

EPIGENETICS



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Cayman Chemical Company

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Epigenetics is an amazingly complex and fascinating area of research that captures the interest of scientists worldwide as well as many scientists here at Cayman Chemical. “Epigenetics” most simply and broadly means ‘above the genome’ (*epi* in Greek meaning over, above). In an almost equally broad sense, it refers to the study of heritable changes in gene activity that are not caused by changes in DNA sequence. In practice, these studies have centered on those chromatin (*i.e.*, histone) and DNA modifications that directly affect gene regulation.

In my discussions with research scientists, I’m often asked how Cayman, a company generally thought of as a supplier of lipids and lipid mediator-related products, became involved in the field of epigenetics. My response is that we have been creating tools in cell signaling disciplines for at least 15 years, including those for nuclear receptor and transcription factor research. As scientists who are truly interested in general cell biology discovery and keep abreast of the relevant literature, we readily witnessed the increased publications related to epigenetics research beginning in the early 2000s. We were eager to provide assays, antibodies, proteins, and biochemicals to further this line of study, first with HDAC and SIRT assays kits, and more recently with a full line of recombinant proteins for epigenetic ‘writers’, ‘erasers’ and ‘readers’ and high-throughput screening assays using TR-FRET technology. This Cayman Currents issue highlights some of that product line, with a primary focus on ‘readers’ of chromatin modification. Dr. Levi Blazer, one of Cayman’s resident epigenetics experts, has contributed an excellent article on these readers on page 4.

Our scientists will continue to develop new and innovative products for epigenetics research. If we don’t have what you need, please let us know so we can create those products that will **help make your research possible** in this exciting field. ■

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Methyl marks are often heritable in cellular lineages. For that reason, they may represent true epigenetic factors, in that they can drive heritable changes in gene activity that are not caused by alterations in DNA sequence. For the same reason, methyl marks are also pivotal to many diseases, including cancers. Methyl readers find and bind methyl marks using methyl binding domains. There are distinct readers for methylated proteins (histones and others) and methylated nucleic acids, in particular at CpG sites. Methyl binding domains and their interactions with methylated proteins are emerging as important areas of contemporary research, in part because of their relevance to disease. However, methyl readers and their

domains are intrinsically interesting. Protein methyl binding domains come in a variety of shapes (see figure 2 on page 5), from propellers (WDs) to flexible cups (Tudors) and fingers (PHDs). These may occur alone or in multiples and seek out distinct methylated epitopes. Given what we know about bromodomains (which bind acetylated lysine residues), some methyl binding domains will be specific for one to a few targets while others will be promiscuous. Methyl binding domains can be components of enzymes, as in certain histone demethylases, which contain Tudors. More commonly, these domains are found in linker proteins as part of larger complexes that regulate chromatin remodeling and gene expression. ■

Tudor Domains and Associated PHD Zinc Finger Domains

Commonly interact with *di*- and *tri*-methylated lysines; when found in pairs, referred to as 'tandem Tudors'; also recognize symmetric methylated arginine

Item No.	Product Name	Recognition Site(s)	Amino Acids	Purity
14134	JMJD2A tudor domains (human recombinant)	Histone H3K4me, H3K9me3, and H3K20me2/3	888-1,023 (N- and C-terminal truncations; contains the tandem tudor domains of JMJD2A)	≥90%
14136	SMN tudor domain (human recombinant)	Methylated Sm proteins, which bind small nuclear RNA	1,472-1,613 (N- and C-terminal truncations)	≥90%
14073	TP53BP1 tudor-like region (human recombinant)	Dimethylated lysine 382 on p53 and histone H4K20me2	1,472-1,613 (N- and C-terminal truncations)	≥90%
14777	UHRF1 PHD domain (human recombinant)	Histone H3R2me0 (unmethylated)	298-366 (partial protein)	≥95%
14778	UHRF1 tudor-like region (human recombinant; GST-tagged)	Histone H3K9me3	126-285 (partial protein)	≥95%
14779	UHRF1 tudor-like region (human recombinant; His-tagged)	Histone H3K9me3	126-285 (partial protein)	≥95%

Chromodomains and Heterochromatin Proteins

Bind *mono*-, *di*-, and *tri*-methyl H3K4/9/27

Item No.	Product Name	Recognition Site(s)	Amino Acids	Purity
14768	CBX1 (human recombinant)	Chromodomain recognizes histones H3K9me2 and H3K9me3, with a preference for H3K9me3, while the chromoshadow domain is responsible for homodimerization and interaction with a number of non-histone chromatin-associated proteins	2-185 (full-length)	≥95%
14769	CBX2 chromodomain (human recombinant)	Histone H3K9me3 or H3K27me3	2-90 (C-terminal truncation)	≥90%
11235	CBX5 (human recombinant)	Chromodomain recognizes histones H3K9me2 and H3K9me3, with a preference for H3K9me3, while the he chromoshadow domain facilitates protein-protein interactions	2-191 (full-length)	≥95%
14772	CHD1 chromodomains (human recombinant)	Histone H3K4me2/3	269-452 (N- and C-terminal truncations; contains the 2 chromodomains of CHD1)	≥95%
14773	CHD2 chromodomains (human recombinant)	Histone H3K4me	259-455 (N- and C-terminal truncations; contains the 2 chromodomains of CHD2)	≥90%

Malignant Brain Tumor (MBT) Domain

Primarily bind *mono*- and *di*-methylated lysines; commonly occur as multiple MBT-repeat sequences (2, 3, or 4), which only bind methyllysines

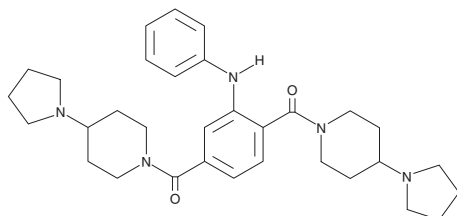
Item No.	Product Name	Recognition Site(s)	Amino Acids	Purity
14775	L3MBTL1 MBT domains (human recombinant; GST-tagged)	Histones H3K4me1 and H4K20me2	191-530 (partial protein; contains the MBT repeat region of L3MBTL1)	≥90%

WD40 Repeat-containing Proteins

One WD domain forms a propeller-type structure with 7 "vanes"; also called WD40 domains, as they contain ~40 amino acids; repeating adjacent WD domains usually form the binding domain

