



Digest

Nucleoside protein arginine methyltransferase 5 (PRMT5) inhibitors

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ABSTRACT

Protein Arginine Methyltransferase 5 (PRMT5) is known to symmetrically dimethylate numerous cytosolic and nuclear proteins that are involved in a variety of cellular processes. Recent findings have revealed its potential as a cancer therapeutic target. PRMT5 selective inhibitors, **GSK3326595**, a substrate competitive inhibitor, and **JNJ64619178**, a SAM (S-adenosyl-L-methionine) mimetic/competitive inhibitor, have entered clinic trials for multiple cancer types. This review focuses on the recent developments in SAM mimetic nucleoside PRMT5 inhibitors, their SAR and structural insight based on published co-crystal structures.

Protein arginine methylation, catalyzed by a family of arginine methyltransferases, has emerged as one of the most common post-translational modifications (PTMs). As the major type II methyltransferase in mammals, PRMT5 forms a hetero-octamer with MEP50, catalyzes the formation of symmetrical dimethylarginines (sDMA) using SAM (S-adenosyl-L-methionine) as a co-factor on histones and non-histone substrates,^{2,3} and plays important roles in various cellular processes, including gene expression and RNA splicing.^{4,5} PRMT5 has been shown to be upregulated in many types of cancers.^{6–8} Loss of methylthioadenosine phosphorylase (MTAP) confers a selective dependence on PRMT5 and its binding partner MEP50 (WDR77).^{9–11} MTAP is frequently lost due to its proximity to the commonly deleted tumor suppressor gene, CDKN2A. Together, these findings reveal PRMT5 as a potential therapeutic target for cancer. Indeed, PRMT5 selective inhibitors have recently been discovered and have demonstrated potent antitumor activity in preclinical models.¹² Two molecules, **GSK3326595**,¹³ a substrate competitive inhibitor, and **JNJ64619178**,^{14,24,25} a SAM mimetic/competitive inhibitor, have entered clinic trials for multiple cancer types. Most recently, **PF-06939999**¹⁵ (structure not disclosed) entered the clinical trial as this review is completed.

PRMT5 has been a drug target for many pharmaceutical and biotechnology companies, as well as academic research institutions, due to its critical function in cancer. Several reviews have been reported recently, summarizing its biological functions in the fields of oncology and immune inflammation, as well as describing the small molecule inhibitors that have been investigated over the past years.^{16–22} Still, many patent applications have been published since late 2016 reporting novel nucleoside mimetics with very promising potency. This review focuses on these nucleoside inhibitors, which are SAM competitive inhibitors with a mode of action different from previously reported

substrate competitive inhibitors such as **GSK3326595**. Key SAR will be summarized, and the binding mode will be discussed based on published X-ray co-crystal structures and our own modeling exercises.

Before disclosure of **LLY-283**²³ and **JNJ64619178**,^{24,25} a major concern among scientists in the field was whether it was possible to obtain SAM mimetic nucleoside analogs with selectivity vs. different PRMTs. This is not different from the assumption, several decades ago, about the potential difficulties to identify ATP-competitive kinase inhibitors. Needless to say, years of research in the kinase field have provided a plethora of potent and selective kinase inhibitors, thus dispelling any reservations in the community about the possibility to achieve specificity at the ATP site. Indeed, many of such inhibitors, a few of them even acting via covalent mode of action, have become very successful marketed drug and are already becoming the standard of care for some therapies. As will be described below, the report of **LLY-283** and **JNJ64619178** as selective SAM-competitive, nucleoside inhibitors of PRMT5 is bound to stimulate the interest in the field and will potentially lead to a new era of epigenetic targets in drug discovery.

