Curr. Opin. Cell Biol. **14,** 286 (2002)*

Enzymes

CARM1 (human), (rec.) (His-tag)

[Coactivator-associated arginine methyltransferase 1; PRMT4; Protein arginine N-methyltransferase 4]

ALX-201-283-C002 2 µg

Produced in SF21 cells. Fused to a His-tag.

LIT: Arginine methyltransferase CARM1 is a promoter-specific regulator of NF-κB-dependent gene expression: M. Covic, et al.; EMBO J. **24,** 85 (2005)

PRMT1 (human), (rec.) (His-tag)

[Protein arginine N-methyltransferase 1]

ALX-201-282-C002 2 µg

Produced in SF21 cells. Fused to a His-tag.

SET7/9 (human), (rec.)

[Histone H3-K4 methyltransferase]

ALX-201-178-C100 100 µg

Produced in *E. coli*.

LIT: Mechanism of histone lysine methyl transfer revealed by the structure of SET7/9-AdoMet: T. Kwon, et al.; EMBO J. 22, 292 (2003)

Antibody

SET7/9 (human), mAb (s4E5)

ALX-804-567-R050 50 µl

ALX-804-567-R100 100 µl

CLONE: s4E5. **ISOTYPE:** Mouse IgG2b. **IMMUNOGEN:** Recombinant human SET7/9. **SPECIFICITY:** Recognizes human SET7/9. **APPLICATION:** ELISA, WB.

LIT: Mechanism of histone lysine methyl transfer revealed by the structure of SET7/9-AdoMet: T. Kwon, et al.; EMBO J. **22,** 292 (2003) ▪ Structure and catalytic mechanism of the human histone methyltransferase SET7/9: B. Xiao, et al.; Nature **421,** 652 (2003)

Arginine and Lysine Methyltransferases

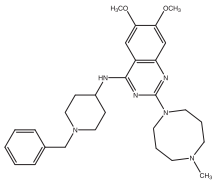
Inhibitors

BIX 01294

ALX-270-473-M002 2 mg

Selective and cell permeable inhibitor of G9a histone methyltransferase.

LIT: Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase: S. Kubicek, et al.; Mol. Cell **25,** 473 (2007)

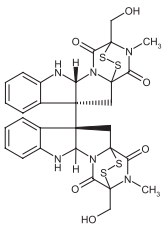


Chaetocin

BML-GR349-0200 200 µg

Chaetocin is a thiodioxopiperazine natural product produced by *Chaetomium* species. It is the first known inhibitor of the lysine-specific histone methyltransferase. It displays potent antimyeloma activity in IL-6-dependent myeloma cell lines. Its antimyeloma activity appears to be due to induction of oxidative stress and consequent apoptosis.

LIT: Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3-9: D. Greiner, et al.; Nat. Chem. Biol. **1,** 143 (2005) ▪ Chaetocin: a promising new antimyeloma agent with in vitro and in vivo activity mediated via imposition of oxidative stress: C.R. Isham, et al.; Blood **109,** 2579 (2007)



Lysine Demethylases

LSD1 Fluorometric Drug Discovery Kit

- Sensitive measurement of LSD1 demethylase activity using the Histone H3 Dimethyl Lysine-4 Peptide
- CELLestial™ Red Substrate allows real-time fluorometric or colorimetric detection
- Single-step, homogenous assay ideal for HTS applications
- >1000U of LDS1 supplied with each kit

Product	Prod. No.	Size
LSD1 Fluorometric Drug Discovery Kit	BML-AK544-0001	1 Kit

Enzyme

LSD1 (human), (rec.)

[Lysine-specific histone demethylase 1]

BML-SE544-0050 50 µg

Produced in *E. coli*. Human LSD1 (lysine-specific histone demethylase 1) (aa 151-852). Highly active in a peroxidase-coupled assay with the Histone H3 Dimethyl Lysine-4 Peptide (Prod. No. BML-P256).

Substrate

Histone H3 dimethyl lysine-4 peptide

BML-P256-0500 0.5 mg

Residues 1-21 from the N-terminal tail of human histone H3, dimethylated on the side-chain (ε-amino function) of Lys[4]. Useful as a substrate for histone lysine demethylases, for example LSD1.

Inhibitor

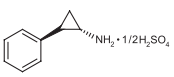
Tranylcypromine

BML-EI217-0001 1 g

BML-EI217-0005 5 g

Inhibits prostacyclin synthase. Monoamine oxidase inhibitor. Effective small molecule inhibitor of histone demethylation. Inhibits the demethylase BHC110/LSD1 (IC_{50}<2µM).

LIT: Histone H3 lysine 4 demethylation is a target of nonselective antidepressive medications: M.G. Lee et al.; Chem. Biol. **13,** 563 (2006) ▪ Mechanism of inactivation of monoamine oxidase by 1-phenylcyclopropylamine: R.B. Silverman & Zieske, P. A.; Biochemistry **24,** 2128 (1985)



Ubiquitin & SUMO Modifications

Core histones are monoubiquitinylated and SUMOylated, but in many cases the specific site of modification or functional consequence the modification is unknown [1,2]. Perhaps the best exception is ubiquitinylation of Lys^{119} of histone H2A and Lys^{120} of histone H2B, marks which are involved in crosstalk with other epigenetic modifications in the chromatin, particularly methylation of Lys^4 of histone H3, and known to regulate transcription and DNA repair [3]. Ubiquitinylation of H2A and H2B result in different outcomes, with ubiquitinylation of H2B being required for Lys^4 H3 methylation and having roles in both transcriptional activation and repression whereas ubiquitinylation of H2A inhibits Lys^4 H3 methylation and is strictly a repressive mark. Ubiquitinylation and deubiquitinylation of H2A and H2B occurs through the action of distinct enzymes, but in both cases it is common that the ubiquitinylation and deubiquitinylation machinery is associated with complexes having histone acetyltransferase, histone methylase and RNA polymerase activities. These associations suggest that the histone ubiquitinylation is connected to a wide range of epigenetic and transcriptional mechanisms.