Item No.	Product Name	Relevance	Amino Acids	Purity
10628	EED (human recombinant)	Forms part of the Polycomb repressive complex 2; transcriptional repression by PRC2-mediated H3K27me3 is dependent on EED binding to repressive histone marks	1-441 (full-length)	≥95%
10947	RbBP5 (human recombinant)	Binds directly to with underphosphorylated tumor suppressor retinoblastoma protein; interacts directly with WDR5 contributing to the activation of the MLL1 core protein complex	2-538 (full-length)	≥90%
10944	WDR5 (human recombinant)	Contains seven WD40 repeats and binds histone H3 by recognizing the first three amino acids of the N-terminal tail; binding to a conserved arginine-containing motif in MLL-1 promotes the assembly and activity of the MLL core complex	23-334 (N-terminal truncation)	≥95%

[1415800-43-9]

MF: C₃₂H₄₃N₅O₂ **FW:** 529.7 **Purity:** ≥98%A crystalline solid **Stability:** ≥2 years at -20°C**Summary:** A potent, selective chemical probe for the methyl lysine reading function of L3MBTL3 (K_d = 120 nM; IC₅₀ = 40 nM) that competitively displaces mono- or dimethyl-lysine containing peptides1 mg
5 mg
10 mg
50 mgask
Cayman

How does Cayman Chemical test the activity of its proteins?

- Methyltransferases are tested using radiometric methyltransferase assays or using our Methyltransferase Colorimetric Assay Kit (Item No. 700140).
- Demethylases are tested using the FDH coupled demethylase assays. Our new Strep-tagged demethylases and other improvements to the assay have increased the robustness of the assays, which are now available for screening in high-throughput format by our Epigenetics Screening Lab.
- Deacetylase enzymes are tested using HDAC activity assays or SIRT Direct Fluorescent assay kits.

Epigenetics Screening Library (96-Well) 11076

10 mM solutions in DMSO **Stability:** ≥2 years at -20°C**Summary:** The Epigenetics Screening Library contains various molecules that are known to modulate the activity of a variety of epigenetic 'writers and erasers' and 'reader' proteins in a 96-well Matrix tube rack format as 10 mM stocks in DMSO.

It may include compounds that modulate the activity of methyltransferases, demethylases, histone acetyltransferases, histone deacetylases, and acetylated histone binding proteins.

50 µl • 100 µl • 200 µl



DNA Methylation Readers

Methyl Binding Domains

Item No.	Product Name	Recognition Site(s)	Amino Acids	Purity
11286	MBD2 (human recombinant; methyl binding domain aa 150-220)	5-Methylcytosine in promoters on CpG islands	150-220 (partial protein)	≥95%
11287	MeCP2 (human recombinant; methyl binding domain aa 77-166)	5-Methylcytosine in promoters on CpG islands; high affinity binding is facilitated by DNA fragments containing A/T bases ([A/T] ≥4) adjacent to the methyl-CpG	77-166 (partial protein)	≥95%

Methyl Binding Domain Antibodies

Item No.	Product Name	Antigen	Host	Isotype	Cross Reactivity	Application(s)
13771	MBD1 Monoclonal Antibody (Clone 100B272.1)	Human MBD1 amino acids 391-405	Mouse, clone 100B272.1	IgG ₁	(+) Human MBD1	WB
13772	MBD1 Polyclonal Antibody	Mixture of synthetic peptides corresponding to amino acids 98-113 and 391-405 of human MBD1	Rabbit		(+) Human MBD1	WB
13777	MBD2 Binding Zinc Finger Polyclonal Antibody	Mixture of synthetic peptides corresponding to amino acids 180-194, 331-346, and 371-388 of human MIZF	Rabbit		(+) Human MIZF	WB
13773	MBD2/3 Monoclonal Antibody (Clone 106B691)	Human MBD3 amino acids 215-230	Mouse, clone 106B691	IgG _{1κ}	(+) Human MBD2/3	WB
13775	MeCP2 Polyclonal Antibody	Mixture of synthetic peptides corresponding two amino acids 11-25 and 181-195 of human MeCP2	Rabbit		(+) Human MeCP2	WB

Protein-Protein Interactions (PPIs) are notoriously difficult to inhibit with small molecules. However, pharmacological targeting of PPIs is quite attractive, due both to the sheer volume of PPIs that exist in biology and the physiological importance of these interactions. While there are examples of small molecule PPI inhibitors in the literature, few have progressed to clinical relevancy.^{1,2} The inability to effectively prosecute this class of targets is predominantly due to the intrinsic properties of PPI interaction interfaces. Most PPI binding surfaces are large, shallow pockets or grooves with a series of diffuse, low-energy interactions, whereas small molecules generally bind to smaller, more distinct, sites using a few high-energy interactions.¹ In spite of the challenges associated with targeting PPIs, drugs that target these interactions successfully could be extremely beneficial to human health. Accordingly, the number of laboratories focused on targeting PPIs has been steadily increasing. Concomitant with this growth, the field has made substantial gains in understanding the fundamental nature of PPIs and in how to approach drug discovery for these challenging targets. This issue of Cayman Currents will introduce one of the most promising new areas of PPI inhibitor development and introduce some of the tools that Cayman has developed to help make chemical biology research in this area possible.

Epigenetics and the Histone Code

The regulation of gene transcription is dependent upon proper recruitment of transcription factors to the promoter regions of target genes. While the signals that regulate gene transcription are multivariate, one major mechanism is mediated by the posttranslational modifications of DNA and of the histones that package DNA into chromatin. These marks, and the repertoire of proteins that place and remove them, form a foundational cornerstone for transcriptional regulation. Recent work using mass spectrometry has identified hundreds of different posttranslational modifications (PTMs) that occur on histones.³ The majority of PTMs occur on the N-terminal tails of histone proteins, which protrude from the nucleosome into the nuclear milieu. While the physiological relevance of the majority of these marks is still unknown, there are several key posttranslational modifications that possess defined biological activities.

