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PRMT Inhibitors

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Contents

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Abstract The methylation of arginine residues in numerous protein targets is a post-translational modification that has gained increased interest in the scientific community over the past two decades. Arginine methylation is performed by the dedicated family of protein arginine methyltransferases and is known to be involved in a plethora of cellular pathways and biochemical mechanisms in both healthy and disease states. The development of inhibitors for these enzymes for use as biological tools can lead to a more detailed understanding of the functions of the different members of the PRMT family. In addition, a number of recent studies point towards PRMTs as therapeutic targets for a number of diseases and the first clinical trials with compounds inhibiting PRMTs are now underway. We here provide a broad overview of the current status of the inhibitors that have been developed against PRMTs using both high-throughput screening and rational design approaches.

Keywords Activity, Inhibition, Methylation, Protein arginine *N*-methyltransferase, Therapeutics

Abbreviations

aDMA	Asymmetrically dimethylated arginine
AdoHcy	*S*-adenosyl-L-homocysteine
AdoMet	*S*-adenosyl-L-methionine
Adox	Adenosine dialdehyde
AMI	Arginine methyltransferase inhibitor
AML	Acute myeloid leukaemia
CARM1	Coactivator-associated arginine methyltransferase
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EC50	Half maximal effective concentration
GAR	Glycine-arginine rich
HEK293T	Human embryonic kidney cell line
HepG2	Hepatocellular carcinoma cell line
HIV	Human immunodeficiency virus
IC_{50}	Half maximal inhibitory concentration
Ki	Inhibition constant

LNCaP	Lymph node carcinoma of the prostate, prostate cancer cell line
MCF7	Michigan Cancer Foundation-7, breast cancer cell line
MCL	Mantle cell lymphoma
MEP50	Methylosome protein 50
MLL	Mixed lineage leukaemia
MMA	Monomethylated arginine
MTA	Methylthioadenosine
MTAP	5-Methylthioadenosine phosphorylase
PABP1	Poly(A)-binding protein-1
PAD	Protein arginine deiminase
PGM	Proline, glycine, methionine-rich
PK/PD	Pharmacokinetic/pharmacodynamic
PRMT	Protein arginine *N*-methyltransferase
RNA	Ribonucleic acid
RSF1	Repressor splicing factor
SAH	*S*-adenosyl-L-homocysteine
SAHH	*S*-adenosyl-L-homocysteine hydrolase
SAM	*S*-adenosyl-L-methionine
SAR	Structure-activity relationship
sDMA	Symmetrically dimethylated arginine
SET7	SET domain containing protein 7
SGC	Structural genomics consortium
Tat	Trans-activator of transcription

1 Introduction

The methylation of arginine residues in proteins is an important post-translational modification, performed by the family of protein arginine *N*-methyltransferases (PRMTs). The enzymes use nature's ubiquitous methyl donor *S*-adenosyl-L-methionine (AdoMet, also known as SAM) as a cofactor to form the methylated protein product with concomitant release of *S*-adenosyl-L-homocysteine (AdoHcy, also known as SAH) as a byproduct. Mechanistically, target arginine residues are first monomethylated by all types of PRMTs and subsequently dimethylated asymmetrically by type I PRMTs and symmetrically by type II PRMTs. Type III PRMTs produce only monomethylated arginine (MMA). The PRMTs share highly conserved active site sequences, including a number of residues involved in AdoMet cofactor recognition as well as two glutamate residues that hydrogen bond with the guanidine moiety of the substrate peptide. These glutamate residues are part of the so-called double E-loop which ensures that the guanidine group is positioned in close proximity to the AdoMet cofactor to facilitate the methyl transfer via an S_N2-like substitution reaction (Fig. 1).

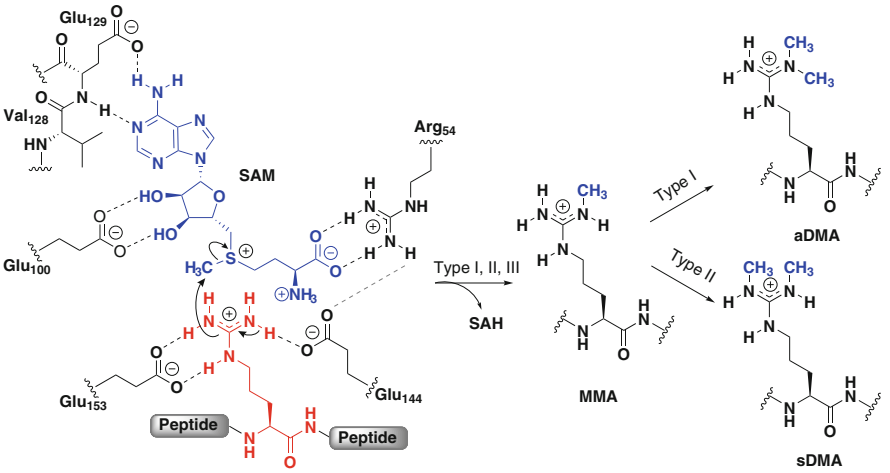


Fig. 1 Schematic overview of the PRMT-binding site (residue numbering PRMT1) showing the hydrogen bonding interactions of the double E-loop (residues Glu144 and Glu153) with the guanidine moiety of the arginine sidechain. Cofactor *S*-adenosyl-L-methionine (SAM) is depicted in blue and the protein arginine sidechain in red. First monomethylarginine (MMA) is formed releasing *S*-adenosyl-L-homocysteine (SAH). MMA is directly converted to asymmetrically dimethylated arginine (aDMA) by type I PRMTs and to symmetrically dimethylated arginine (sDMA) by type II PRMTs

The presence of (di-)methylated arginines in proteins and an enzyme responsible for this process were discovered 50 years ago [1, 2]. A decade later it was found that the majority of proteins with methylated arginines are nuclear proteins that contain asymmetrically dimethylated arginine (aDMA) sidechains [3]. Subsequently, it became clear that not one enzyme but a distinct family of enzymes was responsible for this post-translational modification [4–8]. The seminal study of Herschman and co-workers published in 1996 [9] clearly identified and characterized the first member of the family, PRMT1. In the following decade, new PRMT family members were identified on a near yearly basis resulting in the now known total of nine PRMTs [10–18].

Whether or not arginine methylation is a dynamic process remains to be proven unambiguously. Protein arginine deiminases (PADs) are known to hydrolyse the guanidine moiety of arginine side chains in proteins into citrulline. However, PADs are not able to convert dimethylated arginines to citrulline [19]. In addition, while in vivo data suggested the possibility that PADs can convert monomethylated arginines to citrulline, in vitro studies have not been able to reproduce this activity [19]. In addition, citrulline can only be converted back into arginine after proteolysis, but not in the context of an intact protein. Given that it is unlikely that PADs are the active player in removing methyl groups from methylated arginines, much effort has been spent at trying to identify a true arginine demethylase. In 2007, the lysine demethylating enzyme JMJD6 was reported to also demethylate arginines in histones making it the first example of an arginine demethylase [20]. These findings

have, however, become the source of some debate as the results could not be reproduced by other groups [21–26]. Recently, other members of the Jumonji-domain containing lysine demethylases (KDM4E and KDM5C) were also shown to demethylate arginines in histones in vitro, suggesting the process is indeed dynamic [27]. However, at this point in time, in vivo proof for the presence of arginine-specific demethylases is still lacking. In this regard, the only certain way to control arginine methylation remains via inhibition of the PRMTs.

A great number of protein substrates have been identified for the different PRMTs, ranging from general substrates to others that are only acted upon by one specific PRMT [28–30]. Arginine methylation by PRMTs is involved in many cellular processes, including RNA processing, gene transcription, signal transduction and DNA repair [31, 32]. In terms of their biological roles, the PRMTs have little redundancy as indicated by the dramatic phenotypes observed in knockout mice [31]. A growing body of evidence implicates dysregulated arginine methylation in a variety of diseases, including numerous cancers [32], cardiovascular [33], pulmonary [34–36] and viral diseases [37, 38]. In an attempt to address the connection between aberrant PRMT activity and human disease, the Bedford group published the first report describing PRMT inhibitors in 2004 [39]. Since this time, the number of publications describing new, more potent and selective PRMT inhibitors has steadily increased.

The recent interest in the field of PRMTs has led to growing amounts of data along with reviews describing the progress made in different areas of research [40–46]. We here provide an overview on the current status of the development of compounds aimed at inhibiting PRMTs. Starting with a brief summary of general methyltransferase inhibitors, we then address the development of inhibitors specific for each of the nine PRMTs, followed by a brief discussion on their biological relevance. In cases where a compound has inhibitory activity against more than one PRMT, it will be discussed in the context of the enzyme that is inhibited with the highest activity and selectivity.