We have examined all the PRMT5 co-crystal structures from Protein Data Bank and overlaid their catalytic sites focusing on the residues around the cofactor, namely SAM or its mimetics which were used in the crystallization studies (SAH, sinefungin or MTA). Based on how the ribose C5' substituents interact/displace the substrate Arg, or interact with Glu444 (a catalytic residue which facilitates methyl transfer from SAM to the Arg) (see Fig. 1A), we have categorized all the published nucleoside PRMT5 inhibitors into three classes: A) ribose or carboribose analogs not engaging with the Arg tunnel or interacting with Glu444 (such as **LLY-283**); B) ribose or carboribose analogs reaching into the Arg tunnel and interacting with Glu444, thus potentially acting competitively vs. both SAM and the substrate (such as **JNJ64619178**); C) ribose and carboribose analogs with basic amines which, although

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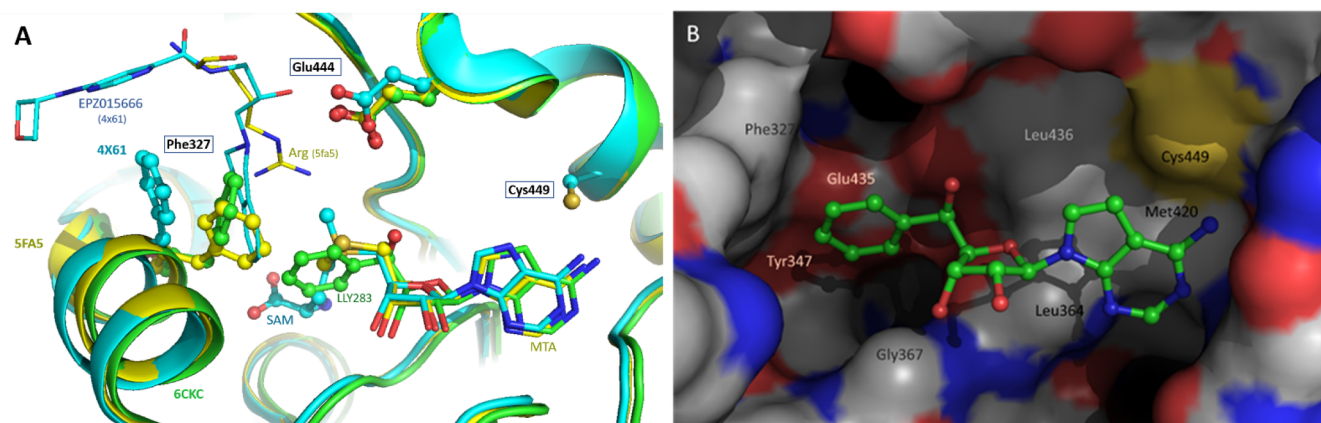


Fig. 1. (A) overlay of X-ray co-crystal structures of MTA (PDB code: **5FA5**) in yellow, **EPZ015666** (PDB code: **4X61**) in cyan, and **LLY-283** (PDB code: **6CKC**) in green at the SAM binding pocket.; (B) close look of the surface of **LLY-283** binding pocket (some residues are removed for better visibility of the ligand). Graphics were generated using PyMOL™ Molecular Graphics System, version 1.8.2.1.

potentially engaging with Glu444, do not extend into the Arg tunnel. In addition, PRMT5 contains a unique cysteine (C449) in proximity to its active site (see Fig. 1), which presents an opportunity for covalent modification. This provides a fourth class of inhibitors, i.e., covalent nucleoside inactivators of PRMT5, as those herein described.

MTA has been shown to be a potent, selective PRMT5 inhibitor, thus providing an excellent starting point for a potential approach based on SAM-mimetics. A number of X-ray crystal structures of PRMT5 bound with different small-molecule ligands are already available in the literature. The structures show that Phe327 is a key residue, located right in the middle of the enzyme active site, between cofactor and peptide substrate. Interestingly, Phe327 is highly flexible and can adopt a variety of distinct conformations in order to interact with these ligands. Thus, as seen in Fig. 1A, in the structure with MTA (PDB code: **5FA5**), Phe327 forms a π -cation interaction with the substrate arginine. On the other hand, with substrate competitive inhibitors such as **EPZ015666** (PDB code: **4X61**), Phe327 moves away in order to enlarge the arginine tunnel and engage with the tetrahydroisoquinoline ring. Lastly, with **LLY-283** (PDB code: **6CKC**) the Phe327 pivots to create a lipophilic pocket that accommodates the phenyl ring at the C-5' position of the LLY inhibitor.