Arguably, the best-studied histone PTM to date is lysine acetylation. Through its ability to weaken the DNA-histone interaction and to recruit transcription factors, this common modification is a hallmark of genes undergoing active transcription.⁴ Acetyllysine is selectively recognized by a small protein motif called a bromodomain.⁵ There are at least 43 bromodomain-containing proteins (Figure 1) that have been identified in the human genome, the majority of which are important transcriptional regulators. Bromodomains are small (~120 amino acid) motifs formed by a left-handed bundle of 4 alpha helices linked by loop domains of varying length.⁵ The acetyllysine binding pocket is formed by a deep hydrophobic groove at the apex of the four helices.⁵ Often appearing in tandem, bromodomains selectively recognize acetyllysine through a hydrogen bond to an asparagine located inside the hydrophobic binding pocket.⁶ Mutations in bromodomain-containing proteins have been identified in human disease. For example, the translocation t(15:19) that fuses the N-terminal region of BRD4 with NUT (nuclear protein in testes) is known to play a major transformative role in NUT midline carcinomas, a particularly aggressive and lethal cancer.⁷ Through recruitment of P-TEFb, BRD4-NUT fusions function to repress expression of *c-fos*, an important mediator of epithelial differentiation. This blockade induces the carcinogenesis and rapid metastasis that is characteristic of undifferentiated NUT midline carcinomas.^{8,9}

While bromodomains have garnered a significant amount of attention from the biomedical community, aberrations in other types of reader domains can possess striking physiological effects. Genomic translocations leading to the fusion of methyllysine reader domains from JARID1A or PHF23 to Nucleoporin-98 can induce oncogenesis by causing the overexpression of several genes required to maintain pluripotency.¹⁰ The family of methyllysine reader domains contains at least 200 members in several different subfamilies. Members of this large class of protein domains vary widely from a

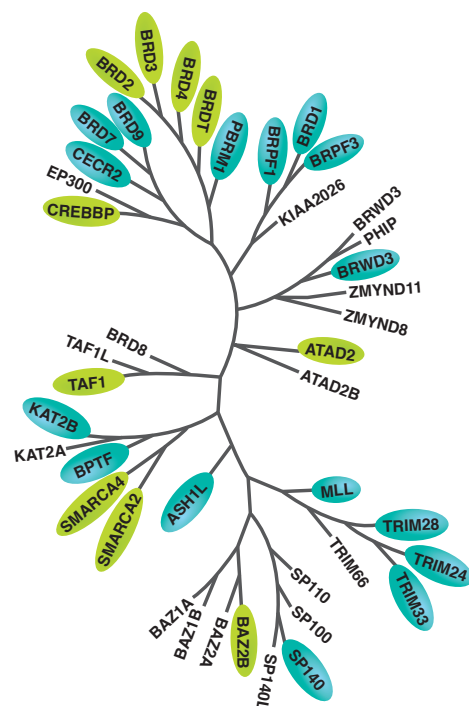


Figure 1. Phylogenetic Analysis of Bromodomain-containing proteins. Cayman Chemical has purified single or tandem bromodomains from the proteins highlighted on the tree. In addition to purified bromodomains, Inhibitor Screening Assay Kits are also available for proteins highlighted in lime green.

“Cayman Chemical will be there every step of the way to provide high-quality research reagents to make discovery possible.”

structural perspective (**Figure 2**) and in specificity for methylated lysine context and methylation status. The binding modality with which these domains recognize methylated lysine is generally formed through conserved interactions of the methylammonium residue with several aromatic residues in the binding pocket.¹¹ In contrast to lysine acetylation, histone methylation happens in an exquisitely site-specific manner. Likewise, many methyllysine readers also interact with the residues surrounding the modified residue to provide sequence specificity to the domain.¹¹ This context-dependence is understandably important given the role of lysine methylation in the recruitment of transcription factors and other chromatin-modifying enzymes, and will likely provide chemical biologists a foothold from which they will develop selective chemical probes that target methyllysine reader domains.

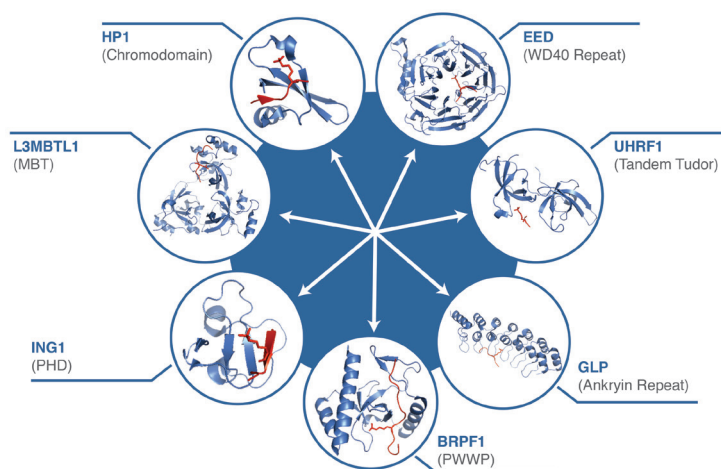
There are several other histone PTM recognition domains that have yet to be fully explored, including the phosphoserine-binding BRCT family, the methyl-DNA binding domains (*e.g.*, MBD domains) and the methylarginine binding domains (*e.g.*, ADD domains). Mutations in some of these reader domains are also linked to disease. For example, mutations in Methyl CpG binding protein 2 (MeCP2) that disrupt the DNA-binding motif lead to an X-linked mental and physical retardation known as Rett Syndrome.¹² BRCT domains, which recognize phosphorylated histone tails, are heavily involved in recruitment of DNA damage response elements,¹³ and mutations in the BRCT domain of the BRCA1 protein are associated with increased familial risk for breast and ovarian cancer.^{12,14,15}

Searching for Drugs in the Epigenetic Landscape

Due to the physiological importance of epigenetic reader domains, pharmacological manipulation of these PPIs may provide a novel approach to disease management. The most advanced class of inhibitors targeting epigenetic reader domains came from phenotypic screens designed to identify molecules that increased the production of Apolipoprotein A-1 by hepatocyte model cell lines.¹⁶ Mechanistic studies identified the lead compound from this screen, and the related compound (+)-JQ1 (Item No. 11187), as inhibitors of the BET-family of bromodomains.¹⁶ While the cardiovascular benefit of raising ApoA-1 levels is debatable,¹⁷⁻¹⁹ BET bromodomain inhibition has proven to be particularly effective at slowing the growth of NUT midline carcinomas and a variety of other cancers.²⁰⁻²⁴ This initial work led to the development of IBET-762, which is currently in Phase I clinical trials as a novel chemotherapeutic agent for midline carcinomas (NCT01587703). Another BET bromodomain inhibitor, RVX-208, has also entered Phase II trials on glucose metabolism in pre-diabetic patients (NCT01728467).