While SUMOylation can have diverse effects on transcription, SUMOyation is most often correlated with transcriptional repression [4]. Many of the enzymes that modify histones are SUMOylated (see table 2) [5] suggesting a complex interplay between SUMOylation and chromatin structure. In particular, corepressors such as HDACs are effectors, substrates and regulators of SUMOylation suggesting a role for SUMOylation in regulating the acetylation state of chromatin. Targeting the SUMO E2 conjugating enzyme Ubc9 to chromatin represses transcription, and SUMOylation of histone H4 increases its association with HDAC1 and HP1, two transcriptional corepressors [2]. Further study is required to characterise the changes in chromatin structure that result from SUMOylation and to determine the proteins that contribute to SUMO-dependent inhibition of transcription.

LIT: [1] Regulation of histone H2A and H2B ubiquitylation: M.A. Osley; Brief Funct. Genomic Proteomic. **5,** 179 (2006) ▪ **[2]** Histone sumoylation is associated with transcriptional repression: Y. Shiio & R.N. Eisenman; PNAS **100,** 13225 (2003) ▪ **[3]** Histone ubiquitination: triggering gene activity: V.M. Weake & J.L. Workman; Mol. Cell. **29,** 653 (2008) ▪ **[4]** Something about SUMO inhibits transcription: G. Gill; Curr. Opin. Genet. Dev. **15,** 536 (2005) ▪ **[5]** SUMO and ubiquitin in the nucleus: different functions, similar mechanisms?: G. Gill Genes Dev. **18,** 2046 (2004)

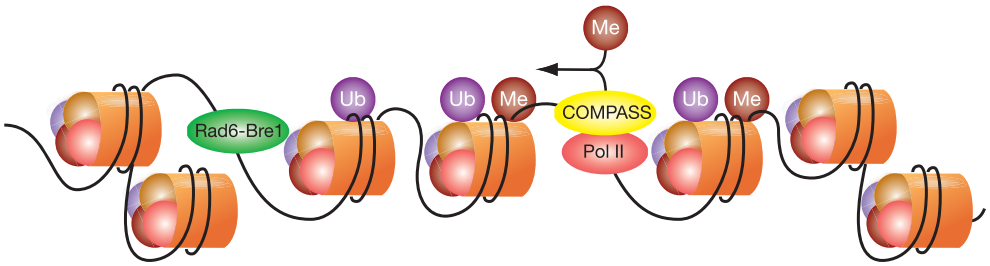


FIGURE 13: Cross-talk between H2B ubiquitinylation and H3K4 methylation. Methylation of H3K4 by COMPASS requires that H2B is ubiquitinylated by the ubiquitin conjugating enzyme Rad6 and ubiquitin ligase Bre1.

Adapted from: Histone cross-talk in stem cells: E. Smith and A. Shilatifard; Science. ***323,*** *221 (2009)*

Genome maintenance and structure	Transcription cofactors
Topoisomerase 1	CtBP
Topoisomerase 2	HDAC1
γPCNA	p300
Histone H4	TIF1α
WRN helicase	GRIP-1
Thymidine-DNA glycosylase enzyme (TDG)	HDAC4
	SRC-1

TABLE 2: SUMO-modified proteins involved in genome maintenance and structure and transcription.

Purified (PF) = Purified (Preservative free); FC = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

Ubiquitin & SUMO Modifications

Ubiquitinylation and SUMOylation Kits

Ubiquitinylation kit

BML-UW9920-0001 1 Kit

For generation of ubiquitin-E2 thioesters for use in ubiquitinylation experiments. **QUANTITY:** Provides sufficient material for 50 x 50µl reactions.

Suggested uses

- Generation of ubiquitin-E2 thioesters for use in ubiquitinylation experiments
- Ubiquitinylation of target proteins in the presence of a dedicated E3 ligase
- Activation of ubiquitin for thioester conjugation to novel E2 enzymes
- Use of cell lysate or crude fractions/preparations as source of E3 ligases to facilitate ubiquitinylation
- Substrate (target) independent *in vitro* ubiquitinylation reactions

SUMOylation kit

BML-UW8955-0001 1 Kit

This kit provides a means of generating SUMOylated proteins *in vitro*. **QUANTITY:** Provides sufficient material for 20 x 20µl reactions.

Suggested uses

- For SUMO-modification of specific proteins *in vitro*
- To demonstrate that novel proteins are potential targets for SUMOylation under *in vitro* conditions
- To generate substrates for deSUMOylating enzymes, such as SENP1 and SENP2
- To test proteins for SUMO E3 ligase activity.

Ubiquitin conjugating kit (HeLa lysate-based)

BML-UW9915-0001 1 Kit

For generation of ubiquitin conjugated proteins. **QUANTITY:** Provides sufficient material for 20 × 50µl reactions.

Suggested uses

- Generation of ubiquitin conjugated proteins. Exogenous or endogenous HeLa lysate proteins (tagged/radio-labeled/immuno-detectable) can be ubiquitinylated followed by immediate detection/analysis. Subsequent analysis could include proteasomal degradation, ubiquitin modification site mapping (by mass spectrometry), and the effect of ubiquitin modification on enzyme interactions, activity and function
- Ubiquitinylation of proteins of interest from cell or tissue extracts
- Modification of proteins using ubiquitin derivatives or ubiquitin mutants for improved detection, analysis or investigation of alternative (non-proteasomal) ubiquitin signaling pathways

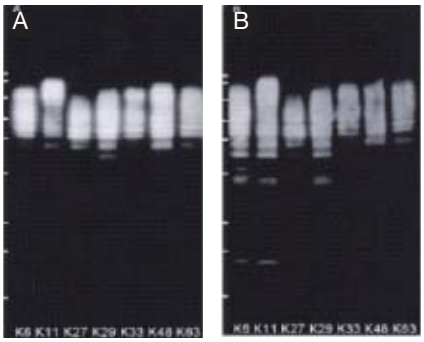


FIGURE 14: Immunodetection of single lysine linked polyubiquitin chains by western bloting following SDS-PAGE using [A] BML-PW8805 (clone FK1) and [B] BML-PW8810 (clone FK2).