2 Nonspecific Protein Methyltransferase Inhibitors

2.1 Background

The enzymatic reaction performed by PRMTs and other methyltransferases using AdoMet as the methyl donor is self-regulating due to the inhibitory properties of cofactor byproduct, AdoHcy (**1**). Structurally similar compounds include Aza-AdoMet (**2**), where the sulphur of AdoMet is replaced by a nitrogen atom, and the bacterially produced natural product sinefungin (**3**, Fig. 2). These compounds are known to inhibit all AdoMet-dependent methyltransferases by competition with AdoMet. It is due to this mechanism of action that the AdoMet analogues lack specificity of inhibition. Therefore, the primary use of these analogues is as reference inhibitors in both biochemical and cellular assays. Adenosine dialdehyde

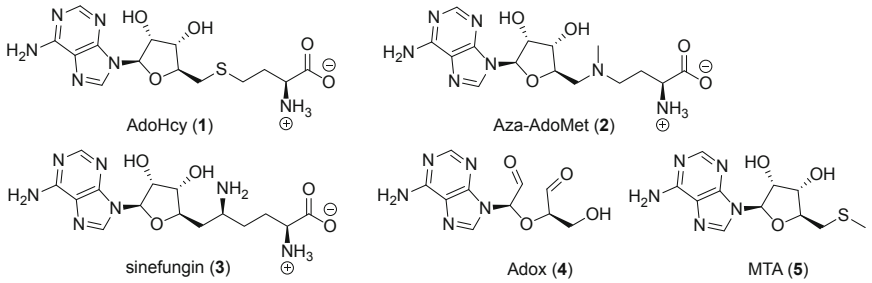


Fig. 2 General methyltransferase inhibitors *S*-adenosyl-L-homocysteine (AdoHcy, **1**), nitrogen analogue Aza-AdoMet (**2**), bacterial compound sinefungin (**3**), indirect inhibitor adenosine dialdehyde (Adox, **4**) and methylthioadenosine (MTA, **5**)

(Adox, **4**) is a known inhibitor of *S*-adenosyl-L-homocysteine hydrolase (SAHH) which leads to a build-up of AdoHcy. Via this mechanism Adox is an indirect methyltransferase inhibitor that can be used in cellular assays. Methylthioadenosine (MTA, **5**) is sometimes mentioned as a general methyltransferase inhibitor, but recent publications show MTA is rather specific in inhibiting PRMTs (see subchapter on PRMT5) [47–49].

2.2 Inhibitors: In Vitro and Cell-Based Activities

Recently, a more targeted general PRMT inhibitor was reported by Jin and co-workers [50]. The compound, designated as MS023 (**6**, Fig. 3), inhibits all type I PRMTs. Its design was based on the structures of other PRMT inhibitors specific for PRMT4 and PRMT6 also containing an ethylenediamino group which was found to function as an arginine mimetic. Building upon structure-activity relationship (SAR) studies, MS023 was developed and, along with the negative control compound MS094 (lacking the ethylenediamino moiety), tested against a panel of methyltransferases. The results showed potent inhibition of all type I PRMTs with activities from 4 to 119 nM with no inhibition of type II and III PRMTs, lysine methyltransferases, DNA methyltransferases, histone demethylases and methyl-readers [50]. A co-crystal structure of MS023 bound to PRMT6 in the presence of AdoHcy (Fig. 3) shows the interactions of the ethylenediamino group in the active site where the arginine moiety would be expected to bind. Replacement of the terminal amine with a hydroxyl group as in MS094 (**7**, Fig. 3) leads to complete loss of inhibitory activity. The cellular assays performed with MS023 showed a decrease in asymmetric dimethylation of histone H4R3 by PRMT1 with an IC_{50} value of 9 nM and of H3R2 by PRMT6 with an IC_{50} value of 56 nM. Also of note, the decrease in cellular aDMA levels was accompanied by a measurable increase in MMA and symmetrically dimethylated arginine (sDMA) levels. Interestingly, no explanation has yet been provided for the striking selectivity observed for MS023 in

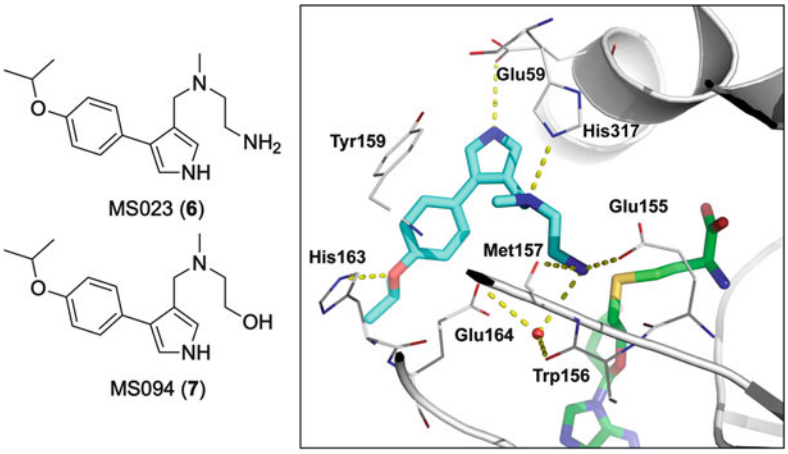


Fig. 3 Left, structures of general type I PRMT inhibitor MS023 (**6**) and its negative control MS094 (**7**). Right, co-crystal structure of MS023 (blue) bound to PRMT6 in the presence of AdoHcy (green). MS023 binds in the substrate-binding site with the ethylenediamino moiety interacting with His317 of the "THW-loop" and Glu155 and Glu164 of the "double E-loop" (PDB ID: 5E8R) [50]

the inhibition of type I over type II/III PRMTs. While such type I-specific PRMT inhibitor can be of value when looking into the role of aDMA in complex systems, their therapeutic potential remains to be demonstrated. Nevertheless, in order to investigate the specific roles of the individual PRMTs in detail, a comprehensive toolbox of inhibitory compounds is essential.

3 PRMT1

3.1 Background

PRMT1 was the first identified member of the protein arginine methyltransferase family and the most abundant [9, 51, 52]. Alternative splicing results in the possibility of at least seven variants (v1–v7) with differences in the length of the N-terminal tail [53]. These variants can differ in localization and substrate specificity. PRMT1 produces asymmetrically dimethylated arginine (aDMA) and is responsible for more than 85% of all arginine methylations [52]. As expected, knockout of PRMT1 in cells results in a significant decrease in aDMA and a corresponding increase in MMA and sDMA [54].

In its target substrates, PRMT1 preferentially methylates the RGG motif, although this is not a strict prerequisite [6, 8, 55]. Known PRMT1 substrates are diverse and include histone H4R3, Sam68 (sarcoma associated in mitosis of 68 kDa), MRE11 (meiotic recombination 11), 53BP1 (p53-binding protein 1) and hnRNP A1 (heterogeneous nuclear ribonucleoprotein A1), among many others which are involved in

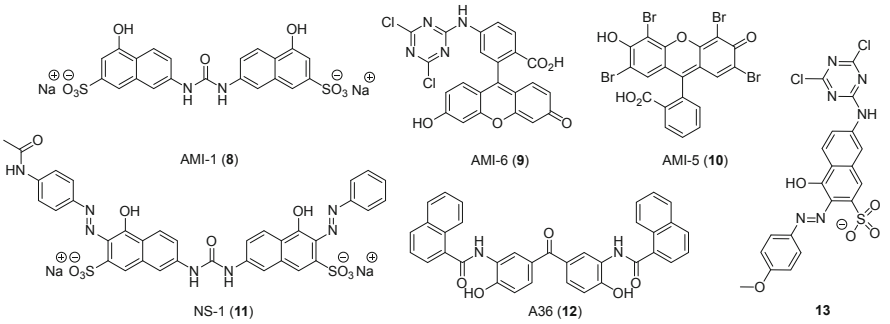


Fig. 4 PRMT1 inhibitors **8**–**13** interacting with the histone H4 and glycine-arginine-rich (GAR) substrates

gene transcription, nuclear transport, DNA repair and RNA processing, respectively [32, 56]. PRMT1 is overexpressed in breast [53, 57], prostate [58], lung [59], colon [60, 61], head and neck [62] and bladder cancer [63] and in leukaemia [32, 64, 65]. It is further involved in a number of other serious conditions ranging from pulmonary disease [35, 66], cardiovascular disease [33] and diabetes [67] to cocaine addiction [68].

3.2 Inhibitors: In Vitro and Cell-Based Activities

The first report on the development of PRMT inhibitors was published in 2004, indicating the relative infancy of the field. A screening campaign resulted in the identification of *a*rginine *m*ethyltransferase *i*nhibitors AMI-1 (**8**) and AMI-6 (**9**, Fig. 4) with IC_{50} values against PRMT1 of 8.8 and 5.1 μM, respectively [39]. Small molecules based on AMI scaffolds, including AMI-5 (**10**, Fig. 4), were studied via docking and binding studies [69], and a follow-up study with simplified AMI-5 analogues showed inhibitory effects on different methyltransferases, including PRMT1 and PRMT4 and lysine methyltransferase SET7 (SET domain containing protein) [70]. For several active compounds, cellular activity in human leukaemia U937 cell lines was found with specific effects on cell cycle arrest, apoptosis and granulocyte differentiation [70].

Follow-up studies revealed analogues of the AMI series, including naphthyl-sulfo derivatives, such as compound NS-1 (**11**, Fig. 4), [71] and pharmacophore-based small molecule inhibitors, like analogue A36 (**12**, Fig. 4), both of which exhibit IC_{50} values in the low μM range [72]. Interestingly, it was later discovered that the mechanism of action of these inhibitors is not by interaction with the PRMT but rather with the histone H4 substrate and other glycine- and arginine-rich (GAR) substrates, explaining – at least in part – the observed (lack of) specificities for these compounds. Inspired by the findings of the AMI compounds, Mowen and co-workers combined structural features of the different AMIs to generate new

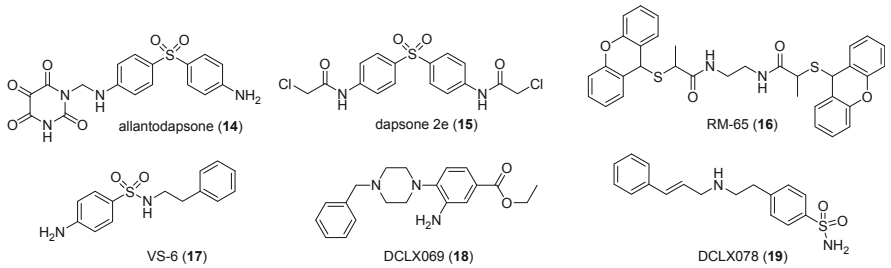


Fig. 5 Virtual screening hits **14**–**19** for PRMT1

PRMT inhibitors [73]. Compound **13** of this series showed an IC_{50} value of 4.2 μM against PRMT1 and 2.7 μM against PRMT4 and also demonstrated activity against PRMT3, PRMT5, PRMT6 and PRMT8 as shown by Western blot analysis. The same inhibitor was only slightly active against lysine methyltransferase SET7 and was found to be cell-permeable and decrease levels of H3R17 methylation and lowers the secretion of interferon IFN-gamma and interleukin IL-4 from T helper cells [73].