Encouraged by the serendipitous discovery of BET-family bromodomain inhibitors, a number of academic and industrial groups have undertaken the challenge of targeting other bromodomains. In a joint academic-industrial collaboration, the Structural Genomics Consortium has crystallized a large number of the bromodomains and has developed selective small molecule inhibitors for several different bromodomains (www.thesgc.org). These structures and related tool compounds many of which are available or in development at Cayman Chemical) will be invaluable in the development of next-generation of bromodomain inhibitors. Small molecule inhibitors of other reader domains have also been identified, including molecules targeting methyllysine binding domains of L3MBTL1 and L3MBTL3 (*e.g.*, UNC1215, Item No. 13968), suggesting that bromodomains are not

Figure 2. Structural diversity of methyllysine reader domains. Unlike the consistent architecture of bromodomains, there is vast structural diversity in the methyllysine reader class of protein domains. Structures from PDB IDs: 1KNE, 2PQW, 2QIC, 2X4W, 3B95, 3DB3, 3IIW.



unique among this family of protein motifs in being amenable to small molecule inhibition.^{25,26}

Given the importance of epigenetic reader domains in gene transcription, this broad family of protein motifs represents a promising new area for drug discovery against a broad array of disease states. Indeed, BET bromodomain inhibitors are currently being pursued beyond their anti-proliferative effects, including anti-viral and anti-inflammatory indications.²⁷⁻³⁰ To help make reader domain research possible, Cayman Chemical offers a broad panel of purified reader domains, inhibitor screening assay kits, and chemical probes. As this field progresses, expect to see novel inhibitors of epigenetic reader domains driving early stage drug discovery across a broad scope of disease states. Cayman Chemical will be there every step of the way to provide high-quality research reagents to make discovery possible. ■

References

- Blazer, L.L. and Neubig, R.R. *Neuropsychopharmacology* **34**(1), 126-141 (2009).
- Arkin, M.R. and Whitty, A. *Curr. Opin. Chem. Biol.* **13**(3), 284-290 (2009).
- Arnaudo, A.M. and Garcia, B.A. *Epigenetics Chromatin* **6**(1), 24 (2013).
- Bannister, A.J. and Kouzarides, T. *Cell Res.* **21**, 381-395 (2011).
- Sanchez, R. and Zhou, M.M. *Curr. Opin. Drug Discov. Devel.* **12**(5), 659-665 (2009).
- Filippakopoulos, P., Picaud, S., Mangos, M., *et al.* *Cell* **149**(1), 214-231 (2012).
- French, C.A., Miyoshi, I., Kubonishi, I., *et al.* *Cancer Res.* **63**(2), 304-307 (2003).
- French, C.A. *Cancer Genet. Cytogenet.* **203**(1), 16-20 (2010).
- Jang, M.K., Mochizuki, K., Zhou, M., *et al.* *Mol. Cell* **19**(4), 523-534 (2005).
- Wang, G.G., Song, J., Wang, Z., *et al.* *Nature* **459**(7248), 847-851 (2013).
- Yun, M., Wu, J., Workman, J.L., *et al.* *Cell Res.* **21**(4), 564-578 (2013).
- Amir, R.E., Van den Veyver, I.B., Wan, I.M., *et al.* *Nat. Genet.* **23**(2), 185-188 (1999).
- Woods, N.T., Mesquita, R.D., Sweet, M., *et al.* *Sci. Signal.* **5**(242), rs6 (2012).
- Glover, J.N. *Fam. Cancer* **5**(1), 89-93 (2006).
- Clapperton, J.A., Manke, I.A., Lowery, D.M., *et al.* *Nat. Struct. Mol. Biol.* **11**(6), 512-518 (2004).
- Mirguet, O., Gosmini, R., Toum, J., *et al.* *J. Med. Chem.* **56**(19), 7501-7515 (2013).
- Landmesser, U. *Curr. Vasc. Pharmacol.* **10**(6), 718-719 (2012).
- Nicholls, S.J., Gordon, A., Johansson, J., *et al.* *Cardiovasc. Drugs Ther.* **26**(2), 181-187 (2012).
- Joy, T.R. *Pharmacol. Ther.* **135**(1), 18-30 (2012).
- Filippakopoulos, P., Qi, J., Picaud, S., *et al.* *Nature* **468**(7327), 1067-1073 (2010).
- Roos, G. and Messens, J. *Free Radic. Biol. Med.* **51**(2), 314-326 (2011).
- Hawkins, C.L., Morgan, P.E., and Davies, M.J. *Free Radic. Biol. Med.* **46**(8), 965-988 (2009).
- Lockwood, W.W., Zejnnullahu, K., Bradner, J.E., *et al.* *Proc. Natl. Acad. Sci. USA* **109**(47), 19408-19413 (2013).
- Cheng, Z., Gong, Y., Ma, Y., *et al.* *Clin. Cancer Res.* **19**(7), 1748-1759 (2013).
- Herold, J.M., Wigle, T.J., Norris, J.L., *et al.* *J. Med. Chem.* **54**, 2504-11 (2011).
- James, L.I., Barsyte-Lovejoy, D., Zhong, N., *et al.* *Nat. Chem. Biol.* **9**(3), 184-191 (2013).
- Belkina, A.C., Nikolajczyk, B.S., and Denis, G.V. *J. Immunol.* **190**(7), 3670-3678 (2013).
- Boehm, D., Calvanese, V., Dar, R.D., *et al.* *Cell Cycle* **12**(3), 452-462 (2013).
- Wang, X., Li, J., Schowalter, R.M., *et al.* *PLoS Pathog.* **8**(11), e1003021 (2012).
- Zhu, J., Gaiha, G.D., John, S.P., *et al.* *Cell Rep.* **2**(4), 807-816 (2012).

