Pharmacology & Therapeutics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: B. Teicher

Epigenetic targets and drug discovery Part 1: Histone methylation

Yanli Liu [a,b,1], Ke Liu [a,b,1], Su Qin [b], Chao Xu [b], Jinrong Min [a,b,c,*]

[a] Hubei Key Laboratory of Genetic Regulation and Integrative Biology, College of Life Science, Central China Normal University, Wuhan 430079, PR China
[b] Structural Genomics Consortium, University of Toronto, 101 College Street, Toronto, Ontario M5G 1L7, Canada
[c] Department of Physiology, University of Toronto, Toronto, Ontario M5S 1A8, Canada

a r t i c l e i n f o

Keywords:
Epigenetics
Histone methylation
Inhibitor
Chemical probe
Cancer

a b s t r a c t

Dynamic chromatin structure is modulated by post-translational modifications on histones, such as acetylation, phosphorylation and methylation. Research on histone methylation has become the most flourishing area of epigenetics in the past fourteen years, and a large amount of data has been accumulated regarding its biology and disease implications. Correspondingly, a lot of efforts have been made to develop small molecule compounds that can specifically modulate histone methyltransferases and methylation reader proteins, aiming for potential therapeutic drugs. Here, we summarize recent progress in chemical probe and drug discovery of histone methyltransferases and methylation reader proteins. For each target, we will review their biological/biochemical functions first, and then focus on their disease implications and drug discovery. We can also see that structure-based compound design and optimization plays a critical role in facilitating the development of highly potent and selective chemical probes and inhibitors for these targets.

Contents

Abbreviations: PTM, posttranslational modification; HMTs, histone methyltrans-ferases; SET, Suv39, E(z) and Trithorax; SUV39, Su(var)3-9; SUV420, histone-lysine N-methyltransferase SUV420; G9a, euchromatic histone-lysine N-methyltransferase 2; GLP, G9a-like protein 1; Wiz, widely-interspaced zinc finger-containing protein; CDYL1, chromodomain Y-like protein; ACIN1, apoptotic chromatin condensation inducer in the nucleus; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosylmethionine; Dot1, disruptor of telomeric silencing; Dot1L, DOT1-Like protein; EZH1, Enhancer of zeste homolog 1; EZH2, Enhancer of zeste homolog 2; Hox, homeotic box; PcG, Polycomb group; PRC1, Polycomb Repressive Complex 1; PRC2, Polycomb Repressive Complex 2; DZNep, 3-Deazan- eplanocin A; SMYD2, SET and MYND domain-containing protein 2; PRMT, protein arginine methyltransferases; AMI-1, arginine methyltransferase inhibitor-1; MBT, Malignant Brain Tumor; PEV, position-effect variegation; HSPC, hematopoietic stem and progenitor cells; ChIP, chromatin immunoprecipitation; PK, pharmacokinetic.

* Corresponding author at: Structural Genomics Consortium, University of Toronto, 101 College Street, Toronto, On M5G 1L7, Canada. Tel.: +1 416 9463868; fax: +1 416 9460580.
E-mail address: jr.min@utoronto.ca (J. Min).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.pharmthera.2014.03.007
0163-7258/

1. Introduction

Gene expression is tightly controlled by chromatin structure spatio-temporally, and chromatin structure is dynamically regulated by various chromatin modifications, including DNA methylation, histone modifications, nucleosome positioning and chromatin remodeling. Chromatin modifying activities are recruited to their target regions through various mechanisms, such as DNA binding (for example, CpG islands), histone binding, transcription factors and non-coding RNA (ncRNA) (Nayak, Xu & Min, 2011). Therefore, gene expression is controlled hierarchically by multi-layer regulation systems, and any alterations in these epigenetic control mechanisms will lead to abnormal gene expression patterns and contribute to human diseases including cancers. Without any doubt, proteins involved in chromatin modifications would be attractive therapeutic targets for drug discovery, and tremendous progress has been made in this regard so far, for instance, three epigenetic drugs have been approved for cancer treatment and a few more are in clinical trials in recent years (Baylin & Jones, 2011; Nebbioso et al., 2012).

In this review series, we will focus on families of proteins involved in covalent chromatin modifications, including "writers", "readers" and "erasers" of chromatin modifications. The "writers" are enzymes, such as histone methyltransferases (HMTs), which can add different posttranslational modifications (PTMs) on specific substrates; while the "erasers" are comprised of enzymes that can remove these PTMs from specific substrates, such as histone demethylases; "readers" are proteins that can specifically recognize these PTMs, such as MBT proteins. In the first part of this review series, we shall discuss the recent progress regarding histone methyltransferases and histone methyllysine reader proteins in terms of their biological/biochemical functions, disease implications and drug discovery.

2. G9a/GLP: a pair of closely homologous histone H3K9me1/2 methyltransferases

In the human genome, there exists at least 60 histone methyltransferases, including histone lysine methyltransferases and arginine methyltransferases, which could also methylate proteins (Fig. 1). Many of these enzymes exist as closely homologous pairs, such as Suv39H1/2, Suv420H1/2 and EZH1/2. G9a (KMT1C, EHMT2, BAT8) and GLP (KMT1D, EHMT1) is also a pair of closely homologous SET-domain histone H3K9 methyltransferases (Fig. 1). They share a 77% sequence identity between their catalytic domains. In vitro, they are able to methylate histone H3K9, H3K27 and H1K26, yielding mono-, di- and trimethylation products. However, they are mainly mono- and dimethyltransferases in vivo (Shinkai & Tachibana, 2011). In addition to histones, they are also able to methylate non-histone proteins, including self-methylation. Interestingly, the target lysine on most of those reported non-histone substrates, such as G9a ($ARK_{185}T$), Wiz ($ARK_{1162}M$), CDYL1 ($ARK_{135}Q$), ACIN1 ($SRK_{654}S$), is located in an ARKS-like sequence motif, similar to that of H3K9/27 and H1K26 (Sampath et al., 2007; Rathert et al., 2008). Biochemically, both G9a and GLP could form homodimer via their catalytic domain (Wu et al., 2010), however, G9 and GLP always exist as a stoichiometric G9a–GLP heteromeric complex when purified endogenously from different cells, and knockout of either of them causes severely reduced H3K9me1/2 levels in vivo (Shinkai & Tachibana, 2011). An ANK repeat domain, which precedes the catalytic SET domain in G9a/GLP, was reported to bind to mono- and di-methylated histone H3K9 (Collins et al., 2008). HP1, an H3K9me2/3 binder, is also present in the G9a/GLP complexes and able to bind to self-methylated G9a/GLP via its ARKS motif (Sampath et al., 2007). The ANK repeat domain and HP1 in the G9a–GLP complex have been proposed to facilitate the spreading of H3K9me2 at their target loci (Shinkai & Tachibana, 2011).

The G9a–GLP complex is ubiquitously expressed and functions as the major euchromatic H3K9me1 and H3K9me2 histone methyltransferases, leading to transcriptional repression (Shinkai & Tachibana, 2011). The G9a–GLP complex plays multiple biological roles ranging from germ cell development and meiosis, embryonic development, DNA replication, cell proliferation and tumorigenesis. G9a overexpression is observed in many different cancers, such as prostate, hepatocellular, colon, lung cancers and lymphocytic leukemia. Knockdown of G9a in bladder and lung cancer cell lines inhibits cancer cell growth (Shinkai & Tachibana, 2011). Therefore, it is highly desirable to develop G9a/GLP specific inhibitors.

The first G9a/GLP specific inhibitor BIX-01294 was identified by screening G9a against a library of 125,000 preselected compounds (Kubicek et al., 2007) (Table 1). BIX-01294 is able to modulate the H3K9me2 methylation in cellular assays. Strikingly, the BIX-01294 inhibitor could also compensate for critical transcription factors, such as Sox2 or Oct4 in inducing somatic cells into pluripotent stem cells (iPSCs) (Shi et al., 2008). On the basis of our complex structure of GLP in complex with a histone H3K9 peptide and cofactor SAM (Wu et al., 2010), the cocrystal structure of GLP and BIX-01294 was determined by the group of Dr. Cheng from the Emory University, which confirmed that BIX-01294 is not a SAM-competitive inhibitor (Chang et al., 2009). Instead, it occupies the histone substrate-binding site. However, BIX-01294 was toxic to cells at high concentration, which limits its application in cellular studies. On the basis of the GLP and BIX-01294 complex structure, an early SAR study on the basis of the 2,4-diamino-6,7-dimethoxyquinazoline template of BIX-01294 by the group of Dr. Jin from the University of North Carolina at Chapel Hill and the Structural Genomics Consortium at the University of Toronto led to a potent and selective inhibitor of G9a/GLP, UNC0224 (F. Liu et al., 2009) (Table 1 and Fig. 2). The complex crystal structure of G9a-UNC0224 was further utilized for inhibitor design and optimization, yielding the highly potent compound UNC0321 (Morrison K_i = 63 pM) (F. Liu et al., 2010) (Table 1). In the meantime, by adding a lysine mimic to the BIX-01294 compound, a potent and less toxic compound E72 was also reported by the group of Dr. Cheng (Chang et al., 2010) (Table 1). Nevertheless, both UNC0321 and E72 are less potent than BIX-01294 in cellular activity (F. Liu et al., 2011). Therefore, further structure-based design and synthesis was carried out, which identified UNC0638 (Table 1 and Fig. 2) as a potent, selective and cell-penetrant chemical probe for G9a and GLP (Fig. 3A). It has significantly improved toxicity/function ratio compared to BIX-01294 (Vedadi et al., 2011). UNC0638 specifically reduced K9me2 levels with little effects on other modifications. This compound was also reported to suppress BON cell proliferation through inhibition of Wnt/β-catenin pathway in an H3K9me2-dependent manner (Kim et al., 2013). After continuously treated with UNC0638, adult human hematopoietic stem and progenitor cells (HSPC) could retain stem cell-like phenotypes better, hence delaying lineage commitment of HSPC cells (Chen et al., 2012). Notably, the group of Dr. Jin also made a biotinylated version of UNC0638 (UNC0965), which facilitates purifying G9a from whole cell lysates by chemiprecipitation (Konze et al., 2014).

Although UNC0638 is a highly potent and selective G9a/GLP cellular chemical probe, this compound has poor pharmacokinetic (PK) properties, making it not suitable for animal studies. To this end, another generation of compounds were synthesized and tested to try to improve the in vivo PK properties, which leads to the discovery of UNC0642 (Liu et al., 2013a) (Table 1 and Fig. 2). UNC0642 is highly potent in vitro (IC_{50}: b2.5 nM), and highly selective over other histone methyltransferases (over 2000-fold selective over PRC2 − EZH2 and over 20000-fold selective over 13 other methyltransferases). It also displayed improved in vitro and in vivo PK properties.

Very recently, a lead compound with a distinct chemotype from BIX-0192 and UNC0638 was discovered by a group of scientists from the Abbvie Inc. and the Structural Genomics Consortium. This compound, spiro [cyclobutane-1, 3′-indol]-2′-amine (IC_{50}: 153 nM against G9a), was screened from a chemically distinct library of compounds by means of a peptide-based AlphaLISA screening. Although this compound has a distinct chemotype from BIX-0192 and UNC0638, it is

Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007

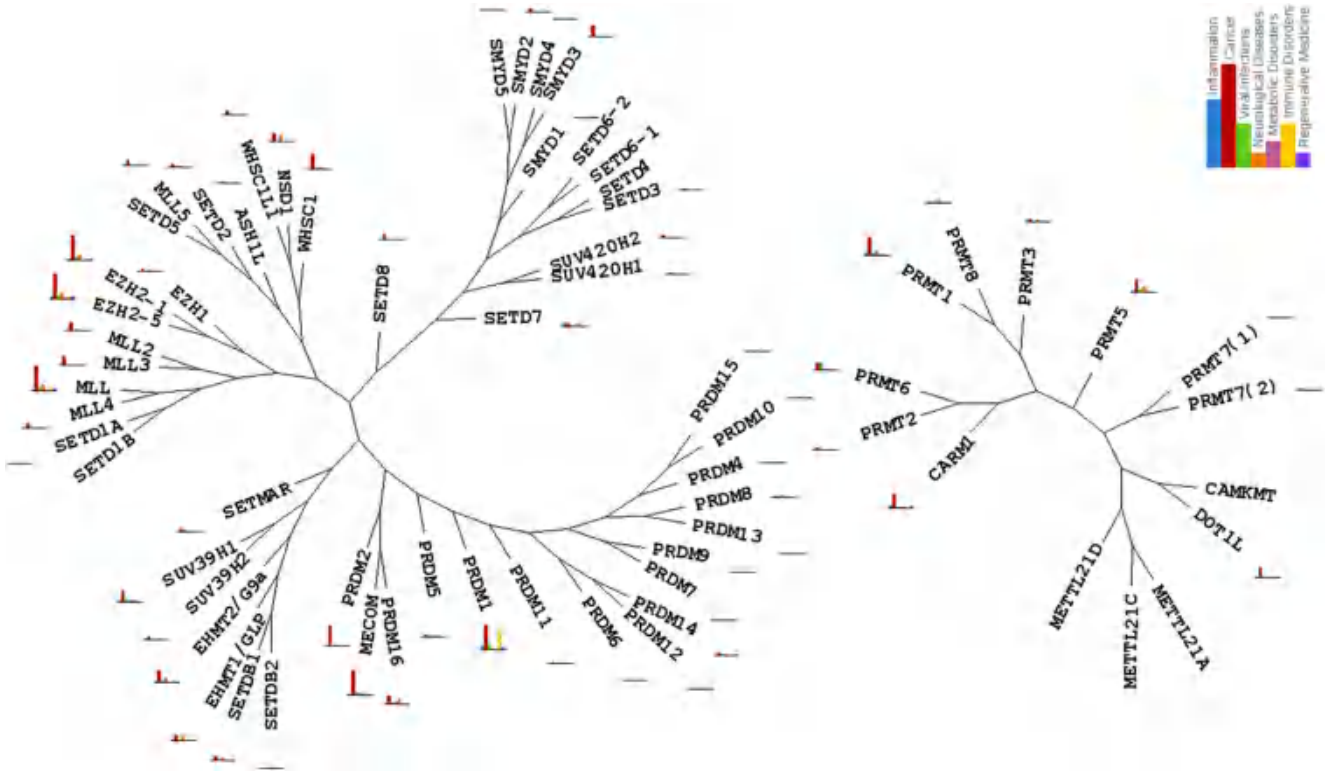


Fig. 1. Phylogenetic tree and disease association of human histone Methyltransfe-Rases.
This figure is drawn based on the website server: http://apps.thesgc.org/resources/phylogenetic_trees/index.php.

also peptide-competitive. By iterative medicinal chemistry to incorporate a propyl-pyrrolidine group into the enzyme lysine channel, a potent inhibitor of G9a, A-366 (IC_{50}: 3.3 nM), was identified, which displays over 1000-fold selectivity over 21 other methyltransferases (Sweis et al., 2014) (Table 1 and Fig. 3B).

3. Suv39H1/2: a pair of closely homologous histone H3K9me3 methyltransferases

Drosophila Su(var)3-9 gene was first discovered through modifier mutations of position-effect variegation (PEV) in 1994, which showed that Su(var)3-9 exhibited a strong suppressor effect in the regulation of PEV (Tschiersch et al., 1994). Later on, its homolog in fission yeast Clr4 was found to be essential for silencing of centromeres and mating-type loci (Ivanova et al., 1998), and its mammalian homolog SUV39H1 played a critical role in heterochromatin organization and transcriptional silencing (Aagaard et al., 1999; Firestein et al., 2000; Melcher et al., 2000). Therefore, the Su(var)3-9 gene has a conserved function from fission yeast to human, which is essential for establishing higher order chromatin structure and transcriptional silencing. Nevertheless, the molecular mechanism of how Su(var)3-9 functioned was unclear until the group of Dr. Jenuwein found that human SUV39H1 and murine Suv39h1 are histone H3K9 methyltransferases (Rea et al., 2000). Sequence analysis revealed that this protein contained an evolutionarily conserved motif of 130 amino acids, i.e., the SET domain (named after Suv39, E(z) and Trithorax), which was later shown to be the catalytic domain responsible for histone lysine methylation (Rea et al., 2000) and attracted enormous attention since then (Martin & Zhang, 2005). In addition to the SET domain, these H3K9 methyltransferases also contain an evolutionarily conserved chromodomain, which could bind to their methylation product H3K9me3 (Zhang et al., 2008; T. Wang et al., 2012). The ability of SUV39H1 and its homologs to both 'write' and 'read' H3K9me3 facilitates the spreading of the repressive H3K9me3 mark along the chromatin for assembly of heterochromatic domains and heterochromatin maintenance during cell divisions in Schizosaccharomyces pombe and probably other organisms (Zhang et al., 2008).