These examples demonstrate the plasticity of Phe327 to undergo induced-fit conformational changes, thus controlling the binding of ligands and acting as a gate keeper of the active site. This Phe is also unique for PRMT5, as the other PRMT enzymes contain a Met at that position.³³ This is likely one of the main reasons whereby it is possible to achieve selectivity for PRMT5 vs. the other members of the PRMT family, a factor that can be exploited for rational design of selective inhibitors.

Fig. 1B clearly shows the space that is available between the protein and the phenyl ring of **LLY-283**. This explains some of the activity trends observed in the SAR studies reported in the public domain for the nucleoside analogs, namely, that halogen atoms at the para- and meta-positions enhance the potency (see examples listed in Table 1). Table 1 contains biological activities from different sources, measured by different methods, and/or in different cell lines. Caution should be taken when SAR data is interpreted across references. Example numbers in the table are the same as those in the cited references.

Compared to no substitution, 4-Cl improves potency by 3-fold (**1** vs **2**), 3,4-difluoro has the same impact on potency (**2** vs **3**). 4-Cl-3-F improves potency further (**4** (**PF-06855800**) vs. **2** or **3**) when comparing potency from the same assays. Polar groups on the phenyl ring are less tolerable (structures not shown).²⁷ Likewise, carbocycle and heteroaryl replacements of the phenyl group are less potent or not tolerated (structures not shown).²⁷ In addition to the SAR around the phenyl ring, Table 1 also summarizes the SAR of the heterocyclic substitution of the

purine ring of SAM (**5–8**),^{27,30} the substituents at 6-position of the pyrrolopyrimidine (**9–11**),^{27,31} and the C5' of the ribose ring including two additional carboribose examples (**12** and **13**).³²

Pyrrolopyrimidine (PRP) appears to be the preferred base mimetics for PRMT5. Among the Class A inhibitors (Table 1), several heteroaryl groups were surveyed, but all are less potent than PRP. For example, compared to compound **3** with PRP, compound **5** bearing a purine ring is 3-fold less potent in the biochemical assay and 8-fold less potent in the cellular assay. Other heteroaryls such as furopyrimidine and thienopyrimidine are less tolerated (structures not shown).²⁷ The loss of potency could be due to a change in the conformation as a result of subtle differences in bond lengths among C–N, C–C, C–O and C–S bonds. Small substituents on PRP (atom A in Table 1), such as F, are tolerated (~2-fold loss of in potency comparing **6** vs. **3**). Acetylene (**7**) at this position seems to be potent in biochemical assay but its potency decreases in cellular assay. With atom A as acetylene, a pyrazole derivative was made but lost potency significantly (**8** vs **7**).

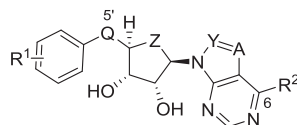
In general, 6-NH₂ gives the best potency. However, 6-Me is also tolerated and only a ~3-fold loss of potency was observed in both biochemical and cellular assays (**9** vs. **3**). This change reduces the number of H-bond donors and polar surface area (97.9 vs. 123.9, tPSA of **9** vs. **3** calculated by ChemDraw v18.0), which could allow blood-brain barrier (BBB) penetration, therefore for the potential treatment of brain tumors. Other small substituents, such as hydrazone in compound **11**, are also tolerated.

C5'-OH appears to be important for potency, likely attributed to a pre-organized ground state conformation being similar to the binding conformation, due to the Gauche effect. The preferred stereochemistry in ribose is *R* (*S* for carboribose due to the change of substitution priority). Compounds lacking OH or with F/OH replacement are much less potent (structures not shown). C5' tolerates a tertiary Me group (not shown), with only a ~3-fold reduction in potency. Interestingly, NH₂ replacement of OH only causes a 3-fold loss of biochemical potency but a much more profound loss of potency in cells (**10** vs **9**). This might be due to the lower permeability imparted by the presence of a basic amine. In carboribose examples, compound **12** is equally potent as its ribose counterpart compound **3**, but loses cell potency significantly. On the other hand, the chemistry to make carboribose allows the replacement of the C5' with a heteroatom such as the sulfur atom, for example in compound **13**, which is less potent but has consistent cellular potency.