Histone Acetylation Readers: Bromodomains

Item No.	Product Name	Function	Amino Acids	Purity
14489	ASH1L bromodomain (human recombinant)	ASH1L is a histone methyltransferase that regulates mammalian Hox gene expression, which plays an important role in haematopoietic development in mammals.	2,438-2,561	≥90%
14490	ATAD2 bromodomain (human recombinant)	ATAD2 is an AAA+ ATPase-containing nuclear transcriptional coactivator for estrogen and androgen receptors. Its bromodomain associates with acetylated lysine 14 on histone H3 to regulate the genes required for cell cycle progression.	981-1,108	≥90%
11917	BAZ2B bromodomain (human recombinant)	A rare allele of BAZ2B has been identified to be a predictor of Sudden Cardiac Death.	2,064-2,168	≥80%
11918	BPTF bromodomain (human recombinant)	BPTF is the largest component of the NURF chromatin remodeling complex. Its bromodomain recognizes acetylation of lysines in histone 4.	2,796-2,907	≥80%
11507	BRD1 bromodomain (human recombinant)	BRD1 has been identified as a susceptibility gene in neurological disorders, such as schizophrenia and bipolar affective disorder.	556-680	≥95%
11071	BRD2 bromodomain 1 (human recombinant; GST-tagged)	A member of the BET protein family, which plays a key role in many cellular processes. The bromodomains of BRD2 bind acetylated histone tails, coupling histone acetylation marks to the transcriptional regulation of target promoters.	65-187	≥95%
11070	BRD2 bromodomain 2 (human recombinant; GST-tagged)	See Item No. 11071 for details.	339-459	≥95%
11069	BRD2 bromodomains 1 and 2 (human recombinant)	See Item No. 11071 for details.	65-459	≥90%
11285	BRD3 bromodomain 1 (human recombinant)	The BET proteins play a key role in many cellular processes, including inflammatory gene expression, mitosis, and viral/host interactions. The bromodomains of BRD3 bind acetylated histone tails, coupling histone acetylation marks to the transcriptional regulation of target promoters.	24-144	≥95%
14658	BRD3 bromodomain 2 (human recombinant)	See Item No. 11285 for details.	306-416	≥95%
14864	BRD3 bromodomains 1 and 2 (human recombinant)	See Item No. 11285 for details.	2-434	≥95%
11068	BRD4 bromodomain 1 (human recombinant; GST-tagged)	The BET proteins play a key role in many cellular processes, including inflammatory gene expression, mitosis, and viral/host interactions. The bromodomains of BRD4 bind acetylated histone tails, serving to couple histone acetylation marks to the transcriptional regulation of target promoters.	49-170	≥60%
11720	BRD4 bromodomain 1 (human recombinant; His-tagged)	See Item No. 11068 for details.	49-170	≥95%
11066	BRD4 bromodomain 2 (human recombinant; GST-tagged)	See Item No. 11068 for details.	342-460	≥95%
11721	BRD4 bromodomain 2 (human recombinant; His-tagged)	See Item No. 11068 for details.	342-460	≥95%
14822	BRD4 bromodomains 1 and 2 (human recombinant; aa 2-477)	See Item No. 11068 for details.	2-477	≥70%
11052	BRD4 bromodomains 1 and 2 (human recombinant; aa 49-460)	See Item No. 11068 for details.	49-460	≥90%
14491	BRD7 bromodomain (human recombinant)	BRD7 is a subunit of the polybromo-associated BRG1-associated factor (PBAF)-specific component of the switch/sucrose non-fermentable chromatin-remodeling complex. It has a tumor suppressor role by acting as a cofactor for p53 and regulating breast cancer tumorigenicity.	129-252	≥90%
11509	BRD9 bromodomain (human recombinant)	Human BRD9 contains a single bromodomain and has five isoforms that are produced by alternative splicing.	21-137	≥95%
11548	BRDT bromodomain 1 (human recombinant)	BRDT shares homology with the RING3 protein. The two bromodomains of BRDT recognize acetylated histone H4. Loss of BRDT leads to defects in spermatogenesis.	21-137	≥95%
11649	BRDT bromodomain 2 (human recombinant)	See Item No. 11548 for details.	259-379	≥95%
14492	BRDT bromodomains 1 and 2 (human recombinant)	See Item No. 11548 for details.	21-379	≥95%
11284	BRG1 bromodomain (human recombinant)	BRG1 is a member of the SWI/SNF protein family, which forms part of a large ATP-dependent chromatin remodeling complex that is required for transcriptional activation of genes normally repressed by chromatin.	1,448-1,575	≥95%
11289	BRM bromodomain (human recombinant)	BRM is a member of the SWI/SNF protein family, which forms part of a large ATP-dependent chromatin remodeling complex that is required for transcriptional activation of genes normally repressed by chromatin.	1,367-1,511	≥95%