Ubiquitin Antibodies

Product	Application	Prod. No.	Size
Ubiquitin-protein conjugates, pAb	IHC, WB	BML-UG9510-0025 BML-UG9510-0100	25 µl 100 µl
Polyubiquitinylated conjugates, mAb (FK1)	IHC, WB	BML-PW8805-0500	500 µg
Mono- and polyubiquitinylated conjugates, mAb (FK2)	IHC, IP, WB	BML-PW8810-0500	500 µg
Mono- and polyubiquitinylated conjugates, mAb (FK2) (HRP conjugate)	IHC, WB	BML-PW0150-0025 BML-PW0150-0100	25 µg 100 µg
Mono- and polyubiquitinated conjugates, mAb (FK2) (Biotin conjugate)	WB	BML-PW0755-0025 BML-PW0755-0100	25 µl 100 µl
Polyubiquitin (K^{63}-linkage-specific), mAb (HWA4C4)	ELISA, IHC, ICC, WB	BML-PW0600-0025 BML-PW0600-0100	25 µl 100 µl
Polyubiquitin (K^{63}-linkage-specific), mAb (HWA4C4) (HRP conjugate)	WB	BML-PW0605-0025	25 µg 100 µg

SUMO Antibodies

Product	Specificity	Application	Prod. No.	Size
SUMO-1 (human) (CT), pAb	Human	IP, WB	BML-PW9460-0025 BML-PW9460-0100	25 µl 100 µl
SUMO-1 (human) (NT), pAb	Human	IP, WB	BML-PW8330-0025 BML-PW8330-0100	25 µl 100 µl
SUMO-2/3 (human) (NT), pAb	Human	IP, WB	BML-PW9465-0025 BML-PW9465-0100	25 µl 100 µl

Ubiquitin Isolation and Purification

UbiQapture™-Q kit

BML-UW8995-0001 1 Kit

A kit specifically developed for the isolation and enrichment of ubiquitinylated proteins. The kit facilitates the isolation of both mono- and poly-ubiquitinylated proteins (independent of lysine residue chain linkage) from cell extracts, tissue lysates and *in vitro* assay solutions through the use of a broad spectrum affinity matrix. Captured proteins may be analyzed by Western blotting using the highly sensitive ubiquitin-conjugate specific antibody provided, using antibodies to specific proteins of interest, or eluted from the matrix for subsequent biochemical characterization. The UbiQapture™-Q matrix supplied with the kit has superior binding characteristics compared to other commercially available matrices and is compatible with a wide range of lysate buffers and cell/tissue samples from a variety of species. The kit provides sufficient material for approximately 25 binding assays.

Suggested uses

- Isolation and detection of ubiquitinylated protein conjugates from a specific cell/tissue lysate.
- Capture and analysis of specific ubiquitinylated protein conjugates of interest from particular cell/tissue lysates.
- Release of free proteins in their active/native form by cleavage of ubiquitin/ubiquitin chains from the UbiQapture™-Q matrix using a deubiquitinylating enzyme.
- Release of ubiquitinylated proteins in their active/native form by elution from the UbiQapture™-Q matrix using high salt buffer.

Ubiquitinylation and SUMOylation Enzymes

Ubiquitin activating enzyme UBE1L (human), (rec.) (His-tag)

BML-UW9410-0050 50 µg

Produced in *E. coli*.

SUMO activating enzyme (human), (rec.) (His-tag)

BML-UW9330-0025 25 µg

Produced in *E. coli*. Full length heterodimeric human SUMO-activating enzyme.

hHR6A (human), (rec.) (His-tag)

BML-UW9635-0100 100 µg

BML-UW9635-1000 1 mg

Produced in *E. coli*. Ubiquitin-conjugating DNA repair enzyme.

hHR6B (human), (rec.) (His-tag)

BML-UW9640-0100 100 µg

BML-UW9640-1000 1 mg

Produced in *E. coli*. Ubiquitin-conjugating DNA repair enzyme.

UbcH9 (human), (rec.) (Untagged)

BML-UW9320-0100 100 µg

Produced in *E. coli* as an untagged protein. SUMO-conjugating enzyme.

These are just a sampling of a panel of E1, E2 and E3 enzymes available from Enzo Life Sciences. Visit www.enzolifesciences.com for a complete listing.

Lysine Modification Antibodies

The availability of antibodies specific for various histone modifications are essential for epigenetics research. Enzo Life Sciences offers a portfolio of highly-characterised modification-specific antibodies to the various lysine modifications useful for immunoblotting, immunoprecipitation or immunostaining.

Acetyl-lysine Antibodies

Acetyl-lysine, pAb
BML-SA615-0100 100 µl

From rabbit. **IMMUNOGEN:** Acetylated KLH. **SPECIFICITY:** Recognizes acetyl-lysine. Species independent. **APPLICATION:** WB.

Acetyl-lysine, pAb (affinity purified)
BML-SA627-0100 100 µl

From rabbit. **IMMUNOGEN:** Acetylated KLH. **SPECIFICITY:** Recognizes acetyl-lysine. Species independent. **APPLICATION:** WB.

Acetyl-lysine, pAb
BML-SA440-0400 400 µl

From rabbit. **IMMUNOGEN:** Acetylated KLH. **SPECIFICITY:** Recognizes acetyl-lysine in a wide range of species. **APPLICATION:** ELISA, ICC, IP, WB.

[K(Ac)^{40}]α-Tubulin, pAb
BML-SA452-0100 100 µl

From rabbit. **IMMUNOGEN:** Synthetic acetylated peptide (K(Ac)40) corresponding to aa 35-45 (Q^{35}MPSD-K(Ac)-TIGGG^{45}) of human α-tubulin. **SPECIFICITY:** Recognizes primate, rodent, chicken, *Xenopus* and zebrafish α-tubulin. **APPLICATION:** WB.

Methyl-lysine Antibodies

Dimethyl-lysine, pAb
BML-SA667-0100 100 µl

From rabbit. Immunogen: Dimethylated bovine serum albumin. **SPECIFICITY:** Recognizes dimethyl-lysine. Species independent. **APPLICATION:** WB.