In 2005, by screening natural products, Chaetocin, a fungal metabolite, was identified as the first inhibitor of histone methyltransferases, and was shown to specifically target the Su(var)3-9 histone H3K9 methyltransferases (Greiner et al., 2005) (Table 4). Kinetic results revealed that it was competitive with the cofactor SAM. However, later studies demonstrated that Chaetocin was a non-specific inhibitor on the basis of the following lines of evidence: 1. Chaetocin could inhibit many totally unrelated enzymes and the disulfide functionality is required for inhibition. 2. Compounds derived from Chaetocin lacking the disulfide functionality lost activity in histone H3K9 methyltransferases inhibition, but other similar natural products retaining the disulfide functionality could still inhibit histone H3K9 methyltransferases (Iwasa et al., 2010; Cherblanc et al., 2013). Because all of the histone H3K9 methyltransferases contain Zinc-binding domains, the inhibition may arise from breaking the Zinc-binding domains by the disulfide functionality of Chaetocin, and this hypothesis requires further validation, particularly structural studies (Greiner et al., 2013).

A pair of synthetic compounds (BIX-01338 and BIX-01337) was identified for SUV39H1 from the same screen in which the G9a specific BIX-01138 compound was identified (Kubicek et al., 2007) (Table 4). These two compounds acted broadly on all enzymes tested, including SUV39H1, G9a and PRMT1 (Kubicek et al., 2007). Consistently, they are SAM-competitive, because all the histone lysine/arginine methyltransferases use SAM as the methyl group donor. Since the initial report of these inhibitors for the SUV39H1/2 enzymes, no further optimization for these compounds has been reported.

4. DOT1L: a histone H3K79 methyltransferase

The Dot1 (Disruptor of telomeric silencing) gene was first identified in a genetic screen for genes whose overexpression disrupted telomeric silencing in Saccharomyces cerevisiae (Singer et al., 1998). Dot1 and its mammalian homolog DOT1L (DOT1-Like protein) were later found to

Table 1
Inhibitors of G9a/GLP.

s	Chemical structure	IC_{50} (μM) G9a/GLP	PDB code of Complex structure	PMID
BIX-01294		1.7/38	–/3FPD	17289593 (Kubicek et al., 2007)
UNC0224		0.015/0.05	3K5K/–	19891491 (Liu et al., 2009)
UNC0321		0.009/0.015		20614940 (Liu et al., 2010)
E72		–/0.1	–/3MO5	20434463 (Chang et al., 2010)
UNC0638		0.015/0.019	3RJW/–	21743462 (Vedadi et al., 2011)
UNC0925		b0.0025/–		24443078 (Konze et al., 2014)
UNC0642		0.0037/0.0025		24102134 (Liu et al., 2013)
spiro[cyclobutane-1,3′-indol]-2′-amine		0.153/–		(Sweis et al., 2014) (Ramzi F. Sweis & B. Soni, 2014)
A-366		0.0033/0.038	4NVQ/–	Sweis et al., 2014 (Ramzi F. Sweis & B. Soni, 2014)
BIX-01338		4.7/–		17289593 (Kubicek et al., 2007)
BRD9539		6.3/–		22536950 (Yuan et al., 2012)
E67		–/0.273	–/3MO2	20434463 (Chang et al., 2010)
E70		–/1.3		20434463 (Chang et al., 2010)
E11		–/0.154 (Kd)	–/3MO0	20434463 (Chang et al., 2010)

methylate histone H3K79 on the nucleosome core (Feng et al., 2002; Lacoste et al., 2002; Ng et al., 2002; van Leeuwen et al., 2002), and this activity is essential for telomeric silencing and Sir protein localization at discrete loci in yeast, because, in Dot1 deletion cells, the Sir proteins were redistributed along the genome with compromised telomeric silencing (Lacoste et al., 2002; Ng et al., 2002; van Leeuwen et al., 2002). Dot1-mediated H3K79 methylation is dependent on monoubiquitination of histone H2B catalyzed by the Rad6-Bre1 complex, and the histone H2B monoubiquitination is facilitated by the Paf1 complex, a complex associated with RNA polymerase II mediated transcription elongation (Briggs et al., 2002; Nishioka et al., 2002; Krogan et al., 2003; Ng et al., 2003; Wood et al., 2003). Dot1/DOT1L is the only known enzyme responsible for H3K79 methylation, and carries out mono-, di- and trimethylation in a non-processive manner (Frederiks et al., 2008). Our high resolution crystal structure of human DOT1L showed that DOT1L contains a non-SET domain catalytic domain, and

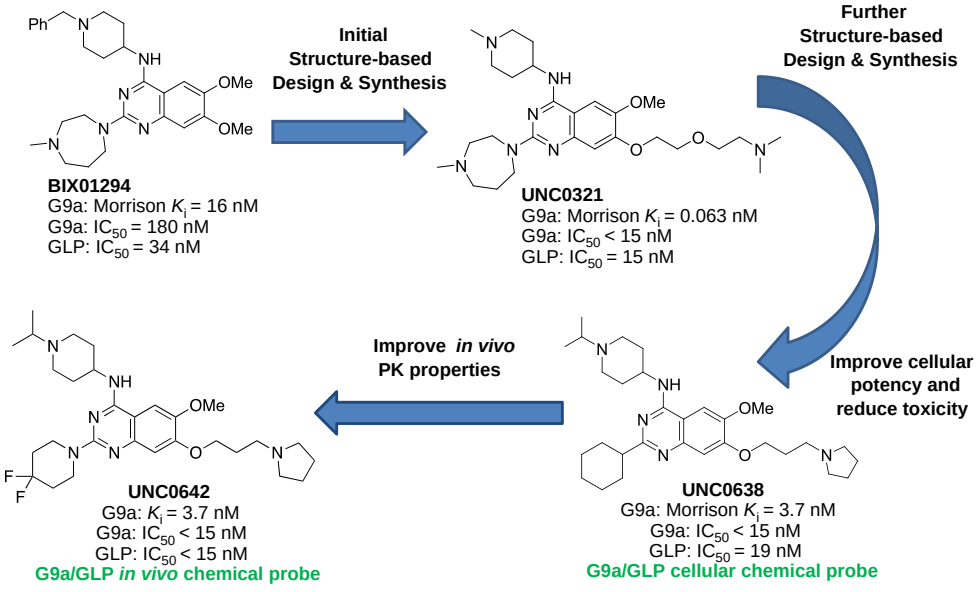


Fig. 2. Structure-based design and synthesis of G9a/GLP inhibitors.

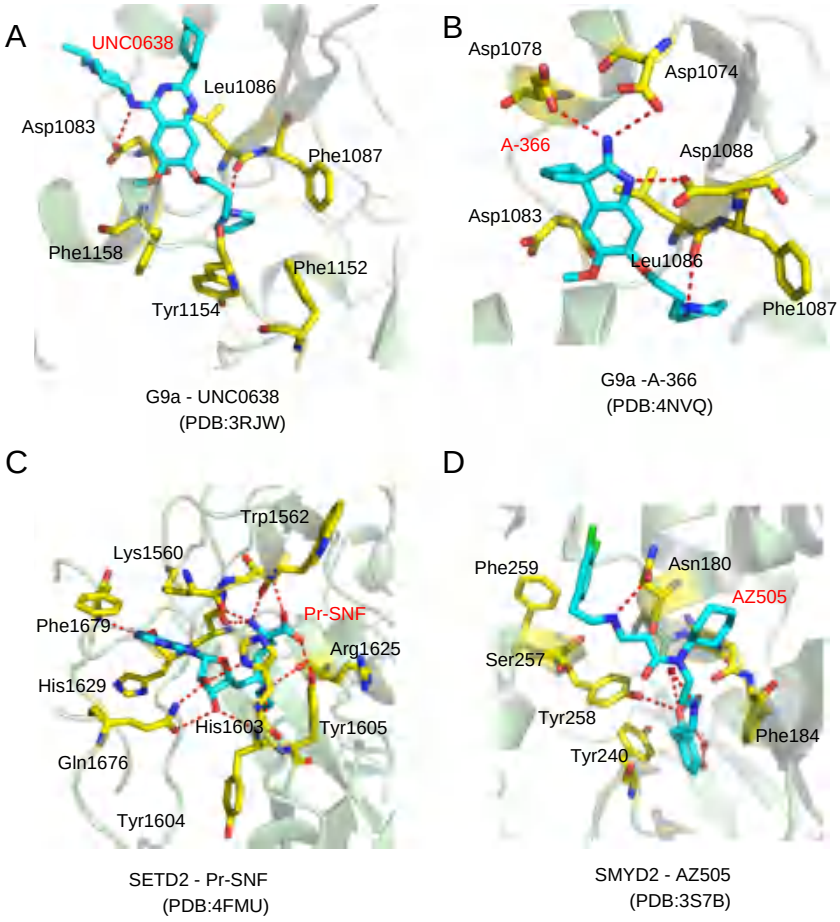


Fig. 3. Inhibitor binding in the G9a, SMYD2 and SETD2 complex structures. (A) UNC0638 in G9a, (B) A-366 in G9a, (C) Pr-SNF in SETD2, (D) AZ505 in SMYD2. Protein residues in contact with inhibitors are depicted by stick models with their carbon atoms colored as yellow. Inhibitors are shown as stick models with their carbon atoms colored as aquamarine. Hydrogen bonds are marked with a red dashed line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

its catalytic domain adapts a class I SAM-dependent methyltransferase fold (Min et al., 2003a), a fold also observed in histone/protein arginine methyltransferases and DNA methyltransferases (Schubert et al., 2003).

Histone H3K79 methylation is generally associated with transcriptional activation. Dot1/DOT1L-mediated histone H3K79 methylation plays an important role in various biological and cellular processes, such as transcriptional regulation, cell cycle regulation, DNA repair, myocardial genesis, embryonic development and hematopoiesis (Nguyen & Zhang, 2011). Knockout of DOT1L in mice results in cardiovascular defects, including heart enlargement and vasculature (Jones et al., 2008), and the cardiac-specific knockout of mouse DOT1L was believed to suppress the expression of Dystrophin, which is a critical component of the dystrophin–glycoprotein complex (DGC). DGC is involved in signal force transmission and relieving mechanical stress during heart contraction (Nguyen & Zhang, 2011). The DOT1L-mutant mice also displayed anemia and defects in embryonic hematopoiesis (Feng et al., 2010b). Most importantly, DOT1L was shown to interact with a subset of MLL translocation fusion proteins, including AF10, ENL, AF9 and AF4 (Nguyen & Zhang, 2011). MLL fusion proteins, such as AF10, AF9, ENL and AF4 are involved in two-thirds of all MLL-associated leukemias (Ayton & Cleary, 2001). These MLL fusion proteins would interact with DOT1L and recruit the DOT1L-mediated H3K79 methylation to their target genes, including proleukemogenic genes (such as Hoxa9 and Meis1) (Nguyen & Zhang, 2011). Therefore, DOT1L is implicated in various human diseases, leukemia in particular, which raises the possibility of DOT1L as a potential therapeutic target for drug discovery, including inhibiting the enzymatic activity of DOT1L and disrupting its interaction with the MLL fusion proteins.

In 2011, Epizyme Inc., a startup pharmaceutical company, reported a potent and selective DOT1L inhibitor EPZ004777 (Table 2), which could specifically inhibit H3K79 methylation and suppress leukemogenic genes (Daigle et al., 2011). Remarkably, this compound selectively killed leukemic cells bearing the MLL translocation genes, but virtually not non-MLL-translocated cells. An extended survival was also observed in a mouse MLL xenograft model after in vivo administration of EPZ004777 (Daigle et al., 2011). Following this exciting discovery, over 20 DOT1L inhibitors have been reported in the literature and many more are patented in less than 3 years (Yao et al., 2011; Anglin et al., 2012; Basavapathruni et al., 2012; Yu et al., 2012; Copeland, 2013; Daigle et al., 2013; Deng et al., 2013; Yu et al., 2013; Basavapathruni et al., in press). Four routes were followed in design and optimization of the DOT1L inhibitors, i.e., SAH-based, mechanism-based, carbamate-based and urea/benzimidazole-containing inhibitors (Anglin & Song, 2013), but all of these inhibitors are SAM-competitive inhibitors (Fig. 4). The EPZ004777 compound and its optimized version EPZ-5676 belong to the urea/benzimidazole-containing inhibitors (Daigle et al., 2013) (Table 2). EPZ-5676 is a highly potent and selective inhibitor of DOT1L with K_i of 70 pM, and has over 10,000-fold selectivity against other HMTs. Superposition of the crystal structures of DOT1L in complex with SAM, EPZ-5676 and TT8 (a SAH-based inhibitor) showed that EPZ-5676 forms hydrogen-bonds with residues Asp222, Glu186, Gly163 and Asp161 from DOT1L, and the side chains of Thr139 and Phe239 move away from the SAM binding site, which open up to form a hydrophobic pocket for binding the 4-tert-butylphenyl group of EPZ-5676 and contribute to the superior potency and selectivity (Daigle et al., 2013) (Fig. 4C). Now EPZ-5676 is in phase I clinical trials

Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007

for advanced hematologic malignancies, including acute leukemia with rearrangement of the MLL gene, which is a first-in-human study.

5. EZH2 and the PRC2 complex: a histone H3K27 methyltransferase

The Drosophila E(z) (Enhancer of zeste) gene belongs to a group of genes called Polycomb group genes, which were originally identified to be responsible for maintaining the spatial expression pattern of homeotic box (Hox) genes during development in Drosophila. Mutation of any of them would cause homeotic transformation. Polycomb group proteins are transcriptional repressors, antagonistic to Trithorax group proteins, a group of transcriptional activators. This cellular memory system is conserved in nearly all multicellular eukaryotes (Pirrotta & Gross, 2005). PcG proteins often function as complexes, and the two best-characterized complexes are the Polycomb Repressive Complex 1 (PRC1) and 2 (PRC2). PRC2 exhibits histone methyltransferase activity on H3K27 via its catalytic subunit E(z) or mammalian homologs EZH1/EZH2 (Enhancer of zeste homolog 1 or 2) (Cao et al., 2002; Czermin et al., 2002; Muller et al., 2002; Cao & Zhang, 2004). PRC1 contains a chromodomain protein, Polycomb, which preferentially recognizes the histone H3K27me3 mark (Fischle et al., 2003; Min et al., 2003b). One hypothesis is that H3K27 methylation by PRC2 creates a binding site for the chromodomain of Polycomb, which in turn maintains transcriptional silencing through an unknown mechanism (Wang et al., 2004).

The PRC2 complex consists of four core components: EZH2/EZH1, EED, SUZ12 and RbAp46/48 (Czermin et al., 2002; Kuzmichev et al., 2002; Muller et al., 2002). EZH2/EZH1 has virtually no histone methylation activity on its own, but exhibits robust methylation activity as a complex (Czermin et al., 2002; Muller et al., 2002). The N-terminal domain of EED is also essential for histone H3K27 trimethylation of H3K27 because an N-terminally truncated EED can still form a complex with EZH2, but this complex is not active any more (Tie et al., 2007). In addition to binding to EZH2, others and we have also shown that EED is able to bind to histone H3K27me3, suggesting a mechanism for PRC2 to propagate and spread the H3K27me3 mark to daughter strands during cell division (Margueron et al., 2009; Xu et al., 2010; Nayak et al., 2011). In addition to silencing Hox genes, EZH2-mediated H3K27 methylation is also involved in X-inactivation, germline development, stem cell pluripotency and differentiation, and cancer metastasis (Cao & Zhang, 2004).

