



Bisubstrate Strategies to Target Methyltransferases

Rostom Ahmed-belkacem, Françoise Debart, Jean-jacques Vasseur

► To cite this version:

Rostom Ahmed-belkacem, Françoise Debart, Jean-jacques Vasseur. Bisubstrate Strategies to Target Methyltransferases. European Journal of Organic Chemistry, 2022, 2022 (21), pp.e202101481. 10.1002/ejoc.202101481 . hal-03727961

HAL Id: hal-03727961

<https://cnrs.hal.science/hal-03727961>

Submitted on 19 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

VIP Very Important Paper

Special
Collection

Bisubstrate Strategies to Target Methyltransferases

Rostom Ahmed-Belkacem,^{*,[a]} Françoise Debart,^[a] and Jean-Jacques Vasseur^{*,[a]}

Bisubstrate strategies address the lack of specificity encountered with *S*-adenosyl-L-methionine (SAM) analogues targeting methyltransferases (MTases). Specifically, a bisubstrate inhibitor mimics the transition state complex associating a substrate and the SAM methyl group donor to a particular methyltransferase. These potential inhibitors contain both parts of the substrate

and the methyl group donor. In this review, bisubstrate inhibitors of several MTases including DNA, RNA, protein, catechol or nicotinamide MTases are discussed in order to evaluate the interest of these promising molecules for the development of selective therapeutics against cancers, neuro-psychiatric disorders and viral infections.

1. Introduction

Methyltransferases (MTases) that methylate DNA, RNA, proteins, carbohydrates and small molecule metabolites play key roles in biological processes such as epigenetic, epitranscriptomic, as well as in a variety of human diseases, including neuro-degenerative, cardiovascular, and cancer diseases, as well as viral and parasitic diseases.^[1] Regardless of biochemical substrates, most MTases use *S*-adenosyl-L-methionine (SAM) as a cofactor to methylate them. MTases harbor two pockets: a SAM-binding pocket and a substrate-binding pocket.^[2] During methylation, SAM and substrate are close enough into their respective recognition sites in the MTase to allow transfer of the methyl group from the SAM to the substrate, resulting in a high-energy transition state. This unstable transition state lasts only few femtoseconds and evolves spontaneously to a lower energy state where the methylated substrate is released from the MTase.

SAM analogues such as sinefungin and *S*-adenosyl-L-homocysteine (SAH) are non-specific pan-MTase inhibitors due to the similarity of the SAM binding pocket of these enzymes.^[3] The bisubstrate strategy could circumvent this lack of specificity because bisubstrate inhibitors, which mimic the transition state of substrate methylation by SAM, simultaneously target both MTase pockets. Therefore, bisubstrate inhibitors may have increased selectivity compared to compounds targeting only the SAM binding pocket. This is because these compounds are designed to interact specifically with the MTase of interest because they lack the structural element required to interact with other MTases. Another advantage of the bisubstrate strategy is that, unlike a ligand that targets only one MTase site, bisubstrate analogues targeting two sites at the same time result in increased affinity. This advantage is due to a reduced energy cost to assemble both the bisubstrate inhibitor and MTase in a binary system compared to the less entropic ternary

complex bringing together SAM, substrate and MTase. The higher specificity and affinity for their targets make bisubstrate inhibitors more effective in targeting MTases.

Structurally, a bisubstrate analogue is constructed with a chemical unit mimicking SAM covalently linked to another unit simulating the methylated substrate (Figure 1).^[4] The linkage between the two parts of the bisubstrate molecule carries at least one carbon atom representing the methyl group of SAM. By modifying the length of the linker between both units or by inserting other chemical motifs, chemists are able to design various bisubstrate structures.

This bisubstrate strategy has shown particular interest in the inhibition of methyltransferases involved in the development of many diseases and viral infections. Several examples will be presented in this review.

2. Bisubstrate Inhibitors Designed for Targeting DNA Methyltransferases (DNMTs)

Mutations, deletions or overexpression of genes encoding the DNMTs could give rise to specified cancers.^[5] These genetic incidents result in methylation defects provided by DNMTs in epigenetic processes.

In 2001, Stephen J. Benkovic's group pioneered the synthesis and evaluation of bisubstrate inhibitors of DNA MTases, in particular the cell cycle regulated DNA MTase (CcrM), a methyltransferase involved in the survival of certain bacteria

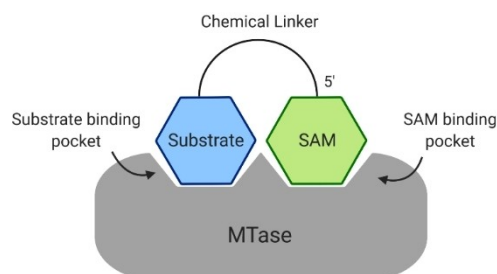


Figure 1. Mode of action of a bisubstrate inhibitor of MTases.

[a] Dr. R. Ahmed-Belkacem, Dr. F. Debart, Dr. J.-J. Vasseur
IBMM, Univ Montpellier, CNRS, ENSCM,
Montpellier, France
E-mail: rostom.ahmed-belkacem@umontpellier.fr
jean-jacques.vasseur@umontpellier.fr

such as *Brucella abortus*, *Helicobacter pylori*, and *Haemophilus influenza*.^[6] CcrM catalyzes the transfer of the methyl group from SAM to the N6 site of a deoxyadenosine residue within the recognition sequence GANTC. Thus, the group designed SAM analogues where the N6 atom of the adenine base was covalently linked to the homocysteine chain of SAM (Figure 2).^[7] Of four compounds, nucleotide **1** inhibited both *Brucella abortus* and *Caulobacter crescentus* CcrMs with a K_i in the micromolar range. Furthermore, this bisubstrate strategy proved to be efficient as nucleotide **1** did not inhibit bacterial C5 cytosine DNA MTase (HhaI) whereas sinefungin inhibited CcrM and HhaI in the same order of magnitude.

In 2018, Paola B. Arimondo's group^[8] reported the synthesis of bisubstrate inhibitors mimicking the transition state of DNA methylation at the C5 position of 2'-deoxycytidine to target DNMTs involved in the formation of certain cancers.^[9] The reaction mechanism in C5-MTases begins with the nucleophilic addition of a cysteine placed in the active site to the C6 atom of the cytosine ring (Figure 3). Methyl transfer from the SAM cofactor to the C5 position takes place and the excess H5 proton is extracted by β -elimination, resulting in the methylated DNA released from the enzyme.^[10] To increase the chemical stability of the bisubstrate inhibitors, the sulfur atom of SAM was replaced by a nitrogen atom (compounds **2–3**) or even deleted (compound **4**) and the SAM analogue was attached to the C5 position of a cytosine by a methylene linkage. The length and/or the position of the spacer between the SAM analogue (in green) and the cytosine derivative (in blue) were

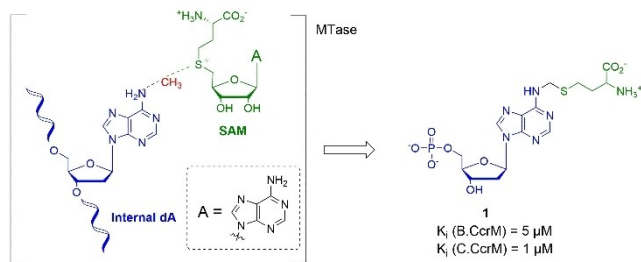


Figure 2. Transition state of internal 2'-deoxyadenosine N6-methylation by a DNMT and bisubstrate SAM analogue **1** developed by Stephen J. Benkovic and co-workers.^[7]

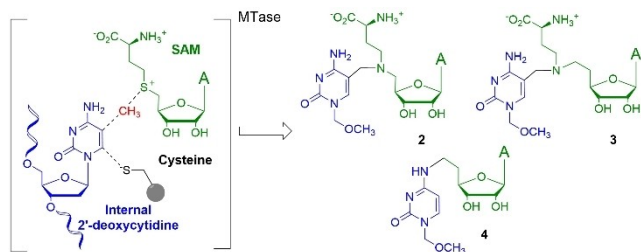


Figure 3. Transition state of the methylation of 2'-deoxycytidine in DNA by DNMT and bisubstrate inhibitors **2–4** developed by Arimondo and co-workers.^[8]



Rostom Ahmed-Belkacem is a postdoctoral researcher at the Institute of Biomolecules Max Mousseron (Montpellier, France). In 2020, he received his PhD degree in Organic Chemistry from Montpellier University under the supervision of Dr. Françoise Debart and Dr. Jean-Jacques Vasseur. Targeting emerging viruses, he designed and developed several series of bisubstrate nucleosides and capped oligoribonucleotides as inhibitors of viral RNA N7- and 2'-O-methyltransferases (MTases). Currently, his main research interests focus on the optimization of hits compounds to fight SARS-CoVs, Zika and Dengue infections. Using molecular docking, he designs, proposes, and synthesizes novel nucleosides.