Dimethyl-lysine, pAb (affinity purified)
BML-SA668-0050 50 µg

From rabbit. **IMMUNOGEN:** Dimethylated KLH. **SPECIFICITY:** Recognizes proteins containing dimethylated lysine residues in SDS-PAGE immunoblots. Does not cross-react with monomethylated or trimethylated lysines in peptides. **APPLICATION:** WB.

Dimethylated protein standard (bovine)
BML-SW126-0100 100 µg

Methylated bovine serum albumin for use as a Western positive control or for peptide blocking experiments for antibodies to dimethyl-lysine (Prod. No. BML-SA667 and BML-SA668).

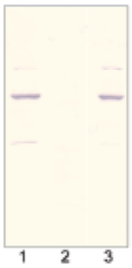


FIGURE 15: Detection of dimethylated proteins in HeLa nuclear extract using the polyclonal antibody to dimethyl-lysine (Prod. No. BML-SA668). HeLa cell nuclear extract was subjected to SDS-PAGE and then transferred to nitrocellulose. The blot was probed with the polyclonal dimethyl lysine antibody. Lane 1: antibody alone. Lane 2: Antibody preincubated with dimethylated BSA. Lane 3: Antibody preincubated with untreated BSA.

[K^9-trimethyl]Histone H3, mAb (6F12-H4)
ALX-804-673-C050 50 µg

CLONE: 6F12-H4. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Branched peptide of the sequence (QTARK(Me)3STGGKA)2-KC. **SPECIFICITY:** Recognizes human and mouse K^9-trimethylated histone H3. Cross-reacts with K^9 dimethylated and weakly with K^9 monomethylated histone H3. Does not cross-react with unmethylated histone H3. **APPLICATION:** ELISA, ICC, IP, WB.

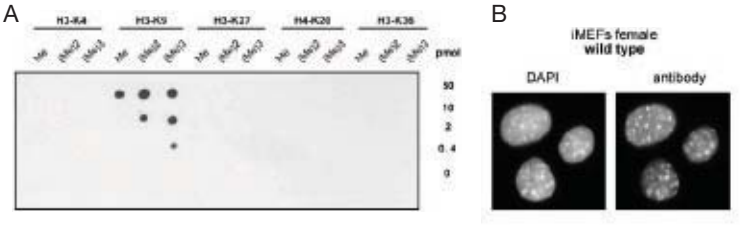


FIGURE 16: Characterization of the Histone H3 (K9 methylated) antibody.

A: Dot-blot analysis using MAb to Histone H3 (K9 methylated) (6F12-H4) (Prod. No. ALX-804-673).

B: Immunocytochemistry of irradiated mouse embryonic fibroblasts (iMEFs) stained with DAPI and MAb to Histone H3 (K9 methylated) (6F12-H4) (Prod. No. ALX-804-673).*Figures courtesy of Susanne Opravil and Thomas Jenuwein, Institute of Molecular Pathology (IMP) Vienna, Austria.*

HIGHLIGHT

For Ubiquitin & SUMO Antibodies see Page 20-21.

Histones

Histones are the major protein constituent of chromatin in the eukaryotic nucleus. These proteins undergo a host of different post-translational modifications, including phosphorylation, acetylation, and methylation, which have profound effects on the remodeling of chromatin. Histone modifications can function either individually or in combination to govern such processes as transcription, replication, DNA repair, and apoptosis.

In response to DNA damage or structural alterations of chromatin, histone H2AX gets phosphorylated on Ser139 by phosphoinositide 3-kinase related protein kinases (PIKKs) such as ataxia telangiectasia mutated (ATM), ATM and Rad-3 related (ATR) kinase, or by DNA dependent protein kinase (DNA-PKcs). One role of γ-H2AX is to accumulate repair factors near DSB sites, thus serving as staging areas for the components of the DNA DSB repair machinery. Constraining broken DNA ends is another suggested role of γ-H2AX. Antibodies against γ-H2AX represent valuable tools for the detection of DSB.

LIT: Histone H2AX in DNA damage and repair: O.A. Sedelnikova, et al.; Cancer Biol. Ther. **2,** 233 (2003) ▪ DNA repair: the importance of phosphorylating histone H2AX: N.F. Lowndes & G.W. Toh; Curr. Biol. **15,** R99 (2005) ▪ Constitutive histone H2AX phosphorylation and ATM activation, the reporters of DNA damage by endogenous oxidants: T. Tanaka, et al.; Cell Cycle **5,** 1940 (2006)

Histone Modification-specific Antibodies

[pSer^{139}]Histone γ-H2AX (human), pAb

ALX-210-390-R050 50 µl

From rabbit. **IMMUNOGEN:** Synthetic peptide corresponding to human histone γ-H2AX (K^{134}ATQApSQEY^{142}) phosphorylated at Ser^{139}. **SPECIFICITY:** Recognizes Ser^{139}-phosphorylated human histone γ-H2AX. Does not cross-react with non-phosphorylated human histone γ-H2AX. **APPLICATION:** ICC, WB.

LIT: DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139: E.P. Rogakou, et al.; J. Biol. Chem. **273,** 5858 (1998) ▪ Hyperphosphorylation of histone H2A.X and dephosphorylation of histone H1 subtypes in the course of apoptosis: H. Talasz, et al.; Cell Death Differ. **9,** 27 (2002) ▪ Local DNA damage by proton microbeam irradiation induces poly(ADP-ribose) synthesis in mammalian cells: L. Tartier, et al.; Mutagenesis **18,** 411 (2003) ▪ Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin: M.D. Meneghini, et al.; Cell **112,** 725 (2003)

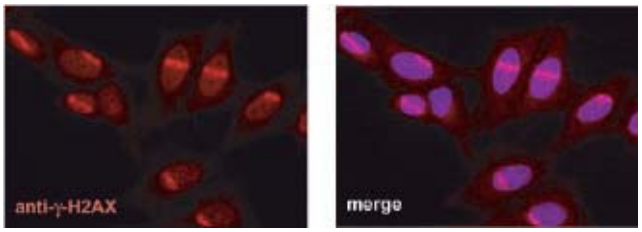


FIGURE 17: Detection of DNA damage in HeLa cells induced locally with anti-γ-H2AX PAb (Prod. No. ALX-210-390). Right: merged image of anti-γ-H2AX PAb and HOE 33258 (Prod. No. ALX-620-051) staining.

