MTA-Cooperative PRMT5 Inhibitors: Mechanism Switching Through Structure-Based Design

Kevin M. Cottrell, Douglas A. Whittington, Kimberly J. Briggs, Haris Jahic, Janid Ali, Deepali Gotur, Matthew R. Tonini, Wenhai Zhang, Alan Huang and John P. Maxwell

Abstract

MTAP deletions occur in 10-15% of all human cancers, providing one of the largest precision oncology patient populations[1]. MTA-cooperative PRMT5 inhibitors, including clinical stage compounds TNG908, TNG462, AMG 193, and MRTX1719[1-4], leverage the well-characterized synthetic lethal relationship between PRMT5 inhibition and *MTAP*-deletion for the treatment of *MTAP*-deleted solid tumors. Prior to running a high throughput screen (HTS) that identified the hits that led to TNG908 and TNG462, we embarked on a medicinal chemistry effort using structure-based design to identify inhibitors that could bind PRMT5 cooperatively with MTA and not with SAM, therefore making them selective for *MTAP*-deleted cancers. We leveraged structures of known first generation, SAM-cooperative PRMT5 inhibitors as starting points, taking advantage of the structural differences between the PRMT5•SAM and PRMT5•MTA complexes, and modified them to switch their mechanism of inhibition. These inhibitors became the first disclosed proof of concept that PRMT5 inhibitors could be designed to selectively kill *MTAP*-deleted cancer cells while sparing MTAP WT cells[5].

MTAP-deletion is synthetic lethal with PRMT5 and provides a significant opportunity in the treatment of cancer

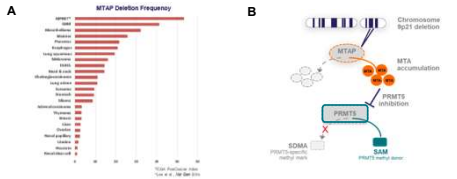


Figure 1: *MTAP*-deletion is a common genetic event in human cancer. (A) *MTAP* deletion frequency in a subset of human cancers[6-8] (B) Biological rationale for sensitivity of MTAP^{null} cells to PRMT5 inhibition.

The space created in PRMT5•MTA relative to PRMT5•SAM offers an opportunity to design selective MTA-cooperative PRMT5 inhibitors

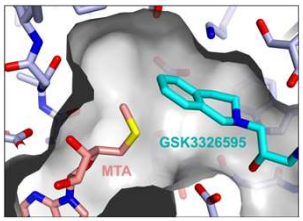


Figure 2: GSK3326595 (pemrametostat, 1) bound in the PRMT5•MTA complex. When MTA is bound to PRMT5, the absence of the $CH_2CH_2CH(COOH)NH_2$ group that is present in SAM provides a new pocket that can be leveraged to design inhibitors that bind selectively with PRMT5•MTA and not with PRMT5•SAM. Addition of appropriate binding elements to previous SAM-cooperative inhibitors could switch their mechanism to MTA-cooperative.

Using a known SAM-cooperative, substrate competitive inhibitor as a starting point for design

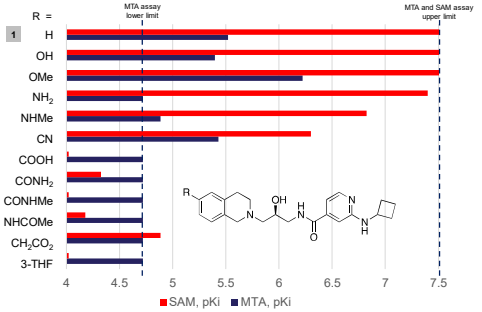


Table 1: Using GSK3203591/EPZ015866[9] as a starting point for design. Exploration of linkers at the 6 position of the tetrahydroisoquinoline. With increased activity in the presence of MTA, ethers are identified as preferred linkage.

Identification of first analogs with selective PRMT5•MTA inhibition

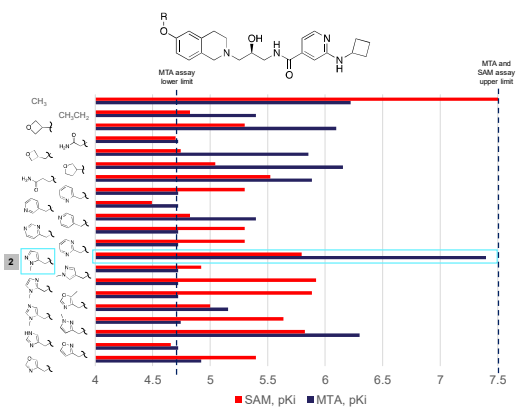


Table 2: Exploration with 6-ether identifies first analogs with MTA/SAM selectivity

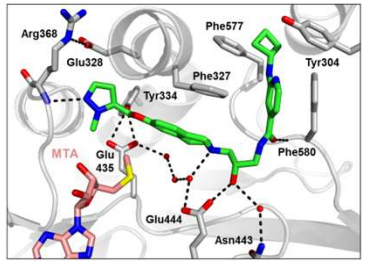


Figure 3: X-ray crystal structure of pyrazole containing 2 with PRMT5•MTA. Crystal structure confirms that the extension off the tetrahydroisoquinoline (THIQ) reaches into the SAM binding pocket. A hydrogen bond of the pyrazole nitrogen to the backbone NH of Arg368 is likely providing increased affinity, and competition with SAM is likely driving selectivity.