Françoise Debart obtained her Ph.D. in Chemistry from University of Montpellier in 1989 in the laboratory of Prof. J.-L. Imbach under the guidance of Dr. B. Rayner. In the same year, she joined the French National Center of Scientific Research (CNRS) as an Associate Researcher. From 1990 to 1992, she was a post-doctoral fellow at IONIS Pharmaceuticals (California, USA) in the field of antisense oligonucleotides. She then moved back to Montpellier in Imbach's group and she worked on backbone-modified DNA oligonucleotides. Since 2011, she has been a Research Director at the Institute of Biomolecules Max Mousseron (IBMM). For around



15 years, her research interests have focused on the synthesis of modified ribonucleosides and RNA oligonucleotides as valuable tools for therapeutic applications and for understanding the RNA machinery. Her expertise in RNA chemistry is well renowned and makes her involved in many pluridisciplinary projects in the field of emerging viruses, epitranscriptomics, RNA arrays.

Jean-Jacques Vasseur received his Ph.D. in 1988 from the University of Montpellier, France working on the reactivity of DNA apurinic sites under the guidance of Prof. Jean-Louis Imbach and Dr. Bernard Rayner. From 1990 to 1992, he was a visiting scientist at IONIS Pharmaceuticals in Carlsbad, CA (USA) where he developed phosphorus-free backbone analogues of oligonucleotides. In 1993, he returned to Montpellier and was promoted Research Director at the CNRS in 1998. He co-authored more than 260 publications on various aspects of nucleic acid chemistry focusing notably on the design of chemically modified DNAs and RNAs and their conjugates for therapeutic and diagnostic applications. He is currently vice-director of the Max Mousseron Institute of Biomolecules (IBMM) in Montpellier (France). In 2020, he was elected vice-president of the International Society of Nucleosides, Nucleotides and Nucleic Acids (IS3NA).

then changed to lead to several transition state analogues 2–4 (Figure 3).

However, unexpectedly none of these bisubstrate nucleosides 2–4 showed inhibitory activity on DNMTs. These SAM analogues were then tested on several viral and human RNA MTases (RNMTs) displaying structural similarities with the initially targeted DNMTs. Among the eight RNMTs tested, only the human *N7*-methyltransferase hRNMT was inhibited.^[11] This enzyme is involved in the methylation of the *N7* position of the cap-guanosine in messenger RNAs (mRNA). More surprisingly, by expanding the range of methyltransferases tested, several analogues were identified as good inhibitors of protein arginine methyltransferases (PRMTs) with IC_{50} in the micromolar range (see section 4).

In another study, Arimondo and co-workers developed non-nucleoside analogues as bisubstrate inhibitors of DNMT3A and DNMT1 in cancer cells. The adenosine of SAM was replaced by a base mimic: aminoquinazoline, 2'-deoxycytidine was changed by different quinoline groups and several linkers were investigated (Figure 4).^[12] The most potent inhibitors induced demethylation of the *CDKN2A* promoter in HCT116 colon carcinoma cells. In addition, these quinoline-quinazoline conjugates were found to be potent growth inhibitors of all asexual blood stages of *Plasmodium falciparum* and reduce DNA methylation in *Plasmodium*. The main advantage is the ability of these bisubstrate inhibitors of DNA methylation to kill multi-drug-resistant *P. falciparum* at the nanomolar level (with an IC_{50} of $60\text{ nM} \pm 14$ and a selectivity index of 42 for the best compound 5 against human HepG2 cells), including artemisinin-resistant strains (Figure 4).^[13]

3. Bisubstrate Inhibitors Designed for Targeting RNA Methyltransferases (RNMTs)

3.1. Targeting 2'-O-RNA MTases

Viral RNA methyltransferases play a crucial role in catalyzing the methylation of the mRNA cap structure. This cap structure at the 5'-end of mRNAs is essential for translation into proteins. Viral mRNAs are primarily methylated at the nitrogen *N7*-position of cap-guanosine and at the 2'-O-position of the first nucleotide of the mRNA (adenosine or guanosine). These methylations are essential for RNA stability, protection against innate immune system and stimulation of translation into viral

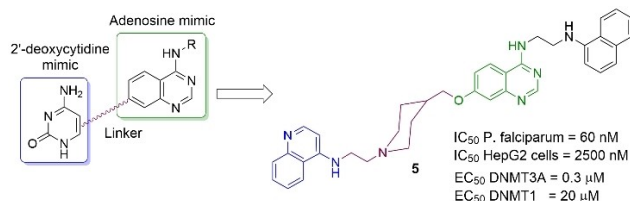


Figure 4. Quinoline–quinazoline derivative 5 as bisubstrate DNMTs inhibitor.^[12]

proteins. Small-molecule inhibitors of RNA MTases, such as SAM analogues, have already been described, but they show insufficient selectivity due to the high homology of the SAM binding domain of different RNA MTases.^[14]

To overcome this lack of selectivity, our group recently reported the synthesis of bisubstrate nucleoside analogues 6–8 as potential inhibitors of 2'-O-MTases by mimicking the transition state of 2'-O-methylation of RNA with each substrate (Figure 5). Several dinucleosides were designed with an adenosine in place of the SAM adenosine, linked to the 2'-OH of an adenosine unit *via* linkers of different sizes containing various heteroatoms (S,^[15] N^[16]), groups of atoms, and even the amino acid side chain of SAM.

Surprisingly, none of the dinucleosides inhibited 2'-O-MTases, including NS5 (Dengue), nsp10/nsp16 (SARS-CoV) and VP39 (vaccinia). Unexpectedly, inhibition of the SARS-CoV *N7*-MTase nsp14 by certain dinucleosides bearing a benzenesulfonamide moiety in the linker was observed with an IC_{50} in the micromolar and submicromolar range (Figure 6). This inhibition was highly specific because human *N7*-methyltransferase hRNMT and other viral MTases were nearly unaffected. In fact, these dinucleosides appear to act as bisubstrate inhibitors, as molecular docking experiments show that the phenyl ring of nitrobenzenesulfonamide moiety occupies the cap binding pocket and establishes π - π stacking interactions with Phe426 of the viral enzyme that naturally stacks the guanosine of the viral mRNA cap-structure.^[17] Among a small library of synthesized dinucleosides, dinucleoside 9 containing a 4-chloro-3-nitrobenzenesulfonamide moiety displayed the best inhibitory activity with an IC_{50} of 0.6 μ M (Figure 6). Thermal shift assay

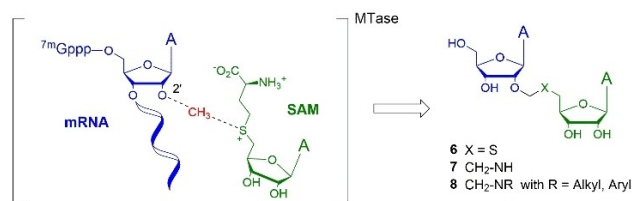


Figure 5. Design of adenine dinucleosides 6–8 as potential bisubstrate inhibitors of RNA 2'-O-MTases.^[15,16]

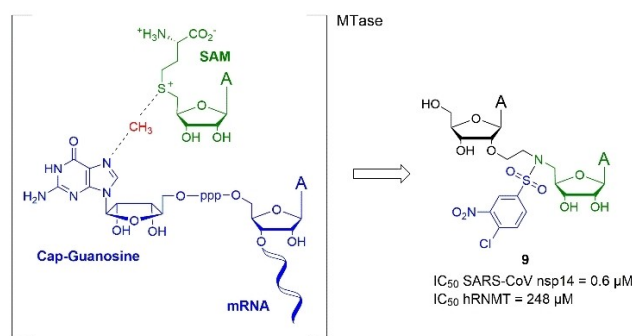


Figure 6. Transition state of *N7*-methylation of mRNA cap structure by SAM and structure of adenine dinucleoside 9 as inhibitor of SARS-CoV *N7*-MTase nsp14.^[16]

experiments to determine affinity with the protein confirmed the value of this new class of bisubstrate inhibitors, as they stabilized the SARS-CoV nsp14 protein better than sinefungin, and for some, better than the natural SAM cofactor. Given that the overall amino acid sequence of the SARS-CoV-2 nsp14 protein has 95.1% identity and 99.1% similarity to the SARS-CoV nsp14 sequence,^[18] this work paves the way for the development of a new class of SARS-CoV-2 inhibitors to fight the Covid-19 pandemic and future coronaviruses.

3.2. Targeting N7-RNA MTases

In 1986, Rottman and co-workers used the bisubstrate strategy to specifically target the Vaccinia virus N7-MTase by synthesizing a dinucleoside analogue mimicking the transition state of N7-guanine methylation of capped mRNA by SAM where the sulfur atom is linked to the N7 position of guanine.^[19] To avoid the formation of a quaternary nitrogen atom therefore a positive charge responsible for the chemical instability of the cap structure, the N7 nitrogen atom of guanine was replaced by a carbon atom (Figure 7). A series of bisubstrate compounds were then developed by chemically modifying the pyrrolo[2,3-d]pyrimidine moieties. The target compound **10** (R = NH₂) could not be obtained despite several attempts, however its structurally related derivative **11** (R = H) was synthesized. Compound **11** showed no inhibitory activity, which is surprising giving its structural similarity to the transition state. In contrast, the C-6 substituted pyrrolo[2,3-d]pyrimidino derivative **12** described in Figure 7 was able to inhibit 81% of capped mRNA methylation at a concentration of 100 μ M (IC₅₀ = 93 μ M).