Pictures courtesy of C. Spenlehauer & G. de Murcia (CNRS, Strasbourg).

[K^9-trimethyl]Histone H3, mAb (6F12-H4)

ALX-804-673-C050 50 µg

CLONE: 6F12-H4. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Branched peptide of the sequence (QTARK(Me)3STGGKA)2-KC. **SPECIFICITY:** Recognizes human and mouse K^9-trimethylated histone H3. Cross-reacts with K^9 dimethylated and weakly with K^9 monomethylated histone H3. Does not cross-react with unmethylated histone H3. **APPLICATION:** ELISA, ICC, IP, WB.

[K^{20}-monomethyl]Histone H4, mAb (5E10-D8)

ALX-804-674-L001 1 ml

CLONE: 5E10-D8. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Branched peptide of the sequence (AKRHRK(Me)VLRDN)$_2$-KC, corresponding to aa 15-25 of human histone H4. **SPECIFICITY:** Recognizes human K^{20} monomethylated histone H4. Predicted to cross-react with K^{20} monomethylated histone H4 of all mammals, *Xenopus*, *Drosophila* and *Arabidopsis*. Does not cross-react with unmethylated or di-/trimethylated histone H4 or with methylated histone H3. **APPLICATION:** ELISA, ChIP, Dot Blot, WB (with endogenous protein).

[K^{20}-trimethyl]Histone H4, mAb (4H1-G3)

ALX-804-675-L001 1 ml

CLONE: 4H1-G3. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Branched peptide of the sequence (AKRHRK(Me)$_3$VLRDN)$_2$-KC, corresponding to aa 15-25 of human histone H4. **SPECIFICITY:** Recognizes human K^{20} trimethylated histone H4. Predicted to cross-react with K^{20} trimethylated histone H4 of all mammals, *Xenopus*, *Drosophila* and *Arabidopsis*. Does not cross-react with unmethylated or mono-/dimethylated histone H4. **APPLICATION:** ELISA, ChIP, Dot Blot.

[K^{20}-trimethyl]Histone H4, mAb (6F8-D9)

ALX-804-676-L001 1 ml

CLONE: 6F8-D9. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Branched peptide of the sequence (AKRHRK(Me)$_3$VLRDN)$_2$-KC, corresponding to aa 15-25 of human histone H4. **SPECIFICITY:** Recognizes human K^{20} trimethylated histone H4. Weakly cross-reacts with human K^{20} dimethylated histone H4. Predicted to cross-react with K^{20} di-/trimethylated histone H4 of all mammals, *Xenopus*, *Drosophila* and *Arabidopsis*. Does not cross-react with methylated histone H3. **APPLICATION:** ELISA, ChIP, Dot Blot, WB (with endogenous protein).

Histone Proteins

Histone H2AX (human), (rec.)

ALX-201-176-M005 5 mg

Produced in *E. coli*.

Histone H2B (29-35)

ALX-158-003-M001 1 mg

ALX-158-003-M005 5 mg

H-Arg-Lys-Arg-Ser-Arg-Lys-Glu-OH. Substrate for cGMP-dependent protein kinase corresponding to residues 29-35 of histone H2B. For the corresponding alanine-for-serine substituted peptides see Prod. No. ALX-158-002 and Prod. No. ALX-158-004.

LIT: Comparison of the substrate specificity of adenosine 3':5'- monophosphate- and guanosine 3':5'-monophosphate-dependent protein kinases. Kinetic studies using synthetic peptides corresponding to phosphorylation sites in histone H2B: D.B. Glass & E.G. Krebs; J. Biol. Chem. **254,** 9728 (1979)

DNA Methyltransferases

Gene silencing through methylation is a well conserved and important feature of gene expression in virtually all eukaryotic and many prokaryotic organisms. In animals, the process is complex and essential in growth and development. There is additional evidence that inappropriate silencing can lead to serious genetic defects as well as cancer. Specifically, there is new evidence to suggest that the balance between growth promoting (oncogenes) and growth suppressing (tumor suppressor) genes is altered by inappropriate methylation. This can lead to aggressive cell growth associated with cancer. Inappropriate silencing can be partially reversed using hypomethylating agents such as the drug aza-deoxycytidine (aza-dC). Aza-dC (also aza-C) is in fact a useful tool to explore methylation events *in vivo* with endogenous methylation enzymes (DNA methyltransferases or DNMTs).

The methylation of mammalian genomic DNA is catalysed by DNA methyltransferases (Dnmts), which play a special role in the initiation of chromatin remodelling and gene expression regulation. The mammalian Dnmts are Dnmt1, Dnmt3, Dnmt3A and Dnmt3B, which are responsible for methylation pattern acquisition during gametogenesis, embryogenesis and somatic tissue development. Dnmt1 and Dnmt3 proteins comprise two domains: the N-terminal "regulatory" domain and C-terminal "catalytic" domain; in contrast Dnmt2 has only the "catalytic" domain. The Dnmts use S-adenosyl-L-methionine (AdoMet) as a donor of methyl groups. The only modification of mammalian genomic DNA is the methylation at the 5- position of the cytosine (C) residue within the cytosine-guanine dinucleotides (CpG) resulting in the formation of 5-methylcytosine (m5C).

Inhibitor

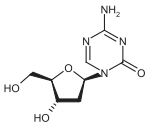
5-Aza-2'-deoxycytidine

[5-Aza-CdR; 5-Aza-dC; 5-Deoxy-2-azacytidine]

ALX-480-096-M005 — 5 mg

Inhibitor of DNA methyltransferase. Restores mRNA and protein expression of caspase-8 and TRAIL (tumor necrosis factor-related apoptosis inducing ligand) sensitivity of resistant cell lines. Enhances apoptosis induced by HDAC (histone deacetylase) inhibitors.