Class B nucleoside PRMT5 inhibitors are represented by a series of compounds such as **JNJ64619178**.³⁴ Table 2 highlights the SAR for this class of compounds. Interestingly, carboribose **15** appears to have comparable potency to ribose **14**. However, a carbon-atom linker seems to result in a better cellular potency (**17** vs **15** or **18**). The more rigid

Table 1

SAR of Class A ribose and carboribose nucleoside PRMT5 inhibitors.



Cmpd.	Example	A	Y	Z	R ¹	R ²	Q	Enzyme IC ₅₀ (nM)	Cell IC ₅₀ (nM)	Refs.
1 LLY-283	2	CH	CH	O	H	NH ₂	CH-(R)-OH	9.5	32 ^a	26
2	1	CH	CH	O	4-Cl	NH ₂	CH-(R)-OH	4.3	11 ^a	26, 27
	87							1	5 ^b	
3	85	CH	CH	O	3,4-F	NH ₂	CH-(R)-OH	1	6 ^b	27
4 PF-0685580	n/a	CH	CH	O	3-F-4-Cl	NH ₂	CH-(R)-OH	0.02 ^c	1.4 ^d	28, 29 ^g
5	84	N	CH	O	3,4-F	NH ₂	CH-(R)-OH	3	48 ^b	27
6	60	CF	CH	O	3,4-F	NH ₂	CH-(R)-OH	2	10 ^b	27
7	119	CCCH	CH	O	4-Cl	NH ₂	CH-(R)-OH	17	131 ^e	30
8	112	CCCH	N	O	4-Cl	NH ₂	CH-(R)-OH	116	n.d.	30
9	9	CH	CH	O	3,4-F	Me	CH-(R)-OH	3	16 ^b	27
10	94	CH	CH	O	3,4-F	Me	CH-(R)-NH ₂	9	1785 ^b	27
11	20	CH	CH	O	4-Cl	=NNH ₂	CH-(R)-OH	0.6	98 ^e	31
12	119	CH	CH	CH ₂	3,4-F	NH ₂	CH-(S)-OH	2	134 ^f	32
13	72	CH	CH	CH ₂	4-Cl	NH ₂	S	26	63 ^f	32

^a Inhibition of A375 cell proliferation.^b Inhibition of A549 cell proliferation.^c Ki value.^d Inhibition of Z-138 proliferation.^e Inhibition of Granta-519 proliferation.^f Inhibition of Granta-519 sDMA (in cell western).^g Structure of PF-0685580 disclosed.

trans double bond analog has similar potency as the one with the sp³ carbon linker, indicating a similar binding conformation for both linkers (**16** vs **17**). A key feature in the series is the substantial increase in activity upon the introduction of an NH₂ group into the quinoline ring (**19** vs **17**). *N*-alkyl analogs such as compound **20** display comparable levels of potency. Halogen substitution at the *ortho*-position is tolerated and among them, compound **21** (**JNJ64619178**) was selected as the clinical candidate in the series.

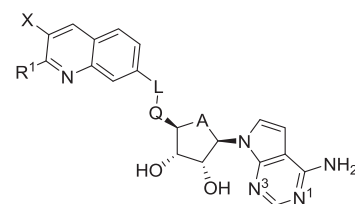
The effect of nitrogen/carbon substitutions at the PRP ring system was also examined for the class B, using similar analogs (structures not shown) as those listed in Table 2. The conclusion was that N¹ of the PRP ring is critical for activity, as replacement of N¹ with CH resulted in ~1000-fold loss of potency, while replacement of N³ with CH had

minimal effect in potency.

Also studied in the same report and the subsequent one³⁵ were several modifications of the quinoline side chain: quinoline/isoquinoline regioisomers, quinazolines, quinoxalines, 5,6-bicyclic heteroaryls, 5-member, 6-member mono-cyclic heteroaryls and others. Likewise, the chain length between the heteroaryl and carboribose was examined as well. Most these modifications led to the loss of potency, except for a few examples in Fig. 2. Thus, substituted imidazo[1,2-*a*]pyridines (**22–25**) maintain potency similar to that of the aminoquinolines in both biochemical and cellular assays (see Table 3). Most interestingly, the phenyl ring of aminoquinoline could be replaced by a two-sp³-carbon linker to still retain potency (see compound **26** in Fig. 2). However, the increase in the number of rotatable bonds might have a

Table 2

SAR of Class B ribose and carboribose nucleoside PRMT5 inhibitors.