Histone Acetylation Readers: Bromodomains *continued*

Item No.	Product Name	Relevance	Amino Acids	Purity
11650	BRPF3 bromodomain 1 (human recombinant)	BRPF3 is a component of the MOZ/MORF histone acetyltransferase (HAT) complex. The addition of BRPF proteins to MOZ/MORF increases its HAT activity. This product contains the first bromodomain of BRPF3.	576-701	≥95%
14133	CECR2 bromodomain (human recombinant)	CECR2 is a transcription factor that forms a heterodimeric complex with the ATP-dependent chromatin remodeler SNF2L forming the CERF, which plays a critical role in neurulation. The bromodomain of CECR2 has strong γ -H2AX inhibition activity suggesting that CECR2 may play a role in DNA damage response.	424-538	≥95%
11288	CREB-binding protein bromodomain (human recombinant)	The CREBBP bromodomain has been shown to modulate the stability and function of the tumor suppressor protein p53. CREBBP bromodomain recognizes the acetylated lysine residue 382 on p53.	1,081-1,197	≥95%
11920	PCAF bromodomain (human recombinant)	p300/CBP-associated factor (PCAF) is a transcriptional coactivator that works both as a histone lysine acetyltransferase, through its HAT domain, and as an acetyl-lysine reader through its conserved bromodomain located directly C-terminal to the HAT domain. The PCAF bromodomain binds acetylated histone H3 and H4 as well as non-histone targets.	714-831	≥95%
11652	Polybromo-1D bromodomain 1 (human recombinant)	PBRM1 contains six bromodomains and is a component of the SWI/SNF complex, PBAF. PBAF is targeted to acetylated sites in chromatin by the PBRM1 bromodomains, where it plays a role in cell cycle regulation and tumor suppression.	23-156	≥95%
14659	SP140 PHD and bromodomain (human recombinant)	The C-terminal PHD and bromodomain regions of SP140 work in concert to bind to chromatin and regulate gene transcription. Nuclear bodies, which are involved in the pathogenesis of acute promyelocytic leukemia and viral infection, consist of several components, such as Sp100, NDP52, PML, and SP140.	687-867	≥95%
11922	TAF1 bromodomain 1 (human recombinant)	TAF1 is a component of transcription factor IID, and binds to core promoter sequences at the transcription start site. TAF1 helps control transcription by both its kinase and histone acetyltransferase enzymatic activities. This protein product contains the first bromodomain of TAF1.	1,371-1,496	≥95%
14495	TAF1 bromodomain 2 (human recombinant)	Contains the second bromodomain of TAF1	1,501-1,635	≥95%
14494	TAF1 bromodomains 1 and 2 (human recombinant)	Contains the first and second bromodomains of TAF1	1,373-1,635	≥90%
11653	TRIM24 bromodomain (human recombinant)	TRIM24 is a transcriptional cofactor whose inactivation leads to hepatocellular carcinoma in mice. The N-terminal TRIM domain of TRIM24 binds ligand-bound nuclear receptors, while its tandem C-terminal plant homeo-domain and bromodomain target TRIM24 to acetylated histones in chromatin.	896-1,014	≥95%
14660	TRIM28 PHD and bromodomain (human recombinant)	TRIM28, a KRAB associated protein, interacts with SETD1 and other methyltransferases for gene silencing of endogenous retroviruses during embryogenesis. The PHD finger of TRIM28 plays an important role in gene silencing. The PHD domain and bromodomain of TRIM28 work together to facilitate lysine SUMOylation, which is a requirement for TRIM28 activity in gene silencing.	624-811	≥85%
14661	TRIM33 PHD and bromodomain (human recombinant)	TRIM33 is a multi-domain regulator of transcription that is necessary for embryogenesis. TRIM33 is targeted to DNA by its tandem PHD and bromodomain, which bind histone 3 at H3K9me3 and H3K18ac, respectively.	882-1,087	≥80%
11549	WDR9 bromodomain 2 (human recombinant)	WDR9 possesses two bromodomain motifs and eight WD repeats. It is also known to interact with BRG1 (SMARCA4).	1,310-1,426	≥85%



How did Jumonji proteins get their name?

Contrary to popular belief, Jumonji proteins were not named after the 1995 blockbuster hit starring Robin Williams. The word Jumonji is Japanese meaning “cross” and was derived from a mouse mutation that affected neural tube development, which produced a cross-like structure on the neural plate.^{1,2}

1. Takeuchi, T., Yamazaki, Y., Katoh-Fukui, Y., *et al.* Gene trap capture of a novel mouse gene, jumonji, required for neural tube formation. *Genes Dev.* **9**(10), 1211-1222 (1995).
2. Lu, S.X., Knowles, S.M., Webb, C.J., *et al.* The jumonji C domain-containing protein JM30 regulates period length in the *Arabidopsis* circadian clock. *Plant Physiol.* **155**(2), 906-915 (2011).

Inhibitors of Histone Acetylation Readers

I-CBP112 (hydrochloride)

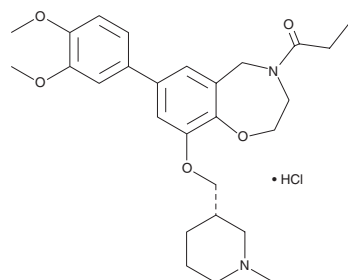
14468

MF: C₂₇H₃₆N₂O₅ • HCl **FW:** 505.1 **Purity:** ≥90%

A crystalline solid **Stability:** ≥2 years at -20°C

Summary: A selective inhibitor of CBP and EP300 that directly binds their bromodomains (K_d = 0.142 and 0.625 μM); shows only weak cross reactivity with the bromodomains of BET proteins and shows no interaction with other bromodomains

1 mg
5 mg
10 mg



PFI-1

11155

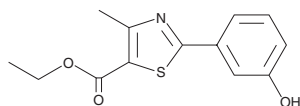
[1403764-72-6] PF-06405761

MF: C₁₆H₁₇N₃O₄S **FW:** 347.4 **Purity:** ≥98%

A crystalline solid **Stability:** ≥2 years at -20°C

Summary: A BET bromodomain inhibitor that exhibits inhibitory activity at BRD2 bromodomain 2 and BRD4 bromodomain 1 with IC₅₀ values of 98 nM and 0.22 μM, respectively

1 mg
5 mg
10 mg
25 mg



NOTE: Sold for research purposes under agreement from Pfizer Inc.

(+)-JQ1

11187

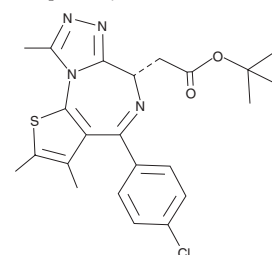
[1268524-70-4]

MF: C₂₃H₂₅ClN₄O₂S **FW:** 457.0 **Purity:** ≥98%

A crystalline solid **Stability:** ≥2 years at -20°C

Summary: Displaces BET proteins from chromatin by competitively binding to the acetyl-lysine recognition pocket of BET bromodomains; binds BRD4 bromodomains 1 and 2 with K_d values of ~ 50 and 90 nM, respectively

1 mg
5 mg
10 mg



NOTE: Manufactured, marketed, and sold with authorization from Tensha Therapeutics, Inc. Patent Pending relating to PCT Publ. No. WO/2011/143669, and any related U.S. and foreign patents and patent applications.