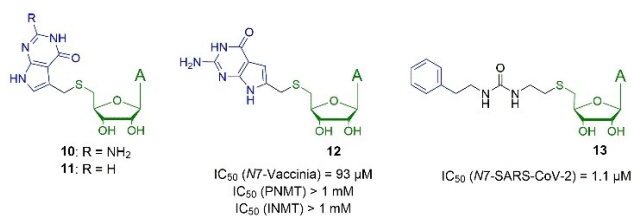


Figure 7. Bisubstrate inhibitor **12** developed by Rottman and co-workers to inhibit Vaccinia virus N7-MTase^[19] and DS0464 **13**, a selective bisubstrate inhibitor of SARS-CoV-2 N7-MTase nsp14 developed by M. Vedadi and co-workers.^[20]

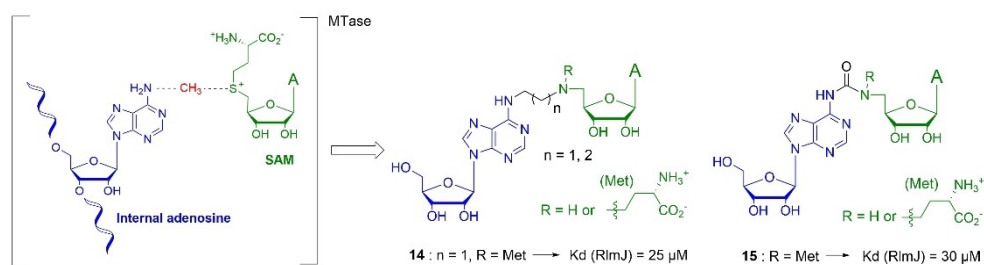


Figure 8. Transition state of adenosine N6-methylation in mRNA by a RNMT and bisubstrate dinucleosides **14–15** developed by Etheve-Quelquejeu and co-workers.^[23]

Remarkably, this inhibitor showed interesting substrate selectivity against the RNA guanine N7-MTase compared with two other SAM-dependent N-MTases: phenylethanolamine-N-methyltransferase (PNMT) and indole-N-methyltransferase (INMT).

In response to the recent SARS-CoV-2 pandemic, M. Vedadi and co-workers screened a library of 161 in-house synthesized SAM analogues in order to discover small-molecule inhibitors of the SARS-CoV-2 N7-MTase nsp14.^[20] Among the 161 compounds, they identified DS0464 (**13**) as a good and selective bisubstrate inhibitor of SARS-CoV-2 nsp14 (IC₅₀ = 1.1 $\pm$ 0.2 μ M), as it barely affected 28 out of 33 human RNA, DNA and protein MTases (Figure 7). This study can be used as a relevant starting point for the development of selective therapeutics against coronaviruses. Indeed, molecular docking experiments revealed that the bisubstrate inhibitor **13** competes with both SAM and mRNA within the enzyme: while the adenosine moiety overlays with the SAM structure, the phenyl-ethyl-urea moiety overlays with the guanosine of the mRNA cap structure and interacts with the surrounding residues.

3.3. Targeting N6A-RNA MTases

Certain RNA methyltransferases catalyze the transfer of the methyl group from the SAM to the N6 position of adenosine residues in the internal sequences of RNAs (including messenger, ribosomal and transfer RNAs). In fact, methylation of adenosine to N6-methyladenosine (m6A) is the most common internal RNA modification in eukaryotes, bacteria and viruses.^[21] For example, in *Escherichia coli*, the m6A modification at position 2030 of rRNA results in improved nucleic acid stability.^[22] In 2018, Etheve-Quelquejeu and co-workers described the synthesis of dinucleosides mimicking the transition state of this N6-methylation.^[23] These dinucleosides are constructed such that the 5' position of the adenosine-mimicking SAM is connected to the N6 position of the adenosine-mimicking mRNA. In these compounds, the 5' sulfur atom of the SAM has been replaced with a nitrogen atom to anchor the α -amino acid chain of the SAM for greater similarity (Figure 8).

The synthesized dinucleosides were evaluated against several human RNA methyltransferases (METTL3/METT14 and METTL16) and Ribosomal RNA large subunit MTase J (RlmJ) in *E. Coli*.^[24] Two dinucleosides **14** and **15** bind to the RlmJ active site with micromolar affinity (Kd = 25 μ M and 30 μ M, respectively).

However, these bisubstrate analogues were not shown to be specific to RlmJ using differential scanning fluorimetry since they were also found to be good ligands for an N1 A-tRNA methyltransferase (TrmK). Nevertheless, these starting scaffolds will be useful for developing future inhibitors against N6A-RNA MTases.

3.4. Targeting N1A-RNA MTases

As an extension of their previous work on bisubstrate analogues targeting N6A-RNA MTases (Figure 8), the same group described in 2020 the synthesis of novel SAM-adenosine con-

jugates **16–19** mimicking the transition state of N1-methylation of adenosine residues.^[25] In these bisubstrate analogues, a 1,2,3-triazole ring was introduced by a copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) to connect the two adenosines, the RNA substrate mimic at the N1-position (in blue) and the SAM analogue (in green) (Figure 9). These dinucleosides could be used as tools for structural studies on RNA N1-methyltransferases.

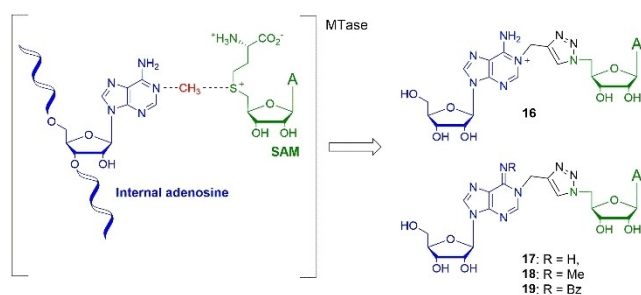


Figure 9. Transition state of adenosine N1-methylation and bisubstrate inhibitors **16–19** developed by Etheve-Quelquejeu and co-workers.^[25]

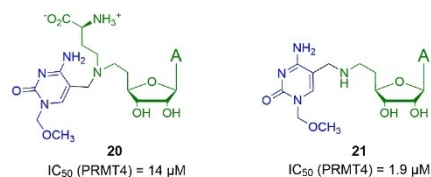


Figure 10. Bisubstrate PRMT4 inhibitors **20–21** developed by Arimondo and co-workers.^[8]

4. Bisubstrate Inhibitors Targeting Arginine N-Methyltransferases (PRMTs)

Arginine methylation is an important process involved in several cellular mechanisms such as transcription, RNA splicing, translation of mRNAs into proteins and even DNA repair. However, overexpression of genes encoding PRMTs can lead to cancer formation.^[26] Thus, these MTases have been investigated by several laboratories for the development of cancer treatments.

As mentioned in section 2, SAM analogues **20–21** developed by Arimondo and co-workers and designed to target DNMTs ultimately showed inhibitions of PRMT4 (also known as CARM1) (Figure 10).^[8] Structural elucidations showed that cytosine occupies the arginine binding pocket, which may provide an explanation for this unexpected result.

More rationally, Martin and co-workers synthesized bisubstrate analogues that mimic the transition state of arginine methylation in peptides by the SAM cofactor.^[27] These analogues were designed to occupy both the SAM binding pocket and the arginine-bearing peptide substrate recognition site, in the MTase protein (Figure 11). This pocket is surrounded by a few glutamic acid residues, which are highly conserved in the eleven PRMTs identified in humans and important for guanidine binding. The bisubstrate compounds were tested against several PRMTs and lysine methyltransferase G9a to assess their

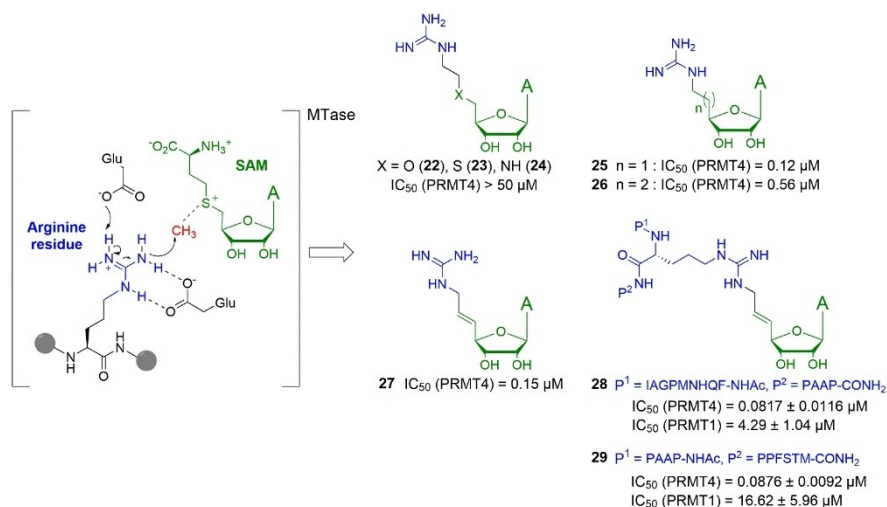


Figure 11. Reaction mechanism of the arginine methylation and bisubstrate PRMT4 inhibitors **22–29** developed in Martin's group.^[27,29]