LIT: Incorporation of a potent antileukemic agent, 5-aza-2';-deoxycytidine, into DNA of cells from leukemic mice: J. Vesely and A. Cihak; Cancer Res. **37,** 3684 (1977) ▪ Resistance to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in neuroblastoma cells correlates with a loss of caspase-8 expression: A. Eggert, et al.; Cancer Res. **61,** 1314 (2001) ▪ 5-Aza-2';-deoxycytidine induces histone hyperacetylation of mouse centromeric heterochromatin by a mechanism independent of DNA demethylation: S. Takebayashi, et al.; BBRC **288,** 921 (2001) ▪ DNA methyltransferase inhibition enhances apoptosis induced by histone deacetylase inhibitors: W.G. Zhu, et al.; Cancer Res. **61,** 1327 (2001) ▪ **For a comprehensive bibliography please visit our website.**



Dnmt2

Dnmt2 is the smallest mammalian DNA methyltransferase. Dnmt2 enzymes have been widely conserved during evolution and contain all of the signature motifs of DNA (cytosine-5)-methyltransferases; however, the DNA methyltransferase activity of these proteins is comparatively weak and their biochemical and functional properties remain enigmatic. Recent evidence now shows that Dnmt2 has a novel tRNA methyltransferase activity, raising the possibility that the biological roles of these proteins might be broader than previously thought.

Dnmt 2, pAb

ALX-210-330-C100 — 100 µg

From rabbit. **IMMUNOGEN:** Synthetic peptides corresponding to aa 39-53 and 361-376 of mouse Dnmt2 (DNA methyltransferase-2). **SPECIFICITY:** Recognizes human and mouse Dnmt2. Detects a band of ~42kDa by Western blot. **APPLICATION:** WB.

LIT: Induction of DNA methylation and gene silencing by short interfering RNAs in human cells: H. Kawasaki and K. Taira; Nature **431,** 211 (2004)

Dnmt3a/Dnmt3b

The methyltransferases Dnmt3a and Dnmt3b are mainly responsible for the de novo methylation of DNA, particularly during embryogenesis. In addition, Dnmt3a and Dnmt3b also interact with Dnmt1 and activate HDAC1, which deacetylates histones and represses gene transcription. This suggests that Dnmt3a and Dnmt3b may be involved in chromatin remodelling associated with the modulation of gene transcription.

Dnmt3a, mAb (mouse) (64B1446)

ALX-804-370-C100 — 100 µg

CLONE: 64B1446. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant mouse Dnmt3a (DNA methyltransferase-3a). **SPECIFICITY:** Recognizes C-terminal epitope aa 705-908 of mouse Dnmt3a [1]. Weakly cross-reacts with human Dnmt3a. **APPLICATION:** IHC, ICC, IP, WB.

LIT: Lsh, a member of the SNF2 family, is required for genome-wide methylation: K. Dennis, et al.; Genes Dev. **15,** 2940 (2001) ▪ De novo DNA methyltransferases Dnmt3a and Dnmt3b primarily mediate the cytotoxic effect of 5-aza-2'-deoxycytidine: M. Oka, et al.; Oncogene **24,** 3091 (2005) ▪ Myc represses transcription through recruitment of DNA methyltransferase corepressor: C. Brenner, et al.; EMBO **24,** 336 (2005)▪ Cell and stage of transformation-specific effects of folate deficiency on methionine cycle intermediates and DNA methylation in an in vitro model: J.M. Stempak, et al.; Carcinogenesis **26,** 981 (2005) ▪ **For a comprehensive bibliography please visit our website.**

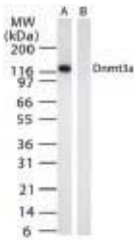


FIGURE: Western blot analysis of Dnmt3a using ALX-804-370 at 2µg/ml against 10µg of 293 cell lysate transfected with either mouse Dnmt3a (lane A) or mouse Dnmt3b (lane B).

Dnmt3b, mAb (52A1018)

ALX-804-233-C100 — 100 µg

CLONE: 52A1018. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant mouse Dnmt3b (DNA methyltransferase-3b). **SPECIFICITY:** Recognizes human and mouse Dnmt3b. Detects a band of ~100kDa by Western blot. Does not cross-react with Dnmt3a. **APPLICATION:** ICC, IP, WB.

LIT: The nucleolar remodeling complex NoRC mediates heterochromatin formation and silencing of ribosomal gene transcription: R. Santoro, et al.; Nat. Genet. **32,** 393 (2002) ▪ Induction of DNA methylation and gene silencing by short interfering RNAs in human cells: H. Kawasaki and K. Taira; Nature **431,** 211 (2004) ▪ Stage-specific induction of DNA methyltransferases in olfactory receptor neuron development: J.L. MacDonald, et al.; Dev. Biol. 288, 461 (2005) ▪ Reduced genomic cytosine methylation and defective cellular differentiation in embryonic stem cells lacking CpG binding protein: D.L. Carlone, et al.; Mol. Cell. Biol. **25,** 4881 (2005) ▪ **For a comprehensive bibliography please visit our website.**

Telomerase

Telomeres are specialised structures that cap the ends of eukaryotic chromosomes and have several important functions such as: i) protecting chromosome ends from exonuclease attack and degradation, ii) positioning the chromosomes in the nucleus, and iii) regulating proper alignment of chromosomes for recombination during meiosis [1]. Telomeres consist of TTAGGG tandem repeats ending in a single-stranded 3´overhang (the G-strand overhang), which can be folded into a duplex T loop structure by telomere- binding proteins. The G-strand overhang is the substrate for the telomerase, which consists of a reverse transcriptase (TERT) that is able to synthesize telomeric repeats de novo and add them to chromosome ends using an associated RNA molecule (TERC) as a template [2]. In most somatic cells, a portion of telomeric DNA is lost every time a cell divides, due to the incomplete replication of linear chromosomes by conventional DNA polymerases. In the absence of special telomere maintenance mechanisms, linear chromosomes shorten progressively with every round of DNA replication, eventually leading to cellular senescence or apoptosis. In germ cells and some cancer cells, high levels of telomerase are expressed which prevents critical telomere shortening and maintains cell viability [3, 4]. Thus telomere function may have contrasting roles: inducing replicative senescence and promoting tumourigenesis and these roles may vary between cell types depending on the expression of the enzyme telomerase, the level of mutations induced, and efficiency/deficiency of related DNA repair pathways [1, 5]