As another approach to discovering PRMT inhibitors, virtual screening methods have also been employed. Such strategies have revealed allantodapsone (**14**) [74]; dapsone analogues, including compound **15** [75]; thioglycolic amides, including RM-65 (**16**) [76]; and other virtual screening hits such as VS-6 (**17**) [77] (Fig. 5). These compounds were evaluated for their inhibitory activity against PRMT1 using a target-based virtual screening approach. Compound **15** was found to be cytotoxic in MCF7 breast cancer cells and LNCaP (lymph node carcinoma of the prostate) prostate cancer cells, while RM-65 was demonstrated to result in hypomethylation of proteins in HepG2 hepatocellular carcinoma cancer cells. Similar virtual screening approaches by other research groups led to the discovery of compounds DCLX069 (**18**) and DCLX078 (**19**) [78] both of which exhibit micromolar inhibition of PRMT1 (IC_{50} 17.9–26.2 μM) with some selectivity over PRMT4 and PRMT6. The compounds also showed antiproliferative action in three different cancer cell lines (HepG2, MCF7 and the monocytic leukaemia cell line THP1). These compounds represent promising starting points for hit-to-lead optimizations in pursuit of selective PRMT inhibitors.

Applying a bisubstrate approach, Dowden and co-workers designed PRMT inhibitors linking structural features of the AdoMet cofactor to a guanidine or amine moiety (**20**–**22**, Fig. 6) [79, 80]. This approach resulted in inhibitors that are active against PRMT1 with low micromolar potency (IC_{50} values ranged from 2.9 to 6.2 μM) and inactive against SET7 and PRMT4. No cellular assays were performed with these bisubstrates.

Working together with Frankel and co-workers, our group has also investigated N^{η}-substituted arginine-containing peptides as PRMT inhibitors (**23–25**, Fig. 6) [81–84]. Using an established PRMT substrate derived from a fibrillarin peptide sequence, incorporation of fluorinated ethyl groups on the side chain of the target

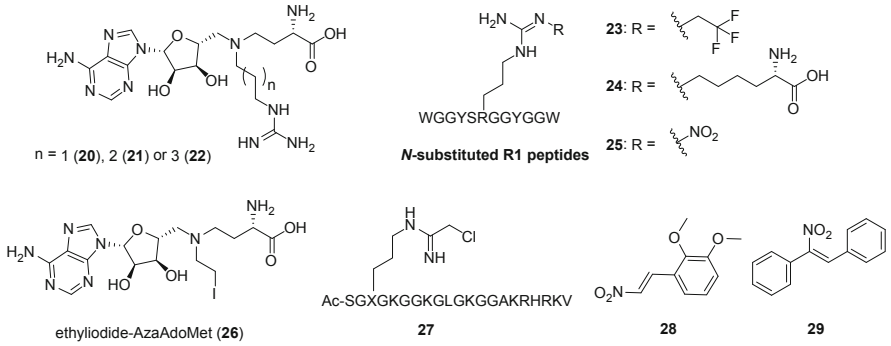


Fig. 6 Bisubstrate inhibitors **20**–**25** and covalent inhibitors **26**–**29** of PRMT1

arginine as in compound **23** (Fig. 6) resulted in micromolar IC_{50} values for PRMT1 (27.5 μM) and PRMT6 (9.4 μM) with no significant inhibition of PRMT4 (168 μM) [81]. By attaching an amino acid moiety to the side chain of the target arginine, peptidic partial bisubstrate **24** (Fig. 6) was obtained that showed inhibition against PRMT1 (13.9 μM), PRMT4 (35.7 μM) and PRMT6 (29.0 μM) [82]. A similar effect was observed for N^{η}-nitro-substituted arginine **25** (IC_{50} 26–47 μM for PRMT1, 4 and 6) when studying the effects of substitution on the methylation kinetics of PRMTs [84]. Small modifications of the side chain of an arginine residue in an HIV Tat peptide sequence resulted in micromolar K_i values against PRMT1 (2.7–7.6 μM) and PRMT6 (19.9–100 μM) with no significant inhibition of PRMT4 [83].

An in situ bisubstrate approach was also applied by Thompson and co-workers using ethyliodide-Aza-AdoMet (**26**, Fig. 6) [85]. This *N*-mustard-containing AdoMet analogue rearranges to form an aziridinium ion which will react with the substrate but only in the presence of the methyltransferase enzyme. In this way the histone H4 (1–21) substrate was enzymatically linked to the AdoMet cofactor. The conjugate was found to inhibit PRMT1 with an IC_{50} of 11.9 μM and a 4.4-fold specificity over PRMT4. In another covalent inhibitor approach, applied to inhibiting PRMT1, a chloroacetamidine warhead was incorporated in a H4 peptide substrate (**27**, Fig. 6). This approach was inspired by the success obtained for inhibiting PAD4, an arginine deiminase, that bears an active site cysteine [86]. Compound **27** inhibited PRMT1 and PRMT6 with IC_{50} values of 1.8 μM and 8.8 μM, respectively, and was >250-fold selective over PRMT3 and PRMT4 [87, 88]. Although a specific target cysteine was not discussed in these papers, other studies found a reactive cysteine (C101) in the active site of PRMT1 involved in binding AdoMet [89, 90]. The reactivity of this cysteine was confirmed for compounds **28** and **29** (Fig. 6) when testing against both a wild-type PRMT1 and the corresponding C101A mutant [91]. These covalent inhibitors were also found to be active against PRMT8 and inactive against PRMT4 and SET7 (tested at 10 and 100 μM only).

Recently, a small library of diamidine compounds, structurally similar to stilbamidine (**30**, Fig. 7), was screened for activity against PRMT1 [92]. The results revealed furamidine (**31**), a known antiparasitic agent, to be a fairly active (9.4 μM)

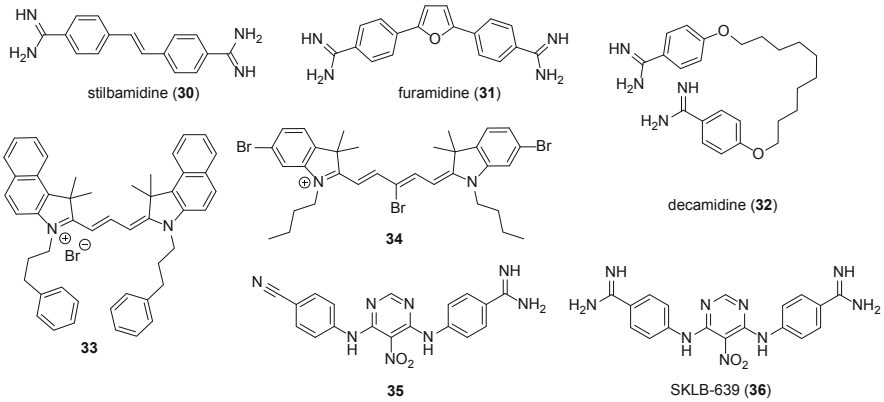


Fig. 7 Diamidines **30**–**32**, carbocyanine dyes **33**–**34** and nitropyrimidines **35**–**36** as inhibitors of PRMT1

and selective (18-, 30- and >42-fold over PRMT5, PRMT6 and PRMT4, respectively) inhibitor of PRMT1. Interestingly, furamidine was found to inhibit proliferation in leukaemia cell lines with higher sensitivity in cell lines derived from Down's syndrome patients and individuals diagnosed with mixed lineage leukaemia (MLL) [92]. Subsequent investigation of spacer length in diamidines revealed decamidine (**32**), bearing a 10-carbon spacer, to be a slightly more active (13 μM IC_{50} compared to 22 μM found for furamidine) but less specific PRMT1 inhibitor compared to furamidine. No additional analogues containing longer spacers were reported so the optimal spacing has yet to be confirmed [93]. The same group also investigated cyanine dyes as PRMT inhibitors and found compound **33** (Fig. 7) as an active hit with IC_{50} values of 0.61–1.74 μM against PRMT1, PRMT3, PRMT4, PRMT5, PRMT6 and PRMT8. This study also revealed compound **34** as a partially selective hit with an IC_{50} of 3.38 μM against PRMT1 with 6-, 10- and 25-fold selectivity over PRMT4, PRMT5 and PRMT8, respectively, but with no selectivity for PRMT3 or PRMT6 [94, 95]. Compound **34** was also tested in three different leukaemia cell lines and showed significant cell growth inhibition at 100–200 nM.

Also recently, an SAR study was reported by Yang and co-workers based upon a nitropyrimidine hit from a screening campaign [96]. This work showed that the amidine moiety was important for PRMT1 inhibition with modelling studies suggesting that it binds with the double E-loop of the enzyme. The optimized nitropyrimidine compound **35** (Fig. 7) showed an IC_{50} of 2.0 μM for PRMT1 with fivefold selectivity over PRMT4 and no activity against PRMT5 and PRMT6. Cellular assays revealed low micromolar IC_{50} activity against colon cancer (4.4 μM), bladder cancer (13.1 μM) and neuroblastoma (11.4 μM) tumour cell lines [96]. In a parallel study, the same group reported nitropyrimidine-diamidine compound SKLB-639 (**36**, Fig. 7) with similar activity against PRMT1 (2.4 μM) and selectivities of 15-fold over PRMT3, 30-fold over PRMT4 and no activity against

PRMT5, PRMT6 and PRMT8 [68]. In vivo studies show a decrease of H4R3me2a, but not H3R2me2a, H3R17me2a or sDMA in cocaine treated mice.