EZH2 is linked to various human cancers through distinct mechanisms, including overexpression, activating mutations and inactivating mutations. Overexpression of EZH2 was first linked to prostate cancer and breast cancer by microarray analysis over a decade ago (Varambally et al., 2002; Kleer et al., 2003). EZH2 overexpression was also observed in other cancers and could serve as a prognostic marker (Chase & Cross, 2011; Takawa et al., 2011). Point mutations at Tyr641, a residue located in the catalytic SET domain of EZH2, was recently found to exist in 8–24% of non-Hodgkin lymphomas, and this EZH2 mutant is always heterozygous (Morin et al., 2010; Sneeringer et al., 2010; Morin et al., 2011; Yap et al., 2011; Lohr et al., 2012). Hence, tumor cells with this mutation would have one copy of wild type EZH2 and one copy of mutated EZH2. Interestingly, enzymatic assays showed that the wild-type EZH2 is more efficiently in generating monomethylation than di or tri-methylation of histone H3K27, whereas the mutant EZH2 is inactive with unmodified H3K27 as a substrate, but very efficient in generating H3K27me2 and especially H3K27me3 marks (Sneeringer et al., 2010; Yap et al., 2011). Therefore, the wild type and Tyr641 mutant EZH2 would cooperatively generate and maintain hypertrimethylation of H3K27, which contributes to lymphomagenic proliferation by abnormal silencing of the PRC2 target genes (McCabe et al., 2012). On the other hand, homozygous inactivating mutations of EZH2, including missense, nonsense and premature stop codons, would lead to various myeloid neoplasms with poor prognosis (Ernst et al., 2010; Nikoloski et al., 2010). Therefore, both activating/overexpressing and inactivating EZH2 lead to abnormal PRC2 functions and carcinogenesis.

In 2012, three groups reported potent and selective small molecule inhibitors for EZH2 independently, which could diminish histone

Table 2
Inhibitors of Dot1L.

Compound Name	Chemical structure	IC_{50} (μM)	PDB code of complex structure	PMID
EPZ004777		0.0004 0.3 (Ki) 0.0001 (Kd)	4EKI 4ER34ER5	21741596 (Daigle et al., 2011) 22978415 (Basavapathruni et al., 2012)
EPZ-5676		8.00E-05	4HRA	23801631 (Daigle et al., 2013)
SGC0946		0.0003	4ER6	23250418 (Yu et al., 2012)
yao11_cmpd_4		0.038		21936531 (Yao et al., 2011)
yao11_cmpd_2		1.1		21936531 (Yao et al., 2011)
EPZ003696		0.0017 (Kd)	4EKG	22978415 (Basavapathruni et al., 2012)
EPZ000004			4EK9	22978415 (Basavapathruni et al., 2012)
FED2			4EQZ	
SGC0947			4ER7	
FED1		0.066–0.55 0.00046 (Ki)	4ER0	
TT8		0.29 (Ki)	3SR4	21936531 (Yao et al., 2011)
5ID		18.2	3UWP	

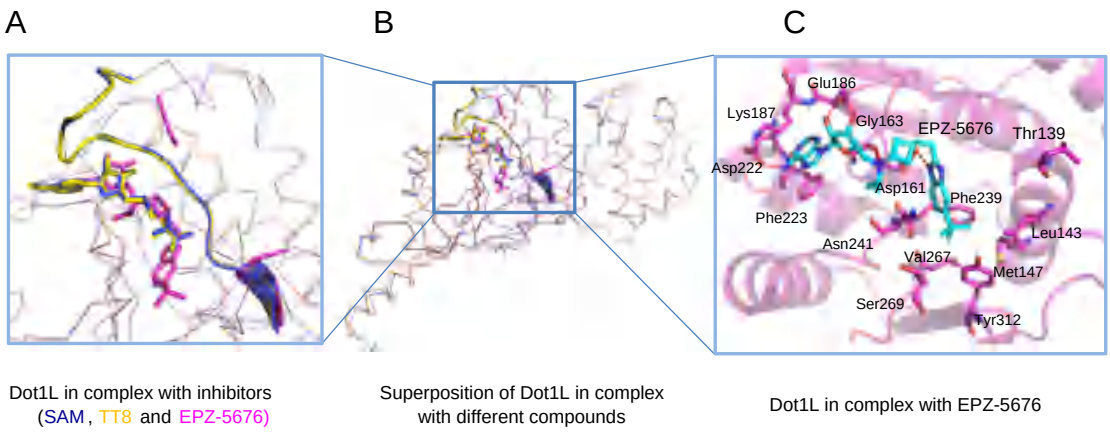


Fig. 4. Distinct binding mode of EPZ-5676 by DOT1L. (A) A large conformational change of a loop near SAM (depicted in cartoon) is introduced by the 4-tert-butylphenyl group of EPZ-5676. SAM (blue, PDB: 1NW3), TT8 (yellow, PDB: 3SR4) and EPZ-5676 (pink, PDB: 4HRA) are shown as stick models. (B) Superposition of crystal structures of SAM, TT8 and EPZ-5676 in complex with DOT1L. SAM, TT8 and EPZ-5676 are shown as stick models. (C) Detailed interactions between EPZ-5676 and DOT1L. EPZ-5676 is shown as stick model in aquamarine, and binding residues from DOT1L are shown as stick models in pink. Hydrogen bonds are marked with a red dashed line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

H3K37 methylation and inhibit diffuse large B-cell lymphoma with EZH2-activating mutations (Knutson et al., 2012; McCabe et al., 2012; Qi et al., 2012). These three inhibitors, EPZ005687, GSK126 and El1 are all SAM-competitive (Table 3), and share the same chemotype pyridines. They exhibited about 500-fold to 10,000 fold selectivity over other histone methyltransferases and about 100-fold selectivity for EZH2 over its closely homologous EZH1. Based on sequence alignment and structural modeling, a stretch of 4 residues difference between EZH2 and EZH1, which is located in the post-SET domain, may contribute to the decreased potency for EZH1 (McCabe et al., 2012). On the basis of the first series of EZH2 inhibitors, the group of Dr. Jin from the UNC developed an inhibitor UNC1999 (Konze et al., 2013) (Table 3). UNC1999 retains the key structural features of EPZ005687 and GSK126, especially the pyridone indazole scaffold, but has the morpholinomethyl region modified to obtain improved physicochemical properties. UNC1999 has low selectivity for EZH2 over EZH1 (about 10 fold) (Table 3), but keeps the high selectivity over other histone methyltransferases (N10,000-fold). More importantly, UNC1999 has better oral bioavailability than the previously reported inhibitors (Konze et al., 2013). Simultaneously, a group of scientists from the Constellation Pharmaceuticals, Inc. reported another series of compounds that bear a benzamide core (Table 3). These inhibitors are also SAM-competitive (Garapaty-Rao et al., 2013), and compound 3 reported in the paper exhibited submicromolar in vitro inhibition for EZH2 (wild type and mutant) and EZH1, and selectively suppress H3K27 methylation globally in cells (Konze et al., 2013).

Table 3
Inhibitors of EZH2/EZH1.

Compound Name	Chemical structure	IC_{50} (μM) EZH2/EZH1	PDB code of complex structure	PMID
EPZ005687		0.054/1		23023262 (Knutson et al., 2012)
GSK126		0.0005/0.089		23051747 (McCabe et al., 2012)
EI1		0.0094/1.34		23236167 (Qi et al., 2012)
UNC1999		0.0046/0.045		23614352 (Konze et al., 2013)
garapatyrao13_cmpd3		0.032/0.21		24183969 (Garapaty-Rao et al., 2013)
EPZ-6438		0.011/0.4		23620515 (Knutson et al., 2013)
copeland12_75				Patent US 20120071418A1
1346702-70-2		0.004/–		WO2011140325
GSK343		0.004/0.24		DOI: 10.1021/ml3003346
BRD9539		10/–		22536950 (Yuan et al., 2012)

By means of iterative medicinal chemistry, Epizyme Inc. developed a second-generation compound EPZ-6438 (Table 3), which has superior potency and pharmacokinetic properties over EPZ005687. Specifically, EPZ-6438 displayed a 35-fold selectivity versus EZH1 and N4500-fold selectivity over 14 other HMTs (Knutson et al., 2013). In June 2013, Epizyme initiated a Phase 1/2 clinical trial of EPZ-6438 in patients with advanced solid tumors or with relapsed or refractory B-cell lymphoma.

6. Other histone/protein methyltransferases and inhibitors

6.1. General SAM-dependent methyltransferase inhibitors: SAH, SNF and DZNep

SAM-dependent methyltransferases transfer the methyl group from the methyl donor SAM to substrates, producing SAH (S-adenosyl-L-homocysteine) and methylated substrates. Therefore, SAH can act as a general inhibitor of SAM-dependent methyltransferases. Sinefungin, which is an antifungal antibiotics isolated from Streptomyces griseolus and was first identified as a potent inhibitor of virion mRNA methyltransferases in 1978 (Pugh et al., 1978), is also potent to all SAM-dependent methyltransferases. DZNep (3-Deazan-eplanocin A) is a potent inhibitor of SAH hydrolase (Glazer et al., 1986), which hydrolyzed SAH to homocysteine and adenosine. Inhibition of the SAH hydrolase will cause accumulation of SAH, inhibiting SAM-dependent methyltransferases indirectly. Although these three compounds and their analogs have been used as general SAM-dependent methyltransferase inhibitors, recent studies show that they also exhibit strong selectivity among histone lysine methyltransferases. By profiling against a panel of histone methyltransferases, Sinefungin shows over 3- to 400-fold selectivity for SMYD2 over other methyltransferases tested (Qi et al., 2012). So far, over half of human histone methyltransferases have been determined structurally by us and others (Shah et al., 2014), which would aid us in designing target-specific SAM-competitive inhibitors on the basis of structural differences around the SAM binding pocket. Indeed, recently, we reported a series of N-alkyl sinefungin analogs, which selectively inhibits SETD2 over other histone methyltransferases tested, and our cocrystal structure shows that the N-propyl Sinefungin (Pr-SNF) compound interacts preferentially with SETD2 by matching the distinct transition-state features of SETD2 in its catalytically active conformation (Zheng et al., 2012) (Table 4 and Fig. 3C).

In 2007, the group of Dr. Yu from the Genome Institute of Singapore found that DZNep could inhibit histone H3K27 methylation and reactivate a set of genes selectively repressed by PRC2 in breast cancer. Reactivation of these genes induces apoptosis in breast cancer cells, but not in normal cells (Tan et al., 2007). Although DZNep could also inhibit other histone methylation marks (Miranda et al., 2009; Lee & Kim, 2013), it seems to have much more profound effect on the PRC2 complex and histone H3K27 methylation. Distinct from other HMT inhibitors, DZNep inhibits the PRC2 complex-mediated histone H3K27 methylation through increased degradation of the PRC2 complex. In addition to killing breast cancer cells, DZNep has also been shown to promote apoptosis in other types of cancers, such as, tumor-initiating hepatocellular carcinoma (Chiba et al., 2012), acute myeloid leukemia (Zhou et al., 2011), non-small cell lung cancer (Kikuchi et al., 2012), Head and neck squamous cell carcinoma (HNSCC) (Gannon et al., 2013), Colon Cancer (Benoit et al., 2013), tongue cancer (Li et al., 2013), pancreatic cancer (Hung et al., 2013) and ovarian cancer (Shen et al., 2013) in a PRC2 and H3K27 methylation-dependent manner.

6.2. SMYD2: a histone and protein lysine methyltransferase

SMYD2 (SET and MYND domain-containing protein 2) is a histone and protein lysine methyltransferase (Fig. 1). It has been reported to methylate both histone (H2B, H3, and H4) and nonhistone proteins, such as tumor suppressors p53 and Rb (Brown et al., 2006; J. Huang et al., 2006; Abu-Farha et al., 2008; J. Wu et al., 2011). Enzymatic analysis shows that SMYD2 is a monomethyltransferase that prefers nonmethylated p53 peptide as a substrate among different histone and protein substrates tested in vitro (J. Wu et al., 2011). SMYD2 plays a critical role during early development and in human ES cell differentiation (Sese et al., 2013). Overexpression of SMYD2 was frequently detected in esophageal squamous cell carcinoma (ESCC) patients with a worse survival rate than those with non-expressing tumors (Komatsu et al., 2009). By means of the high throughput AlphaScreen technology to screen against a 1.23 million compound library, a potent and selective inhibitor of SMYD2, AZ505 (K_d of 0.5 μM) (Table 4), was discovered. It is noteworthy that it also exhibited strong selectivity over its close homologue SMYD3 and presumably other members in the subfamily (Ferguson et al., 2011). AZ505 is a substrate-competitive inhibitor, occupying a similar location in the substrate-binding groove of SMYD2 like G9a/GLP inhibitors do in the G9a/GLP complex structures (Ferguson et al., 2011) (Fig. 3D).

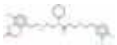
6.3. Arginine methyltransferases

Arginine methylation is a very abundant covalent post-translational modification in cells, which regulates diverse cellular processes, including transcriptional regulation, RNA processing, signal transduction and DNA repair (Bedford & Richard, 2005). Nine protein arginine methyltransferases (PRMT) have been identified in the human genome, which can be grouped into three classes. Type I PRMTs (PRMT1, 2, 3, 4, 6, and 8) generate both monomethylarginine and asymmetric dimethylarginine marks. PRMT5 is the only confirmed Type II arginine methyltransferase generating monomethylarginine and symmetric dimethylarginine marks. PRMT7 is a type III arginine methyltransferase generating only monomethylarginine mark. The enzymatic activity of PRMT9 is not characterized yet (Bedford & Richard, 2005). Protein arginine methyltransferases can methylate a variety of targets, including histones, Sm proteins and transcription factors (Bedford & Richard, 2005; Di Lorenzo & Bedford, 2011). Many of these target proteins contain glycine and arginine-rich (GAR) motifs, such as SmD1/3 and MIWI/PIWI proteins (Friesen et al., 2001; Siomi et al., 2010). Some target proteins harbor PGM motifs (Cheng et al., 2007). Overexpression, aberrant splicing or mutations of these different PRMTs have been implicated in various types of cancer, which have been nicely reviewed recently (Yang & Bedford, 2013), so here we will just focus on the advances in developing PRMT inhibitors as chemical probes and therapeutic reagents.

The first series of arginine methyltransferase inhibitors were reported by the group of Dr. Bedford from the University of Texas M.D. Anderson Cancer Center in 2004. One of these inhibitors, AMI-1 (arginine methyltransferase inhibitor-1) (Table 4), was shown to be non-SAM-competitive and specifically inhibit arginine methylation, not lysine methylation. Since then, many groups have devoted a great deal of effort to developing arginine methyltransferase inhibitors, which can be classified as AM1-1-like (Castellano et al., 2010; Dowden et al., 2010), substrate-competitive inhibitors (Purandare et al., 2008; Huynh et al., 2009; Wan et al., 2009; Sack et al., 2011), bisubstrate inhibitors (Dowden et al., 2010; Lakowski et al., 2010; t Hart et al., 2011), substrate-targeting inhibitors (inhibitors targeting substrates directly, such as histone H3 or H4 other than PRMTs) (Feng et al., 2010a; Selvi et al., 2010; J. Wang, et al., 2012) and allosteric inhibitors of PRMT3 (Siarheyeva et al., 2012; Liu et al., 2013b) (Table 4). Most of these inhibitors have modest inhibition activity and poor cell permeability with no selectivity among the PRMT members. It is worth noting that the indole and pyrazole inhibitors of CARM1, which occupy the substrate-binding groove, have decent selectivity over PRMT1/3 (over 300 folds) (Sack et al., 2011) (Table 4). Structural analysis revealed that the specificity arises from structural/sequence differences from the putative substrate binding groove, particularly from the N-domain among these members (Sack et al., 2011). Allosteric inhibitors are another interesting set of

Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007

Table 4
Inhibitors of SUV39H1 and other methyltransfearses.

