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Methyltransferase inhibitors for modulation of the epigenome and beyond

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Over the past two years tremendous progress has been made in the discovery of new inhibitors of protein lysine and arginine methyltransferases, establishing this class of epigenetic enzymes, along with DNA methyltransferases, as druggable protein families. New inhibitors of protein methyltransferases have been described with a variety of mechanisms of action including cofactor competitive, substrate competitive, allosteric inhibitors and disruptors of protein–protein interactions. Inhibitors have been used extensively in oncology studies, and inhibitors of EZH2, and DOT1L are currently in clinical trials. Finally, advances in understanding the clinical mechanism of action of 5-azacytidine and related DNA hypomethylation agents were reported, revealing a likely role for a cell autonomous innate immune response.

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Introduction

The human genome contains approximately 208 methyltransferases, of which approximately 129 are known or likely to be relevant to epigenetic chromatin and transcriptional regulation, namely protein lysine methyltransferases (PKMTs), protein arginine methyltransferases (PRMTs), DNA methyltransferases (DNMTs), and RNA methyltransferases (RNMTs). These enzymes are key regulators of histone and DNA methyl marks that regulate chromatin and epigenetic states, as well as methylation of many other important factors such as transcription factors (p53, Rb, ER, etc.), splicing factors, cytosolic signaling cascades and coding and noncoding RNAs. Of these, histone and DNA methylation is the best characterized.

DNMT inhibitory compounds have been known since the 1960's and are now approved for clinical use [1], and protein methyltransferases (PMTs) as a class are thought to be a promising new therapeutic target family for oncology and other indications [2,3]. Therefore, this review focuses on potent, selective and cell-active inhibitors of the human methyltransferases of most relevance to epigenetic regulation, namely SET domain PKMTs, PRMTs, and DNMT1 as outlined in Table 1. While RNA methylation likely plays a role in epigenetic regulation, as of yet there are no known specific inhibitors of RNA methyltransferases.

Recent progress in discovery of protein methyltransferase inhibitors

The majority of human PMTs mono-, di- or tri-methylate lysine side-chains and are organized around a catalytic SET domain (named after the proteins where it was first observed: Su(var)3-9, Enhancer-of-zeste and Trithorax) where both cofactor and protein substrate bind. Structurally distinct, PRMTs are Rossmann fold enzymes that monomethylate, symmetrically or asymmetrically dimethylate arginine side-chains. A growing number of Rossmann fold proteins are also found to methylate lysines, as was originally shown for the H3K79 PKMT, DOT1L (Figure 1).

While little progress has been made toward new chemical inhibitors of DNMTs over the past 40 years (all advanced compounds are analogs of the azanucleosides azacytidine and decitabine, which were developed as cytostatic agents in the 1960s, and were characterized as DNMT1 inhibitors in 1980 [4]), the chemical coverage of PMTs has rapidly advanced in recent years (Figure 1). BIX-01294 was the first chemical tool to probe a specific PMT function in cells [5]. BIX-01294 selectively inhibits EHMT1/GLP and EHMT2/G9a, two closely related H3K9 di-methylases. Structure-based optimization of its quinazoline scaffold resulted in UNC0638, a more potent and specific inhibitor, and UNC0642, an analog with improved pharmacokinetics [6,7]. The recently published A-366 is the first non-quinazoline G9a/GLP chemical probe and avoids some potential off-target toxicities of the quinazoline scaffold [8]. These studies established the chemical tractability of SET domain PKMTs, paving the way for rapid advances over the past 5 years.