3.3 Biological Relevance of Inhibitors and Current Outlook

As the predominant member of the arginine methyltransferase family, PRMT1 has been the subject of thorough investigation and aims at identifying potent and selective inhibitors. Thus far, limited success has been achieved. To date, the most potent inhibitors reported show activity in the micromolar range, and the compounds are often active against at least one more PRMT. At present, no potent and selective inhibitors have been described for PRMT1. Studies investigating the role of PRMT1 in healthy and disease states show its upregulation in malignant cell lines. Cellular assays performed with a number of the inhibitors described above show their efficacy in killing tumour cell lines. Such promising results underscore the importance of developing potent and selective PRMT1 inhibitors.

4 PRMT2

4.1 Background

The second member identified as part of the PRMT family [10], PRMT2, is among the least studied PRMTs. PRMT2 is a type I PRMT, producing both MMA and aDMA. While it exhibits weak activity for histone H4 methylation [97], only few unique substrates for PRMT2 have been identified to date [98]. PRMT2 resides mainly in the nucleus, interacts with splicing factors and is a coactivator of nuclear hormone receptors, including the androgen and oestrogen α receptors [99–101]. PRMT2 regulates leptin signalling by methylation of STAT3 (signal transducer and activator of transcription 3) [102] and is downregulated under high glucose conditions leading to increased atherosclerosis through reduced cholesterol efflux [103]. PRMT2 is also associated with survival outcome and tumour grade in breast cancer via transcriptional activation of oestrogen receptor α [43, 104, 105].

4.2 Inhibitors: In Vitro and Cell-Based Activities

In addition to its weak methylating activity towards histones and the limited knowledge on nonhistone substrates, very few PRMT2 inhibitors have been reported. In a recent paper describing the crystal structure of PRMT2 [98], repressor splicing factor 1 (RSF1) was identified as a new nonhistone substrate. PRMT2 was found to methylate RSF1 much more efficiently than histone H3 or H4. Crystal structures

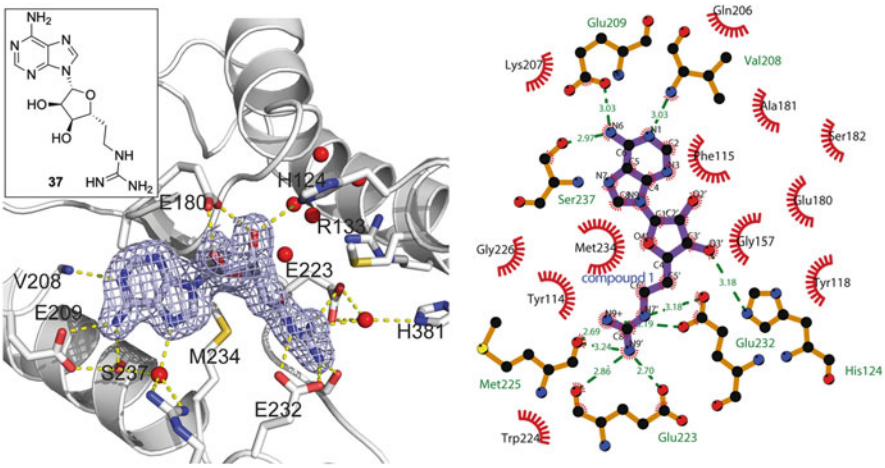


Fig. 8 Co-crystal structure of compound **37** bound to mouse PRMT2 (PDB ID: 5FWA), showing the interactions in the active site [98]. The "double E-loop" residues E223 and E232 and "THW-loop" residue H381 interact with the guanidine moiety of **37**. In addition, the adenosine moiety of **37** interacts with the conserved active site residues E180, E209, V208 and S237 in the cofactor-binding site

of PRMT2 from zebrafish and from mice were solved in complex with AdoHcy, sinefungin or a small molecule bisubstrate inhibitor known as Cp1 [106] (**37**, Fig. 8). Thermal shift assays of PRMT2 demonstrated an increased stability for the enzyme when bound to compound **37** compared to sinefungin or AdoHcy, indicating a stronger binding affinity. Furthermore, when using RSF1 as a substrate, the IC_{50} values for AdoHcy and inhibitor compound **37** could be determined. Compound **37** was found to inhibit PRMT2 with an IC_{50} of 16.3 μM, similar to AdoHcy (18.3 μM). In this regard, **37** is the first synthetic compound described as an inhibitor of PRMT2. However, compound **37** is not selective for PRMT2 as it displays much more potent inhibition of PRMT4.

4.3 Biological Relevance of Inhibitors and Current Outlook

PRMT2 is an elusive target with potential roles in a wide range of diseases, including obesity [102], diabetes [103] and cancer [104, 105]. The structural information now available combined with the discovery of an efficient nonhistone substrate for PRMT2 should provide the tools needed for future inhibitor development.

5 PRMT3

5.1 Background

PRMT3 was identified by Herschman and co-workers in 1998 based on sequence similarity with PRMT1 [11]. The crystal structure of the core of PRMT3, resolved shortly thereafter, revealed the highly conserved active site residues and its dimeric nature [107]. PRMT3 is a type I PRMT, predominantly present in the cytoplasm. PRMT3 contains a zinc finger for substrate recognition with known substrates including the 40S ribosomal protein S2, tumour protein p53 and (at least in vitro) histone H4R3 [108]. A recent screening via bio-orthogonal profiling of protein methylation using engineered methyltransferases resulted in the identification of over 80 substrates of PRMT3, 70% of which were cytoplasmic [109]. However, their functions in a biological setting remain to be elucidated. The human tumour suppressor protein DAL-1/4.1B (differentially expressed in adenocarcinoma of the lung) has also been shown to interact with PRMT3, inhibiting its methylating activity and leading to apoptosis in breast cancer cells [110].

5.2 Inhibitors: In Vitro and Cell-Based Activities

In 2012, the groups of Vedadi and Schapira published the crystal structure of PRMT3 bound to an allosteric inhibitor, identified through virtual screening [111]. The initial hit compound (**38**, Fig. 9) was reported to inhibit PRMT3 with an IC_{50} of 2.5 μM. As revealed in the co-crystal structure in Fig. 9, it binds close to the dimerization arm, preventing the enzyme to form a catalytically active state. The inhibitor was optimized through extensive SAR studies to yield compound SGC707

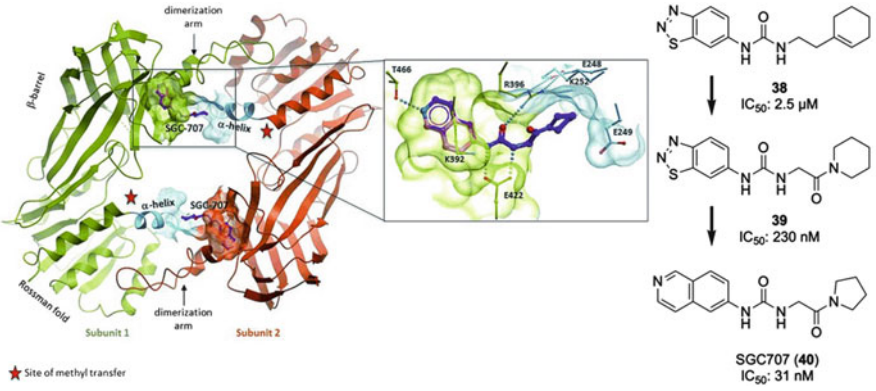


Fig. 9 Co-crystal structure of SGC707 (**40**) bound to PRMT3 (PDB ID: 4RYL), showing the interactions of the allosteric inhibitor in a pocket near the dimerization arm of PRMT3 [113]. The star indicates the site of methyl transfer. On the right side, the SAR optimization from hit compound **38** to SGC707 is presented [113]

(**40**, Fig. 9), a potent allosteric inhibitor of PRMT3 with an IC_{50} of 31 nM and high selectivity for PRMT3 over almost 300 other methyltransferase enzymes, including PRMT1 and PRMT4–PRMT8 [112, 113]. SGC707 is cell-active and non-toxic, except for high concentrations and long exposures. Initial PK data in mice show it can be used in animal models. No disease relevant studies have yet been reported.

5.3 Biological Relevance of Inhibitors and Current Outlook

The nanomolar activity of SGC707 combined with its high selectivity provides a strong tool for probing the biological role(s) of PRMT3. While links with human disease are currently limited, its interaction with tumour suppressor DAL-1/4.1B suggests PRMT3 as a potential target in breast cancer.

6 CARM1 (PRMT4)

6.1 Background

First identified as *c*oactivator-*a*ssociated a*r*ginine *m*ethyltransferase [12], CARM1 – or PRMT4 – was the fourth PRMT to be found through its sequence homology with PRMT1, PRMT2 and PRMT3. Unlike the other PRMTs, CARM1 doesn't recognize the GAR motif but prefers proline-, glycine- and methionine-rich (PGM) motifs [114, 115]. CARM1 asymmetrically dimethylates histone residues H3R17, H3R26 and H3R42 [116–118] and a large number of nonhistone substrates, including splicing factors, RNA-binding proteins, transcription factors, coactivators and itself [114, 119, 120].

In a well-characterized example of crosstalk between post-translational modifications, the methylation of Arg17 in the histone H3-tail peptide is regulated by the acetylation state of the neighbouring Lys18. Specifically, acetylation of Lys18 makes the H3 tail a better substrate for CARM1 [121]. Automethylation and O-GlcNAcylation of CARM1 is also known to regulate substrate specificity [122]. Upregulation of CARM1 is associated with a variety of diseases, including breast [43, 123], colon [124, 125], prostate [124, 126] and liver [127] cancers. Therefore, CARM1 has received increasing attention as a therapeutic target.