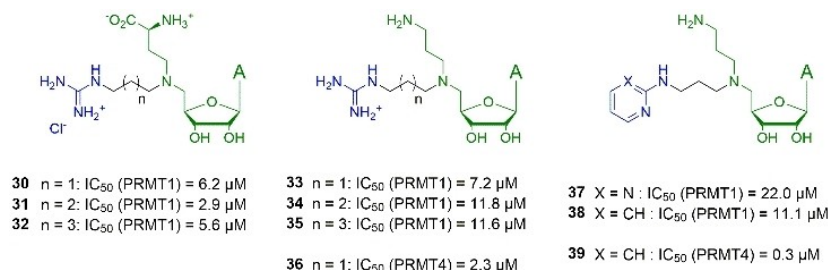


Figure 12. Bisubstrate PRMT1 inhibitors described by Ward and co-workers^[30] (30–32) and by Dreveny and co-workers^[32] (33–39).

specificity. While compounds **22–24** were barely active against PRMT4, compounds **25–27** showed submicromolar inhibitory activities, which has been identified as the cause of some forms of cancer such as colorectal and prostate cancers.^[28]

Interestingly, the bisubstrate inhibitors exhibited significant PRMT / G9a specificity as their IC_{50} towards G9a was greater than 50 μM . In comparison, SAH inhibited both PRMT4 (IC_{50} = 0.8 μM) and G9a (IC_{50} = 16.6 μM) in the same range. Although these compounds did not display any inhibitory effect on cancer cell proliferation, they may serve as starting building blocks for the development of more specific bisubstrate analogues for PRMT inhibition.

In continuation of this work, the same group reported the synthesis and evaluation of bisubstrate inhibitors **28–29** where the adenosine scaffold was linked to an arginine residue located in a short peptide sequence.^[29] Also designed to target CARM1, some of these nucleoside-peptide hybrid compounds showed potent inhibitions with IC_{50} in the low nanomolar range (IC_{50} < 100 nM) while affecting 50- to 200-fold less PRMT1 activity (Figure 11).

Ward and co-workers also developed bisubstrate analogues to target PRMTs.^[30] By replacing the sulfur atom of SAM with a nitrogen atom, they synthesized several compounds in which the nitrogen atom was substituted with both a guanidine moiety and the SAM amino acid chain (Figure 12). Three of them, compounds **30–32** bearing guanidine attached by 3-, 4- and 5-carbon alkyl linkers showed micromolar inhibitory activities against PRMT1 (IC_{50} between 2.9 μM and 6.2 μM), and they barely inhibit the SET-domain lysine methyltransferase (SET7) (IC_{50} > 50 μM), indicating a good discrimination between these proteins.

The same authors extended the original set of PRMT1 inhibitors by replacing the guanidine with a primary amine or an aryl guanidine or they changed the SAM amino acid chain to a propyl chain terminated by a carboxylic acid, an amine or a guanidine group.^[31] With the exception of the compound bearing two *N*-propyl chains terminated by a carboxylic acid or a guanidine group, all synthesized nucleoside analogues inhibited PRMT1 with similar micromolar IC_{50} values. However, some of them with an amine and a guanidine *via* the propyl linker, or a symmetrical bis-amine, or a symmetrical bis-guanidine inhibited PRMT4 (also known as CARM1 cofactor-associated arginine methyltransferase) with the same efficiency.

In contrast, none of these compounds had inhibitory activity on SET7.

Recently, Dreveny and co-workers developed novel bisubstrate inhibitors where the carboxylate group of the amino acid chain was removed.^[32] In some compounds, the guanidine moiety was replaced by 2-aminopyridine and 2-aminopyrimidine scaffolds to decrease polarity and validate a new isosteric approach (Figure 12). In addition, the number of linker carbon atoms was set to three based on preliminary inhibition results. The inhibition data showed on the one hand, with compounds **33–35**, that the carboxylate group provided a slight increase in PRMT1 inhibition and on the other hand, with compounds **37–39**, that the aromatic motifs were less effective than the original guanidine motif. Nevertheless, it should be noted that 2-aminopyridine and 2-aminopyrimidine scaffolds are more beneficial modifications for CARM1 inhibition than PRMT1 (Figure 12).

Finally, in 2020, Huang and co-workers reported the synthesis of bisubstrate inhibitors of PRMTs where the SAM moiety was linked to an arginine motif, carrying various peptide sequences to explore the effect of the substrate peptide moiety on inhibition (Figure 13).^[33] More interestingly, their best compound **40** did not affect the activity of various other methyltransferases including N-terminal methyltransferases (NTMTs), histone methyltransferases (HMTs) and DNMTs. Unfortunately, these compounds have shown poor cell penetration. Thus, in 2021, to improve cell permeability, Huang R. and co-workers developed new nucleoside analogues with peptide sequences replaced by various alkyl/aryl groups increasing lipophilicity.^[34] This novel series resulted in the pan-PRMTs inhibitor **41** with an IC_{50} in the nanomolar range and specificity towards other MTases (Figure 13).

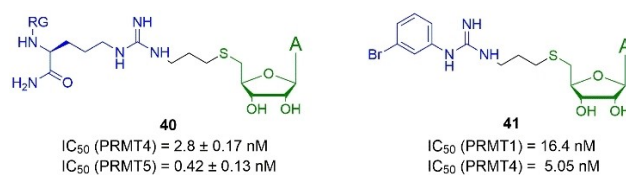


Figure 13. Bisubstrate PRMTs inhibitors **40–41** developed in Huang's group.^[33,34]

5. Bisubstrate Inhibitors Designed for Targeting N-Terminal Methyltransferase NTMT1

Using SAM as a methyl donor, certain α -N-terminal amines of proteins undergo methylation catalyzed by the protein N-terminal methyltransferase (NTMT1). Specifically, NTMT1 recognizes and methylates proteins that possess a canonical X-Pro-Lys motif (X = Ala, Gly, Pro, or Ser) at the N-terminus. Although they play an important role in the regulation of mitosis and DNA repair, their overexpression in various tissues of cancer patients, including malignant melanoma, as well as colorectal and brain cancers, make them attractive targets for the development of potent and selective anticancer therapies.

Over the past five years, Huang and co-workers have developed several bisubstrate inhibitors designed to specifically interact with NTMT1. Structurally, these compounds are constructed such that a SAM analogue is covalently linked to a short peptide. In 2015, they reported the synthesis and evaluation of NAH (N-adenosylhomocysteine) bisubstrate analogues where the peptide was attached to the nucleoside scaffold *via* a triazole ring using CuAAC conditions (Figure 14).^[35]

For the first time, a specific inhibition of NTMT1 was noticed. Indeed, NAH-TZ-SPKRIA **42** was found to be a potent inhibitor with an IC_{50} of $0.81 \pm 0.13 \mu M$ (fluorescence assay) without affecting the lysine methyltransferase G9a and the arginine methyltransferase PRMT1. To assess the importance of the bisubstrate structure, both NAH-TZ **43** and TZ-SPKRIA **44** monosubstrates were tested as control compounds for their ability to inhibit NTMT1 and finally showed no detectable

inhibition at $100 \mu M$, thus supporting the bisubstrate approach for the development of effective inhibitors (Figure 14).

One year later, the same group reported the synthesis of NAH-TZ-SPKRIA analogues where the triazole (TZ) ring was replaced by a propyl chain (C3) in the linker (Figure 15).^[36] Moreover, the peptide sequence was modified and starts with a Gly residue instead of a Ser residue. A fluorescence-based assay showed that the NAH-C3-GPRRRS analogue inhibited NTMT1 with an IC_{50} of $0.94 \pm 0.16 \mu M$ in a range similar to inhibition by NAH-TZ-SPKRIA. In 2019, the synthesis of a modified analogue of NAH-C3-GPRRRS was described with a Pro residue replacing the N-terminal Gly residue of the peptide.^[37] Indeed, the peptide substrate starting with Pro showed the highest binding affinity among all peptide substrates tested in a recent study.^[38] In this study, three bisubstrate inhibitors **45–47** were synthesized: NAH-C3-PPRRRS, NAH-C3-PPKRIA^[37] and NAH-C3-PPKR.

Of the three compounds, NAH-C3-PPRRRS **46** exhibited a two-fold higher inhibition with an IC_{50} of 485 ± 74 nM against NTMT1 compared to NAH-C3-GPRRRS **45** previously described by Zhang and Huang.^[37] This result supports the relevance of a Pro residue at the N-terminus of the peptide.^[38] Nevertheless, the bisubstrate NAH-C3-PPKRIA **47** was the best inhibitor with an IC_{50} of 158 ± 20 nM. Its selectivity profile is noteworthy as the activity of other methyltransferases were not affected ($IC_{50} > 10 - 100 \mu M$) including the protein lysine methyltransferases PKMTs (G9a and SETD7), protein arginine methyltransferases (PRMT1 and TbPRMT7) and nicotinamide N-methyltransferase (NNMT).