Table 1

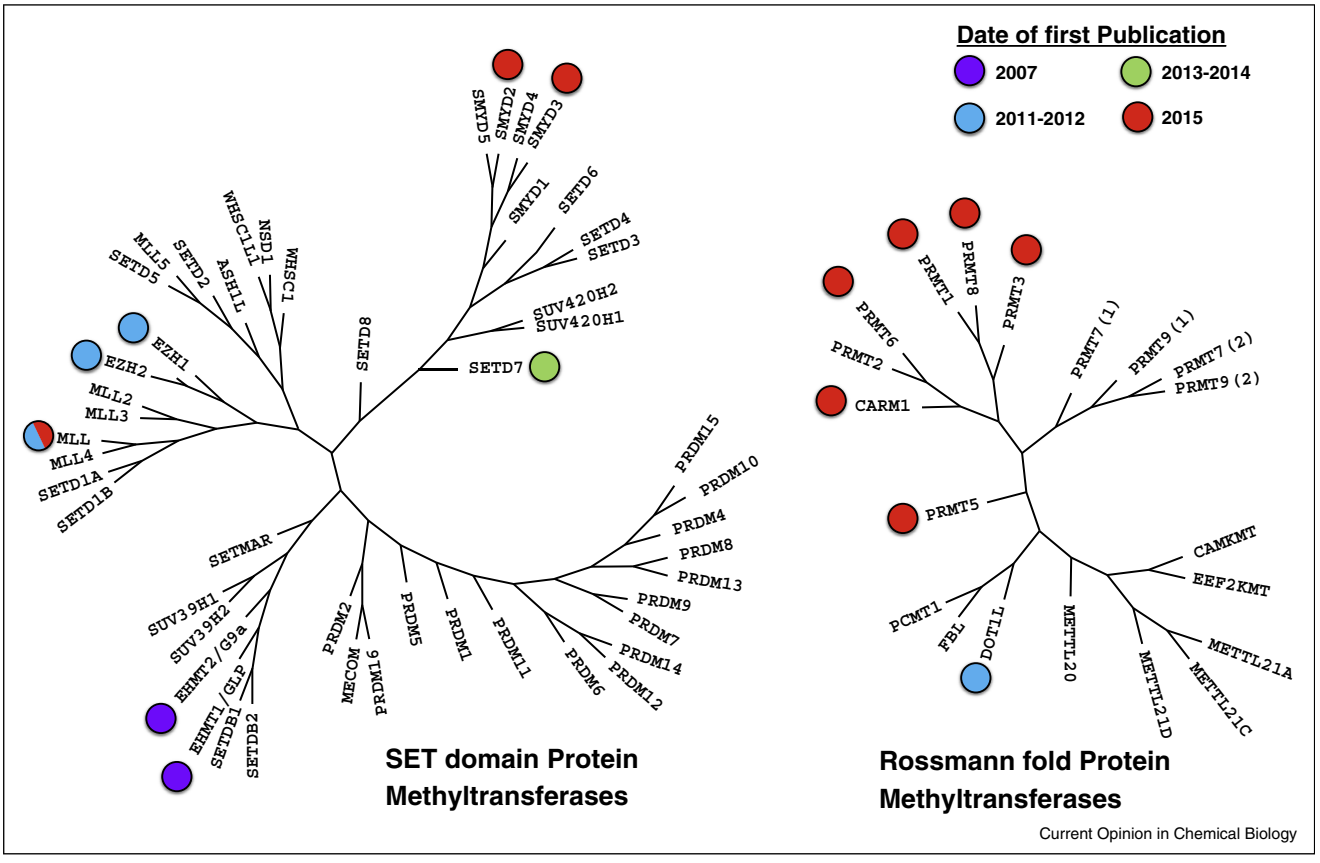
Mechanism of action of PMT inhibitors. Only potent, selective, cell-active molecules are shown

Mechanism of action	Target	Inhibitors[a]	Ref.
Competition with substrate Lys/Arg	G9a/GLP	BIX01294, UNC0638, A-366*	[5,7,8]
	SETD7	PFI-2*	[15•]
	SMYD2	AZ-505, LLY507*, A-893*	[16–18]
	SMYD3	EPZ031686*	[19]
	PRMT5	EPZ015666**, GSK591	[23••,27]
	PRMT6	EPZ020411*	[26]
	Type I PRMTs	MS023*	[24]
Competition with cofactor	DOT1L	EPZ-5676/pinometostat EPZ004777, SGC0946	[20–22]
	EZH2	GSK126, EPZ-6438/tazemetostat, GSK343, UNC1999, EPZ011989	[9–14]
Allosteric	PRMT3	SGC707*	[25•]
Disruption of protein–protein interaction	MENIN-MLL	MI-503**	[33••]
	WDR5-MLL	OICR-9429**	[32••]
DNA incorporation followed by covalent target binding	DNMT1	Azacitidine, decitabine	[28]

[a] Compounds of special and outstanding interest published in the past two years are respectively indicated with * and **.

Additional SET domain PKMTs have subsequently been targeted with highly selective inhibitors (Figure 1, Table 1). Several groups identified a class of compounds sharing a pyridone pharmacophore as highly selective inhibitors of EZH2 [9,10,11,12,13,14]. Recently published chemical probes of other SET domain PKMTs include PFI-2, an inhibitor of SETD7 (one of the first characterized PMT) [15•], the SMYD2 inhibitors LLY-507 and A-893 (an

Figure 1



Chemical coverage of human PMTs. The date of first publication of chemical inhibitors.