6.2 Inhibitors: In Vitro and Cell-Based Activities

Through a series of high-throughput screens and hit-to-lead SAR optimizations, different pyrazole (**40**–**42**, **47** and CMPD-2 (**43**)), benzo[d]imidazole (**48**) and indole-type (CMPD-1 (**50**) and **53**) inhibitors were developed with IC_{50} values as

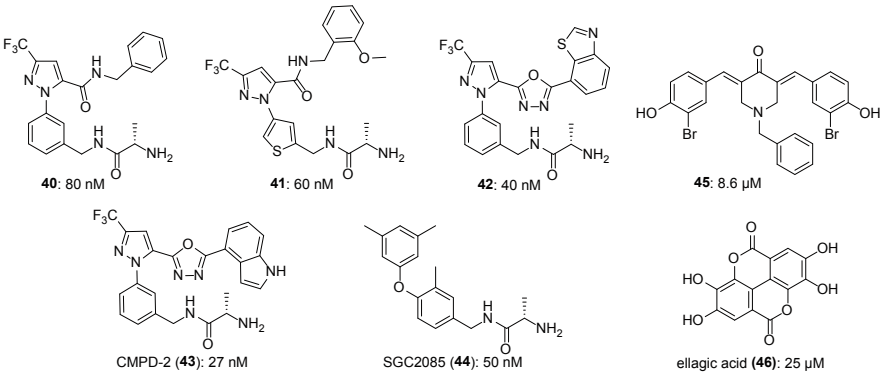


Fig. 10 CARM1 inhibitors **40**–**44** containing the alanine-amide moiety as a mimic of the guanidine of the arginine substrate. Compound **45** is a curcumin analogue, and ellagic acid (**46**) is a natural product extracted from pomegranates

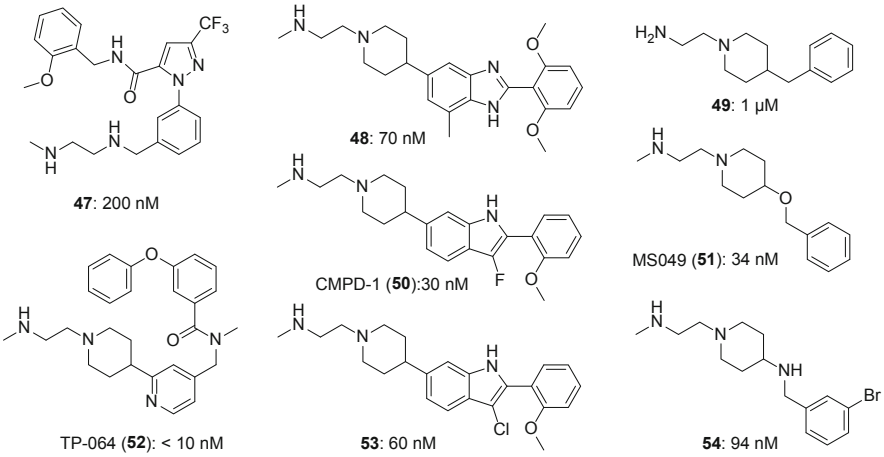


Fig. 11 CARM1 inhibitors **47**–**54** containing the ethylenediamino moiety mimicking the guanidine of the arginine substrate

low as 27 nM against CARM1 (Figs. 10 and 11) [128–132]. These inhibitors were found to be selective for CARM1 over PRMT1 and PRMT3 (other PRMTs were not tested). The most active compounds (CMPD-1 and CMPD-2) were co-crystallized with CARM1 in the presence of AdoHcy or sinefungin showing that they compete for the substrate-binding site (Fig. 12) [133].

Based upon the structural features present in the potent CARM1 inhibitors described above, it was recognized that the ethylenediamino (Fig. 10) and alanine-amide moieties (Fig. 11) are good mimics of the guanidine moiety which interacts with the double E-loop in the active site of PRMTs. Using a fragment-based approach, initially aimed at developing inhibitors of PRMT6, the ethylenediamino-containing

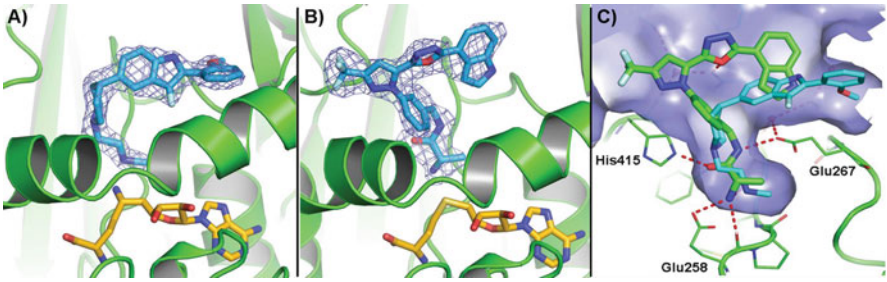


Fig. 12 Co-crystal structures of CARM1 bound to CMPD-1 (**50**) and CMPD-2 (**43**) (PDB ID: 2Y1W and 2Y1X). (**a**) CMPD-1 bound to CARM1 in the presence of sinefungin, (**b**) CMPD-2 bound to CARM1 in the presence of AdoHcy, (**c**) overlay of CMPD-1 and CMPD-2 showing their interactions with the glutamate residues of the double E-loop (Glu258 and Glu267) and the histidine residue of the THW-loop (His415). In CMPD-1, the ethylenediamino group is mimicking the guanidine, whereas in CMPD-2 the guanidine is mimicked by the alanine-amide moiety [133]

fragment **49** (Fig. 11) was identified [134]. This fragment exhibited the low micromolar IC_{50} values against type I PRMTs, PRMT1 (12 μM), PRMT3 (19 μM), CARM1 (1 μM), PRMT6 (0.3 μM) and PRMT8 (2.1 μM), and was not active against PRMT5 and PRMT7. In the same study, compound **53** (Fig. 11), a chlorine analogue of the previously described CMPD-1, was also found to be equally active against CARM1 (60 nM) and PRMT6 (70 nM), while IC_{50} values against PRMT1, PRMT3 and PRMT8 ranged from 1.7 to 4.1 μM. Further optimization of fragment **49** led to compound MS049 (**51**) [135], a dual inhibitor of CARM1 and PRMT6 with activities similar to compound **53**, CARM1; $IC_5$34 nM, PRMT6; IC_{50} 43 nM, PRMT8; and IC_{50} 1.6 μM, while no activity was detected against PRMT1, PRMT3, PRMT5 and PRMT7. Cellular activity of MS049 was detected in HEK293T human embryonic kidney cells where the compound exhibited an IC_{50} value of 0.97 μM for the PRMT6-mediated methylation of H3R2 and 1.4 μM for the methylation of Med12 by CARM1 [135].

Additional SAR studies, starting from fragment **49**, revealed that substitutions on the aromatic ring were generally well tolerated [136]. This led to the identification of the structurally similar but more specific CARM1 inhibitor compound **54** (Fig. 11) with an IC_{50} of 94 nM and 23-fold selectivity over PRMT6 (2.2 μM). However, no cellular assays were reported with this compound. A more complex analogue of MS049 is TP-064 (**52**, Fig. 11) discovered in a collaboration between Takeda Pharmaceutical and the Structural Genomics Consortium (SGC) [137]. TP-064 has an IC_{50} of $<$10 nM, a 100-fold selectivity over other histone methyltransferases and potent cellular activity with an IC_{50} of 43 nM for CARM1/Med12-Rme2a and growth inhibition observed in multiple myeloma cell lines. A similar hit-to-lead approach was undertaken by the group of Shapira and Vedadi focusing on the alanine-amide moiety as a guanidine mimic [138]. This SAR study yielded compound SGC2085 (**44**) with an IC_{50} of 50 nM against CARM1 and 100-fold selectivity over PRMT6 (5.2 μM). However, the compound did not show any appreciable cellular activity, presumably due to poor cell permeability.

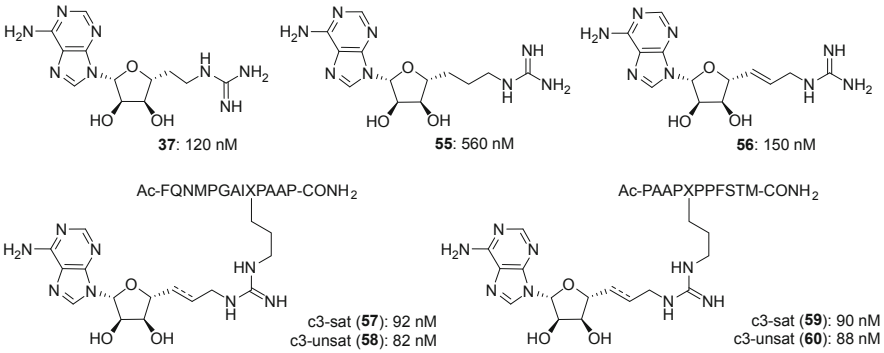


Fig. 13 Bisubstrate inhibitors **37** and **55**–**60** mimicking parts of the transition state of the methylation reaction

Curcumin-like structures (**45**, Fig. 10) have also been found to act as moderate and somewhat selective CARM1 inhibitors with IC_{50} values in the low micromolar range and no inhibition of PRMT1 or the lysine methyltransferase SET7 [139]. PRMT3 and PRMT6 were inhibited to some extent when **45** was tested at 100 μM. In human LNCaP cells, the prostate-specific antigen promoter was decreased in a dose-dependent manner by compound **45**. Pomegranate-derived compound ellagic acid (**46**, Fig. 10) was found to be a site-specific inhibitor of CARM1, inhibiting methylation of H3R17 but not of H3R26 [140]. Modelling suggests ellagic acid binds the KAPRK motif present around H3R17. Treatment of DNA-damaged HEK293T cells with ellagic acid showed a significant decrease in H3R17 methylation and p21 expression.