In 2020, the synthesis of NAH-C4-GPKRIA, in which the linker between the SAM analogue and the peptide was extended with an additional carbon atom, was reported.^[39] Gly was chosen as the starting amino acid of the peptide due to its synthetic versatility and, therefore, the synthesis strategy was similar to that described previously.^[36] Fluorescence-based assay proved that NAH-C4-GPKRIA compound containing a 4-C linker was 1.5-fold better inhibitor than NAH-C3-GPKRIA with a 3-C linker (IC_{50} of 82 ± 17 nM and 130 ± 40 nM, respectively). The selectivity of NAH-C4-GPKRIA was further examined and the activity of other methyltransferases was not affected ($IC_{50} > 33 - 100 \mu M$).

Finally, in 2021, Huang's group reported a chemoproteomic study to evaluate the selectivity of NTMT1 bisubstrate inhibitors. Thus, biotinylated NAH-Cn-GPK and NAH-Cn-GPKK compounds ($n = 3$ or 4) were synthesized.^[40] Although inhibition was confirmed for all substrates ($5.0 \text{ nM} < K_i < 44 \text{ nM}$), the bisubstrate NAH-C3-GPKK was found to inhibit HemK2/KMT9 methyltransferase (a lysine and glutamine methyltransferase with the

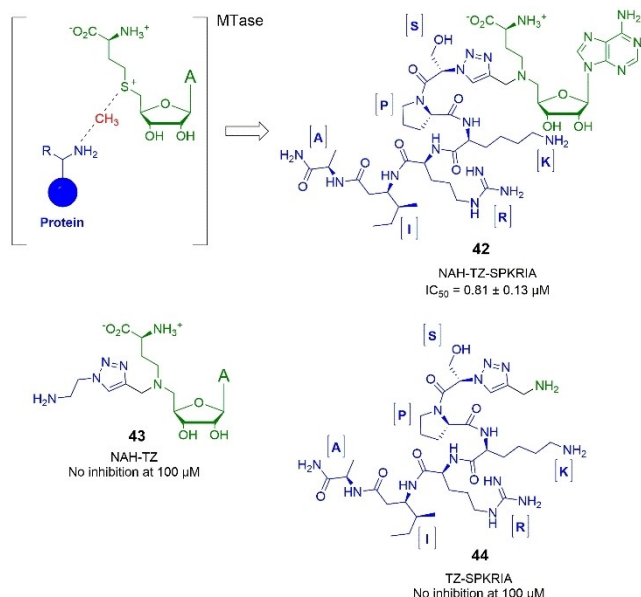


Figure 14. Transition state of the methylation at N-terminus of proteins, first generation of bisubstrate NTMT1 inhibitor **42** and control compounds **43–44** derived from the bisubstrate inhibitor NAH-TZ-SPKRIA **42**.^[35]

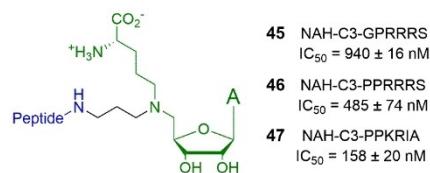


Figure 15. Second generation of NTMT1 inhibitors **45–47**.^[37]

cofactor Trm112) overexpressed in prostate cancer lines.^[41] Therefore, this compound remains a solid starting point for the development of selective HemK2/KMT9 inhibitors. This last result strongly validates the selectivity profile of NAH-peptide analogues developed by several teams led by R. Huang, a pioneer in this field.

6. Bisubstrate Inhibitors Designed for Targeting Histone Lysine Methyltransferases (HKMTs)

Histone lysine methyltransferases (HKMTs) play an important role in the regulation of gene expression. Recently, various reports have suggested that HKMTs are involved in the development of various diseases, including cancer.^[42] In 2010,

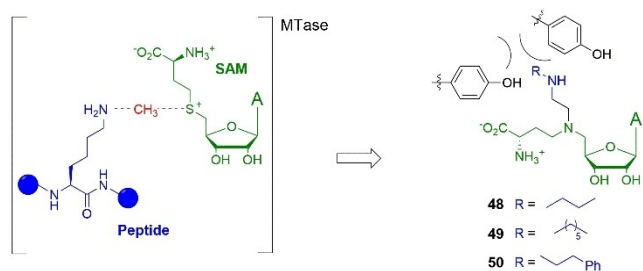


Figure 16. Transition state of the methylation of lysine-containing peptide and bisubstrate inhibitors 48–50 of SET7/9 developed by Hirano and co-workers.^[43]

Hirano reported the design and synthesis of bisubstrate inhibitors 48–50 of the histone lysine methyltransferase SET7/9.^[43] As the crystal structure of SET7/9 revealed the presence of an aromatic amino acid such as tyrosine surrounding the substrate binding site, the secondary amine representing the lysine was attached to various hydrophobic linkers (Figure 16). An ethylene linker between the secondary amine and the 5'N atom was chosen to best mimic the methyl group of SAM. Similar inhibitory activity (at 100 μ M) of SET7/9 to that of the non-specific inhibitor sinefungin was observed, making these compounds good starting points for the development of potent and specific SET7/9 inhibitors.

7. Bisubstrate Inhibitors Designed for Targeting Catechol-O-methyltransferase (COMT)

Catechols are compounds with a 1,2-dihydroxybenzene unit. Adrenaline, norepinephrine and dopamine are among the best known biological catechols. Dopamine is a neurotransmitter that transfers information in the central nervous system. However, its methylation by SAM-dependent catechol-O-methyltransferase leads to its degradation which can cause Parkinson's disease.^[44] In 2003, Diederich and co-workers developed a class of bisubstrate analogues that mimic the methylation transition state of catechols (Figure 17).^[45] The bisubstrate inhibitors 51–55 were designed to occupy both the SAM recognition site and the motif recognition site of catechol of neurotransmitters. As nitro-substituted catechols have previ-

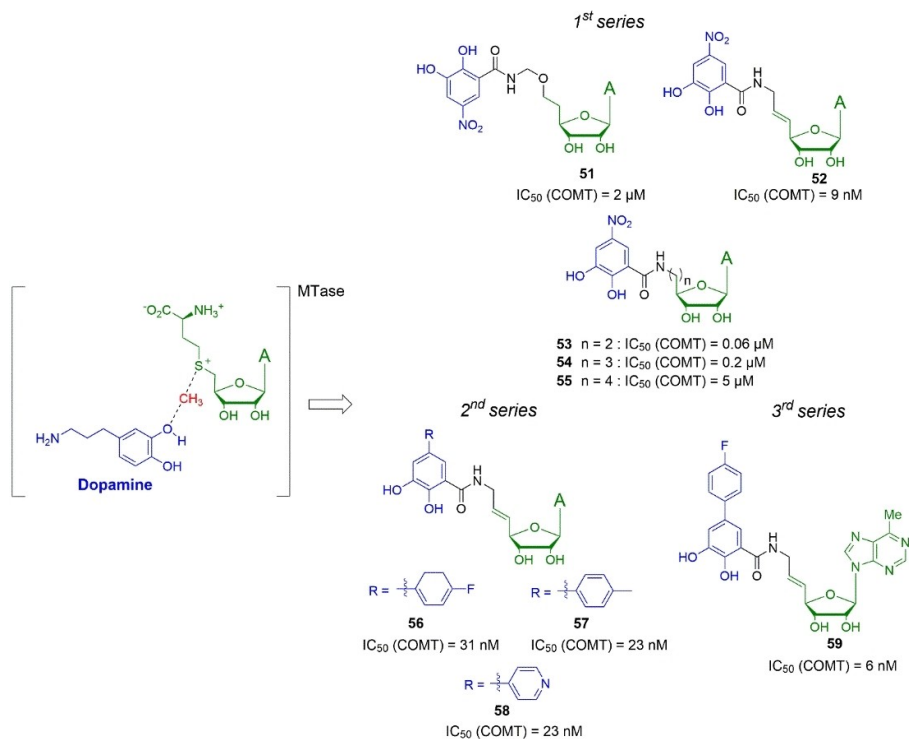


Figure 17. Transition state of the dopamine methylation and three series of bisubstrate COMT inhibitors 51–59 developed by Diederich and co-workers.^[45,48,49]

ously shown efficacy in COMT inhibition and two drugs with such a motif (Comptan® and Tasmar®) are on the pharmaceutical market, Diederich and co-workers have also developed inhibitors with a nitro group in the catechol unit.^[45]

It is noteworthy that the introduction of a rigidified olefinic linker in **52** between the two parts of the substrate led to a significant increase in inhibition (from 0.2 μ M to 9 nM) due to a reduction in entropy loss during binding for the constrained analogue. However, it should be noted that Tasmar® has shown significant liver toxicity limiting the use of this drug.^[46] As it was assumed that the nitro group of the catechol was responsible for this toxicity,^[47] Diederich's group developed a 2nd series of bisubstrate analogues, based on the structure of the best inhibitor of the 1st series, where the nitro group was replaced by different substituents.^[48] The three compounds described in Figure 17 with replacements of the NO₂ group by phenyl or pyridyl rings in **56–58** led to IC₅₀ values in the nanomolar range. Compared to the initial compound **52** containing a nitro group (IC₅₀ = 9 nM), the three most potent compounds showed inhibitions of the same order of magnitude. This result demonstrates that high inhibitory activity can be maintained while removing the nitro group.