Compound	Example	A	L-Q	R ¹	X	Enzyme IC ₅₀ (nM)	Cell IC ₅₀ ^a (nM)
14	1	O	OCH ₂	H	H	15.8	79.4
15	46	CH ₂	OCH ₂	H	H	20	125
16	55	CH ₂	trans-CH=CH	H	H	7.9	51.3
17	56	CH ₂	CH ₂ CH ₂	H	H	6.3	7.9
18	64	CH ₂	CH ₂ O	H	H	125	1070
19	76	CH ₂	CH ₂ CH ₂	NH ₂	H	0.25	0.16
20	74	CH ₂	CH ₂ CH ₂	NHCH ₂ c-Pr	H	0.79	0.40
21 JNJ64619178	80	CH ₂	CH ₂ CH ₂	NH ₂	Br	0.13	0.25

^aInhibition of sDMA in A549; Ex.# and data in Ref. 34; IC₅₀ values converted from pIC₅₀ values in Ref. 34.

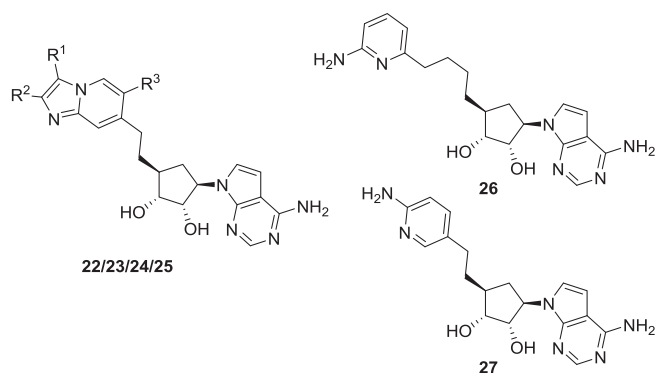


Fig. 2. Class B PRMT5 inhibitors: aminoquinoline replacements.

Table 3
SAR of compounds 22–27.

Cmpd.	Ex.	R ¹ /R ² /R ³	Enzyme IC ₅₀ (nM)	Cell IC ₅₀ ^a (nM)
22	1	Me/H/H	0.80	1.26
23	2	Me/H/Cl	0.16	6.31
24	11	Me/Me/H	< 0.20	3.98
25	12	Me/H/F	0.32	2.00
26	55	n/a	0.40	n.d.
27	27	n/a	5.01	6.31

^a Inhibition of sDMA in A549, IC₅₀ values converted from pIC₅₀ values in Ref. 35; Ex.# and data in Ref. 35.

negative impact in cell permeability and other drug-like properties. Compound 27 is the only aminopyridine with a shorter linker that still maintains good potency.

We modeled Class B inhibitors, such as compound 20, in the MTA structure (5FA5) as shown in Fig. 3A. It appears that the aminoquinoline and other heteroaryl groups likely occupy the substrate Arg site, thus also interacting with the catalytic Glu444. Since *N*-alkyl and even *N*-benzyl substituents are well tolerated, they likely extend into the arginine tunnel. In our model, the distance between the NH of the aminoquinoline to Glu444 carboxylate is only 2.8 Å and the quinoline ring forms π - π stacking with Phe327, as already discussed above. Such a molecular model would imply that this class of inhibitors could be competitive with both substrate and co-factor.³⁶ This unique binding mode results in a unique, pseudo-irreversible mode of action on target and subsequent in vitro and in vivo pharmacodynamics (PD), disclosed at AACR annual conferences in 2017 and 2018. JNJ64619178 (21) showed long residence time on the target, extended PD effect, namely sDMA inhibition in cells after washout of the compound, and in a

xenograft model after dosing of the compound was stopped.^{24,25} It remains to be demonstrated in the clinic whether this pseudo covalent characteristics would lead to superior efficacy or unexpected toxicity.