Recently, we described the development of inhibitors designed to mimic the transition state of the CARM1 methylation reaction [106]. To do so, bisubstrate-based inhibitors **37**, **55** and **56** (Fig. 13) were prepared by linking an adenosine moiety (mimicking that of AdoMet) to a guanidine moiety via different spacers. Although these compounds were initially expected to be nonspecific PRMT inhibitors, surprising selectivity was found. While three carbon-spaced inhibitor **55** was found to be equally active towards PRMT1, PRMT4 and PRMT6 (IC_{50} 0.56–1.30 μM), the two carbon-spaced inhibitor **37** and the unsaturated three carbon-spaced inhibitor **56** showed 34- to 169-fold selectivity for CARM1. All compounds were inactive against lysine methyltransferase G9a.

In order to increase the specificity of the bisubstrate compounds, a peptidic fragment of the poly(A)-binding protein-1 (PABP1), a well-known substrate of CARM1 [114], was appended to the guanidine group [141]. These peptidic transition state mimics (**57–60**, Fig. 13) showed potent inhibition of CARM1 (IC_{50} 82–92 nM) with high selectivity over PRMT1 (no other PRMTs were tested). Importantly, during co-crystallization studies, it was found that these transition state mimics stabilize the enzyme-substrate complex, thereby greatly facilitating crystallization. Figure 14 shows the complex formed between transition state mimics

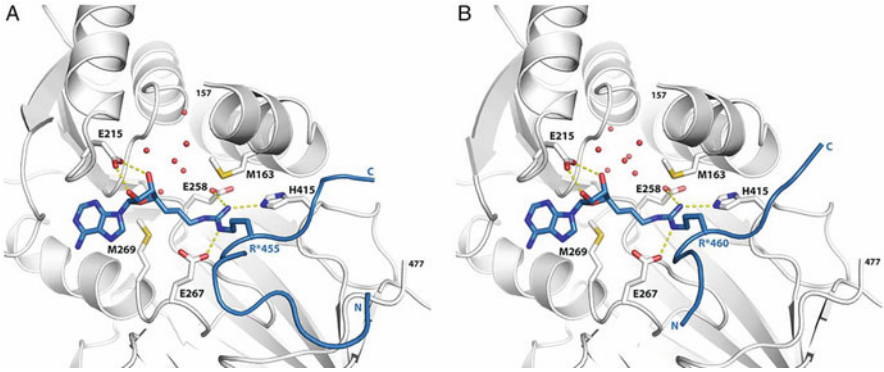


Fig. 14 Co-crystal structures of transition state mimics **57** and **59** bound to CARM1 (PDB ID: 5LGP and 5LGQ). (**a**) Compound **57** consists of residues 447–459 of poly(A)-binding protein-1 (PABP1) linked to adenosine via a fully saturated three-carbon linker; (**b**) compound **59** consists of residues 456–466 of PABP1 linked to adenosine via a fully saturated three-carbon linker. The most important interactions with active site residues have been indicated, including E215 interacting with the hydroxyls of the ribose and E258/E267 of the double E-loop and His415 of the THW-loop interacting with the guanidine [141]

57 and **59** and CARM1, clearly revealing the expected active site interactions with the adenosine and guanidine groups.

6.3 Biological Relevance of Inhibitors and Current Outlook

The involvement of CARM1 in a wide range of human cancers has led to increasing interest in the discovery of a potent and selective CARM1 inhibitor. The first inhibitors described for CARM1 came from high-throughput screening campaigns and showed selectivity for CARM1 over PRMT1 and PRMT3 but were not tested against other PRMTs. Results of later studies showed that structurally similar compounds were equally active against CARM1 and PRMT6. Both CARM1 and PRMT6 are involved in a variety of cancers, and synergy between the two has been described in stimulating oestrogen receptor α-dependent transcription. Dual active compounds, like MS049, are great tool compounds to investigate this synergy in detail. TP-064 is the most potent and selective CARM1 inhibitor described to date, showing activity in enzymatic assays and cell proliferation assays. In addition, the potent and selective peptidic transition state mimics developed by our group are valuable tools for structural studies to investigate PRMT-substrate interactions. The structural information made possible by this approach may yield new insights for the discovery of new PRMT inhibitors.

7 PRMT5

7.1 Background

Initially identified as a Janus kinase-binding protein [142], PRMT5 is the first identified and most abundant member of the type II PRMTs. The type II PRMTs preferentially produce symmetrically dimethylated arginine (sDMA) [13]. PRMT5 recognizes both the GAR and PGM motifs in a wide variety of substrates, including histone arginines H2AR3, H4R3, H3R2 and H3R8 (in vivo) [13, 143, 144]. Nonhistone substrates include ribosomal proteins (RPS10) [145], nuclear factor NF-κB, tumour suppressor protein p53, transcription factor E2F-1 [143, 146, 147], tumour suppressor PDCD4 (programmed cell death protein 4) [148] and the MAPK/ERK pathway (mitogen-activated protein kinase/extracellular signal-regulated kinase) [149], among many others [56]. PRMT5 has multiple associations with binding partners of which MEP50 (methylosome protein 50) is known to be necessary for regulating its specificity in methylating H2A and H4 [150, 151]. Other binding partners regulate the activity and substrate specificity of PRMT5 [152].

PRMT5 is upregulated in wide variety of human cancers, including breast [148], colorectal [153], lung [154, 155] and epithelial ovarian cancer [156], lymphomas [157–159] and melanoma [160]. In addition, recent studies have shown PRMT5 to be a unique anticancer target [47–49]. These recent findings suggest that methylthioadenosine (MTA) plays a role in regulating the activity of PRMT5. This finding originated with the discovery that 5-methylthioadenosine phosphorylase (MTAP) is often co-deleted with a commonly deleted tumour suppressor gene, CDKN2A (cyclin-dependent kinase inhibitor 2A), through close chromosomal proximity [161]. This leads to the accumulation of MTA, which in turn inhibits PRMT5 in a surprisingly specific manner. Through this pathway the MTAP/CDKN2A-deleted tumours have a hypomorphic PRMT5 state, making them sensitive towards further inhibition of PRMT5.

7.2 Inhibitors: In Vitro and Cell-Based Activities

The majority of inhibitors developed against PRMT5 have only been described in recent years. A hit-to-lead optimization study was recently published by Epizyme Inc. describing in detail the optimization of compounds identified through HTS [162]. Extensive SAR studies were performed with compound EPZ007345 (**61**, IC_{50} of 326 nM), yielding compound EPZ015666 (**62**, Fig. 15a) [163]. EPZ015666 was found to inhibit PRMT5:MEP50 with an IC_{50} of 22 nM with no activity detected against a panel of 20 other methyltransferases [162]. Furthermore, the compound was found to be substrate-competitive and AdoMet-uncompetitive. The co-crystal structure confirms EPZ015666 binds in the substrate-binding pocket of PRMT5 (see Fig. 15c). Interestingly, binding to the PRMT5:MEP50 complex was observed only

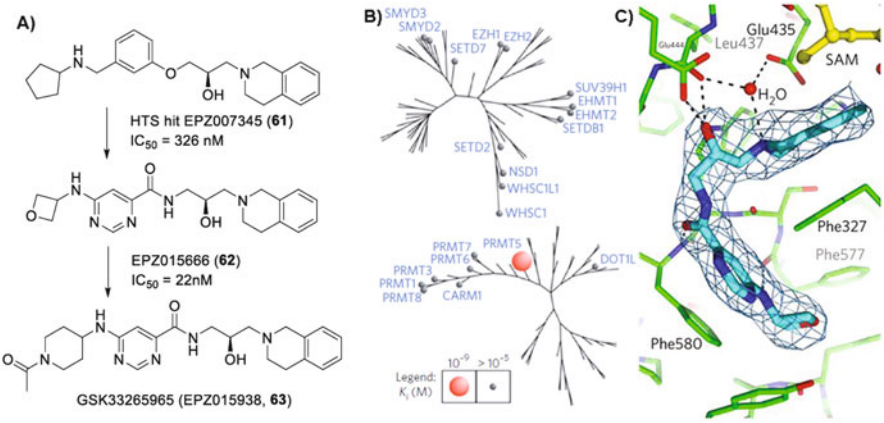


Fig. 15 Overview of the results of the studies performed for compound EPZ015666 (**62**) [163]. (**a**) Hit-to-lead optimization from EPZ007345 (**61**) to GSK3326595 (**63**). GSK3326595 is currently in phase I clinical trials, (**b**) arginine and lysine methyltransferase family trees showing the selectivity of EPZ015666, (**c**) co-crystal structure of EPZ015666 with PRMT5 (PDB ID: 4X61) showing the interactions with the glutamate residues of the "double E-loop" and the substrate-competitive nature of the compound [162]

in the presence of AdoMet or a cofactor analogue like AdoHcy or sinefungin. When testing the compound in mantle cell lymphoma (MCL) cells, EPZ015666 showed concentration-dependent antiproliferative effects, with IC_{50} values of 96 nM and 450 nM against Z-138 and Maver-1 MCL cells, respectively. In addition, upon oral dosing in mice, the compound showed dose-dependent antitumour activity in MCL xenograft models. The correlating decrease in sDMA strongly suggests a direct link with PRMT5 inhibition. Compound EPZ015666 has subsequently been further improved to compound GSK3326595 (previously EPZ015938, **63**, Fig. 15a) in collaboration with GlaxoSmithKline (GSK) and has entered phase I clinical trials with patients that have advanced or recurrent solid tumours and non-Hodgkin's lymphoma.

