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Design and synthesis of benzylpiperidine inhibitors targeting the menin–MLL1 interface

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ABSTRACT

Menin is an essential oncogenic cofactor for mixed lineage leukemia (MLL)-mediated leukemogenesis, functioning through its direct interaction with MLL1 protein. Therefore, targeting the menin–MLL1 protein–protein interface represents a promising strategy to block MLL-mediated leukemogenesis. On the basis of co-crystal structure analysis, starting from thienopyrimidine chemotype, we have investigated the detailed structure–activity relationship of the piperazinyl-dihydrothiazole moiety. Several compounds were found with potent inhibitory activity against menin and better activities in cell-based experiments than MI-2-2. Molecular docking analysis revealed a less explored subpocket, which could be used for the design of new menin–MLL1 inhibitors.

Mixed lineage leukemia (MLL) is a very aggressive blood cancer that predominantly occurs in pediatric patients. Chromosomal translocations involving the MLL gene at *11q23* are observed in more than 70% of infant acute lymphoblastic leukemia (ALL) and 5–10% of acute myeloid leukemia (AML) in adults.[1,2] Studies showed that fusion of MLL gene with one out of over 60 partner genes results in expression of chimeric MLL fusion proteins, which enhance proliferation of hematopoietic cells, block hematopoietic differentiation, and ultimately lead to acute leukemia.[3,4] In addition, patients with mixed lineage leukemia have very poor prognosis and respond poorly to currently available treatments,[5,6] emphasizing the urgent need for developing novel therapies.

The leukaemogenic activity of mutant MLL proteins depends on their interactions with menin, a product of the multiple endocrine neoplasia type 1 (MEN1) tumor suppressor gene.[7] As a regulator of target gene expression, menin is a highly specific binding partner of MLL1 and MLL1 fusion proteins.[8–10] MLL1 interacts with menin through two N-terminal motifs: The high-affinity binding part called menin-binding motif 1 (MBM1) and the low-affinity part called menin-binding motif 2 (MBM2).[11] Since the N terminal of most MLL1 fusion proteins remain unchanged, disruption of the protein–protein interaction between menin and MLL1 fusions could treat many types of mixed lineage leukemia. Therefore, menin was regarded as a critical oncogenic cofactor of MLL1 fusion proteins in acute leukemia, and intercepting the protein–protein interaction between menin and MLL1 consequently became a very attractive therapeutic strategy for new drug design for the MLL leukemia patients.[12]

Grembecka's group pioneered the development of menin–MLL1 inhibitors and identified the thienopyrimidine MI-2-2 (**1**) as the first small molecule targeting this protein–protein interaction reported to date.[13,14] Very recently, they further reported the optimized inhibitor MI-503 (**2**) with high potency in cellular studies and good in vivo efficacy in mice models of castration-resistant tumors and MLL leukemia.[15,16] On the basis of amino methyl piperidine chemotype, Grembecka et al. also discovered MIV-6 (**3**), which binds to the same binding site of menin to block the menin–MLL1 protein–protein interaction.[17] In addition, the MLL1 derived peptidomimetic (MCP-1) (**4**) were recently reported as a potent inhibitor to block the menin–MLL1 interaction. However, the cellular activity of this peptidomimetic compound was not disclosed (Fig. 1).[18]

On the basis of the crystal structure analysis of menin MI-2-2 (**1**) complex, we first identified the close interaction of thienopyrimidine ring and the protein exemplified by a favorable C—F—C═O dipolar interaction between the fluorine atom in MI-2-2 and

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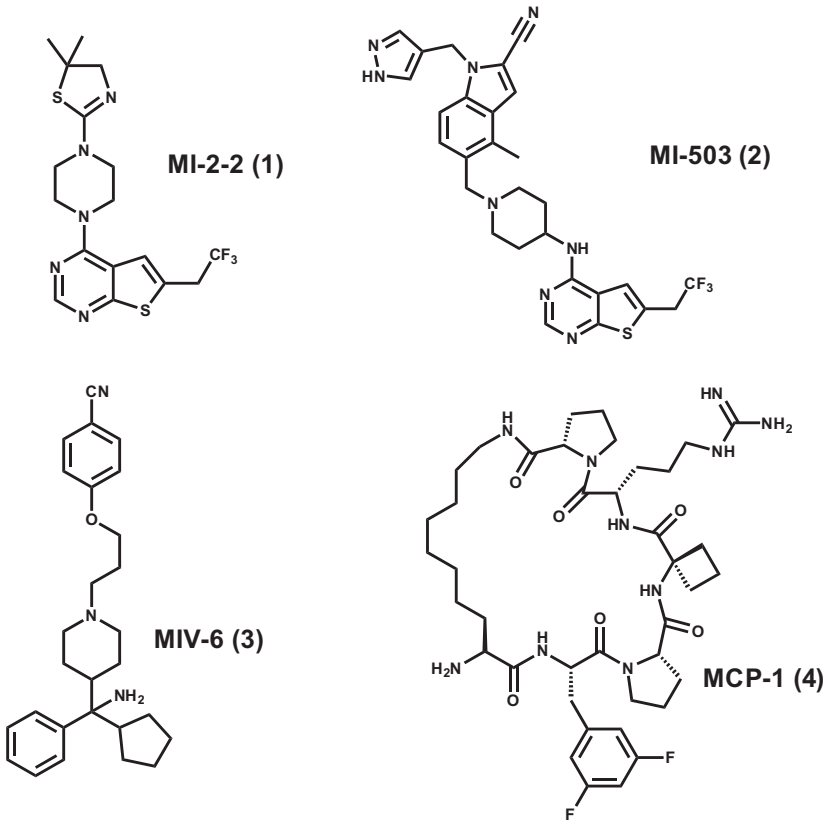