Class C nucleoside PRMT5 inhibitors all have a basic nitrogen atom that is expected to interact with Glu444, but without extending into the arginine tunnel. Both ribose 28 and carboribose 29 in Table 4 are indeed very potent PRMT5 inhibitors.³⁷ Carboribose 30 with a simple oxygen atom as the linker is equally potent. This is an interesting finding, as building blocks needed for SAR studies are likely more readily accessible for compounds related to 30, than for compounds related to 28 and 29.

However, these compounds lose cellular potency quite significantly, presumably due to poor cell permeability, usually an indication for high polarity. Indeed, compounds 28–30 all have cLog P values (calculated using ChemDraw v18.0) lower than 1. A variety of halogen atoms were introduced (F/Cl/Br (Ex. 31)/I), as well as CF₃/CN Ex. 32/33. Although no real difference was found in their biochemical potency, these analogs exhibited a wide range of cellular activities. The difference in cellular potency cannot be simply explained by an increase of lipophilicity since both Br and CN seem to give some improvement in cellular potency but compound 31, with Br, and compound 33, with CN, have cLogP values which are 1.24 log units apart.

Remarkably, introduction of a CHF₂ group was found to dramatically improve the cellular potency, with many compounds bearing such substitution at the R¹ position displaying low nM potency in the cellular assay. A key factor at play here could be efflux transporter-mediated resistance to cell penetration, since cancer cells are known to over express the multi-drug transporter P-gp. Compounds bearing CHF₂ might not act as efflux transporter substrates, therefore being more effective at inhibiting PRMT5 within cells. Another example of a unique property of the CHF₂ group is provided by compound 37 (an OCHF₂ derivative), which also has much improved cellular potency compared to its OMe counterpart 36. Bulkier alkoxy groups are not tolerated here.

In this series of analogs, R¹ tolerates a variety of 5-member heteroaryl groups such as pyrazole and oxazole (not shown),³⁷ but still suffering from loss of cellular potency. R² can accommodate small substitution such as F, as seen for compound 35, which is equipotent in the biochemical assay and only ~3-fold less potent in cells comparing to 34. A Me group at R³ is tolerated, without a very strong stereochemistry preference (isomer 2, 39, is only 7-fold more potent than isomer 1, 38). Me groups next to the tetrahydroisoquinoline nitrogen reduce potency. Modification at R² and R³ position might provide a handle to modulate metabolic stability.

We modeled compound 34 in the structure of 4X61 as shown in Fig. 3B. Compared to 5FA5 or 6CKC, Glu444 in the structure of 4X61 shifted away by 1–2 Å (see Fig. 1A). This flexibility can accommodate a

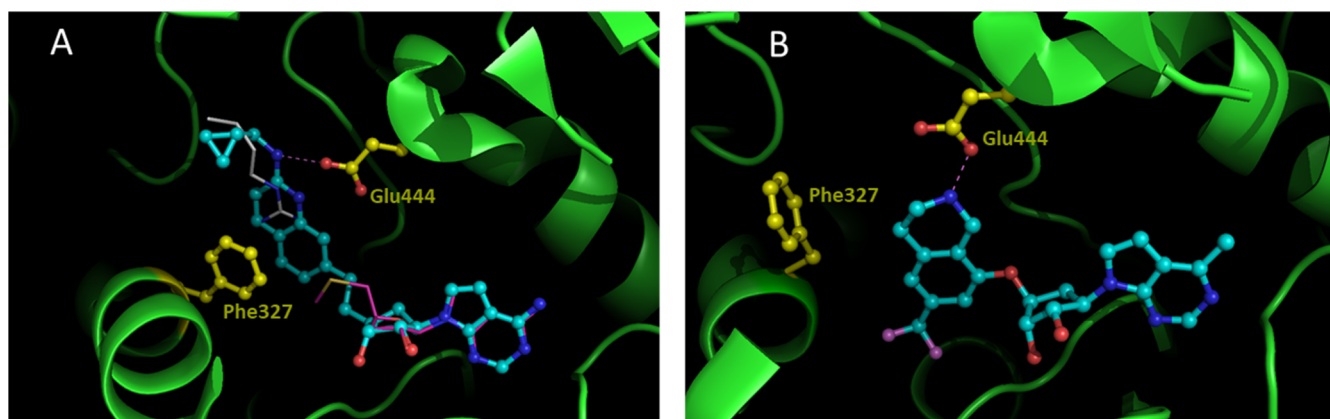
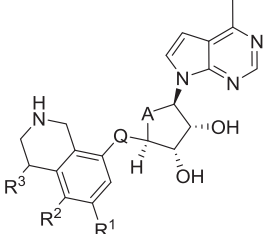