PRMT5 overexpression was also found in Epstein-Barr virus (EBV)-induced B-cell transformation by the group of Baiocchi and co-workers [38]. The PRMT5 expression was limited to EBV-transformed cells and not found in resting or activated B lymphocytes. From a virtual screening approach, compound **64** (Fig. 16) was identified as a selective inhibitor of PRMT5 over PRMT1, PRMT4 and PRMT7 (tested at a fixed concentration only). Compound **64** was capable of blocking EBV-induced B-cell transformation and survival without affecting the viability of normal B cells. In addition, chromatin immunoprecipitation assays show a decrease of PRMT5 overexpression and its histone marks at H3R8 and H4R3 upon treatment with **64**. No effect on asymmetrically dimethylated arginine at H4R3 was found. Compound **64** was optimized to compound **65** (Fig. 16), by replacing the pyridine ring with an ortho-methoxyphenyl group [164]. Its activity was shown at 10 μM against PRMT5 with no inhibition of PRMT1, PRMT4 and PRMT7. PRMT5 was shown to be upregulated in

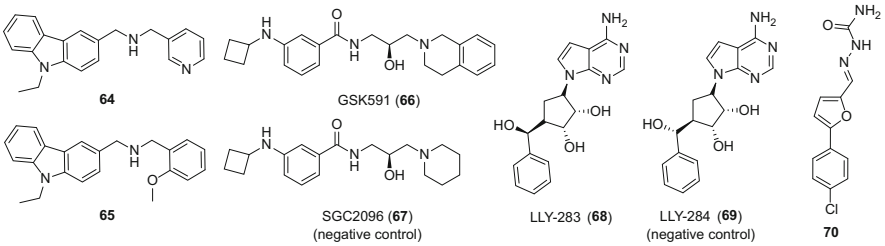


Fig. 16 Chemical structures of PRMT5 inhibitors **64–70**

acute myeloid leukaemia (AML) [164]. When tested against AML samples, compound **65** inhibited H4R3me2s and H3R8me2s methylation while decreasing cell viability in a dose-dependent manner with IC_{50} values of 7.2–21.5 μM for AML cell lines and 4.0–8.7 μM for AML patient blasts.

The SGC has also recently reported two chemical probes for PRMT5. One of the probes (GSK591 [165], **66**, Fig. 16) derives from a compound series explored by Epizyme and GSK, and the other was developed in collaboration with Eli Lilly (LLY-283 [166], **68**, Fig. 16). Probe compound GSK591 inhibits PRMT5:MEP50 methylation of histone H4 in vitro with an IC_{50} of 11 nM and in Z-138 lymphoma cells; it also inhibits the methylation of PRMT5 substrate Sm protein D3 with EC_{50} of 56 nM [167]. Compound LLY-283 has an IC_{50} of 20 nM in vitro (against H4R3 methylation), and in cellular assays, LLY-283 inhibited the methylation of RNA-associated Sm proteins B/B′ with an IC_{50} of 25 nM in MCF7 cells and also affected MDM4 (mouse double minute 4 protein) splicing with a relative IC_{50} of 40 nM in A375 cells [166].

A virtual screening campaign and subsequent SAR studies performed by Ji et al. [168] led to the discovery of a new PRMT5 inhibitor (**70**, Fig. 16), which showed an IC_{50} value of 0.57 μM with selectivity for PRMT5 over all other PRMTs tested in biochemical assays (all but PRMT2 and PRMT9). In DLD-1 colorectal cancer cells, a time- and dose-dependent growth inhibition was demonstrated using a cell viability assay. In addition, a decrease in sDMA marks on H4R3 and H3R8 was observed, but no change in aDMA on H4R3. No cellular IC_{50} or EC_{50} values were calculated.

7.3 Biological Relevance of Inhibitors and Current Outlook

Recently, there has been a significant increase in the number of published reports aimed at identifying new roles of PRMT5 in different disease states as well as the development of inhibitors against PRMT5. With the first clinical trial for a PRMT5 inhibitor against non-Hodgkin's lymphoma currently underway, the biological relevance and therapeutic potential of PRMT5 inhibition will become clearer. With a growing body of knowledge highlighting the involvement of PRMT5 in different

cancers and the steady increase in the development of novel PRMT5 inhibitors, PRMT5 is likely the most interesting therapeutic target among the PRMTs at this time.

8 PRMT6

8.1 Background

PRMT6 is a nuclear type I PRMT that methylates histone residues H2AR3, H2AR29, H3R2, H3R42 and H4R3 [14, 169, 170]. PRMT6-mediated aDMA methylation of H3R2 blocks the mixed lineage leukaemia (MLL) complex-mediated di- and tri-methylation of H3K4 and vice versa [171]. Other substrates of PRMT6 include HMGA1a, involved in chromatin structure organization [172] and DNA polymerase β, involved in DNA base excision repair [173]. Furthermore, automethylation increases the stability and anti-HIV-1 activity of PRMT6, and methylation of HIV-Tat protein reduces HIV-1 production and viral replication [37, 174, 175]. PRMT6 has also been found to be overexpressed in a variety of cancers, including bladder and lung cancer [63] and prostate cancer [176], but is downregulated in melanoma [177]. In addition, PRMT6 dysregulation was also recently found to be associated in pulmonary disorders [35].

8.2 Inhibitors: In Vitro and Cell-Based Activities

The ethylenediamino compounds discussed in the section above on CARM1/PRMT4 generally demonstrated similar potency towards both CARM1 and PRMT6. This includes the potent dual CARM1-PRMT6 inhibitor MS049 (**51**, Fig. 11) [135]. The same moiety is also present in compound **71** (Fig. 17), recently developed in our group. Compound **71** showed selective inhibitory activity

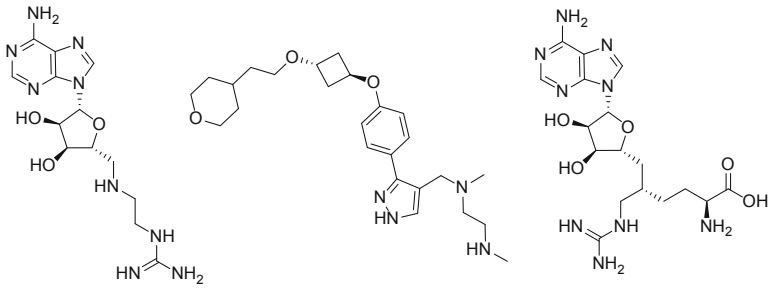


Fig. 17 Structures of PRMT6 inhibitors **71**–**73**

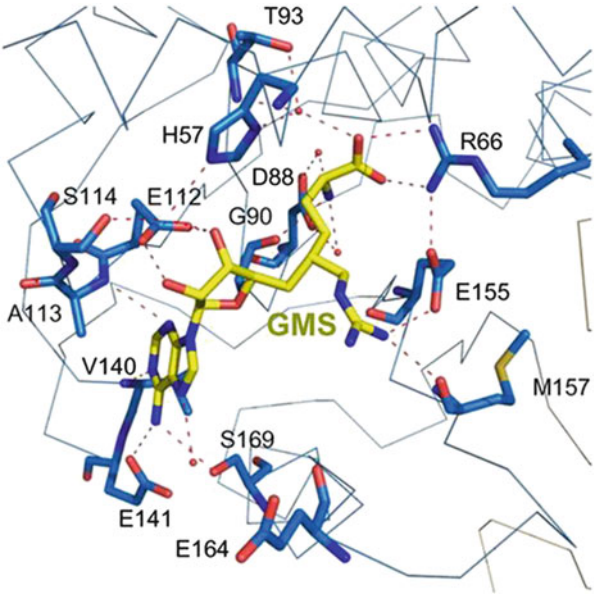


Fig. 18 Co-crystal structure of PRMT6 with bisubstrate compound GMS (**73**), showing the interactions with the active site residues. The adenosine binds with conserved active site residues, such as Arg66, Glu112, Val140 and Glu141 in the AdoMet cofactor-binding pocket, and the guanidine moiety interacts with Glu155 of the "double E-loop" [179]

(IC_{50} 3.2 μM) towards PRMT6 over PRMT1, CARM1 and lysine methyltransferase G9a [106].

Epizyme developed compound EPZ020411 (**72**, Fig. 17), which exhibited an IC_{50} of 10 nM against PRMT6 [178]. It also showed IC_{50} values of 119 nM against PRMT1 and 223 nM against PRMT8 but was more than 100-fold selective over PRMT3, PRMT4, PRMT5 and PRMT7. In fact, most of the ethylenediamino aryl pyrazole compounds tested showed high affinity for PRMT1, PRMT6 and PRMT8. The crystal structure obtained with compound EPZ020411 showed the interactions in the active site of PRMT6, although the structure of the inhibitor was not fully resolved. Treatment of A375 cells with EPZ020411 resulted in a dose-dependent decrease in H3R2 methylation (IC_{50} 0.64 μM).

In a recent study investigating the structural basis of PRMT6-mediated asymmetric dimethylation [179], a bisubstrate guanidine-sinefungin analogue (GMS, **73**, Fig. 17) was synthesized. GMS showed an IC_{50} value of 90 nM for PRMT6 but, not surprisingly, was also active against most other PRMTs. In the co-crystal structure of GMS with PRMT6, the compound binds in the cofactor-binding site with the guanidine moiety interacting with residues in the substrate-binding pocket as depicted in Fig. 18.

8.3 Biological Relevance of Inhibitors and Current Outlook

PRMT6 has been shown to be a valid therapeutic target for a range of diseases. Many compounds developed for PRMT6 (or other PRMTs) show low nanomolar

inhibition but often lack PRMT6 selectivity. To date, only limited cellular assays have been performed with the aim of establishing PRMT6 inhibition and its potential role in specific disease relevance. In this regard, more work is necessary in the development of potent, selective, and biologically relevant inhibitors of PRMT6.

9 PRMT7

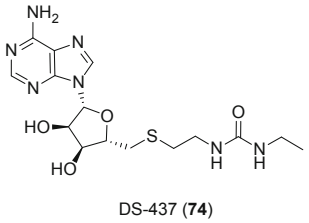
9.1 Background

In 2004, the first type III PRMT, PRMT7, was identified by Clarke and co-workers [16]. As a type III arginine methyltransferase, PRMT7 produces only monomethylarginine (MMA) and preferentially methylates RxR motifs in lysine- and arginine-rich regions of target proteins [180]. Known substrates for PRMT7 include histones H2AR3, H2BR29, H2BR31 H2BR33, H4R3, H4R17 and H4R19 [180]. In addition, in conjunction with PRMT5, PRMT7 aids in the sDMA methylation of Sm proteins non-redundantly [181]. Interestingly, mutation of the Glu181 residue to Asp in the double E-loop in the PRMT7 active site switched the type III PRMT activity into type I, producing aDMA [182]. Furthermore, the additional mutation of Gln329 to Ala in the canonical THW-loop converted PRMT7 into a type II PRMT, capable of producing sDMA [183].