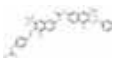
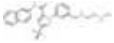
Protein name	Compound name	Chemical structure	IC_{50} (μM)	PDB code of complex structure	PMID
SUV39H1	Chaetocin				16408017 (Greiner et al., 2005)
	BIX-01338		1.1		17289593 (Kubicek et al., 2007)
	BIX-01337		3.7		17289593 (Kubicek et al., 2007)
SETD2	zheng12_cmp3d		0.48		23043551 (Zheng et al., 2012)
	0UM		8.2 0.36 (Kd)	4FMU	23043551 (Zheng et al., 2012)
SETD7	compound 4		63.6		17458842 (Mai et al., 2007) 18348515 (Mai et al., 2008)
	compound 8		37.1		17458842 (Mai et al., 2007)
	compound 9		34.6		17458842 (Mai et al., 2007) 18348515 (Mai et al., 2008)
	compound 10		19.1		17458842 (Mai et al., 2007) 18348515 (Mai et al., 2008)
	cyproheptadine		20		Norikazu et al. (2012) Kyushu Institute of Technology. Patent JP 2011-224604 20111012. Priority: JP 2010-230916 20101013. AN 2012:768019
	CCT021895		20		22772057 (Francis et al., 2012)
	0N6			4E47	
	IL4			4JDS	
	IL8			4JLG	
	K15			3VUZ	23519668 (Niwa et al., 2013)
	KH3		10	3VV0	23519668 (Niwa et al., 2013)
SETD8	EBI-435				Kodoma et al. WO 2011010715
	EBI-455				Kodoma et al. WO 2011010715
	EBI-099		4.7		Kodoma et al. WO 2011010715
SMYD2	AZ505		0.12	3S7B	21782458 (Ferguson et al., 2011)
PRMT1	AMI-1		8.81		15056663 (Cheng et al., 2004) 17323938 (Ragno et al., 2007) 17432842 (Spannhoff et al., 2007)

(continued on next page)

Table 4 (continued)

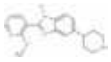
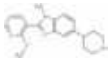
Protein name	Compound name	Chemical structure	IC_{50} (μM)	PDB code of complex structure	PMID
	AMI-2		11.18		15056663 (Cheng et al., 2004)
	AMI-3		16.29		15056663 (Cheng et al., 2004)
	AMI-4		0.19		15056663 (Cheng, et al., 2004)
	AMI-5		1.41		15056663 (Cheng et al., 2004) 17323938 (Ragno et al., 2007) 17432842 (Spannhoff et al., 2007)
	AMI-6		5.11		15056663 (Cheng et al., 2004) 17323938 (Ragno et al., 2007)
	AMI-7		1.61		15056663 (Cheng et al., 2004)
	AMI-8		1.8		15056663 (Cheng et al., 2004)
	AMI-9		0.28		15056663 (Cheng et al., 2004)
	sinefungin		1.63		15056663 (Cheng et al., 2004)
	Bissinger_2e		1.5		21440447 (Bissinger et al., 2011)
	allantodapsone		1.7		17432842 (Spannhoff et al., 2007)
	dowden2010_16		2.9		20219369 (Dowden et al., 2010)
	compound 4		11.8		17458842 (Mai et al., 2007)
	compound 8		3.4		17458842 (Mai et al., 2007)
	compound 9		3		17458842 (Mai et al., 2007)
	compound 10		9.9		17458842 (Mai et al., 2007)
	Bonham10_C4		4.2		20345902 (Bonham et al., 2010)
	BIX-01338		6		17289593 (Kubicek et al., 2007)

Table 4 (continued)

Protein name	Compound name	Chemical structure	IC_{50} (μM)	PDB code of complex structure	PMID
	A36		12		22928876 (J. Wang et al., 2012; T. Wang et al., 2012)
	NS-1		12.7		20666457 (Feng et al., 2010a, 2010b)
	NS-2		43.1		20666457 (Feng et al., 2010a, 2010b)
PRMT3	TDU		2.5	3SMQ	22795084 (Siarheyeva et al., 2012)
	KTD			4HSG	
PRMT5	1313207-04-3				
	A9145C		0.035		23071334 (Antonysamy et al., 2012)
	EPZ004777		0.5		21741596 (Daigle et al., 2011)
CARM1					
	therrien09_2b		0.06		19836951 (Therrien et al., 2009)
	therrien09_12		0.2		19836951 (Therrien et al., 2009)
	therrien09_23d		0.9		19836951 (Therrien et al., 2009)
	therrien09_23m		0.32		19836951 (Therrien et al., 2009)
	wan09_1		0.04		19632837 (Wan et al., 2009)
	wan09_17b		0.07		19632837 (Wan et al., 2009)
	benzo[d]imidazole related analog_17a		0.41		19632837 (Wan et al., 2009)
	benzo[d]imidazole related analog_17b		0.07		19632837 (Wan et al., 2009)
	benzo[d]imidazole related analog_17c		0.26		19632837 (Wan et al., 2009)
	benzo[d]imidazole related analog_17d		0.12		19632837 (Wan et al., 2009)
	Benzo[d]imidazole related analog_17e		4.6		19632837 (Wan et al., 2009)
	Benzo[d]imidazole related analog_2		0.84		19632837 (Wan et al., 2009)

(continued on next page)

Table 4 (continued)

Protein name	Compound name	Chemical structure	IC_{50} (μM)	PDB code of complex structure	PMID
	benzo[d]imidazole related analog_5		17		19632837 (Wan et al., 2009)
	benzo[d]imidazole related analog_8		1.2		19632837 (Wan et al., 2009)
	Benzo[d]imidazole related analog_9		2.9		19632837 (Wan et al., 2009)
	benzo[d]imidazole related analog_14		0.7		19632837 (Wan et al., 2009)
	Bonham10_C4		2.7		20345902 (Bonham et al.)
	cheng_cmpd_7g		8.6		21612300 (Cheng et al., 2011)
	TBDD		25		20022955 (Selvi et al., 2010)
	compound 4		52.7		17458842 (Mai et al., 2007)
	compound 8		35.7		17458842 (Mai et al., 2007)
	compound 9		100.1		17458842 (Mai et al., 2007)
	compound 10		40.8		17458842 (Mai et al., 2007)
	845		0.027	2Y1X	21410432 (Sack et al., 2011)
	849		0.465 (Kd)	2Y1W	21410432 (Sack et al., 2011)

inhibitors for PRMTs (Siarheyeva et al., 2012; Liu et al., 2013b) (Table 4). Structural analyses showed that these allosteric inhibitors reside in a pocket formed mainly by the dimerization arm (Fig. 5A and B). The dimerization arm is a common and essential structure element among the type I PRMTs, which is required for their enzymatic activity. On the other hand, the dimerization arm is also very diverse among the PRMT members in sequence and structure (Fig. 5A and B). Therefore, it provides another venue in developing potent and selective inhibitors for the PRMT enzymes.

7. MBT repeat domain proteins: mono- and di-methyl lysine binding proteins

The MBT (Malignant Brain Tumor) repeat is a structural motif of ~100 amino acids, which often exists as tandem repeats (Wismar et al., 1995). It was originally identified in the Drosophila tumor-suppressor protein L(3)mbt, a putative tumor-suppressor gene. Mutations of L(3)MBT affect synchronous mitotic divisions and nuclear migration of early embryos (Yohn et al., 2003). Based on sequence analysis, the MBT repeat shows structural similarity with the chromodomain, Tudor and PWWP domains, which are jointly referred to as the Tudor domain 'Royal Family' (Maurer-Stroh et al., 2003). The MBT domain is conserved across metazoa. There are 9 MBT domain containing proteins in the human genome. Like other members in the Tudor Royal superfamily, MBT domains in different proteins have been shown to bind to methylated histones, specifically mono and di-methylated histones by us and others (Kim et al., 2006; Li et al., 2007; Min et al., 2007; Trojer et al., 2007; Adams-Cioaba & Min, 2009; Eryilmaz et al., 2009; Guo et al., 2009). Functional studies of human L3MBTL1 have suggested that L3MBTL1 could compact nucleosomal arrays with mono- and di-methylated H4K20, but not trimethylated H4K20 (Trojer et al., 2007). The association of L3MBTL1 with chromatin is abolished by the knockdown of the H4K20 monomethyltranferase SETD8 (Trojer et al., 2007; Kalakonda et al., 2008). L3MBTL2 plays an essential role in pluripotent stem cells and early development (Qin et al., 2012). In Drosophila, Scm of two MBT repeats is a core component in the PRC1 complex, which is essential for the repression of Hox genes. Embryos lacking Scm protein show widespread misexpression of Hox genes and die at the end of embryogenesis (Breen & Duncan, 1986; Bornemann et al., 1998; Grimm et al., 2007).

L3MBTL1 downregulates the expression of a subset of genes controlled by E2F (Trojer et al., 2007), and is essential for genome stability

Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007

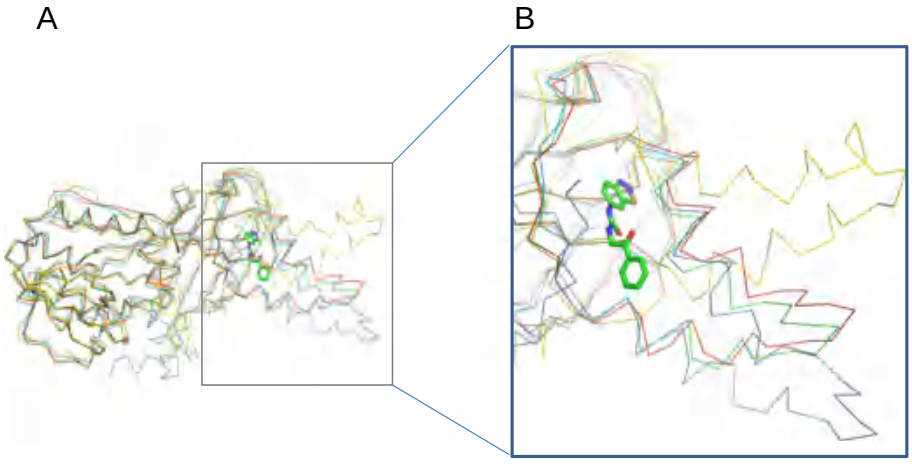


Fig. 5. Superposition of the complex structures of CARM1 (PDB: 2Y1W), PRMT1 (PDB: 1ORI), PRMT3 (PDB: 4HSG) and PRMT6 (PDB: 4HC4). (A) The inhibitor KTD in complex with PRMT3 is shown as stick models with carbon atoms colored as green. Proteins are shown as ribbon models and colored in blue for CARM1, green for PRMT1, red for PRMT3 and yellow for PRMT6, respectively. (B) Zoomed view of the dimerization arm in different PRMT structures. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Gurvich et al., 2010). L3MBTL suppresses erythroid differentiation and is implicated in the pathogenesis of myeloid malignancies associated with 20q deletions (Aziz et al., 2013). In order to facilitate the biological studies of L3MBTL1 and related proteins, the group of Dr. Frye from the University of North Carolina at Chapel Hill launched a small molecule inhibitor screening effort to antagonize the L3MBTL1-histone peptide interactions in collaboration with the Structural Genomics Consortium, University of Toronto. After unsuccessful attempts of virtual screening and diverse compound library screening, they switched to a ligand- and structure-based design approach, starting from a peptide mimics (Herold et al., 2011). Initial successful discovery that only the pyrrolidine mimics of the methyl-lysine side chain contribute to binding affinity persuaded them to focus on non-peptidic small molecules of the pyrrolidine scaffold, which yielded a compound UNC669 (Table 5 and Fig. 6A). UNC669 exhibited a binding affinity of ~5 μM to L3MBTL1, which is a few times stronger than the native histone peptides. In addition, this compound has modest selectivity over some other MBT members (Herold et al., 2011).

Based on structure-guided sequence alignment, we have predicted previously that some MBT members might have more than one MBT repeat capable of binding methylated histones, such as L3MBTL3 (Guo et al., 2009). The potential multivalent binding feature of these MBT proteins could be utilized to develop potent chemical probes. Based on the compound UNC669, a symmetrical compound UNC1215 was synthesized in the hope that this compound was able to bind to the aromatic cages of two different MBT repeats of a MBT protein of two potential methyllysine binding MBT repeats, such as L3MBTL3 (James et al., 2013) (Table 5). Indeed, UNC1215 shows a strong binding affinity of 120 nM to L3MBTL3, which is 40 times more potent than UNC669 to L3MBTL1. It also shows over 50-fold selectivity over other MBT proteins and over 200-fold selectivity over other histone reader domains. As expected, the cocrystal structure shows that UNC1215 binds to L3MBTL3 in a 2:2 bivalend binding mode (James et al., 2013) (Fig. 6B).

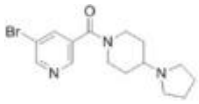
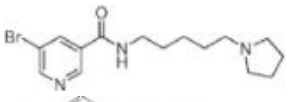
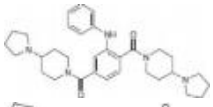
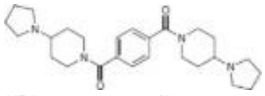
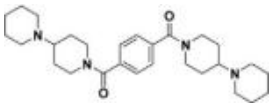
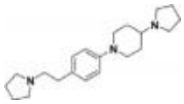
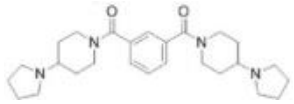
8. WDR5: a binder of histone H3R2 and the Win motif conserved in SETD1A/B and MLL1-4

WDR5 is a core component of the SET1/MLL complexes and mediates ES cell self-renewal and somatic cell reprogramming (Ang et al., 2011). The SET1/MLL family of methyltransferases has virtually no histone methylation activity on its own but becomes active as multi-component complexes (Dou et al., 2006). The non-catalytic components in these complexes play important roles in activation, regulation and recruitment of the SET1/MLL family of methyltransferase complexes. Therefore, it is of fundamental importance to study the composition and function of the SET1/MLL complexes, and how those complexes are recruited to their target genes through specific recruiting elements. In 2005, the group of Dr. Allis from Rockefeller University showed that WDR5 directly associates with histone H3K4me2 in vitro and in vivo, and it is required for targeting the H3K4 methyltransferase complexes to carry out the di- to tri-methylation conversion of histone H3K4 (Wysocka et al., 2005). Structural studies by my group — and other groups show that WDR5 is able to recognize both unmodified and methylated histone H3K4 peptides mainly through an arginine residue (H3R2) (Couture et al., 2006; Han et al., 2006; Ruthenburg et al., 2006; Schuetz et al., 2006). In addition, we also found that WDR5 is able to bind to other arginine containing peptides, such as its own arginine containing N-terminal tail, and the His-tag fused to WDR5 (Schuetz et al., 2006).

Recently, WDR5 was also found to bind a stretch of arginine containing motif, the Win motif (WDR5 interaction motif) conserved in the SETD1A/B and MLL1-4 catalytic subunits, and the binding of WDR5 to the Win motif is critical for assembly and enzymatic activity of the SET1/MLL complexes (Patel et al., 2008a, 2008b; Xu & Min, 2011; Dharmarajan et al., 2012; Zhang et al., 2012). The essential arginine residue in the different peptides inserts into the central channel of the WD40 β-propeller of WDR5 from the top surface and forms extensive interactions with WDR5 through a network of hydrogen bonds and cation-π interactions, whereas the residues surrounding the invariant residue arginine make very few interactions with WDR5. Therefore, WDR5 is able to recognize either histone H3 or its catalytic partner SETD1A/B or MLL1-4.

Genetic aberrations of the SET1/MLL family of histone H3K4 methyltransferases, such as translocation fusion and mutations, cause various genetic diseases and cancers. For instance, MLL1 is involved in chromosomal rearrangements with over 60 different translocation partners, generating MLL1 fusion proteins that account for up to 80% of infant acute leukemia and approximately 5–10% of adult acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) cases (Muntean et al., 2010). In most MLL-rearranged leukemia, one MLL1 allele loses the C-

Table 5
Inhibitors of L3MBTL1/L3MBTL3.