(-)-JQ1

11232

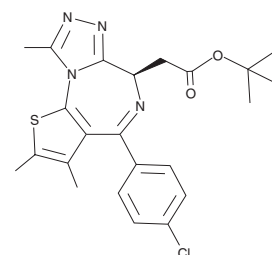
[1268524-71-5]

MF: C₂₃H₂₅ClN₄O₂S **FW:** 457.0 **Purity:** ≥98%

A crystalline solid **Stability:** ≥2 years at -20°C

Summary: The inactive stereoisomer of a selective BET bromodomain inhibitor

1 mg
5 mg
10 mg

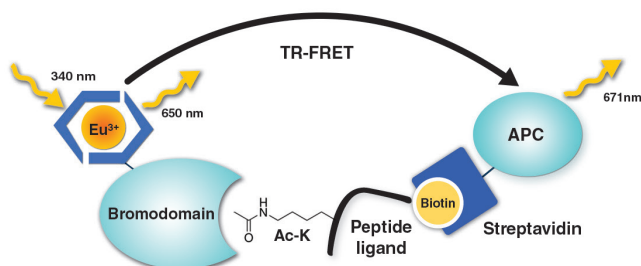


NOTE: Manufactured, marketed, and sold with authorization from Tensha Therapeutics, Inc. Patent Pending relating to PCT Publ. No. WO/2011/143669, and any related U.S. and foreign patents and patent applications.

Bromodomain TR-FRET Assay Kits

For high-throughput, rapid characterization of inhibitors of bromodomain/acetylated peptide interactions

- Homogenous mix & read TR-FRET assay in 384-well format
 - Add inhibitor compound or control sample to well
 - Add Europium-labeled bromodomain to well
 - Add bromodomain ligand/probe mix to well and incubate 60 minutes at room temperature
 - Measure TR-FRET
 - Assay is stable for at least 4 hours
- Substrate-independent, non-kinetic assay
- Miniaturized to final assay volume of 20 μl



Cayman's TR-FRET assays screen compounds that block the interaction between bromodomains and acetylated substrates. The assays utilize a Europium-labeled recombinant bromodomain as the donor and an acetylated histone peptide coupled with streptavidin-APC as a FRET acceptor.

Item No.	Product Name
600710	BAZ2B bromodomain TR-FRET Assay Kit
600500	BRD2 bromodomain 1 TR-FRET Assay Kit
600510	BRD2 bromodomain 2 TR-FRET Assay Kit
600810	BRD2 bromodomains 1 and 2 TR-FRET Assay Kit
600630	BRD3 bromodomain 1 TR-FRET Assay Kit
600820	BRD3 bromodomains 1 and 2 TR-FRET Assay Kit
600640	BRD3 bromodomain 2 TR-FRET Assay Kit
600520	BRD4 bromodomain 1 TR-FRET Assay Kit
600530	BRD4 bromodomain 2 TR-FRET Assay Kit
600830	BRD4 bromodomains 1 and 2 TR-FRET Assay Kit
600650	BRDT bromodomain 1 TR-FRET Assay Kit
600720	BRG1 bromodomain TR-FRET Assay Kit
600730	BRM bromodomain TR-FRET Assay Kit
600850	CBP bromodomain TR-FRET Assay Kit
600870	TAF1 bromodomain 1 TR-FRET Assay Kit
600930	TAF1 bromodomains 1 and 2 TR-FRET Assay Kit

Researcher Spotlight

Where did you earn your PhD? In what field?

I earned my Ph.D. in Biological Sciences from the University of Liverpool (UK).

Tell us a little bit about your journey from the UK to the Dressler lab. What attracted you to the Dressler Lab at the University of Michigan? What do you like most about working in the lab?

During Graduate School I became interested in the kidney field. My PhD thesis project was aimed at characterizing a population of kidney stem cells and assessing their capability to form rudimentary kidney structures *in vitro*. At the beginning of my third and last year of Graduate School, I realized that I wanted to continue working in the kidney field to deepen my knowledge on how the kidney develops in the embryo and to start addressing the unsolved question of how the kidney regenerates after an injury. To investigate these aspects I wanted to find a postdoctoral position in one of the best research labs in the kidney field. Therefore, I contacted Dr. Gregory Dressler of the University of Michigan, who is recognized worldwide as a leading scientist in the kidney field and has made many groundbreaking discoveries for more than 20 years.

There are three main aspects I really like about working in the Dressler lab. First, lab members have the opportunity to get involved in exciting research projects that answer pure developmental biology questions. Additionally, these projects can unveil how diseases initiate and/or progress. I really appreciate the strong support and leadership that Dr. Dressler has dedicated to the project on which I have been involved. Finally, I enjoy working with my colleagues. There's no competition among us. Rather we help each other whenever we can as we believe that such team player attitude will eventually benefit everyone in the lab.

What can you tell us about your current research project?

My current research project encompasses important epigenetic aspects that need to be addressed. It is focused on the role of the transcription factor Pax2 in the specification of renal epithelium during development and its role in renal regeneration after injury. Pax2 is essential for urogenital system development and is expressed in renal progenitor cells during development where it is required to specify the renal epithelium. Pax2 expression must be suppressed in adult proximal and distal tubule cells as abnormal or deregulated Pax2 expression is associated with a variety of disease states. However, Pax2 is reactivated in regenerating epithelia after acute kidney injury. Recently, Pax2 has been linked with epigenetic modifications leading to either gene activation or repression, depending on the availability of specific co-factors. Pax2 mediates gene activation by recruiting a Mll3/4 histone H3K4 methyltransferase complex to a Pax2 DNA binding site through the interaction with the nuclear protein Ptip.¹ Conversely, high levels of the co-repressor Tle4/Grg4 displace Ptip from Pax2 DNA target sites and recruit a histone H3K27 methyltransferase complex thus leading to gene repression.² Despite its importance in development and regeneration and its role in regulating gene expression through histone modifications, many genes directly regulated by Pax2 still remain to be identified. We are making use of unique mouse strains, gene expression profile analysis and chromatin immunoprecipitation followed by next generation sequencing (ChIP-Seq) to define how Pax2 specifies the renal epithelial lineage through the interactions with Ptip and Tle4/Grg4 to imprint positive or negative histone methylation patterns. To date, we have identified many potential target genes of Pax2 in renal progenitor cells that can be crucial for renal epithelium development. This research is very important as it will allow us to define the molecular signals controlling kidney development and to understand whether such signals are also necessary for epithelia regeneration after injury. Our ultimate goal is to define the epigenome of renal progenitor cells, as this will also be relevant to identify what patterns of histone modifications are altered in kidney disease.