To evaluate the importance of the adenine motif in these bisubstrate inhibitors, in 2011, Diederich and co-workers developed a third series of compounds where the nucleobase adenine was replaced by various aromatic heterocycles.^[49] Keeping the 4-fluorobenzene derivative that proved to be an efficient substitute for the nitro group, a library of C6-modified nucleoside analogues was obtained to finally identify the 6-methylpurine analogue **59** with an IC₅₀ of 6 nM, 5-fold better than the reassessed IC₅₀ of 31 nM for the adenine analogue **56** (Figure 17).

8. Bisubstrate Inhibitors Designed for Targeting Nicotinamide N-Methyltransferase (NNMT)

Nicotinamide N-methyltransferase catalyzes the transfer of a methyl group from SAM to N1 position of nicotinamide (also called vitamin B3). In some individuals, the gene encoding this protein is overexpressed, leading to overproduction of NNMT, a phenomenon implicated in the development of certain diseases such as cancer.^[50] In 2017, Martin's group described the synthesis of bisubstrate inhibitors, mimicking the transition state of nicotinamide methylation (Figure 18).^[51] These analogues are designed to occupy both the SAM and nicotinamide binding sites. Among about 30 analogues synthesized, compound **60** that respected the overall transition state structure showed the best inhibitory activity towards NNMT. Later, in line with this work, Jin and co-workers synthesized another bisubstrate analogue **61** where the linker between SAM and nicotinamide was lengthened by the addition of a methylene (Figure 18).^[52] Out of a total of 34 MTases (histone MTases, DNA MTases, RNA MTases), compound **61** showed a significant selectivity since only four of them were inhibited, including the histone methyltransferase DOT1 L in the micromolar range.

In 2019, Martin and co-workers reported the evaluation of novel bisubstrate NNMT inhibitors where the amino acid chain was modified (**62–63**) and the nicotinamide motif was replaced with other aromatic substituents (**64–65**) while keeping the same methylene linker (Figure 18).^[53] The SAM amino acid chain seems to be crucial since compounds with modified amino acid chains barely inhibited the NNMT (IC₅₀ > 250 μ M) while changing the nicotinamide motif with a naphthalene ring was more convincing. Indeed, a 10-fold increase in inhibition was noticed. These observations are partly justified by recent work describing bisubstrate inhibitors containing other amino acid chain substitutions that did not show higher inhibition than the parental compound.^[54] Furthermore, this work identified a bisubstrate inhibitor **64** with 381-fold (IC₅₀ = 3.7 nM) higher inhibitory activity than the naphthalene-containing compound

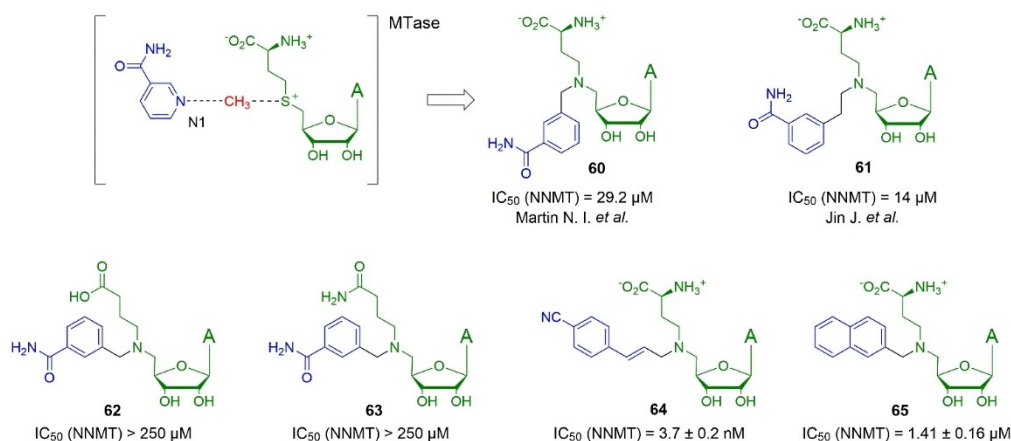


Figure 18. Transition state of nicotinamide methylation and bisubstrate NNMT inhibitors respectively developed by Martin and co-workers^[51,53] (**60**, **62–65**) and Jin and co-workers^[52] (**61**).

65 ($IC_{50} = 1.41 \mu M$) (Figure 18), making it one of the most potent NNMT inhibitors reported to date.^[54]

In the same year, Huang and co-workers continued this work by extending the linker between the 5' nitrogen atom of the nucleoside and the nicotinamide mimic.^[55] This choice to extend the linker by an additional carbon atom is supported by the work of Shair who, based on the crystal structure of NNMT bound to SAM and nicotinamide, showed a distance of 4 Å between the methyl group of SAM and the N1 position of nicotinamide.^[56] Furthermore, as with the compounds developed by Diederich (cited above),^[45] the linker was made more rigid with an alkynyl linker, leading to compounds **66–68** with high affinity with K_i ranging from submicromolar to nanomolar (Figure 19). To evaluate the importance of the different parts of the potent ligands, a deconstruction strategy was conducted where control compounds lacking the nicotinamide motif (**69**), the exact amino acid chain (**70**) or strictly the amino acid chain (**71**) were synthesized (Figure 19). The most striking observations concern the absence of the nicotinamide motif for the 3-C linker (700-fold reduced affinity for **69**) and the absence of the amino acid chain for the alkynyl linker (441-fold reduced affinity for **71**). In addition, a selectivity assay proved the ability of the alkynyl linker-containing compound **68** to not affect other methyltransferases, including PRMTs, lysine methyltransferases and *N*-terminal methyltransferases at 100 μM , which strongly supports the use of a bisubstrate strategy.

The alkynyl linker turned out to be interesting since at the same time, Shair and co-workers reported the synthesis and evaluation of bisubstrate NNMT inhibitors with the same motif.^[56] One of the main differences was the replacement of the nitrogen atom with a *CH* at the 5' position, which allowed them to reduce the linker length between the nucleoside and the nicotinamide scaffold. A 14-step synthesis strategy finally led to the identification of a high affinity inhibitor **72**

characterized by a K_i of 0.5 nM, making this compound the best NNMT inhibitor (Figure 19). A library of approximately 30 control compounds was also obtained (structure not shown). Although some showed affinities of the same order of magnitude, none showed a stronger affinity. Again, these data show the importance of nicotinamide, the purine motif, and the amino acid chain for NNMT inhibition.

9. Bisubstrate Inhibitors Designed for Targeting Phenylethanolamine N-Methyltransferase (PNMT)

Phenylethanolamine *N*-methyltransferase (PNMT) catalyzes the transfer of a methyl group from SAM to the primary amine of norepinephrine to give epinephrine (also called adrenaline) (Figure 20). Secreted during a moment of stress, it causes an increase in heart rate and blood pressure. Thus, PNMT remains a potential drug target for hypertension control.

In 2020, Schramm and co-workers reported the synthesis of the first bisubstrate inhibitor **73** of PNMT, which showed a high binding affinity to hPNMT ($K_i = 12.0$ nM).^[57] Structurally, the NAH moiety was attached to the PNMT inhibitor SK&F 64139 (Figure 20), which showed specificity and permeability issues. According to the crystal structure of hPNMT in complex with SK&F 64139 and SAH (PDB: 1YZ3) supported by the SAM docked structure, the linker between SK&F 64139 and NAH was estimated to be three carbon atoms (Figure 20).