Compound name	Chemical structure	IC_{50} (μM)	PDB code of complex structure	PMID
UNC669		6/35	3P8H/–	21417280 (Herold et al., 2011)
UNC280		46/28		21417280 (Herold et al., 2011)
UNC1215		8.9/0.024	3UWN/4FL6	23292653 (James, Barsyte-Lovejoy, et al., 2013) 24466405 (Camerino et al., 2013)
UNC1021		3/0.04		23292653 (James, Barsyte-Lovejoy, et al., 2013) 24466405 (Camerino et al.)
UNC1079		170/8		23292653 (James, Barsyte-Lovejoy, et al., 2013) 24466405 (Camerino et al.)
UNC2533		14/0.062	–/4L59	24466405 (Camerino et al.)
UNC928		2.8/0.36		24040942 (James, Korboukh, et al.)

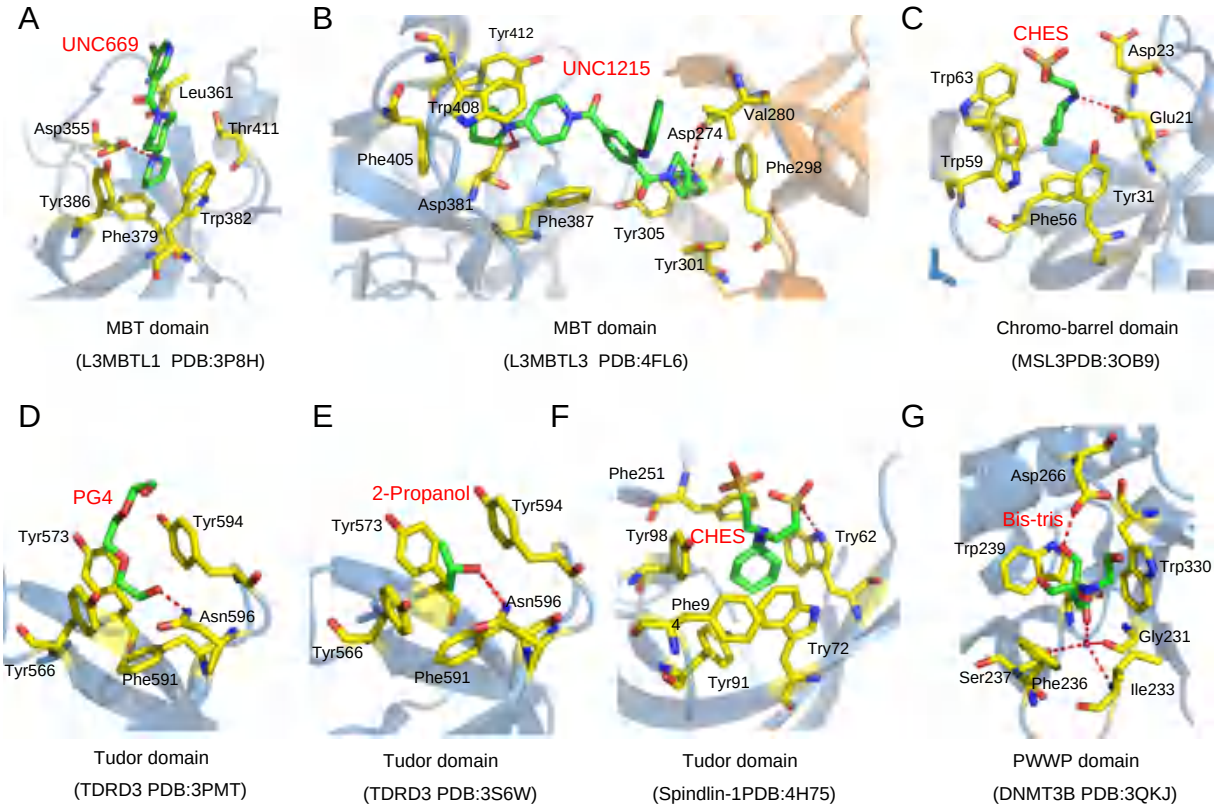


Fig. 6. Proteins of the MBT domain, Chromo-barrel domain, Tudor domain and PWWP domains bind inhibitors or other small molecules in their respective aromatic cages. (A) L3MBTL1 and inhibitor UNC669. (B) L3MBTL3 and UNC1215. (C) MSL3 and a CHES buffer molecule (N-Cyclohexyl-2-aminoethanesulfonic acid). (D) TDRD3 and a PG4 molecule. (E) TDRD3 and a 2-isopropanol molecule. (F) Spindlin1 and a CHES buffer molecule. The CHES molecule binds to the aromatic cage in the first Tudor domain while the K4me3 of histone H3 resides in the aromatic cage located in the second Tudor domain. (G) DNMT3B and a Bis-tris molecule. Protein residues in contact with small molecules are depicted by stick models with their carbon atoms colored as yellow, small molecules are shown as stick models with their carbon atoms colored as green. Hydrogen bonds and salt bridges are marked with a red dashed line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

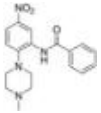
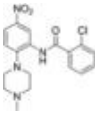
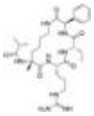
Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007

terminal half, which contains the catalytic SET domain, and fused with one of its translocation partners, and the other MLL1 allele is still intact. In MLL-AF9-induced leukemogenesis, both the wild type MLL1 and oncogenic MLL1-AF9 fusion proteins are required for MLL-AF9-induced leukemogenesis (Thiel et al., 2010). Because the WDR5-MLL1 interaction is critical for the assembly and enzymatic activity of the MLL1 complexes (Dou et al., 2006; Patel et al., 2008a, 2008b; Xu & Min, 2011; Dharmarajan et al., 2012; Zhang et al., 2012), it is tempting to design inhibitors to disrupt the WDR5-MLL1 interaction for the therapy of MLL-rearranged leukemia. Markedly, much progress has been made in developing antagonists disrupting the WDR5-MLL1 interaction.

The first small molecule inhibitor was discovered by the group of Dr. Vedadi from the Structural Genomics Consortium at the University of Toronto by screening a diverse library of 16,000 compounds, which yielded just one promising hit WDR5-0101 (K_{dis}: 12 μM) (Senisterra et al., 2013) (Table 6). 119 compounds from the library of 6 million commercially available compounds have similar chemical structures to WDR5-0101, and WDR5-0102 and WDR5-0103 were confirmed to bind to WDR5 by ITC (Table 6). Of these 3 compounds, WDR5-0103 has the highest binding affinity with K_d of 0.45 μM (Senisterra et al., 2013). Following crystallographic analysis showed that the compounds occupy the central pore in a similar pocket to the arginine-binding pocket in the WDR5-peptide complexes, and the enzymatic assays showed that WDR5-0103 could inhibit the catalytic activity of the MLL1 complex in vitro (Senisterra et al., 2013).

At the same time, another group of scientists from the University of Michigan developed a series of peptidomimetic inhibitors based on the available WDR5 complex structures and the fact that the arginine residue is the major binding affinity contributor in the WDR5-peptide interactions (Cao et al., 2014; Karatas et al., 2013). They started from a minimum binding motif of WDR5: −CO-ARA-NH−, and identified three potent peptidomimetics (MM-101, -102 and -103) (Table 6), which bind to WDR5 with K_i b 1 nM (Karatas et al., 2013). Guided by the complex structures of MM-101 and MM-102 with WDR5, they synthesized a cyclic compound, MM-401 based on the linear peptidomimetic MM-101 (Table 6). MM-401 is over 3-fold more potent than MM-101, and 700-fold more potent than the Win motif. Enzymatic assays showed that MM-401 is able to specifically inhibit MLL1, but not other members of the SET1/MLL family of H3K4 methyltransferase complexes, implying that the MLL1 complex has a distinct regulatory mechanism via the WDR5-MLL1 interaction than other members. Furthermore, MM-401 specifically kills MLL-associated leukemic cells

Table 6
Inhibitors of WD40 domain of WDR5.

Compound Name	Chemical structure	IC_{50} (μM)	PDB code of complex structure	PMID
WDR5-0101		5.5 (Kd)		22989411 (Senisterra et al., 2013)
WDR5-0102		4.0 (Kd)		22989411 (Senisterra et al., 2013)
WDR5-0103		0.45 (Kd)	3UR4	22989411 (Senisterra et al., 2013)
MM-101		0.0029	4GM3	
MM-102		0.0024	4GM8	23210835 (Karatas et al., 2013)
MM-103		0.0045		
MM-401		0.0009	4GM9	24389101 (Cao et al., 2014)
MM-402			4GMB	
bolshan13_47		0.3		(Bolshan et al., 2013)
IA9			4IA9	
NP7			3SMR	

by inducing cell-cycle arrest, apoptosis, and myeloid differentiation, but not normal cells in a MLL1-mediated H3K4 methylation-dependent manner (Cao et al., 2014).

9. Other methyl-lysine/arginine binding proteins

In the human genome, there are about 150 potential methyl-lysine or methyl-arginine binding proteins and this list is still growing. A common feature of these proteins is that they all utilize an aromatic cage to recognize the methyl-lysine or methyl-arginine residue. These proteins have diverse methylation state and sequence selectivity. For example, the MBT domains specifically recognize mono- or dimethylated lysine without much selectivity over its surrounding sequence (Adams-Cioaba & Min, 2009). Different members of the Chromodomain subfamily have been shown to preferentially recognize H3K4me2/3 (Flanagan et al., 2005), H3K9me2/3 (Jacobs & Khorasanizadeh, 2002; Nielsen et al., 2002; Li et al., 2011; Kaustov et al., 2011; Ruan et al., 2012; T. Wang et al., 2012), H3K27me2/3 (Fischle et al., 2003; Min et al., 2003b; Kaustov et al., 2011), H3K36me3 (Sun et al., 2008; Xu et al., 2008) and H4K20me1/2 (Kim et al., 2010; Moore et al., 2010; Gong et al., 2012), respectively. The PWWP domain has been shown to bind to H4K20me3 (H. Wu et al., 2011; Qiu et al., 2012b), H3K36me3 (Vezzoli et al., 2010; H. Wu et al., 2011; Eidahl et al., 2013) and H3K79me3 (H. Wu et al., 2011). The Tudor domain is very diverse in both structures and binding activities. It exists in single (Tripsianes et al., 2011; Liu et al., 2012; Musselman et al., 2012; Cai et al., 2013; Qin et al., 2013), double (Adams-Cioaba et al., 2010; Bian et al., 2011; Botuyan et al., 2006; Huang et al., 2006a; K. Liu et al., 2010; Nady et al., 2011) and triple (N. Yang et al., 2012) Tudor repeats, and could bind both methyl-arginine (H. Liu et al., 2010; K. Liu et al., 2010; Liu et al., 2012; Tripsianes et al., 2011) and methyl-lysine proteins (Adams-Cioaba & Min, 2009). The PHD domain has been shown to bind both histone H3K4me0 and H3K4me3 (Adams-Cioaba & Min, 2009). In addition, a small subset of double PHD domains can also bind unmodified H3R2 (Zeng et al., 2010; Rajakumara et al., 2011; Qiu et al., 2012a). Recently, the Ankyrin repeat domains of G9a/GLP (Collins et al., 2008), WD40 repeat protein EED (Margueron et al., 2009; Xu et al., 2010), the BAH domain of ORC1 (Kuo et al., 2012) have also been shown to specifically recognize methylated histones. Over the past 10 years, many human methyl-lysine/arginine binding proteins have been characterized by others and us in terms of both binding specificity and three-dimensional structures. It provides a rich source of information and reagents in developing target-selective antagonists. It is worth noting that some of these methyl-lysine/arginine binding proteins have been crystallized in complex with small molecules, which could provide some clues in drug design in the future as well (Fig. 6C–G).

10. Conclusion

Since the first histone lysine methyltransferases (Rea et al., 2000), the first protein/histone arginine methyltransferases (Lin et al., 1996) and the first histone code reader HP1 (Lachner et al., 2001) were identified, a large amount of data has been amassed for these protein families in regards to their biological functions and disease implications. Growing evidence over the past two decades supports the concept that epigenetic abnormalities play an important role in the pathogenesis of cancers and other genetic diseases. Therefore, proteins involved in chromatin modifications are attractive therapeutic targets for drug design. A lot of ongoing clinical trials regarding inhibitors of histone methyltransferases, such as E7438 and EPZ-5676, inhibitors of EZH2 and DOT1L respectively, are ongoing (please refer to www.clinicaltrials.gov). In this article, we systematically review the progresses in the discovery of chemical probes and therapeutic reagents for histone methyltransferases and methyllysine readers. These compounds could in turn be used to further study the biological functions and pathogenesis of these epigenetic targets. It is noteworthy that a large number of crystal structures of these protein targets and their co-crystals have been made available, and this rich source of information has been extensively utilized and will facilitate the lead generation and compound optimization for these epigenetic targets.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

We thank Dr. Jian Jin for making Fig. 2 for us. The SGC is a registered charity (number 1097737) that receives funds from AbbVie, Boehringer Ingelheim, the Canada Foundation for Innovation, the Canadian Institutes for Health Research, Genome Canada through the Ontario Genomics Institute [OGI-055], GlaxoSmithKline, Janssen, Lilly Canada, the Novartis Research Foundation, the Ontario Ministry of Economic Development and Innovation, Pfizer, Takeda, and the Wellcome Trust [092809/Z/10/Z]. Due to the limited space, the authors apologize to those whose original work could not be cited in this review.

References

Aagaard, L., Laible, G., Selenko, P., Schmid, M., Dorn, R., Schotta, G., et al. (1999). Functional mammalian homologues of the Drosophila PEV-modifier Su(var)3-9 encode centromere-associated proteins which complex with the heterochromatin component M31. EMBO J 18, 1923–1938.

Abu-Farha, M., Lambert, J. P., Al-Madhoun, A. S., Elisma, F., Skerjanc, I. S., & Figeys, D. (2008). The tale of two domains: proteomics and genomics analysis of SMYD2, a new histone methyltransferase. Mol Cell Proteomics 7, 560–572.

Adams-Cioaba, M.A., Guo, Y., Bian, C., Amaya, M. F., Lam, R., Wasney, G. A., et al. (2010). Structural studies of the tandem tudor domains of fragile X mental retardation related proteins FXR1 and FXR2. PLoS One 5, e13559.

Adams-Cioaba, M.A., & Min, J. R. (2009). Structure and function of histone methylation binding proteins. Biochem Cell Biol 87, 93–105.

Ang, Y. S., Tsai, S. Y., Lee, D. F., Monk, J., Su, J., Ratnakumar, K., et al. (2011). Wdr5 mediates self-renewal and reprogramming via the embryonic stem cell core transcriptional network. Cell 145, 183–197.

Anglin, J. L., Deng, L., Yao, Y., Cai, G., Liu, Z., Jiang, H., et al. (2012). Synthesis and structure–activity relationship investigation of adenosine-containing inhibitors of histone methyltransferase DOT1L. J Med Chem 55, 8066–8074.

Anglin, J. L., & Song, Y. (2013). A medicinal chemistry perspective for targeting histone H3 lysine-79 methyltransferase DOT1L. J Med Chem 56, 8972–8983.

Antonysamy, S., Bonday, Z., Campbell, R. M., Doyle, B., Druzina, Z., Gheyi, T., et al. (2012). Crystal structure of the human PRMT5:MEP50 complex. Proc Natl Acad Sci U S A 109, 17960–17965.

Ayton, P.M., & Cleary, M. L. (2001). Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. Oncogene 20, 5695–5707.

Aziz, A., Baxter, E. J., Edwards, C., Cheong, C. Y., Ito, M., Bench, A., et al. (2013). Cooperativity of imprinted genes inactivated by acquired chromosome 20q deletions. J Clin Invest 123, 2169–2182.