Figure 1. Small-molecular and peptidomimetic inhibitors to block the menin–MLL1 interaction.

the backbone of His181. Next, we noticed that the piperazinyl-dihydrothiazole part formed van der Waals interactions with the flat surface centered at residue Tyr323 of menin. This subpocket has large room to accommodate various chemical groups and can be explored for new chemical skeleton. Thus, we initialized a program to investigate the detailed structure–activity relationship on the piperazinyl-dihydrothiazole part (R group in Fig. 2). It was during this work, that compound (**2**) was reported by Grembecka's group with a similar design rationale. Herein, we would like to report on our detailed SAR study on the piperazinyl-dihydrothiazole moiety, and complement the results of Grembecka et al.

We have designed and assessed several structures with docking study for variations of the R group. Based on the binding interaction analysis, compounds **5**–**8** were selected for chemical synthesis and tested with fluorescence polarization (FP) assay (see the Supporting information for the experimental details).

Compound **5** was designed by merging MI-2-2 (**1**) with compound **3**. Molecular docking showed the thienopyrimidine moiety of **5** adopted a similar binding mode as MI-2-2 (**1**), and the benzonitrile part reached the subpocket of residue Trp341 to form a hydrogen bond. Compound **5** showed potent inhibitory activity (IC_{50} = 0.43 μM) in the FP assay, encouraging us to continue the optimization. Although compounds **6**–**8** have similar structures at the R part, their inhibitory activities against menin showed significant difference. Compounds **7** and **8** were potent inhibitors, while compound **6** did not show activity at 20 μM. To elaborate the SAR of this part, three potent compounds (**5**, **7** and **8**) were further optimized.

Based on compound **5**, compounds **9**–**11** were designed for the investigation of the relationship of the linker and binding activity. We varied the atom type and length of the linker between piperazine and benzonitrile, and found that the most potent inhibitor

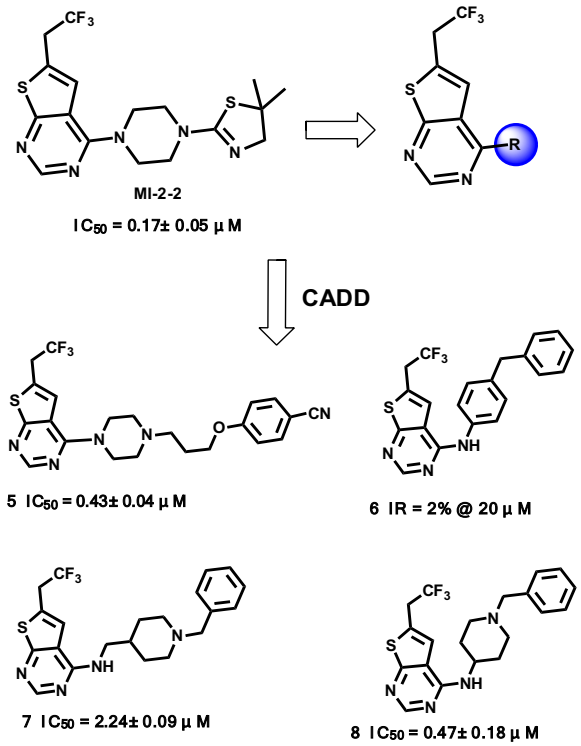

Figure 2. The modification of piperazinyl-dihydrothiazole part (R group).

was compound **5** (n = 3, X = O). Replacing the atom x with N decreased the activity about 3 folds (compound **9**, IC_{50} = 1.54 μM),