Fig. 3. Class B and Class C PRMT5 inhibitors modeled to engage with Glu444. (A) Compound 20 modeled in 5FA5 to show aminoquinoline occupies the space for arginine; (B) Compound 34 modeled in 4X61 to show tetrahydroisoquinoline N making H-bond with Glu444. Graphics were generated using PyMOL™ Molecular Graphics System, version 1.8.2.1.

Table 4
SAR of Class C ribose and carboribose nucleoside PRMT5 inhibitors.



Cmpd.	Ex.	A	Q	R ¹	R ²	R ³	Enzyme IC ₅₀ (nM)	Cell IC ₅₀ (nM) ^a	cLog P
28	112	O	CH-(R)-OH	H	H	H	1	350	0.07
29	56	CH ₂	CH-(S)-OH	H	H	H	2	378	−0.14
30	78	CH ₂	O	H	H	H	1	1615	0.41
31	89	CH ₂	O	Br	H	H	1	195	1.41
32	90	CH ₂	O	CF ₃	H	H	2	948	1.54
33	91	CH ₂	O	CN	H	H	1	119	0.17
34	154	CH ₂	O	CHF ₂	H	H	1	2	0.61
35	190	CH ₂	O	CHF ₂	F	H	1	7	0.89
36	198	CH ₂	O	OMe	F	H	1	3822	0.45
37	200	CH ₂	O	OCHF ₂	F	H	1	140	0.95
38	211	CH ₂	O	CHF ₂	F	Me, isomer 1	1	14	1.41
39	212	CH ₂	O	CHF ₂	F	Me, isomer 2	1	2	1.41

^a Inhibition of A549 proliferation; Ex.# and data in Ref. 37, IC₅₀ converted to nM.

H-bond salt-bridge interaction between acid and amine with good complementarity. In our molecular model, the tetrahydroisoquinoline nitrogen of compound **34** is placed only 2.6 Å away from Glu444. Our modeling can also explain the SAR quite well. For example, it shows that the R¹ position still has room to accommodate a group as big as 5-member heteroaryl such as pyrazole, while R² and R³ positions can only accommodate smaller groups such as F and Me, respectively.

Another series of nucleoside inhibitors contain a spirocycle with a basic N atom in each ring, exemplified by compound **40** (Ex. 20 in Ref. 38a) and **41** (Ex. 29 in ref. 38a) in Fig. 4.^{38a} A variety of 4,4, 4,5, 4,6, 5,5, 5,6 and 6,6 spirocycles were prepared and tested. Both ribose and carboribose were prepared. These compounds are not as potent as any of the above-mentioned scaffolds. Compounds **40** and **41** have IC₅₀ values of 22 nM and 8.7 nM, respectively, in the biochemical assay using mass spectrometric detection; and 81 nM and 51 nM, respectively, measuring inhibition of sDMA using an IHC readout in the A549 cell line.^{38b} In this scaffold, *N*-Me substitution at the piperidine ring also decreases potency compared to those with NH. Similar to inhibitor Classes A and B, a PRP ring system is optimal for this series as well. Replacement of the PRP with 4-aminopyrazolo[1,5-*a*][1,3,5]triazin-8-yl reduces potency by 10–20-fold.

Having a nitrogen atom at 5'-position (i.e., in the 5-membered ring) is likely for ease of synthesis instead of being important for potency. However, the basic N in the piperidine ring of compound **40** and **41** could potentially interact with Glu444 and thus be critical for potency. Since most potent nucleoside mimetics reported far, all have a substituted phenyl ring or heteroaryl ring that could potentially form a face

to face interaction with Phe327 (in the case of aminoquinoline series) or a lipophilic interaction in a pocket formed with Phe327, introduction of a phenyl or a substituted phenyl ring in this spirocyclic chemotype might improve potency.

