Design, Synthesis and Characterization of Small Molecules Targeting MLL1 Methyltransferase in Mixed-Lineage Leukemia

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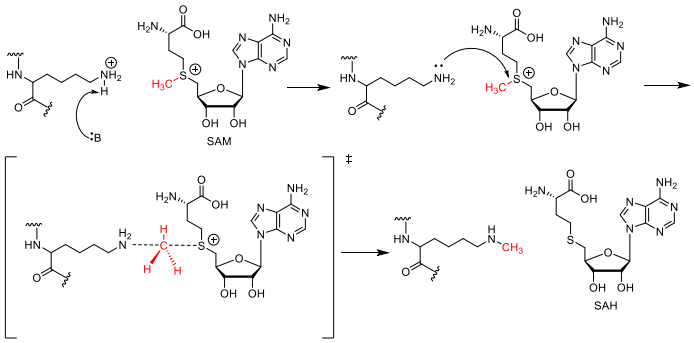
Rearrangements of the Mixed-Lineage Leukemia (*MLL*) gene located on the chromosome 11q23 are responsible for >70% of acute lymphoblastic leukemia (ALL) in infants and ~ 10% of acute myelogenous leukemia (AML) in adults. Compared to other types of blood cancer, Mixed-Lineage Leukemia is associated with poor prognosis even with advanced treatment like stem cell transplantation. There is an urgent need for understanding the molecular biology basis of the pathogenies as well as developing therapeutic strategies based on that knowledge.

The human *MLL1* gene encodes a large multi-domain protein possessing several DNA recognition, transcription activation domain, and the C-terminal SET domain that catalyzes the histone H3 lysine 4 (H3K4) methylation. MLL1 SET domain histone methyltransferase activity requires several stoichiometric components, including WDR5, ASH2L, and RbBp5 (WAR). In normal physiology, *MLL1* is essential to the proper maintenance of the transcriptional regulators *HOX* genes that involved in embryogenesis and hematopoiesis. The *MLL* rearrangements are involved in the translocation of a truncated N-terminus of *MLL* allele fused to more than 60 C-terminus fusion partners to produce the oncogenic MLL fusion proteins (MLL-FPs). The chimeric proteins will cause the consistently high expression of *HOXA* cluster genes (e.g. *HOXA9*) via recruiting elongation complex and DOT1L.

Despite that the gain-of-function of MLL-FPs is the predominant cause of Mixed-Lineage Leukemia, genetic studies show that wild-type MLL is required in MLL leukemogenesis *in vivo.* Our lab has previously developed a cyclic peptide inhibitor **MM-401**, which selectively inhibit the methyltransferase activity of MLL1 by interrupting MLL1/WDR5 protein-protein interaction. **MM-401** did decrease the expression of *HOX* genes and resulted in cell growth arrest, apoptosis, and induction of myeloid differentiation in MLL fusion leukemia cells. This finding encourages us to pursuit chemical probe that targets directly to the MLL1 SET domain.



A mechanism-based approach is applied to design transition state analog based on the co-factor **SAM** (*S*-Adenosyl methionine). There are two binding pockets for histone methyltransferases: the cofactor binding site and substrate binding site. When activated, the enzyme will recruit SAM to cofactor binding site and lysine substrate to the substrate binding site. Two binding sites are connected by a hydrophobic, narrow tunnel that offers proximity for lysine to cofactor SAM via an S_N2 nucleophilic attack to transfer the methyl group. Based on this mechanism, we want to design an SAM-based focused library to systematically probe the lysine tunnel, substrate binding pocket and one solvent exposed pocket. The most potent compound among our focused library, **TC-3307,** has an IC_{50} value of 27 nM against MLl1. Preliminary data also revealed that **TC-3307** exhibit anti-proliferative activity (GI50 = 5.9μM) and downregulation of *HOXA9* gene on MLL-AF4 fusion leukemia cell line MV4-11. In addition, **TC-3307** showed no inhibitory activity against other histone methyltransferases: SET7/9, SET1a, and EZH2 under 1 μM concentration.

Further optimization of this compound will focus on probing the substrate binding site, in which we envision will gain the most potency and selectivity over other histone methyltransferases. This process will be guided by the cocrystal structure of the inhibitor with MLl1 SET domain. We will perform HMT assay on a representative panel of methyltransferases to get the selectivity profiling. To prove the on-target effect of inhibitors, more experiments will be conducted, for example, gene expression profiling, cell cycle and apoptosis analysis on leukemia cell lines.