As related to human disease, PRMT7 has been linked to breast cancer metastasis [184, 185], DNA damage [186] and parasite infection [187]. Interestingly, several studies have also demonstrated the involvement of PRMT7 in increasing the sensitivity of tumour cells to chemotherapeutic agents [186, 188–190].

9.2 Inhibitors: In Vitro and Cell-Based Activities

The group of Vedadi and co-workers developed compound DS-437 (**74**, Fig. 19) [191], an AdoMet analogue, which showed dual inhibition of PRMT5 and PRMT7 (IC_{50} values for both at 6 μM) with no activity against a panel of 29 other methyltransferases, including PRMTs.



DS-437 (**74**)

Fig. 19 Structure of dual PRMT5-PRMT7-inhibitor DS-437 (**74**)

9.3 Biological Relevance of Inhibitors and Current Outlook

Very limited work has been done in the development of PRMT7 inhibitors. Currently, no potent and specific PRMT7 inhibitors are available. However, interesting recent findings suggest PRMT7 is a viable therapeutic target, e.g. for (re-)sensitising tumour cells to chemotherapeutic agents or for the treatment of breast cancer.

10 PRMT8

10.1 Background

The eighth member of the PRMT family is a type I PRMT that is primarily expressed in the brain and is myristoylated at the N-terminal glycine, both unique features within the PRMT family [17]. The myristoylation of PRMT8 results in its association with the plasma membrane. Cleavage of the N-terminal domain results in an increase in methylation activity [192], and a variant (PRMT8v2) lacking the N-terminal glycine was found to be located primarily in the nucleus rather than the cell membrane [193, 194]. PRMT8 has high sequence similarity with PRMT1 (about 80%) and methylates the GAR motif.

Recent studies point to an involvement of PRMT8 in amyotrophic lateral sclerosis (ALS) [195] and high expression levels of PRMT8 are linked to a variety of cancers [196]. Furthermore, knockdown of PRMT8 halted cell proliferation and caused cell death in both healthy human dermal fibroblasts and U87MG glioblastoma cells [194].

10.2 Inhibitors: In Vitro and Cell-Based Activities

No specific inhibitors have been developed against PRMT8. Interestingly, ethylenediamino-containing compounds (fragment **49** [134], MS049 (**51**) [135], Fig. 11) that were active against PRMT4 and PRMT6 were also generally active against PRMT8. In addition, the covalent inhibitors **28** and **29** [91] (Fig. 6) designed to interact with the active site cysteine found in PRMT1 were also found to be active against PRMT8. This is not surprising given the high sequence similarity, including the presence of an active site cysteine, between these two PRMTs.

10.3 Biological Relevance of Inhibitors and Current Outlook

Although relatively little is known about the roles of PRMT8 in both healthy and disease states, recent publications suggest its potential as a therapeutic target. In order to study these in greater detail, the development of specific inhibitors against PRMT8 will be necessary.

11 PRMT9

The most recently added member of the PRMT family, PRMT9, took longer to identify due to its low sequence similarity to other PRMTs [17, 18]. Initially described in the literature as Fbox Only Protein 11 (FBXO11), PRMT9 is the second type II PRMT after PRMT5 and is found to localize mainly in the cytoplasm [18, 197]. Recently, spliceosome-associated protein SAP145 was identified as a substrate for PRMT9 [197]. sDMA methylation of SAP145 results in a Tudor domain-binding site for the survival of motor neuron (SMN) protein, thereby regulating alternative splicing.

Recently, the preferred substrate recognition motif of PRMT9 was described as R-F-(K/R/F)-(R/W)-**R**-(M/F)-P-X-P [198], which may facilitate the identification of new PRMT9 substrates. Interestingly, however, the only substrate identified to date (spliceosome-associated protein 145 (SAP145)) does not bear this motif (CFK**R**KYL). Currently, a very limited number of studies focused on PRMT9 have been reported, and much work is needed to elucidate the roles played by PRMT9 in both healthy and disease states.

12 Conclusions

The protein arginine *N*-methyltransferases play crucial roles in a wide range of biological processes associated with both human health and disease. Their functions concentrate on gene regulation, RNA transcription, splicing processes and DNA repair. Not surprisingly, the dysregulation of PRMTs has been shown to contribute to a variety of diseases, including many different cancers. As a result, the development of PRMT inhibitors has also received increasing attention over the past decade.

Comparing different studies focused on inhibiting the same PRMT can prove challenging as different assay methods can result in different IC_{50} values for the same compound. Furthermore, many factors are involved in obtaining a reliable measure of an inhibitor's potency, including substrate and enzyme concentrations, the reference compounds used, signal readout and many other variable assay-specific conditions. In addition, PRMT inhibitor specificity is rarely tested in the context of a large panel of methyltransferases.

Despite these limitations, the overview of the PRMT inhibitors here presented revealed that potent and highly selective compounds have already been developed against PRMT3 (SGC707 (**40**) [113]), CARM1/PRMT4 (TP-064 (**52**) [137]) and PRMT5 (GSK3326595 (**63**) [199], GSK591 (**66**) [167] and LLY-283 (**68**) [166]). In addition, the dual PRMT4/PRMT6 (**51**) [135] and PRMT5/PRMT7 (**74**) [191] inhibitors that have been developed, as well as the more general type I PRMT inhibitor (**6**) [50], serve to strengthen the toolbox of small molecules needed for detailed investigations of PRMTs. Furthermore, the technology recently developed for the co-crystallization of PRMTs with their respective substrates using transition state mimics [141] is expected to yield key new structural insights of value in the discovery of new PRMT inhibitors.

To date, PRMT5 is the first PRMT for which an inhibitor has been taken into phase I clinical trials, illustrating the rapid progress being made in this field. The increasing number of reports describing the involvements of PRMTs in cellular processes and the development of new PRMT inhibitors can be expected to result in a clearer picture of the role(s) played by this important family of enzymes (Table 1).

Table 1 PRMT substrate(s), function(s) in normal and disease-related processes, and effect(s) of inhibition

PRMT	Type	Substrates	Function/disease relation	Effect by inhibition
PRMT1	Type I	GAR substrates Histone H2A Arg3 Histone H4 Arg3 Splicing factors DNA damage proteins RNA-binding proteins Transcription factors Viral proteins Signalling proteins	Involved in gene transcription, nuclear transport, DNA repair and RNA processing Overexpressed in breast, colon, prostate, lung, head and neck, bladder cancer and leukaemia Involved in pulmonary disease, cardiovascular disease, diabetes and cocaine addiction	Inhibition increases cellular MMA and sDMA, and inhibitors show growth inhibition in a variety of cancer cell lines
PRMT2	Type I, III	Histone 4 Splicing factors Transcription factors	Regulates leptin signalling Interacts with splicing factors and nuclear hormone receptors Involved in atherosclerosis and breast cancer	Unknown
PRMT3	Type I	Histone H4 Arg3 Ribosomal proteins Tumour suppressors	Involved in breast cancer	Unknown
CARM1 (PRMT4)	Type I	PGM motif Histone H3 Arg17 Histone H3 Arg26 Histone H3 Arg42 PRMT4	Involved in gene transcription, nuclear transport, DNA repair and RNA processing Overexpressed in breast, colon, prostate and liver cancer	Inhibition results in a decrease in prostate-specific antigen reporter

(continued)

Table 1 (continued)

PRMT	Type	Substrates	Function/disease relation	Effect by inhibition
		(auto) Splicing factors RNA-binding proteins Transcription factors Coactivators		
PRMT5	Type II	Histone H2A Arg3 Histone H3 Arg8 Histone H4 Arg3 Sm proteins Ribosomal proteins Nuclear factors Transcription factors Tumour suppressors	Involved in embryogenesis and gene transcription Binding partners regulate activity and substrate specificity Upregulated in breast, colorectal, lung, epithelial ovarian cancer, lymphomas and melanoma	Antiproliferative in MCL cells and MCL mice. Phase I clinical trials on solid tumours and non-Hodgkin's lymphoma
PRMT6	Type I	Histone H2A Arg3 Histone H2A Arg29 Histone H3 Arg2 Histone H3 Arg8 Histone H3 Arg42 Histone H4 Arg3 Chromatin proteins DNA-binding proteins PRMT6 (auto) Viral proteins	Overexpressed in bladder, lung and prostate cancer Downregulated in melanoma Associated with pulmonary disorders Reduces HIV-1 production and viral replication	Dose-dependent decrease in H3R2 methylation in A375 cells
PRMT7	Type III	RxR motif Histone H2A Arg3 Histone H2B Arg29 Histone H2B Arg31 Histone H2B Arg33 Histone H4 Arg3 Histone H4 Arg17 Histone H4 Arg19 Sm proteins	Linked to breast cancer metastasis, DNA damage and parasite infection Increases tumour sensitivity to chemotherapeutics	Unknown

(continued)

Table 1 (continued)

PRMT	Type	Substrates	Function/disease relation	Effect by inhibition
PRMT8	Type I	GAR substrates Histone H2A Histone 4 Arg3 Tumour proteins RNA-binding proteins PRMT8 (auto)	Essential for cell proliferation Highly expressed in breast, head and neck, glandular, cervical, prostate and thyroid cancer Involved in ALS	Knockdown halted cell proliferation and caused cell death in fibroblasts and glioblastoma cells
PRMT9	Type II	SAP145	Regulates splicing	Unknown

Compliance with Ethical Standards

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