At the same time, Grunewald and co-workers also reported the synthesis of bisubstrate PNMT inhibitors with low nanomolar affinity.^[58] Here, the amino acid chain was removed to retain the sulfur atom of the SAM structure. A propylamino linker was first

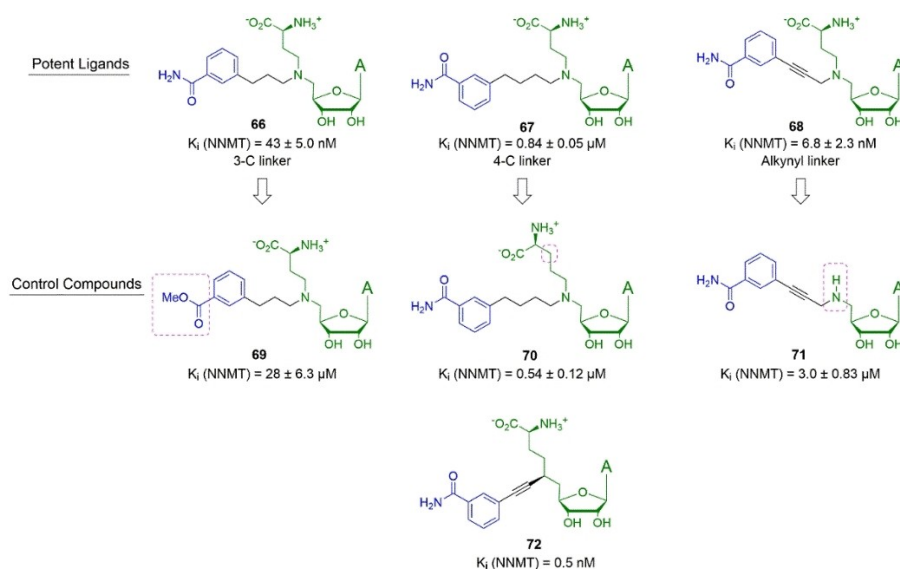


Figure 19. Bisubstrate NNMT inhibitors **66–68** and control compounds **69–71** developed by Huang's group^[55] and NNMT inhibitor **72** developed by Shair and co-workers.^[56]

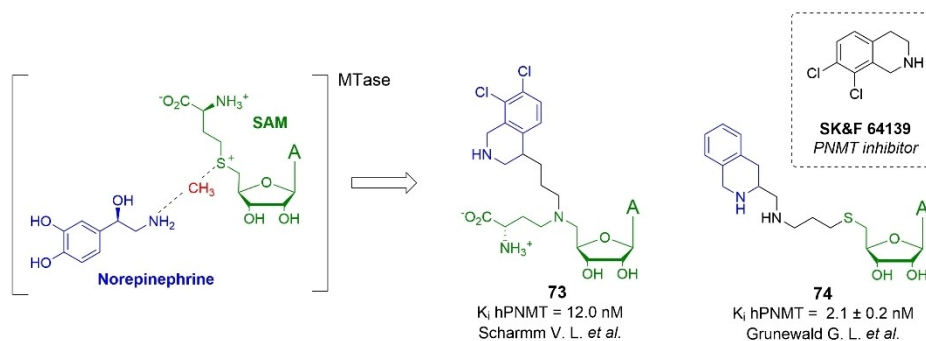


Figure 20. Transition state of norepinephrine methylation yielding epinephrine and bisubstrate inhibitors of hPNMT developed by Schramm and co-workers^[57] (**73**) and Grunewald and co-workers^[58] (**74**).

added to at the 5' end to obtain the desired compound **74** (Figure 20).

10. Bisubstrate Inhibitors Designed for Targeting Mycolic Acid Methyltransferases (MA-MTases)

MA-MTases are involved in the development of *Mycobacterium tuberculosis*, the causative agent of tuberculosis and one of the most widespread infectious diseases in the world. Indeed, MA-MTases catalyze the transfer of a methyl group onto very long chain α -alkylated β -hydroxylated fatty acids, major components of the thick lipid-rich envelope of the genus *Mycobacterium* (Figure 21).^[59] In 2009, Mourey and co-workers highlighted S-adenosyl-N-decyl-aminoethyl (SADAE) **75** as an effective inhibitor of MA-MTases ($0.1 \mu\text{M} < \text{IC}_{50} < 1 \mu\text{M}$) in contrast to the pan-inhibitor SAH and sinefungin which showed no inhibition against MA-MTases. Therefore, SADAE may represent a first step towards the design of anti-tuberculosis therapeutics.

11. Conclusions and Outlook

Methyltransferases that play crucial roles in biological processes are considered promising therapeutic targets for the treatment of various human diseases. Most of them use SAM as the methyl donor and many efforts have been made by research

groups to design bisubstrate MTase inhibitors. DNA, RNA, proteins such as histones for example and smaller molecules such as catechol or nicotinamide have been targeted by these bisubstrate inhibitors. They are very promising as novel therapeutics and valuable tools for biological studies of MTases. Nevertheless, much remains to be investigated for their biological and biomedical applications. For this, cell membrane permeability, stability, selectivity remain the main limitations and challenges to overcome. In this review, more than 80% of the design studies presented are consistent with the observed enzymatic results, emphasizing that careful tuning of the bisubstrate structure is generally worthwhile. However, systematic analysis and modeling studies of the bisubstrate in the binding pockets of different MTases would further justify the selectivity and without dispute, validate this strategy. Of note, for less than 20% of the reported studies, the enzymatic results contradicted the bisubstrate design performed prior to synthesis. These results, although disappointing at first glance, nonetheless suggest new prospects for the design of more potent inhibitors, especially since the researchers would not have discovered these structures if they had thoroughly copied the substrate-SAM binding state during the methylation reaction. In our opinion, solving the structures of MTases with small selective inhibitors will provide the essential structural information to determine their mechanism of action and optimize these inhibitors. Co-crystallization of an MTase and the SAM cofactor is often possible and the SAM binding site can therefore be well characterized. In contrast, the substrate binding site is less known due to the lower affinity of the substrate for MTase and thus the challenge of crystallizing the MTase-substrate complex. The bisubstrate approach would increase the stability of the complex and consequently facilitate its crystallization to allow structural studies. Methyltransferases still have secrets to share and motivate the scientific community to understand their structures and mechanisms. Moreover, all the data described in this review may provide a new starting point in the search for more effective and selective bisubstrate compounds.

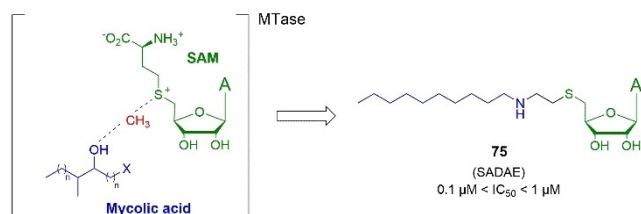


Figure 21. Transition state of mycolic acid methylation and bisubstrate inhibitor SADAE **75** developed by Mourey and co-workers.^[59]

Acknowledgements

Rostom Ahmed-Belkacem thanks University of Montpellier for funding his PhD student grant, CNRS and French National Research Agency (ANR) for financial support of his one-year post-doc (AAP RA-COVID-19V14, Project N° ANR-21-CO14-0004-01).

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Bisubstrate inhibitors • Enzymes • Methylation transition states • Methyltransferases • Nucleoside analogues