Expansion of 6-position ether SAR

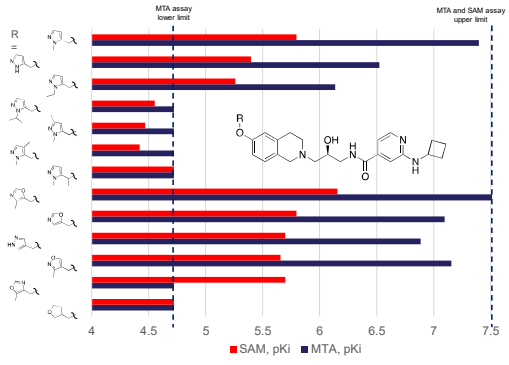


Table 3: Expansion of 6-position SAR. Modification of the ring and exploration of small substitutions identifies other analogs that also show selective inhibition.

Switching to C-linked THIQ leads to improved potency

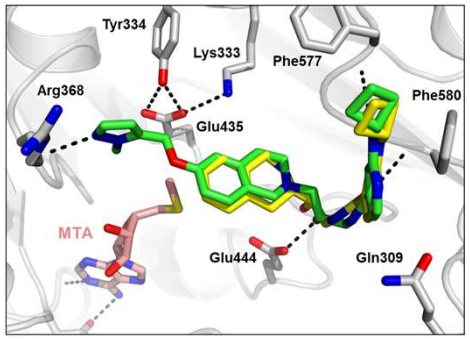
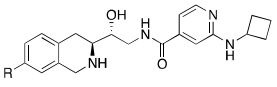


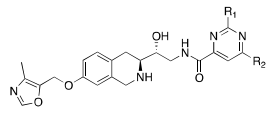
Figure 4: X-ray crystal structure overlay of N-linked (2, green) and C-linked (3, yellow) THIQs. Crystal structure overlays of unsubstituted, SAM-cooperative, C-linked THIQ **3** with MTA-cooperative, 6-ether substituted, N-linked THIQ **2** suggests that the appropriate position to substitute on C-linked compounds to achieve MTA cooperativity is at C7.



R1	Biochemical, $K_{i\ app}$, μM		SDMA ICW Pharmacodynamic Assay, HAP1	
	+MTA	+ SAM	MTAP-null, IC_{50}, μM	Selectivity vs WT
3 H	6	0.03	0.03	1.6X
	0.01	0.3	1.9	> 5X
	0.004	0.05	0.5	> 20X
	0.005	0.3	3	> 3X

Table 4: A subset of analogs demonstrating activity in the C-linked series. The substituents from the most active N-linked compounds were applied to the 7 position in the C-linked series and selective inhibition was achieved, along with cellular PD activity.

Further modification improves potency and leads to selective activity in a viability assay



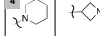
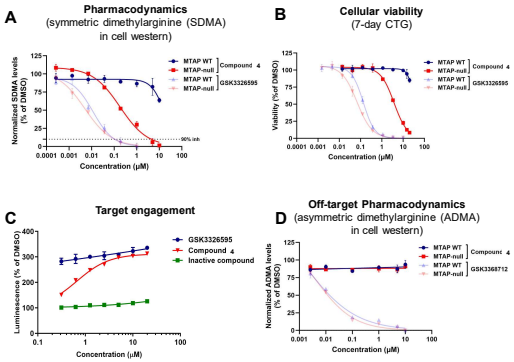
R1	R2	SDMA ICW Pharmacodynamic Assay, HAP1				Viability Assay, HAP1	
		MTAP-null IC_{50}, μM	Selectivity, vs MTAP WT	MTAP-null IC_{50}, μM	Selectivity, vs MTAP WT	MTAP-null GI_{50}, μM	Selectivity, vs MTAP WT
		0.03	52X	0.7	14X	0.3	25X
		0.05	47X	0.9	> 11X	0.3	4.5X
4 		0.1	39X	2.8	> 3.6X	3.3	> 6X
		0.01	30X	0.2	24X	0.1	5X

Table 5: A subset of analogs with varying pyrimidine substitution. Examples of pyrimidine substitution shows selective PD activity in MTAP-null vs MTAP WT cells and selective cell viability effects.

Selective cellular viability effects are driven by on-target on-mechanism activity



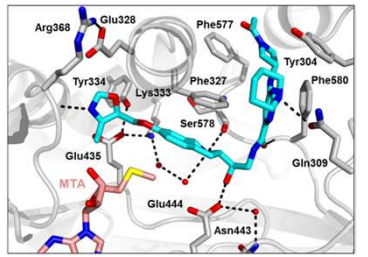


Figure 5: Representative data for tool compound 4. (A) SDMA in cell western (ICW) PD data for HAP1 MTAP-null vs MTAP WT cells. (B) Cellular viability data in HAP1 MTAP-null vs MTAP WT cells. (C) Thermostability target engagement in HAP1 MTAP-null cells (D) Asymmetric dimethylarginine (ADMA) in cell western (ICW) PD data in HAP1 MTAP-null vs MTAP WT cells. (E) X-ray crystal structure with PRMT5•MTA.

Summary

- **A series of inhibitors that bind PRMT5 cooperatively with MTA, are selective with PRMT5•MTA relative to PRMT5•SAM, and selectively kill *MTAP*-deleted cancer cells was discovered using structure-based design to switch the selectivity of known SAM-selective PRMT5 inhibitors.**
- **This series of compounds was the first published demonstration of MTA-cooperative PRMT5 inhibitors.**
- **The most advanced compounds in the series were valuable tools to increase understanding of the biology in the program in advance of hits from a HTS that ultimately led to TNG908 and TNG462, two compounds currently being evaluated in Phase 1/2 clinical trials for *MTAP*-deleted cancers.**

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