What are the next steps in your career? What do you plan/hope to do in the future?

My first priority is to complete my research project and publish my findings in good journals. In future I would like to move to the life science industry with the main reason being that I would like to make use of my background and the research I would be involved in to directly develop a product or drug that can be used by customers or patients for their benefit. I see myself joining a biotech or pharmaceutical company as research scientist working in a team in a R&D division.



Egon Ranghini

Postdoctoral Fellow

University of Michigan
Department of Pathology
Dressler Lab

What piece of advice do you have for fellow postdocs/researchers?

Be as productive as possible because securing a job is becoming more and more competitive. Always keep a Plan B in mind in case Plan A falls apart (and this can happen). Network with people, not only at your institution or at other universities but also outside academia, and attend social events organized in your area by the scientific and/or entrepreneurial community. Last but not least, always have fun doing what you do.

References

1. Patel, S.R., Kim, D., Levitan, I. *et al. Dev. Cell* **13**, 580-592, (2007).
2. Patel, S.R., Bhumbra, S.S., Paknikar, R.S. *et al. Mol. Cell* **45**, (2012).



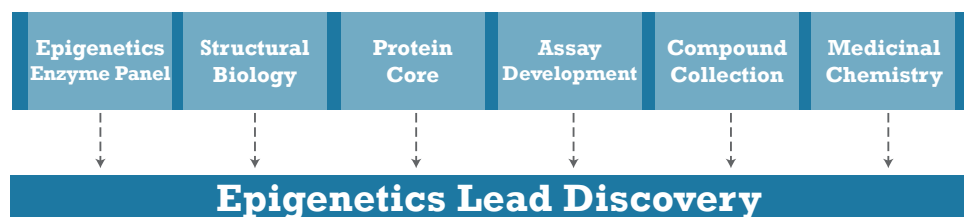
www.linkedin.com/in/egonranghini

Researcher Spotlight

Want to have your research featured in the Cayman Currents?
Send a brief background to
marketing@caymanchem.com

Epigenetics Screening & Lead Discovery

Cayman Chemical offers a dedicated Epigenetic Screening Laboratory designed to be flexible and innovative. High-throughput capabilities allow us to work with you to screen a chemical library against specific epigenetic targets. Alternatively, our broad collection of epigenetic enzymes, substrates, and assays enable profiling the activity of a few compounds against several targets. Our experienced staff and expanding suite of assays are designed to get the results you need in a timely manner. ■



Epigenetics Screening Lab

Our People:

Dedicated staff of Ph.D.-level scientists will:

- Engage partners in discussion of target validation, protein production, assay development, and screening strategies
- Perform screens and collaborate with partners to identify screening hits
- Execute hit validation on freshly resynthesized compounds *via* dose-response analysis and selectivity profiling
- Support hit-to-lead discovery strategies in collaboration with our expert medicinal chemistry team

Our Compound Collection:

Comprised of bioactive compounds from the following in-house collections:

- Maybridge HitFinder Diversity Collection (14,400 compounds)
- Catalog Collection
 - Bioactive Lipid Collection (1,168)
 - Kinase Collection (140)
 - Fatty Acid Collection (84)
 - Epigenetic Collection (80)
 - Prostaglandin Collection (226)
- Rationally-Designed Epigenetics Collection

Cayman Chemical also screens customer and third-party compound collections



Learn more

about our
**Epigenetics Screening
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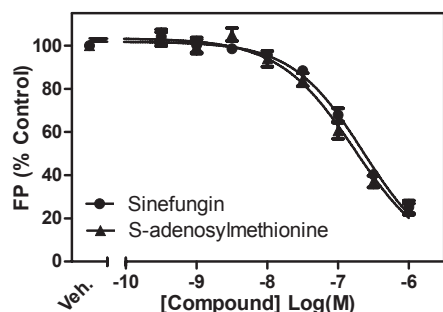
sales@caymanchem.com

www.caymanchem.com/episcreen

High-Throughput Screening Assays

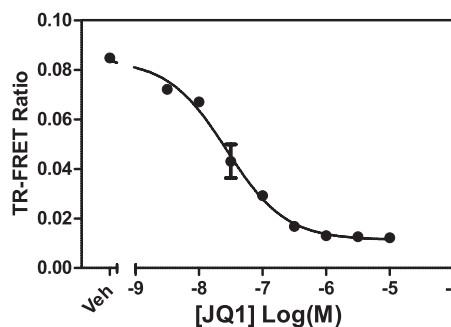
SAM-Binding Site Inhibitor Screening Assay

- Homogenous, one-step fluorescence polarization assay based upon a proprietary small molecule probe that binds to the S-adenosylmethionine (SAM) binding pocket in a variety of histone methyltransferases
- Available for Set7/9, MLL1, and GLP



Bromodomain Inhibitor Screening Assays

- Time-resolved FRET-based assay that monitors the interaction between bromodomain-containing proteins and acetylated lysine histone peptides
- Profiling against our panel of recombinant bromodomains



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DNA Methylation

Bromodomains

Histone Acetylation & Deacetylation

Histone Methylation & Demethylation





QUESTIONS FROM THE FIELD

Do you have any bromodomain inhibitors other than JQ-1

Yes. We also offer bromodomain inhibitors I-CBP112, which is best used for binding CBP and EP300, and PFI-1, which exhibits inhibitory activity at BRD2 and BRD4.

What is the SAM Screener Probe?

SAM Screener is our exclusive technology that directly probes the binding site of SAM-dependent methyltransferases in a substrate-independent manner. Our patented small molecule provides a simple method to identify SAM-competitive compounds in an HTS-compatible fluorescence polarization format and is compatible with several methyltransferases.

How do Cayman's bromodomain inhibitor screening assays compare to other screening assays?

Our bromodomain inhibitor screening assays use Time-Resolved FRET to monitor the binding of an acetylated peptide to a purified bromodomain. Unlike other commercially available assays, our TR-FRET assays do not require costly beads and are not influenced by antioxidants. Furthermore, since we use a time-resolved assay format, our inhibitor screening kits are less susceptible to interference by fluorometric interference by test compounds. Given the benefits of a homogenous TR-FRET assay system and the relatively low price of TR-FRET compared to other commercial assays, our kits provide high-quality results at an affordable price.



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