LIT: [1] DNA repair factors and telomere-chromosome integrity in mammalian cells: M.P. Hande; Cytogenet. Genome Res. **104,** 116 (2004) ▪ **[2]** The epigenetic regulation of mammalian telomeres: M.A. Blasco; Nat. Rev. Genet. **8,** 299 (2007) ▪ **[3]** How telomeres are replicated: E. Gilson & V. Geli; Nat. Rev. Mol. Cell. Biol. **8,** 825 (2007) ▪ **[4]** Replication and protection of telomeres: R.E. Verdun & J. Karlseder; Nature **447,** 924 (2007) ▪ **[5]** Telomeres and human disease: ageing, cancer and beyond: M.A. Blasco; Nat. Rev. Genet. **6,** 611 (2005)

Inhibitors

BPPA

BML-GR321-0005 5 mg

BML-GR321-0025 25 mg

A novel, potent and selective inhibitor of human telomerase (IC_{50}=4.5 µM). Displays potent cytotoxic activity against human carcinoma cell lines (IC_{50}=1-6 µM), an effect which is unrelated to telomerase inhibition.

LIT: 1,4- and 2,6-disubstituted amidoanthracene-9,10-dione derivatives as inhibitors of human telomerase: P.J. Perry et al.; J. Med. Chem. **41,** 3253 (1998)

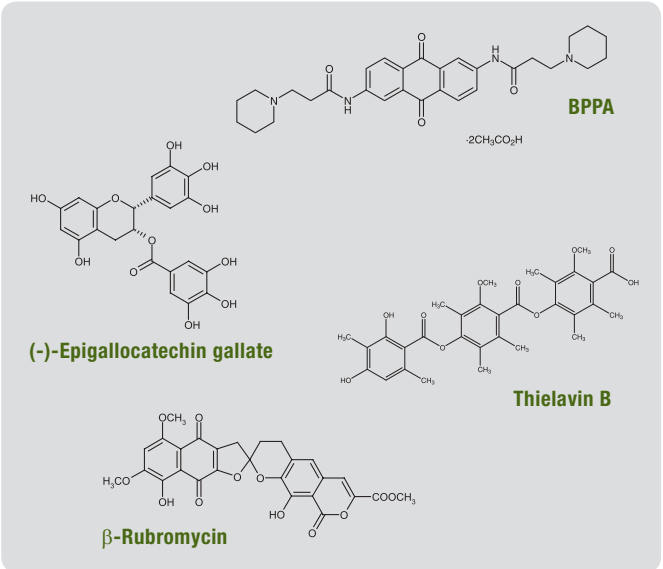
(-)-Epigallocatechin gallate

ALX-270-263-M010 10 mg

ALX-270-263-M050 50 mg

Isolated from green tea. Antitumor reagent. Antioxidant. Protects cells from lipid peroxidation and DNA damage induced by reactive free radicals. Inhibits inducible nitric oxide synthase (iNOS; NOS II). Inhibits angiogenesis. Inhibits telomerase and DNA methyltransferase. Anti-inflammatory agent.

LIT: Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin: M.T. Huang, et al.; Carcinogenesis **13,** 947 (1992) ▪ Telomerase inhibition, telomere shortening, and senescence of cancer cells by tea catechins: I. Naasani, et al.; BBRC **249,** 391 (1998) ▪ Tea flavanols inhibit angiotensin-converting enzyme activity and increase nitric oxide production in human endothelial cells: I.A. Persson, et al.; J. Pharm. Pharmacol. **58,** 1139 (2006) ▪ **For a comprehensive bibliography please visit our website.**



Thielavin B

ALX-350-340-MC05 0.5 mg

Isolated from an unidentified fungus MST-FP1888. Fungal metabolite. Glucose-6-phosphatase inhibitor. Potent inhibitor of phospholipase C. Inhibits telomerase, viral reverse transcriptase, peptidoglycan formation and prostaglandin biosynthesis.

LIT: Thielavin A and B, new inhibitors of prostaglandin biosynthesis produced by Thielavia terricola: N. Kitahara, et al.; J. Antibiot. (Tokyo) **34,** 1562 (1981) ▪ Inhibition of telomerase activity by fungus metabolites, CRM646-A and thielavin B: K. Togashi, et al.; Biosci. Biotechnol. Biochem. **65,** 651 (2001) ▪ **For a comprehensive bibliography please visit our website.**

β-Rubromycin

ALX-380-067-M001 1 mg

ALX-380-067-M005 5 mg

Isolated from *Streptomyces* sp. Antibiotic. Inhibitor of HIV-1 reverse transcriptase (RT). Inhibits human telomerase. Cytostatically active against different tumor cell lines.

LIT: The structure of rubromycin: H. Brockmann, et al.; THL **30,** 3525 (1966) ▪ Inhibition of human telomerase by rubromycins: implication of spiroketal system of the compounds as an active moiety: T. Ueno, et al.; Biochemistry **39,** 5995 (2000) ▪ **For a comprehensive bibliography please visit our website.**

Antibody

Tankyrase-1 (human), mAb (19A449)

ALX-804-234-C100 100 µg

CLONE: 19A449. **ISOTYPE:** Mouse IgG1 . **IMMUNOGEN:** Recombinant human tankyrase-1 (PARP-5a). **SPECIFICITY:** recognizes human tankyrase-1. Detects a band of ~120kDa by Western blot. **APPLICATION:** WB.