PRMT5 possesses a cysteine (C449) in the active site, a feature which is unique for PRMT5 and not present in the other PRMTs (which have a serine at the same position). The co-crystal structure of MTA in PRMT5/MEP50 (5FA5) has revealed a small space that might accommodate a covalent warhead. The distances between C449 to N⁶ and N⁷ atoms of the SAM adenine ring are found to be 3.6 Å and 3.9 Å, respectively. Therefore, a covalent approach may offer potent and selective PRMT5 inhibitor analogues to SAM. On the other hand, C449 is not considered catalytic, so it may not be inherently reactive, which potentially makes this approach challenging.

Acrylamide is a typical covalent pharmacophore that has been used in many covalent kinase inhibitors, such as the EGFR inhibitor afatinib, and the BTK inhibitor ibrutinib that have benefited many cancer patients. Compound **42** (Ex. 2 in Ref. 39) and **43** (Ex. 20 in Ref. 39) in Fig. 5 are acrylamide derivatives of MTA.³⁹ They are the first reported covalent small molecule PRMT5 inhibitors. Although weak (IC₅₀ values 5.01 and 2.38 μM, respectively), they showed evidence of covalent modification of wild type PRMT5 but not C449S mutant in a jump dilution study. That is, wild type PRMT5 enzyme activity did not recover after dialysis, while C449S mutant enzyme activity did recover.

Covalent modification of methyltransferases could be as fruitful as identifying covalent kinase inhibitors, which have successfully

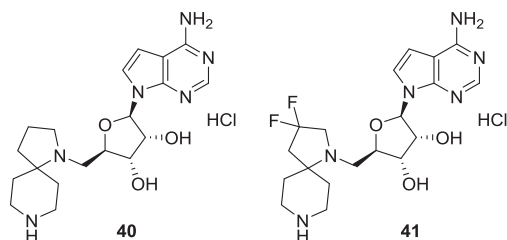


Fig. 4. Examples of spirobicyclic Class C ribose nucleoside.

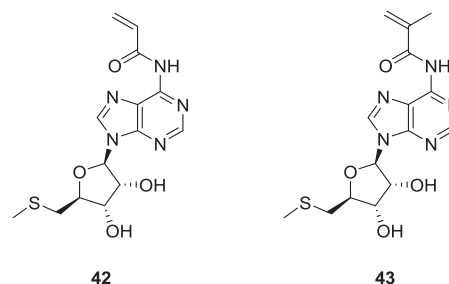


Fig. 5. Covalent inhibitors of PRMT5.

demonstrated their effectiveness in treating drug resistance. Evidence of covalent modification of PRMT5 at C449 is encouraging, since it shows that C449 has adequate reactivity. Other methyltransferases such as PRMT1 and EZH2 also have non-catalytic cysteine residues close to or at the SAM binding pockets. Covalent inhibitors of PRMT1 have been reported.⁴⁰

In summary, PRMT5 has been a highly successful methyltransferase target for the discovery of potent and selective SAM mimetic inhibitors. A right balance of lipophilicity can be achieved by careful substitution at C5' with aryl or heteroaryl groups, resulting in permeable, metabolically stable and oral bioavailable molecules.²³ **JNJ64619178** has been the first oral nucleoside analog to enter the clinical trials and other chemotypes, such as those based on **LLY-283**, also have excellent DMPK properties to potentially follow suit.⁴¹ As a target class, methyltransferases have many similarities to kinases. The structural based approach certainly has accelerated the discovery of potent and selective kinase inhibitors and brought them to the patients. Same type of learnings could be likewise applied to methyltransferase drug discovery as well. This review has been an effort to describe recent reports in the field, and to compare published PRMT5 crystal structures, and their molecular models to highlight the potential to discover novel nucleoside scaffolds. Although this review only focuses on nucleoside SAM mimetic PRMT5 inhibitors, non-nucleoside SAM competitive small molecule PRMT5 inhibitors have been published and modeled in the SAM binding pocket.⁴² Having co-crystal structures of these molecules will certainly accelerate discovery of diverse chemotypes, which was the case with kinase inhibitors. With emerging knowledge on methyltransferases and the critical roles they play in the fields of oncology, immune inflammation, and immuno-oncology, we are bound to see more medicinal chemistry efforts on these potential therapeutic targets in the not too distant future.

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