- [1] C. Bon, L. Halby, P. B. Arimondo, *Epigenomics* **2020**, *12*, 1479–1482.
- [2] D. Lavogina, E. Enkvist, A. Uri, *ChemMedChem* **2010**, *5*, 23–34.
- [3] J. Zhang, Y. G. Zheng, *ACS Chem. Biol.* **2016**, *11*, 583–597.
- [4] P. B. Le Calvez, C. J. Scott, M. E. Migaud, *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 1291–1318.
- [5] B. Jin, K. D. Robertson, *Adv. Exp. Med. Biol.* **2013**, *754*, 3–29.
- [6] R. Wright, C. Stephens, L. Shapiro, *J. Bacteriol.* **1997**, *179*, 5869–5877.
- [7] D. C. Wahnou, V. K. Shier, S. J. Benkovic, *J. Am. Chem. Soc.* **2001**, *123*, 976–977.
- [8] L. Halby, N. Marechal, D. Pechalrieu, V. Cura, D.-M. Franchini, C. Faux, F. Alby, N. Troffer-Charlier, S. Kudithipudi, A. Jeltsch, W. Aouadi, E. Decroly, J.-C. Guillemot, P. Page, C. Ferroud, L. Bonnefond, D. Guianvarc'h, J. Cavarelli, P. B. Arimondo, *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **2018**, *373*, 20170072.
- [9] Ž. M. Svedružić, N. O. Reich, *Biochemistry* **2005**, *44*, 14977–14988.
- [10] J. C. Wu, D. V. Santi, *J. Biol. Chem.* **1987**, *262*, 4778–4786.
- [11] D. Varshney, A.-P. Petit, J. A. Bueren-Calabuig, C. Jansen, D. A. Fletcher, M. Pegg, S. Weidlich, P. Scullion, A. V. Pislakov, V. H. Cowling, *Nucleic Acids Res.* **2016**, *44*, 10423–10436.
- [12] L. Halby, Y. Menon, E. Rilova, D. Pechalrieu, V. Masson, C. Faux, M. A. Bouhleh, M.-H. David-Cordonnier, N. Novosad, Y. Aussagues, A. Samson, L. Lacroix, F. Ausseil, L. Fleury, D. Guianvarc'h, C. Ferroud, P. B. Arimondo, *J. Med. Chem.* **2017**, *60*, 4665–4679.
- [13] F. Nardella, L. Halby, E. Hammam, D. Erdmann, V. Cadet-Daniel, R. Peronet, D. Ménard, B. Witkowski, S. Mecheri, A. Scherf, P. B. Arimondo, *ACS Cent. Sci.* **2020**, *6*, 16–21.
- [14] P. Z. Kozbial, A. R. Mushegian, *BMC Struct. Biol.* **2005**, *5*, 19.
- [15] R. Ahmed-Belkacem, P. Sutto-Ortiz, E. Decroly, J.-J. Vasseur, F. Debart, *Eur. J. Org. Chem.* **2019**, 2019, 6486–6495.
- [16] R. Ahmed-Belkacem, P. Sutto-Ortiz, M. Guiraud, B. Canard, J.-J. Vasseur, E. Decroly, F. Debart, *Eur. J. Med. Chem.* **2020**, *201*, 112557.
- [17] Y. Ma, L. Wu, N. Shaw, Y. Gao, J. Wang, Y. Sun, Z. Lou, L. Yan, R. Zhang, Z. Rao, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 9436–9441.
- [18] F. K. Yoshimoto, *Protein J.* **2020**, *39*, 198–216.
- [19] E. Benghiat, P. A. Crooks, R. Goodwin, F. Rottman, *J. Pharm. Sci.* **1986**, *75*, 142–145.
- [20] K. Devkota, M. Schapira, S. Perveen, A. Khalili Yazdi, F. Li, I. Chau, P. Ghiabi, T. Hajian, P. Loppnau, A. Bolotokova, K. J. F. Satchell, K. Wang, D. Li, J. Liu, D. Smil, M. Luo, J. Jin, P. V. Fish, P. J. Brown, M. Vedadi, *SLAS Discovery* **2021**, *26*, 1200–1211.
- [21] X. Jiang, B. Liu, Z. Nie, L. Duan, Q. Xiong, Z. Jin, C. Yang, Y. Chen, *Sig. Transduct. Target Ther.* **2021**, *6*, 74.
- [22] R. Gao, H. Shi, *Clin. Microbiol.* **2016**, *5*, 1000256.
- [23] C. Atjdian, L. Iannazzo, E. Braud, M. Ethève-Quelquejeu, *Eur. J. Org. Chem.* **2018**, 2018, 4411–4425.
- [24] S. Oerum, M. Catala, C. Atjdian, F. Brachet, L. Ponchon, P. Barraud, L. Iannazzo, L. Droogmans, E. Braud, M. Ethève-Quelquejeu, C. Tisné, *RNA Biol.* **2019**, *16*, 798–808.
- [25] C. Atjdian, D. Coelho, L. Iannazzo, M. Ethève-Quelquejeu, E. Braud, *Molecules* **2020**, *25*, 3241.
- [26] R. S. Blanc, S. Richard, *Mol. Cell* **2017**, *65*, 8–24.
- [27] M. van Haren, L. Q. van Ufford, E. E. Moret, N. I. Martin, *Org. Biomol. Chem.* **2014**, *13*, 549–560.
- [28] S. Greenblatt, N. Man, P.-J. Hamard, C. Martinez, Y. Xu, F. Liu, J. M. Watts, D. G. Tenen, S. D. Nimer, *Blood* **2017**, *130*, 241–241.
- [29] M. J. van Haren, N. Marechal, N. Troffer-Charlier, A. Cianciulli, G. Sbardella, J. Cavarelli, N. I. Martin, *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 3625–3630.
- [30] J. Dowden, W. Hong, R. V. Parry, R. A. Pike, S. G. Ward, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2103–2105.
- [31] J. Dowden, R. A. Pike, R. V. Parry, W. Hong, U. A. Muhsen, S. G. Ward, *Org. Biomol. Chem.* **2011**, *9*, 7814–7821.
- [32] E. A. Gunnell, A. Al-Noori, U. Muhsen, C. C. Davies, J. Dowden, I. Dreveny, *Biochem. J.* **2020**, *477*, 787–800.
- [33] A. A. Al-Hamashi, D. Chen, Y. Deng, G. Dong, R. Huang, *Acta Pharmaceutica Sinica B* **2020**, *11*, 2709–2718.
- [34] I. D. Iyamu, A. A. Al-Hamashi, R. Huang, *Biomol. Eng.* **2021**, *11*, 854.
- [35] G. Zhang, S. L. Richardson, Y. Mao, R. Huang, *Org. Biomol. Chem.* **2015**, *13*, 4149–4154.
- [36] G. Zhang, R. Huang, *RSC Adv.* **2016**, *6*, 6768–6771.
- [37] D. Chen, G. Dong, N. Noinaj, R. Huang, *J. Med. Chem.* **2019**, *62*, 3773–3779.
- [38] R. Wu, Y. Yue, X. Zheng, H. Li, *Genes Dev.* **2015**, *29*, 2337–2342.
- [39] D. Chen, C. Dong, G. Dong, K. Srinivasan, J. Min, N. Noinaj, R. Huang, *J. Med. Chem.* **2020**, *63*, 8419–8431.
- [40] D. Chen, Y. Meng, D. Yu, N. Noinaj, X. Cheng, R. Huang, *ACS Chem. Biol.* **2021**, *16*, 1234–1242.
- [41] E. Metzger, S. Wang, S. Urban, D. Willmann, A. Schmidt, A. Offermann, A. Allen, M. Sum, N. Obier, F. Cottard, S. Ulferts, B.-T. Preca, B. Hermann, J. Maurer, H. Greschik, V. Hornung, O. Einsle, S. Perner, A. Imhof, M. Jung, R. Schüle, *Nat. Struct. Mol. Biol.* **2019**, *26*, 361–371.
- [42] A. Spannhoff, W. Sippl, M. Jung, *Int. J. of Biochem. Cell Biol.* **2009**, *41*, 4–11.
- [43] S. Mori, K. Iwase, N. Iwanami, Y. Tanaka, H. Kagechika, T. Hirano, *Bioorg. Med. Chem.* **2010**, *18*, 8158–8166.
- [44] T. Müller, *Drugs* **2015**, *75*, 157–174.
- [45] C. Lerner, B. Masjost, A. Ruf, V. Gramlich, R. Jakob-Roetne, G. Zürcher, E. Borroni, F. Diederich, *Org. Biomol. Chem.* **2003**, *1*, 42–49.
- [46] C. W. Olanow, *Arch. Neurol.* **2000**, *57*, 263–267.
- [47] K. Nepali, H.-Y. Lee, J.-P. Liou, *J. Med. Chem.* **2019**, *62*, 2851–2893.
- [48] R. Paulini, C. Lerner, R. Jakob-Roetne, G. Zürcher, E. Borroni, F. Diederich, *ChemBioChem* **2004**, *5*, 1270–1274.
- [49] M. Ellermann, R. Paulini, R. Jakob-Roetne, C. Lerner, E. Borroni, D. Roth, A. Ehler, W. B. Schweizer, D. Schlatter, M. G. Rudolph, F. Diederich, *Chem. Eur. J.* **2011**, *17*, 6369–6381.
- [50] C. Dong, Y. Mao, W. Tempel, S. Qin, L. Li, P. Loppnau, R. Huang, J. Min, *Genes Dev.* **2015**, *29*, 2343–2348.
- [51] M. J. van Haren, R. Taig, J. Kuppens, J. S. Toraño, E. E. Moret, R. B. Parsons, D. Sartini, M. Emanuelli, N. I. Martin, *Org. Biomol. Chem.* **2017**, *15*, 6656–6667.
- [52] N. Babault, A. Allali-Hassani, F. Li, J. Fan, A. Yue, K. Ju, F. Liu, M. Vedadi, J. Liu, J. Jin, *J. Med. Chem.* **2018**, *61*, 1541–1551.
- [53] Y. Gao, M. J. van Haren, E. E. Moret, J. J. M. Rood, D. Sartini, A. Salvucci, M. Emanuelli, P. Craveur, N. Babault, J. Jin, N. I. Martin, *J. Med. Chem.* **2019**, *62*, 6597–6614.
- [54] Y. Gao, M. J. van Haren, N. Buijs, P. Innocenti, Y. Zhang, D. Sartini, R. Campagna, M. Emanuelli, R. B. Parsons, W. Jespers, H. Gutiérrez-de-Terán, G. J. P. van Westen, N. I. Martin, *J. Med. Chem.* **2021**, *64*, 12938–12963.
- [55] D. Chen, L. Li, K. Diaz, I. D. Iyamu, R. Yadav, N. Noinaj, R. Huang, *J. Med. Chem.* **2019**, *62*, 10783–10797.
- [56] R. L. Policarpo, L. Decultot, E. May, P. Kuzmič, S. Carlson, D. Huang, V. Chu, B. A. Wright, S. Dhakshinamoorthy, A. Kannt, S. Rani, S. Dittakavi, J. D. Panarese, R. Gaudet, M. D. Shair, *J. Med. Chem.* **2019**, *62*, 9837–9873.
- [57] N. Mahmoodi, R. K. Harijan, V. L. Schramm, *J. Am. Chem. Soc.* **2020**, *142*, 14222–14233.
- [58] J. Lu, A. G. Bart, Q. Wu, K. R. Criscione, M. J. McLeish, E. E. Scott, G. L. Grunewald, *J. Med. Chem.* **2020**, *63*, 13878–13898.
- [59] J. Vaubourgeix, F. Bardou, F. Boissier, S. Julien, P. Constant, O. Ploux, M. Daffé, A. Quémard, L. Mourey, *J. Biol. Chem.* **2009**, *284*, 19321–19330.

Manuscript received: December 8, 2021

Revised manuscript received: February 1, 2022