Basavapathruni, A., Jin, L., Daigle, S. R., Majer, C. R., Therkelsen, C. A., Wigle, T. J., et al. (2012). Conformational adaptation drives potent, selective and durable inhibition of the human protein methyltransferase DOT1L. Chem Biol Drug Des 80, 971–980.

Basavapathruni, A., Olhava, E. J., Daigle, S. R., Therkelsen, C. A., Jin, L., Boriack-Sjodin, P. A., et al. (2014). Nonclinical pharmacokinetics and metabolism of EPZ-5676, a novel DOT1L histone methyltransferase inhibitor. Biopharm Drug Dispos. http://dx.doi.org/10.1002/bdd.1889 (in press).

Baylin, S. B., & Jones, P. A. (2011). A decade of exploring the cancer epigenome — biological and translational implications. Nat Rev Cancer 11, 726–734.

Bedford, M. T., & Richard, S. (2005). Arginine methylation an emerging regulator of protein function. Mol Cell 18, 263–272.

Benoit, Y. D., Laursen, K. B., Witherspoon, M. S., Lipkin, S. M., & Gudas, L. J. (2013). Inhibition of PRC2 histone methyltransferase activity increases TRAIL-mediated apoptosis sensitivity in human colon cancer cells. J Cell Physiol 228, 764–772.

Bian, C., Xu, C., Ruan, J., Lee, K. K., Burke, T. L., Tempel, W., et al. (2011). Sgf29 binds histone H3K4me2/3 and is required for SAGA complex recruitment and histone H3 acetylation. EMBO J 30, 2829–2842.

Bissinger, E. M., Heinke, R., Spannhoff, A., Eberlin, A., Metzger, E., Cura, V., et al. (2011). Acyl derivatives of p-aminosulfonamides and dapsone as new inhibitors of the arginine methyltransferase hPRMT1. Bioorg Med Chem 19, 3717–3731.

Bolshan, Y., Getlik, M., Kuznetsova, E., Wasney, G. A., Hajian, T., Poda, G., Kong, T., et al. (2013). Synthesis, optimization, and evaluation of novel small molecules as antagonists of WDR5-MLL interaction. ACS Med Chem Lett 4, 353–357.

Bonham, K., Hemmers, S., Lim, Y. H., Hill, D. M., Finn, M. G., & Mowen, K. A. (2010). Effects of a novel arginine methyltransferase inhibitor on T-helper cell cytokine production. FEBS J 277, 2096–2108.

Bornemann, D., Miller, E., & Simon, J. (1998). Expression and properties of wild-type and mutant forms of the Drosophila sex comb on midleg (SCM) repressor protein. Genetics 150, 675–686.

Botuyan, M. V., Lee, J., Ward, I. M., Kim, J. E., Thompson, J. R., Chen, J., et al. (2006). Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. Cell 127, 1361–1373.

Breen, T. R., & Duncan, I. M. (1986). Maternal expression of genes that regulate the bithorax complex of Drosophila melanogaster. Dev Biol 118, 442–456.

Briggs, S. D., Xiao, T., Sun, Z. W., Caldwell, J. A., Shabanowitz, J., Hunt, D. F., et al. (2002). Gene silencing: trans-histone regulatory pathway in chromatin. Nature 418, 498.

Brown, M. A., Sims, R. J., III, Gottlieb, P. D., & Tucker, P. W. (2006). Identification and characterization of Smyd2: a split SET/MYND domain-containing histone H3 lysine 36-specific methyltransferase that interacts with the Sin3 histone deacetylase complex. Mol Cancer 5, 26.

Cai, L., Rothbart, S. B., Lu, R., Xu, B., Chen, W. Y., Tripathy, A., et al. (2013). An H3K36 methylation-engaging Tudor motif of polycomb-like proteins mediates PRC2 complex targeting. Mol Cell 49, 571–582.

Camerino, M. A., Zhong, N., Dong, A., Dickson, B. M., James, L. I., Baughman, B. M., et al. (2013). The structure–activity relationships of L3MBTL3 inhibitors: flexibility of the dimer interface. Medchemcomm 4, 1501–1507.

Cao, F., Townsend, E. C., Karatas, H., Xu, J., Li, L., Lee, S., et al. (2014). Targeting MLL1 H3K4 methyltransferase activity in mixed-lineage leukemia. Mol Cell 53, 247–261.

Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., et al. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298, 1039–1043.

Cao, R., & Zhang, Y. (2004). The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr Opin Genet Dev 14, 155–164.

Castellano, S., Milite, C., Ragno, R., Simeoni, S., Mai, A., Limongelli, V., et al. (2010). Design, synthesis and biological evaluation of carboxy analogues of arginine methyltransferase inhibitor 1 (AMI-1). ChemMedChem 5, 398–414.

Chang, Y., Ganesh, T., Horton, J. R., Spannhoff, A., Liu, J., Sun, A., et al. (2010). Adding a lysine mimic in the design of potent inhibitors of histone lysine methyltransferases. J Mol Biol 400, 1–7.

Chang, Y., Zhang, X., Horton, J. R., Upadhyay, A. K., Spannhoff, A., Liu, J., et al. (2009). Structural basis for G9a-like protein lysine methyltransferase inhibition by BIX-01294. Nat Struct Mol Biol 16, 312–317.

Chase, A., & Cross, N. C. (2011). Aberrations of EZH2 in cancer. Clin Cancer Res 17, 2613–2618.

Chen, X., Skutt-Kakaria, K., Davison, J., Ou, Y. L., Choi, E., Malik, P., et al. (2012). G9a/GLP-dependent histone H3K9me2 patterning during human hematopoietic stem cell lineage commitment. Genes Dev 26, 2499–2511.

Cheng, D., Cote, J., Shaaban, S., & Bedford, M. T. (2007). The arginine methyltransferase CARM1 regulates the coupling of transcription and mRNA processing. Mol Cell 25, 71–83.

Cheng, D., Yadav, N., King, R. W., Swanson, M. S., Weinstein, E. J., & Bedford, M. T. (2004). Small molecule regulators of protein arginine methyltransferases. J Biol Chem 279, 23892–23899.

Cheng, D., Valente, S., Castellano, S., Sbardella, G., Di Santo, R., Costi, R., et al. (2011). Novel 3,5-bis(bromohydroxybenzylidene)piperidin-4-ones as coactivator-associated arginine methyltransferase 1 inhibitors: enzyme selectivity and cellular activity. J Med Chem 54, 4928–4932.

Cherblanc, F. L., Chapman, K. L., Brown, R., & Fuchter, M. J. (2013). Chaetocin is a nonspecific inhibitor of histone lysine methyltransferases. Nat Chem Biol 9, 136–137.

Chiba, T., Suzuki, E., Negishi, M., Saraya, A., Miyagi, S., Konuma, T., et al. (2012). 3-Deazaneplanocin A is a promising therapeutic agent for the eradication of tumor-initiating hepatocellular carcinoma cells. Int J Cancer 130, 2557–2567.

Collins, R. E., Northrop, J. P., Horton, J. R., Lee, D. Y., Zhang, X., Stallcup, M. R., et al. (2008). The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyllysine binding modules. Nat Struct Mol Biol 15, 245–250.

Copeland, R. A. (2013). Molecular pathways: protein methyltransferases in cancer. Clin Cancer Res 19, 6344–6350.

Couture, J. F., Collazo, E., & Trievel, R. C. (2006). Molecular recognition of histone H3 by the WD40 protein WDR5. Nat Struct Mol Biol 13, 698–703.

Czermin, B., Melfi, R., McCabe, D., Seitz, V., Imhof, A., & Pirrotta, V. (2002). Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell 111, 185–196.

Daigle, S. R., Olhava, E. J., Therkelsen, C. A., Basavapathruni, A., Jin, L., Boriack-Sjodin, P. A., et al. (2013). Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood 122, 1017–1025.

Daigle, S. R., Olhava, E. J., Therkelsen, C. A., Majer, C. R., Sneeringer, C. J., Song, J., et al. (2011). Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell 20, 53–65.

Deng, L., Zhang, L., Yao, Y., Wang, C., Redell, M. S., Dong, S., et al. (2013). Synthesis, activity and metabolic stability of non-ribose containing inhibitors of histone methyltransferase DOT1L. MedChemcomm 4, 822–826.

Dharmarajan, V., Lee, J. H., Patel, A., Skalnik, D.G., & Cosgrove, M. S. (2012). Structural basis for WDR5 interaction (Win) motif recognition in human SET1 family histone methyltransferases. J Biol Chem 287, 27275–27289.

Di Lorenzo, A., & Bedford, M. T. (2011). Histone arginine methylation. FEBS Lett 585, 2024–2031.

Dou, Y., Milne, T. A., Ruthenburg, A. J., Lee, S., Lee, J. W., Verdine, G. L., et al. (2006). Regulation of MLL1 H3K4 methyltransferase activity by its core components. Nat Struct Mol Biol 13, 713–719.

Dowden, J., Hong, W., Parry, R. V., Pike, R. A., & Ward, S. G. (2010). Toward the development of potent and selective bisubstrate inhibitors of protein arginine methyltransferases. Bioorg Med Chem Lett 20, 2103–2105.

Eidahl, J. O., Crowe, B.L., North, J. A., McKee, C. J., Shkriabai, N., Feng, L., et al. (2013). Structural basis for high-affinity binding of LEDGF PWWP to mononucleosomes. Nucleic Acids Res 41, 3924–3936.

Ernst, T., Chase, A. J., Score, J., Hidalgo-Curtis, C. E., Bryant, C., Jones, A. V., et al. (2010). Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet 42, 722–726.

Eryilmaz, J., Pan, P., Amaya, M. F., Allali-Hassani, A., Dong, A. P., Adams-Cioaba, M.A., et al. (2009). Structural studies of a four-MBT repeat protein MBTD1. PLoS One 4.

Feng, Q., Wang, H., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Struhl, K., et al. (2002). Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr Biol 12, 1052–1058.

Feng, Y., Li, M., Wang, B., & Zheng, Y. G. (2010a). Discovery and mechanistic study of a class of protein arginine methylation inhibitors. J Med Chem 53, 6028–6039.

Feng, Y., Yang, Y., Ortega, M. M., Copeland, J. N., Zhang, M., Jacob, J. B., et al. (2010b). Early mammalian erythropoiesis requires the Dot1L methyltransferase. Blood 116, 4483–4491.

Ferguson, A.D., Larsen, N. A., Howard, T., Pollard, H., Green, I., Grande, C., et al. (2011). Structural basis of substrate methylation and inhibition of SMYD2. Structure 19, 1262–1273.

Firestein, R., Cui, X., Huie, P., & Cleary, M. L. (2000). Set domain-dependent regulation of transcriptional silencing and growth control by SUV39H1, a mammalian ortholog of Drosophila Su(var)3-9. Mol Cell Biol 20, 4900–4909.

Fischle, W., Wang, Y., Jacobs, S. A., Kim, Y., Allis, C. D., & Khorasanizadeh, S. (2003). Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. Genes Dev 17, 1870–1881.

Flanagan, J. F., Mi, L. Z., Chruszcz, M., Cymborowski, M., Clines, K. L., Kim, Y., et al. (2005). Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature 438, 1181–1185.

Francis, N. J., Rowlands, M., Workman, P., Jones, K., & Aherne, W. (2012). Small-molecule inhibitors of the protein methyltransferase SET7/9 identified in a high-throughput screen. J Biomol Screen 17, 1102–1109.

Frederiks, F., Tzouros, M., Oudgenoeg, G., van Welsem, T., Fornerod, M., Krijgsveld, J., et al. (2008). Nonprocessive methylation by Dot1 leads to functional redundancy of histone H3K79 methylation states. Nat Struct Mol Biol 15, 550–557.

Friesen, W. J., Massenet, S., Paushkin, S., Wyce, A., & Dreyfuss, G. (2001). SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. Mol Cell 7, 1111–1117.

Gannon, O. M., Merida de Long, L., Endo-Munoz, L., Hazar-Rethinam, M., & Saunders, N. A. (2013). Dysregulation of the repressive H3K27 trimethylation mark in head and neck squamous cell carcinoma contributes to dysregulated squamous differentiation. Clin Cancer Res 19, 428–441.

Garapaty-Rao, S., Nasveschuk, C., Gagnon, A., Chan, E. Y., Sandy, P., Busby, J., et al. (2013). Identification of EZH2 and EZH1 small molecule inhibitors with selective impact on diffuse large B cell lymphoma cell growth. Chem Biol 20, 1329–1339.

Glazer, R. I., Hartman, K. D., Knode, M. C., Richard, M. M., Chiang, P. K., Tseng, C. K., et al. (1986). 3-Deazaneplanocin: a new and potent inhibitor of S-adenosylhomocysteine hydrolase and its effects on human promyelocytic leukemia cell line HL-60. Biochem Biophys Res Commun 135, 688–694.

Gong, W., Zhou, T., Mo, J., Perrett, S., Wang, J., & Feng, Y. (2012). Structural insight into recognition of methylated histone tails by retinoblastoma-binding protein 1. J Biol Chem 287, 8531–8540.

Greiner, D., Bonaldi, T., Eskeland, R., Roemer, E., & Imhof, A. (2005). Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3-9. Nat Chem Biol 1, 143–145.

Greiner, D., Bonaldi, T., Eskeland, R., Roemer, E., & Imhof, A. (2013). Reply to "Chaetocin is a nonspecific inhibitor of histone lysine methyltransferases". Nat Chem Biol 9, 137.

Grimm, C., de Ayala Alonso, A. G., Rybin, V., Steuerwald, U., Ly-Hartig, N., Fischle, W., et al. (2007). Structural and functional analyses of methyl-lysine binding by the malignant brain tumour repeat protein Sex comb on midleg. EMBO Rep 8, 1031–1037.

Guo, Y., Nady, N., Qi, C., Allali-Hassani, A., Zhu, H., Pan, P., et al. (2009). Methylation-state-specific recognition of histones by the MBT repeat protein L3MBTL2. Nucleic Acids Res 37, 2204–2210.

Gurvich, N., Perna, F., Farina, A., Voza, F., Menendez, S., Hurwitz, J., et al. (2010). L3MBTL1 polycomb protein, a candidate tumor suppressor in del(20q12) myeloid disorders, is essential for genome stability. Proc Natl Acad Sci U S A 107, 22552–22557.

Han, Z., Guo, L., Wang, H., Shen, Y., Deng, X. W., & Chai, J. (2006). Structural basis for the specific recognition of methylated histone H3 lysine 4 by the WD-40 protein WDR5. Mol Cell 22, 137–144.

Herold, J. M., Wigle, T. J., Norris, J. L., Lam, R., Korboukh, V. K., Gao, C., et al. (2011). Small-molecule ligands of methyl-lysine binding proteins. J Med Chem 54, 2504–2511.

Huang, Y., Fang, J., Bedford, M. T., Zhang, Y., & Xu, R. M. (2006). Recognition of histone H3 lysine-4 methylation by the double tudor domain of JMJD2A. Science 312, 748–751.

Huang, J., Perez-Burgos, L., Placek, B. J., Sengupta, R., Richter, M., Dorsey, J. A., et al. (2006). Repression of p53 activity by Smyd2-mediated methylation. Nature 444, 629–632.

Hung, S. W., Mody, H., Marrache, S., Bhutia, Y. D., Davis, F., Cho, J. H., et al. (2013). Pharmacological reversal of histone methylation presensitizes pancreatic cancer cells to nucleoside drugs: in vitro optimization and novel nanoparticle delivery studies. PLoS One 8, e71196.

Huynh, T., Chen, Z., Pang, S., Geng, J., Bandiera, T., Bindi, S., et al. (2009). Optimization of pyrazole inhibitors of Coactivator Associated Arginine Methyltransferase 1 (CARM1). Bioorg Med Chem Lett 19, 2924–2927.

Ivanova, A. V., Bonaduce, M. J., Ivanov, S. V., & Klar, A. J. (1998). The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. Nat Genet 19, 192–195.

Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007

Iwasa, E., Hamashima, Y., Fujishiro, S., Higuchi, E., Ito, A., Yoshida, M., et al. (2010). Total synthesis of (+)-chaetocin and its analogues: their histone methyltransferase G9a inhibitory activity. J Am Chem Soc 132, 4078–4079.