LIT: Tankyrase, a poly(ADP-ribose) polymerase at human telomeres: S. Smith, et al.; Science **282,** 1484 (1998) ▪ Mammalian telomeres end in a large duplex loop: J.D. Griffith, et al.; Cell **97,** 503 (1999) ▪ Cell cycle dependent localization of the telomeric PARP, tankyrase, to nuclear pore complexes and centrosomes: S. Smith & T. de Lange; J. Cell. Sci. **112,** 3649 (1999) ▪ Chromosomal mapping of the tankyrase gene in human and mouse: L. Zhu, et al.; Genomics **57,** 320 (1999) ▪ Tankyrase promotes telomere elongation in human cells: S. Smith & T. de Lange; Curr. Biol. **10,** 1299 (2000) ▪ DNA G-quadruplexes, telomere-specific proteins and telomere-associated enzymes as potential targets for new anticancer drugs: E. Raymond, et al.; Invest New Drugs **18,** 123 (2000) ▪ Tankyrase is a golgi-associated mitogen-activated protein kinase substrate that interacts with IRAP in GLUT4 vesicles: N.W. Chi & H.F. Lodish; J. Biol. Chem. **275,** 38437 (2000)

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Enzo Life Sciences, Leader in Epigenetics

Scientists at BIOMOL and now at Enzo Life Sciences have repeatedly made significant contributions, both commercial and scientific, to the field of epigenetics, specifically with regard to lysine acetylation. Important contributions are listed below:

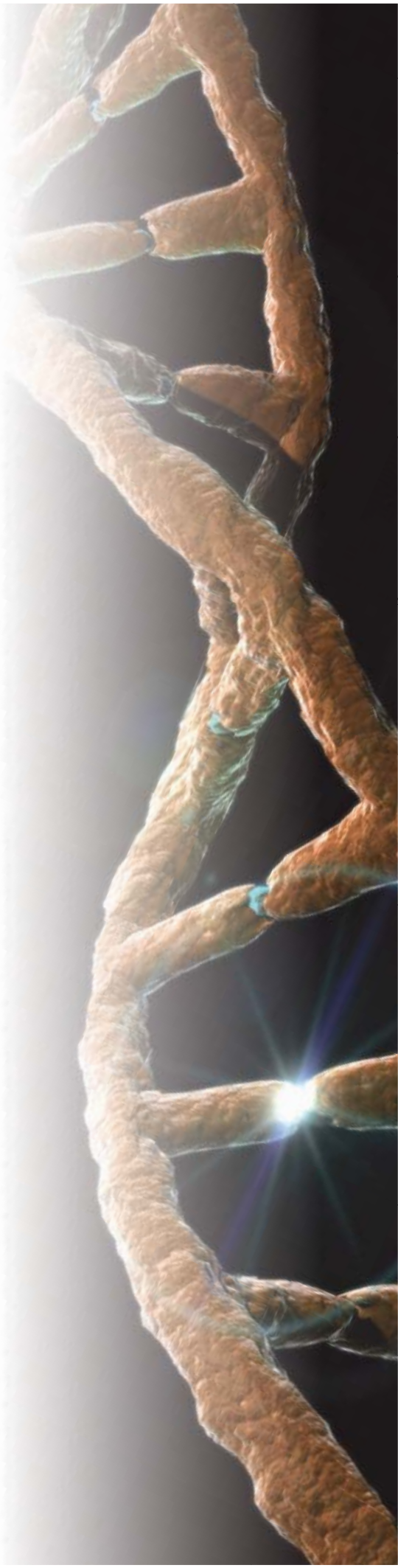
Commercial

- Developed the *Fluor de Lys*™ HDAC assay, the first fluorogenic assay for measuring histone deacetylase activity (see page 8). This assay platform replaced cumbersome radioactive assays, has been adopted widely within the pharmaceutical industry for HDAC drug discovery, and enabled the development of therapeutic HDAC inhibitors.
- Developed the first cell-based fluorogenic HDAC assay (see page 9).
- First to offer recombinant sirtuins (SIRTs 1-3) (see page 9).
- Developed specialised HDAC and sirtuin substrates based on endogenous acetylated sites in histones and other proteins (see page 11). These substrates, as a consequence of their increased sensitivity with specific HDAC and sirtuin enzymes, facilitated the development of the first sirtuin and HDAC8 assays on the market.
- First to develop fluorogenic HDAC substrates with a choice of fluorophores allowing one to avoid quenching and fluorescent interference from compounds absorbing in the near UV and blue range (see page 8).

Scientific

- Discovered that resveratrol and other polyphenols activate SIRT1 and, in subsequent collaboration with researchers at Harvard University, Brown University, and the University of Connecticut, found that activators of sirtuins extend lifespan in yeast and multiple metazoans [1,2]. These studies have inspired both significant research on the mechanism of life extension through sirtuin activation and the development new therapeutics based on sirtuin activation.
- First to explore SIRT1 activator structure-activity relationships, using both natural polyphenols [1] and the first “second-generation” activators, synthetic resveratrol analogs designed and made at BIOMOL [3]. The new compounds display lower toxicity toward human cells, and higher potency with respect to SIRT1 activation and lifespan extension in *Saccharomyces cerevisiae*. These studies demonstrated that it is possible to improve life extension properties of naturally ocurring sirtuin-activating compounds.
- Contributed to studies that demonstrated that yeast lifespan extension by calorie restriction is independent of NAD fluctuation [4] and that calorie restriction increases mammalian cell survival by inducing SIRT1 expression [5].
- Identified suramins as a new structural class of sirtuin inhibitors [1].
- Contributed to a high-throughput screen that identified four novel sirtuin inhibitor scaffolds with micromolar potency [6].

LIT: [1] Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan: K.T. Howitz et al.; Nature **425,** 191 (2003) ▪ **[2]** Sirtuin activators mimic caloric restriction and delay ageing in metazoans: J. G. Wood et al.; Nature **430,** 686 (2004) ▪ **[3]** Design and synthesis of compounds that extend yeast replicative lifespan: H. Yang et al.; Aging Cell **6,** 35 (2007) ▪ **[4]** Yeast life-span extension by calorie restriction is independent of NAD fluctuation: R.M. Anderson et al.; Science **302,** 2124 (2003) ▪ **[5]** Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase: H.Y. Cohen et al.; Science **305,** 390 (2004) ▪ **[6]** Identification and characterization of novel sirtuin inhibitor scaffolds: B.D. Sanders et al.; Bioorg. Med. Chem. **17,** 7031 (2009)



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