Jacobs, S. A., & Khorasanizadeh, S. (2002). Structure of HP1 chromodomain bound to a lysine 9-methylated histone H3 tail. Science 295, 2080–2083.

James, L. I., Barsyte-Lovejoy, D., Zhong, N., Krichevsky, L., Korboukh, V. K., Herold, J. M., et al. (2013). Discovery of a chemical probe for the L3MBTL3 methyllysine reader domain. Nat Chem Biol 9, 184–191.

James, L. I., Korboukh, V. K., Krichevsky, L., Baughman, B. M., Herold, J. M., Norris, J. L., et al. (2013). Small-molecule ligands of methyl-lysine binding proteins: optimization of selectivity for L3MBTL3. J Med Chem 56, 7358–7371.

Jones, B., Su, H., Bhat, A., Lei, H., Bajko, J., Hevi, S., et al. (2008). The histone H3K79 methyltransferase Dot1L is essential for mammalian development and heterochromatin structure. PLoS Genet 4, e1000190.

Kalakonda, N., Fischle, W., Boccuni, P., Gurvich, N., Hoya-Arias, R., Zhao, X., et al. (2008). Histone H4 lysine 20 monomethylation promotes transcriptional repression by L3MBTL1. Oncogene 27, 4293–4304.

Karatas, H., Townsend, E. C., Cao, F., Chen, Y., Bernard, D., Liu, L., et al. (2013). High-affinity, small-molecule peptidomimetic inhibitors of MLL1/WDR5 protein–protein interaction. J Am Chem Soc 135, 669–682.

Kaustov, L., Ouyang, H., Amaya, M., Lemak, A., Nady, N., Duan, S., et al. (2011). Recognition and specificity determinants of the human cbx chromodomains. J Biol Chem 286, 521–529.

Kikuchi, J., Takashina, T., Kinoshita, I., Kikuchi, E., Shimizu, Y., Sakakibara-Konishi, J., et al. (2012). Epigenetic therapy with 3-deazaneplanocin A, an inhibitor of the histone methyltransferase EZH2, inhibits growth of non-small cell lung cancer cells. Lung Cancer 78, 138–143.

Kim, D., Blus, B. J., Chandra, V., Huang, P., Rastinejad, F., & Khorasanizadeh, S. (2010). Corecognition of DNA and a methylated histone tail by the MSL3 chromodomain. Nat Struct Mol Biol 17, 1027–1029.

Kim, J., Daniel, J., Espejo, A., Lake, A., Krishna, M., Xia, L., et al. (2006). Tudor, MBT and chromo domains gauge the degree of lysine methylation. EMBO Rep 7, 397–403.

Kim, J. T., Li, J., Jang, E. R., Gulhati, P., Rychahou, P. G., Napier, D. L., et al. (2013). Deregulation of Wnt/beta-catenin signaling through genetic or epigenetic alterations in human neuroendocrine tumors. Carcinogenesis 34, 953–961.

Kleer, C. G., Cao, Q., Varambally, S., Shen, R., Ota, I., Tomlins, S. A., et al. (2003). EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. Proc Natl Acad Sci U S A 100, 11606–11611.

Knutson, S. K., Warholic, N. M., Wigle, T. J., Klaus, C. R., Allain, C. J., Raimondi, A., et al. (2013). Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. Proc Natl Acad Sci U S A 110, 7922–7927.

Knutson, S. K., Wigle, T. J., Warholic, N. M., Sneeringer, C. J., Allain, C. J., Klaus, C. R., et al. (2012). A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. Nat Chem Biol 8, 890–896.

Komatsu, S., Imoto, I., Tsuda, H., Kozaki, K. I., Muramatsu, T., Shimada, Y., et al. (2009). Overexpression of SMYD2 relates to tumor cell proliferation and malignant outcome of esophageal squamous cell carcinoma. Carcinogenesis 30, 1139–1146.

Konze, K. D., Ma, A., Li, F., Barsyte-Lovejoy, D., Parton, T., Macnevin, C. J., et al. (2013). An orally bioavailable chemical probe of the lysine methyltransferases EZH2 and EZH1. ACS Chem Biol 8, 1324–1334.

Konze, K. D., Pattenden, S. G., Liu, F., Barsyte-Lovejoy, D., Li, F., Simon, J. M., et al. (2014). A chemical tool for in vitro and in vivo precipitation of lysine methyltransferase G9a. ChemMedChem 9, 549–553.

Krogan, N. J., Dover, J., Wood, A., Schneider, J., Heidt, J., Boateng, M.A., et al. (2003). The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. Mol Cell 11, 721–729.

Kubicek, S., O'Sullivan, R. J., August, E. M., Hickey, E. R., Zhang, Q., Teodoro, M. L., et al. (2007). Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. Mol Cell 25, 473–481.

Kuo, A. J., Song, J., Cheung, P., Ishibe-Murakami, S., Yamazoe, S., Chen, J. K., et al. (2012). The BAH domain of ORC1 links H4K20me2 to DNA replication licensing and Meier-Gorlin syndrome. Nature 484, 115–119.

Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P., & Reinberg, D. (2002). Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. Genes Dev 16, 2893–2905.

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., & Jenuwein, T. (2001). Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410, 116–120.

Lacoste, N., Utley, R. T., Hunter, J. M., Poirier, G. G., & Cote, J. (2002). Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. J Biol Chem 277, 30421–30424.

Lakowski, T. M., t Hart, P., Ahern, C. A., Martin, N. I., & Frankel, A. (2010). Neta-substituted arginyl peptide inhibitors of protein arginine N-methyltransferases. ACS Chem Biol 5, 1053–1063.

Lee, J. K., & Kim, K. C. (2013). DZNep, inhibitor of S-adenosylhomocysteine hydrolase, down-regulates expression of SETDB1 H3K9me3 HMTase in human lung cancer cells. Biochem Biophys Res Commun 438, 647–652.

Li, H., Fischle, W., Wang, W., Duncan, E. M., Liang, L., Murakami-Ishibe, S., et al. (2007). Structural basis for lower lysine methylation state-specific readout by MBT repeats of L3MBTL1 and an engineered PHD finger. Mol Cell 28, 677–691.

Li, J., Li, Z., Ruan, J., Xu, C., Tong, Y., Pan, P. W., et al. (2011). Structural basis for specific binding of human MPP8 chromodomain to histone H3 methylated at lysine 9. PLoS One 6, e25104.

Li, Z., Wang, Y., Qiu, J., Li, Q., Yuan, C., Zhang, W., et al. (2013). The polycomb group protein EZH2 is a novel therapeutic target in tongue cancer. Oncotarget 4, 2532–2549.

Lin, W. J., Gary, J.D., Yang, M. C., Clarke, S., & Herschman, H. R. (1996). The mammalian immediate-early TIS21 protein and the leukemia-associated BTG1 protein interact with a protein–arginine N-methyltransferase. J Biol Chem 271, 15034–15044.

Liu, F., Barsyte-Lovejoy, D., Allali-Hassani, A., He, Y., Herold, J. M., Chen, X., et al. (2011). Optimization of cellular activity of G9a inhibitors 7-aminoalkoxy-quinazolines. J Med Chem 54, 6139–6150.

Liu, F., Barsyte-Lovejoy, D., Li, F., Xiong, Y., Korboukh, V., Huang, X. P., et al. (2013). Discovery of an in vivo chemical probe of the lysine methyltransferases G9a and GLP. J Med Chem 56, 8931–8942.

Liu, F., Chen, X., Allali-Hassani, A., Quinn, A.M., Wasney, G. A., Dong, A., et al. (2009). Discovery of a 2,4-diamino-7-aminoalkoxyquinazoline as a potent and selective inhibitor of histone lysine methyltransferase G9a. J Med Chem 52, 7950–7953.

Liu, F., Chen, X., Allali-Hassani, A., Quinn, A.M., Wigle, T. J., Wasney, G. A., et al. (2010). Protein lysine methyltransferase G9a inhibitors: design, synthesis, and structure activity relationships of 2,4-diamino-7-aminoalkoxy-quinazolines. J Med Chem 53, 5844–5857.

Liu, K., Chen, C., Guo, Y., Lam, R., Bian, C., Xu, C., et al. (2010). Structural basis for recognition of arginine methylated Piwi proteins by the extended Tudor domain. Proc Natl Acad Sci U S A 107, 18398–18403.

Liu, K., Guo, Y., Liu, H., Bian, C., Lam, R., Liu, Y., et al. (2012). Crystal structure of TDRD3 and methyl-arginine binding characterization of TDRD3, SMN and SPF30. PLoS One 7, e30375.

Liu, F., Li, F., Ma, A., Dobrovetsky, E., Dong, A., Gao, C., et al. (2013). Exploiting an allosteric binding site of PRMT3 yields potent and selective inhibitors. J Med Chem 56, 2110–2124.

Liu, H., Wang, J. Y., Huang, Y., Li, Z., Gong, W., Lehmann, R., et al. (2010). Structural basis for methylarginine-dependent recognition of Aubergine by Tudor. Genes Dev 24, 1876–1881.

Lohr, J. G., Stojanov, P., Lawrence, M. S., Auclair, D., Chapuy, B., Sougnez, C., et al. (2012). Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. Proc Natl Acad Sci U S A 109, 3879–3884.

Mai, A., Cheng, D., Bedford, M. T., Valente, S., Nebbioso, A., Perrone, A., et al. (2008). Epigenetic multiple ligands: mixed histone/protein methyltransferase, acetyltransferase, and class III deacetylase (sirtuin) inhibitors. J Med Chem 51, 2279–2290.

Mai, A., Valente, S., Cheng, D., Perrone, A., Ragno, R., Simeoni, S., et al. (2007). Synthesis and biological validation of novel synthetic histone/protein methyltransferase inhibitors. ChemMedChem 2, 987–991.

Margueron, R., Justin, N., Ohno, K., Sharpe, M. L., Son, J., Drury, W. J., III, et al. (2009). Role of the polycomb protein EED in the propagation of repressive histone marks. Nature 461, 762–767.

Martin, C., & Zhang, Y. (2005). The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol 6, 838–849.

Maurer-Stroh, S., Dickens, N. J., Hughes-Davies, L., Kouzarides, T., Eisenhaber, F., & Ponting, C. P. (2003). The Tudor domain 'Royal Family': Tudor, plant Agenet, Chromo, PWWP and MBT domains. Trends Biochem Sci 28, 69–74.

McCabe, M. T., Ott, H. M., Ganji, G., Korenchuk, S., Thompson, C., Van Aller, G. S., et al. (2012). EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature 492, 108–112.

Melcher, M., Schmid, M., Aagaard, L., Selenko, P., Laible, G., & Jenuwein, T. (2000). Structure–function analysis of SUV39H1 reveals a dominant role in heterochromatin organization, chromosome segregation, and mitotic progression. Mol Cell Biol 20, 3728–3741.

Min, J. R., Allali-Hassani, A., Nady, N., Qi, C., Hui, O. Y., Liu, Y. S., et al. (2007). L3MBTL1 recognition of mono- and dimethylated histones. Nat Struct Mol Biol 14, 1229–1230.

Min, J., Feng, Q., Li, Z., Zhang, Y., & Xu, R. M. (2003). Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. Cell 112, 711–723.

Min, J., Zhang, Y., & Xu, R. M. (2003). Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. Genes Dev 17, 1823–1828.

Miranda, T. B., Cortez, C. C., Yoo, C. B., Liang, G., Abe, M., Kelly, T. K., et al. (2009). DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. Mol Cancer Ther 8, 1579–1588.

Moore, S. A., Ferhatoglu, Y., Jia, Y., Al-Jiab, R. A., & Scott, M. J. (2010). Structural and biochemical studies on the chromo-barrel domain of male specific lethal 3 (MSL3) reveal a binding preference for mono- or dimethyllysine 20 on histone H4. J Biol Chem 285, 40879–40890.

Morin, R. D., Johnson, N. A., Severson, T. M., Mungall, A. J., An, J., Goya, R., et al. (2010). Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 42, 181–185.

Morin, R. D., Mendez-Lago, M., Mungall, A. J., Goya, R., Mungall, K. L., Corbett, R. D., et al. (2011). Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature 476, 298–303.

Muller, J., Hart, C. M., Francis, N. J., Vargas, M. L., Sengupta, A., Wild, B., et al. (2002). Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell 111, 197–208.

Muntean, A. G., Tan, J., Sitwala, K., Huang, Y., Bronstein, J., Connelly, J. A., et al. (2010). The PAF complex synergizes with MLL fusion proteins at HOX loci to promote leukemogenesis. Cancer Cell 17, 609–621.

Musselman, C. A., Avvakumov, N., Watanabe, R., Abraham, C. G., Lalonde, M. E., Hong, Z., et al. (2012). Molecular basis for H3K36me3 recognition by the Tudor domain of PHF1. Nat Struct Mol Biol 19, 1266–1272.

Nady, N., Lemak, A., Walker, J. R., Avvakumov, G. V., Kareta, M. S., Achour, M., et al. (2011). Recognition of multivalent histone states associated with heterochromatin by UHRF1 protein. J Biol Chem 286, 24300–24311.

Nayak, V., Xu, C., & Min, J. (2011). Composition, recruitment and regulation of the PRC2 complex. Nucleus 2.

Nebbioso, A., Carafa, V., Benedetti, R., & Altucci, L. (2012). Trials with 'epigenetic' drugs: an update. Mol Oncol 6, 657–682.

Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007

Ng, H. H., Dole, S., & Struhl, K. (2003). The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. J Biol Chem 278, 33625–33628.

Ng, H. H., Feng, Q., Wang, H., Erdjument-Bromage, H., Tempst, P., Zhang, Y., et al. (2002). Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. Genes Dev 16, 1518–1527.

Nguyen, A. T., & Zhang, Y. (2011). The diverse functions of Dot1 and H3K79 methylation. Genes Dev 25, 1345–1358.

Nielsen, P. R., Nietlispach, D., Mott, H. R., Callaghan, J., Bannister, A., Kouzarides, T., et al. (2002). Structure of the HP1 chromodomain bound to histone H3 methylated at lysine 9. Nature 416, 103–107.

Nikoloski, G., Langemeijer, S. M., Kuiper, R. P., Knops, R., Massop, M., Tonnissen, E. R., et al. (2010). Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet 42, 665–667.

Nishioka, K., Rice, J. C., Sarma, K., Erdjument-Bromage, H., Werner, J., Wang, Y., et al. (2002). PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. Mol Cell 9, 1201–1213.

Niwa, H., Handa, N., Tomabechi, Y., Honda, K., Toyama, M., Ohsawa, N., et al. (2013). Structures of histone methyltransferase SET7/9 in complexes with adenosylmethionine derivatives. Acta Crystallogr D Biol Crystallogr 69, 595–602.

Patel, A., Dharmarajan, V., & Cosgrove, M. S. (2008). Structure of WDR5 bound to mixed lineage leukemia protein-1 peptide. J Biol Chem 283, 32158–32161.

Patel, A., Vought, V. E., Dharmarajan, V., & Cosgrove, M. S. (2008). A conserved arginine-containing motif crucial for the assembly and enzymatic activity of the mixed lineage leukemia protein-1 core complex. J Biol Chem 283, 32162–32175.

Pirrotta, V., & Gross, D. S. (2005). Epigenetic silencing mechanisms in budding yeast and fruit fly: different paths, same destinations. Mol Cell 18, 395–398.

Pugh, C. S., Borchardt, R. T., & Stone, H. O. (1978). Sinefungin, a potent inhibitor of virion mRNA(guanine-7-)-methyltransferase, mRNA(nucleoside-2′-)-methyltransferase, and viral multiplication. J Biol Chem 253, 4075–4077.

Purandare, A. V., Chen, Z., Huynh, T., Pang, S., Geng, J., Vaccaro, W., et al. (2008). Pyrazole inhibitors of coactivator associated arginine methyltransferase 1 (CARM1). Bioorg Med Chem Lett 18, 4438–4441.

Qi, W., Chan, H., Teng, L., Li, L., Chuai, S., Zhang, R., et al. (2012). Selective inhibition of Ezh2 by a small molecule inhibitor blocks tumor cells proliferation. Proc Natl Acad Sci U S A 109, 21360–21365.

Qin, S., Guo, Y., Xu, C., Bian, C., Fu, M., Gong, S., et al. (2013). Tudor domains of the PRC2 components PHF1 and PHF19 selectively bind to histone H3K36me3. Biochem Biophys Res Commun 430, 547–553.

Qin, J., Whyte, W. A., Anderssen, E., Apostolou, E., Chen, H. H., Akbarian, S., et al. (2012). The polycomb group protein L3mbtl2 assembles an atypical PRC1-family complex that is essential in pluripotent stem cells and early development. Cell Stem Cell 11, 319–332.

Qiu, Y., Liu, L., Zhao, C., Han, C., Li, F., Zhang, J., et al. (2012). Combinatorial readout of unmodified H3R2 and acetylated H3K14 by the tandem PHD finger of MOZ reveals a regulatory mechanism for HOXA9 transcription. Genes Dev 26, 1376–1391.

Qiu, Y., Zhang, W., Zhao, C., Wang, Y., Wang, W., Zhang, J., et al. (2012). Solution structure of the Pdp1 PWWP domain reveals its unique binding sites for methylated H4K20 and DNA. Biochem J 442, 527–538.

Ragno, R., Simeoni, S., Castellano, S., Vicidomini, C., Mai, A., Caroli, A., et al. (2007). Small molecule inhibitors of histone arginine methyltransferases: homology modeling, molecular docking, binding mode analysis, and biological evaluations. J Med Chem 50, 1241–1253.

Rajakumara, E., Wang, Z., Ma, H., Hu, L., Chen, H., Lin, Y., et al. (2011). PHD finger recognition of unmodified histone H3R2 links UHRF1 to regulation of euchromatic gene expression. Mol Cell 43, 275–284.

Rathert, P., Dhayalan, A., Murakami, M., Zhang, X., Tamas, R., Jurkowska, R., et al. (2008). Protein lysine methyltransferase G9a acts on non-histone targets. Nat Chem Biol 4, 344–346.

Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z. W., Schmid, M., et al. (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature 406, 593–599.

Ruan, J., Ouyang, H., Amaya, M. F., Ravichandran, M., Loppnau, P., Min, J., et al. (2012). Structural basis of the chromodomain of cbx3 bound to methylated peptides from histone h1 and g9a. PLoS One 7, e35376.

Ruthenburg, A. J., Wang, W., Graybosch, D.M., Li, H., Allis, C. D., Patel, D. J., et al. (2006). Histone H3 recognition and presentation by the WDR5 module of the MLL1 complex. Nat Struct Mol Biol 13, 704–712.

Sack, J. S., Thieffine, S., Bandiera, T., Fasolini, M., Duke, G. J., Jayaraman, L., et al. (2011). Structural basis for CARM1 inhibition by indole and pyrazole inhibitors. Biochem J 436, 331–339.

Sampath, S.C., Marazzi, I., Yap, K. L., Sampath, S.C., Krutchinsky, A. N., Mecklenbrauker, I., et al. (2007). Methylation of a histone mimic within the histone methyltransferase G9a regulates protein complex assembly. Mol Cell 27, 596–608.

Schubert, H. L., Blumenthal, R. M., & Cheng, X. (2003). Many paths to methyltransfer: a chronicle of convergence. Trends Biochem Sci 28, 329–335.

Schuetz, A., Allali-Hassani, A., Martin, F., Loppnau, P., Vedadi, M., Bochkarev, A., et al. (2006). Structural basis for molecular recognition and presentation of histone H3 by WDR5. EMBO J 25, 4245–4252.

Selvi, B. R., Batta, K., Kishore, A. H., Mantelingu, K., Varier, R. A., Balasubramanyam, K., et al. (2010). Identification of a novel inhibitor of coactivator-associated arginine methyltransferase 1 (CARM1)-mediated methylation of histone H3 Arg-17. J Biol Chem 285, 7143–7152.

Senisterra, G., Wu, H., Allali-Hassani, A., Wasney, G. A., Barsyte-Lovejoy, D., Dombrovski, L., et al. (2013). Small-molecule inhibition of MLL activity by disruption of its interaction with WDR5. Biochem J 449, 151–159.

Sese, B., Barrero, M. J., Fabregat, M. C., Sander, V., & Izpisua Belmonte, J. C. (2013). SMYD2 is induced during cell differentiation and participates in early development. Int J Dev Biol 57, 357–364.

Shah, M.A., Denton, E. L., Liu, L., & Schapira, M. (2014). ChromoHub V2: cancer genomics. Bioinformatics 30, 590–592.

Shen, L., Cui, J., Pang, Y. X., Ma, Y. H., & Liu, P.S. (2013). 3-Deazaneplanocin A is a promising therapeutic agent for ovarian cancer cells. Asian Pac J Cancer Prev 14, 2915–2918.

Shi, Y., Desponts, C., Do, J. T., Hahm, H. S., Scholer, H. R., & Ding, S. (2008). Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell 3, 568–574.

Shinkai, Y., & Tachibana, M. (2011). H3K9 methyltransferase G9a and the related molecule GLP. Genes Dev 25, 781–788.

Siarheyeva, A., Senisterra, G., Allali-Hassani, A., Dong, A., Dobrovetsky, E., Wasney, G. A., et al. (2012). An allosteric inhibitor of protein arginine methyltransferase 3. Structure 20, 1425–1435.

Singer, M. S., Kahana, A., Wolf, A. J., Meisinger, L. L., Peterson, S. E., Goggin, C., et al. (1998). Identification of high-copy disruptors of telomeric silencing in Saccharomyces cerevisiae. Genetics 150, 613–632.

Siomi, M. C., Mannen, T., & Siomi, H. (2010). How does the royal family of Tudor rule the PIWI-interacting RNA pathway? Genes Dev 24, 636–646.

Sneeringer, C. J., Scott, M. P., Kuntz, K. W., Knutson, S. K., Pollock, R. M., Richon, V. M., et al. (2010). Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci U S A 107, 20980–20985.

Spannhoff, A., Heinke, R., Bauer, I., Trojer, P., Metzger, E., Gust, R., et al. (2007). Target-based approach to inhibitors of histone arginine methyltransferases. J Med Chem 50, 2319–2325.

Sun, B., Hong, J., Zhang, P., Dong, X., Shen, X., Lin, D., et al. (2008). Molecular basis of the interaction of Saccharomyces cerevisiae Eaf3 chromo domain with methylated H3K36. J Biol Chem 283, 36504–36512.

Sweis, R. F., Pliushchev, M., Brown, P. J., Guo, Jun, Li, Fengling, Maag, David, et al. (2014). Discovery and development of potent and selective inhibitors of histone methyltransferase G9a. ACS Med Chem Lett 5, 205–209.

t Hart, P., Lakowski, T. M., Thomas, D., Frankel, A., & Martin, N. I. (2011). Peptidic partial bisubstrates as inhibitors of the protein arginine N-methyltransferases. Chembiochem 12, 1427–1432.

Takawa, M., Masuda, K., Kunizaki, M., Daigo, Y., Takagi, K., Iwai, Y., et al. (2011). Validation of the histone methyltransferase EZH2 as a therapeutic target for various types of human cancer and as a prognostic marker. Cancer Sci 102, 1298–1305.

Tan, J., Yang, X., Zhuang, L., Jiang, X., Chen, W., Lee, P. L., et al. (2007). Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev 21, 1050–1063.

Therrien, E., Larouche, G., Manku, S., Allan, M., Nguyen, N., Styhler, S., et al. (2009). 1,2-Diamines as inhibitors of co-activator associated arginine methyltransferase 1 (CARM1). Bioorg Med Chem Lett 19, 6725–6732.

Thiel, A. T., Blessington, P., Zou, T., Feather, D., Wu, X., Yan, J., et al. (2010). MLL-AF9-induced leukemogenesis requires coexpression of the wild-type Mll allele. Cancer Cell 17, 148–159.

Tie, F., Stratton, C. A., Kurzhals, R. L., & Harte, P. J. (2007). The N terminus of Drosophila ESC binds directly to histone H3 and is required for E(Z)-dependent trimethylation of H3 lysine 27. Mol Cell Biol 27, 2014–2026.

Tripsianes, K., Madl, T., Machyna, M., Fessas, D., Englbrecht, C., Fischer, U., et al. (2011). Structural basis for dimethylarginine recognition by the Tudor domains of human SMN and SPF30 proteins. Nat Struct Mol Biol 18, 1414–1420.

Trojer, P., Li, G., Sims, R. J., III, Vaquero, A., Kalakonda, N., Boccuni, P., et al. (2007). L3MBTL1, a histone-methylation-dependent chromatin lock. Cell 129, 915–928.

Tschiersch, B., Hofmann, A., Krauss, V., Dorn, R., Korge, G., & Reuter, G. (1994). The protein encoded by the Drosophila position-effect variegation suppressor gene Su(var)3-9 combines domains of antagonistic regulators of homeotic gene complexes. EMBO J 13, 3822–3831.

van Leeuwen, F., Gafken, P. R., & Gottschling, D. E. (2002). Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell 109, 745–756.

Varambally, S., Dhanasekaran, S. M., Zhou, M., Barrette, T. R., Kumar-Sinha, C., Sanda, M. G., et al. (2002). The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 419, 624–629.

Vedadi, M., Barsyte-Lovejoy, D., Liu, F., Rival-Gervier, S., Allali-Hassani, A., Labrie, V., et al. (2011). A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. Nat Chem Biol 7, 566–574.

Vezzoli, A., Bonadies, N., Allen, M.D., Freund, S. M., Santiveri, C. M., Kvinlaug, B. T., et al. (2010). Molecular basis of histone H3K36me3 recognition by the PWWP domain of Brpf1. Nat Struct Mol Biol 17, 617–619.

Wan, H., Huynh, T., Pang, S., Geng, J., Vaccaro, W., Poss, M.A., et al. (2009). Benzo[d]imidazole inhibitors of Coactivator Associated Arginine Methyltransferase 1 (CARM1)–Hit to lead studies. Bioorg Med Chem Lett 19, 5063–5066.

Wang, L., Brown, J. L., Cao, R., Zhang, Y., Kassis, J. A., & Jones, R. S. (2004). Hierarchical recruitment of polycomb group silencing complexes. Mol Cell 14, 637–646.

Wang, J., Chen, L., Sinha, S. H., Liang, Z., Chai, H., Muniyan, S., et al. (2012). Pharmacophore-based virtual screening and biological evaluation of small molecule inhibitors for protein arginine methylation. J Med Chem 55, 7978–7987.

Wang, T., Xu, C., Liu, Y., Fan, K., Li, Z., Sun, X., et al. (2012). Crystal Structure of the human SUV39H1 chromodomain and its recognition of histone H3K9me2/3. PLoS One 7, e52977.

Wismar, J., Loffler, T., Habtemichael, N., Vef, O., Geissen, M., Zirwes, R., et al. (1995). The Drosophila melanogaster tumor suppressor gene lethal(3)malignant brain tumor encodes a proline-rich protein with a novel zinc finger. Mech Dev 53, 141–154.

Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007

Wood, A., Schneider, J., Dover, J., Johnston, M., & Shilatifard, A. (2003). The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. J Biol Chem 278, 34739–34742.

Wu, J., Cheung, T., Grande, C., Ferguson, A.D., Zhu, X., Theriault, K., et al. (2011). Biochemical characterization of human SET and MYND domain-containing protein 2 methyltransferase. Biochemistry 50, 6488–6497.

Wu, H., Min, J., Lunin, V. V., Antoshenko, T., Dombrovski, L., Zeng, H., et al. (2010). Structural biology of human H3K9 methyltransferases. PLoS One 5, e8570.

Wu, H., Zeng, H., Lam, R., Tempel, W., Amaya, M. F., Xu, C., et al. (2011). Structural and histone binding ability characterizations of human PWWP Domains. PLoS One 6, e18919.

Wysocka, J., Swigut, T., Milne, T. A., Dou, Y., Zhang, X., Burlingame, A. L., et al. (2005). WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. Cell 121, 859–872.

Xu, C., Bian, C., Yang, W., Galka, M., Ouyang, H., Chen, C., et al. (2010). Binding of different histone marks differentially regulates the activity and specificity of polycomb repressive complex 2 (PRC2). Proc Natl Acad Sci U S A 107, 19266–19271.

Xu, C., Cui, G., Botuyan, M. V., & Mer, G. (2008). Structural basis for the recognition of methylated histone H3K36 by the Eaf3 subunit of histone deacetylase complex Rpd3S. Structure 16, 1740–1750.

Xu, C., & Min, J. (2011). Structure and function of WD40 domain proteins. Protein Cell 2, 202–214.

Yang, Y., & Bedford, M. T. (2013). Protein arginine methyltransferases and cancer. Nat Rev Cancer 13, 37–50.

Yang, N., Wang, W., Wang, Y., Wang, M., Zhao, Q., Rao, Z., et al. (2012). Distinct mode of methylated lysine-4 of histone H3 recognition by tandem tudor-like domains of Spindlin1. Proc Natl Acad Sci U S A 109, 17954–17959.

Yao, Y., Chen, P., Diao, J., Cheng, G., Deng, L., Anglin, J. L., et al. (2011). Selective inhibitors of histone methyltransferase DOT1L: design, synthesis, and crystallographic studies. J Am Chem Soc 133, 16746–16749.

Yap, D. B., Chu, J., Berg, T., Schapira, M., Cheng, S. W., Moradian, A., et al. (2011). Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. Blood 117, 2451–2459.

Yohn, C. B., Pusateri, L., Barbosa, V., & Lehmann, R. (2003). l(3)malignant brain tumor and three novel genes are required for Drosophila germ-cell formation. Genetics 165, 1889–1900.

Yu, W., Chory, E. J., Wernimont, A. K., Tempel, W., Scopton, A., Federation, A., et al. (2012). Catalytic site remodelling of the DOT1L methyltransferase by selective inhibitors. Nat Commun 3, 1288.

Yu, W., Smil, D., Li, F., Tempel, W., Fedorov, O., Nguyen, K. T., et al. (2013). Bromo-deaza-SAH: a potent and selective DOT1L inhibitor. Bioorg Med Chem 21, 1787–1794.

Yuan, Y., Wang, Q., Paulk, J., Kubicek, S., Kemp, M. M., Adams, D. J., et al. (2012). A small-molecule probe of the histone methyltransferase G9a induces cellular senescence in pancreatic adenocarcinoma. ACS Chem Biol 7, 1152–1157.

Zeng, L., Zhang, Q., Li, S., Plotnikov, A. N., Walsh, M. J., & Zhou, M. M. (2010). Mechanism and regulation of acetylated histone binding by the tandem PHD finger of DPF3b. Nature 466, 258–262.

Zhang, P., Lee, H., Brunzelle, J. S., & Couture, J. F. (2012). The plasticity of WDR5 peptide-binding cleft enables the binding of the SET1 family of histone methyltransferases. Nucleic Acids Res 40, 4237–4246.

Zhang, K., Mosch, K., Fischle, W., & Grewal, S. I. (2008). Roles of the Clr4 methyltransferase complex in nucleation, spreading and maintenance of heterochromatin. Nat Struct Mol Biol 15, 381–388.

Zheng, W., Ibanez, G., Wu, H., Blum, G., Zeng, H., Dong, A., et al. (2012). Sinefungin derivatives as inhibitors and structure probes of protein lysine methyltransferase SETD2. J Am Chem Soc 134, 18004–18014.

Zhou, J., Bi, C., Cheong, L. L., Mahara, S., Liu, S.C., Tay, K. G., et al. (2011). The histone methyltransferase inhibitor, DZNep, up-regulates TXNIP, increases ROS production, and targets leukemia cells in AML. Blood 118, 2830–2839.

Please cite this article as: Liu, Y., et al., Epigenetic targets and drug discovery, Pharmacology & Therapeutics (2014), http://dx.doi.org/10.1016/j.pharmthera.2014.03.007