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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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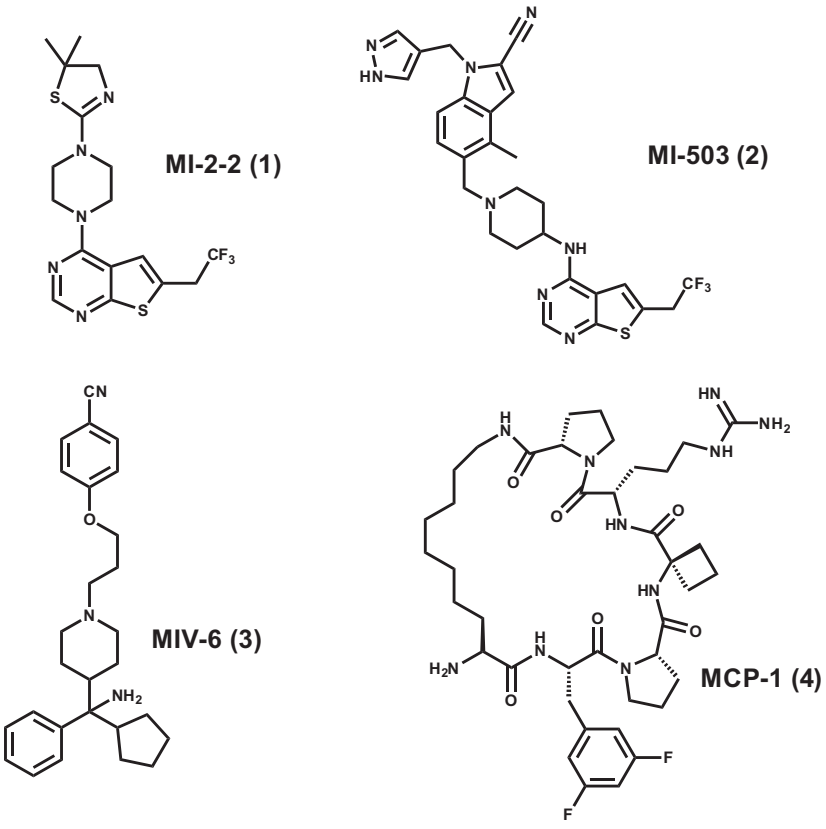


Figure 1. Small-molecular and peptidomimetic inhibitors to block the menin–MLL1 interaction.

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

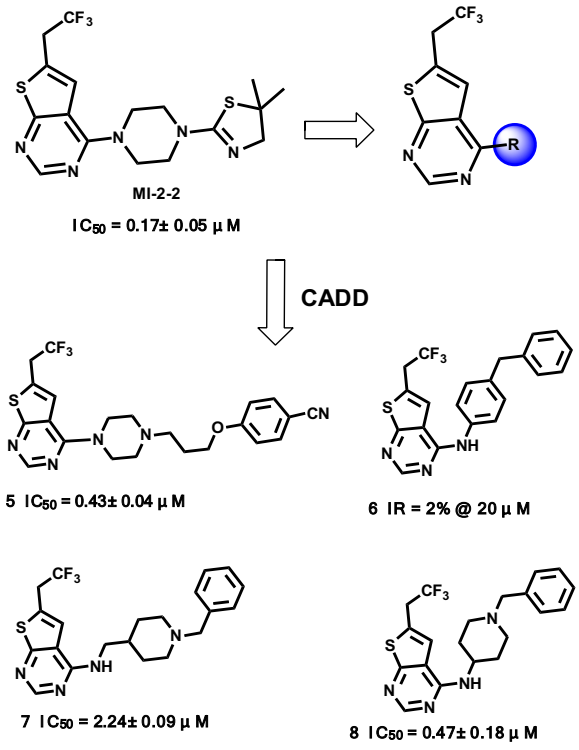


Figure 2. The modification of piperazinyl-dihydrothiazole part (R group).

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),

possibly due to the orientation change of benzonitrile moiety. Reducing the *n* length from 3 to 2 resulted in compounds **10** and **11** (x = O, N respectively) with decreased inhibitory activity about 20 folds, emphasizing the necessity of proper length to maintain the hydrogen bond interaction with residue Trp341 (Table 1).

From compound **7**, we first performed the optimization of the R^1 substituent on the phenyl ring. Introducing electron-donating methyl or methoxy group resulted in compounds (**12** and **13)** with similar activity. In contrast, introducing electron-withdrawing cyano or trifluoromethyl group resulted in compounds (**14** and **15)** with more than 20-fold decrease in the inhibitory activity. Additionally, compound **16** with fluorophenyl ring showed similar activity as compound **7** (Table 2).

Eventually, we focused on the optimization of compound **8**. Molecular docking indicated that the thienopyrimidine part of compound **8** adopted a similar mode with MI-2-2, and the benzyl part attached to piperidine ring interacts with the Pro13 subpocket of menin. We explored the SAR of the benzyl moiety, and found that the absence of benzyl group significantly decreased the inhibitory activity for about 70-fold (compound **17**, IC_{50} = 32.81 μM). Similarly, replacing phenyl ring with more steric–hindered group (**18**–**20**) showed much weaker activity. We replaced the benzyl group with phenyl ethyl group to increase the flexibility, and the resulting compound (**21)** showed slightly decreased activity about 4-fold. Aliphatic moieties were also introduced, yielding compounds **22**–**23** with slightly weaker activity than compound **8**. By using the bioisosterism rules, we replaced the phenyl ring with alkynyl group and heterocyclic rings to produce compounds **24**–**27**, and among which, compound **26** (IC_{50} = 0.22 μM) contained a thiophene ring was about 2-fold more potent than compound **8** (Table 3).

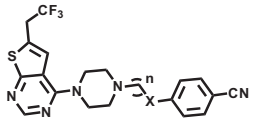
We then explored the substitutions on the phenyl ring of compound **8**. With methoxyl substitution, the inhibitory activity of compound **28** was similar to compound **8**. In contrast, introduction of 4-trifluoromethyl or 4-cyano resulted in compounds **29** and **30** with decreased inhibitory activity by more than 20-fold. But the introduction of fluorine atom (compound **31**) did not show much decrease of activity. Similarly, 3-amino substituent reduced the activity slightly, and 3-nitro lowered the activity significantly. Overall, compounds substituted with electron-donating groups were more potent than that with electron-withdrawing groups (Table 4).

The molecular docking structure of menin with compound **8** revealed several negatively charged residues around the phenyl ring, such as Asp285 and Glu363. On the basis of structure data, we anticipated that introducing an appropriate positively charged group, such as an amino moiety, should result in additional favorable electrostatic interactions with adjacent acidic residues. Different linear amino alkyl groups were introduced to the 3-position of phenyl ring and most of these compounds exhibited preferable activity except for compound **37**, which may be due to its steric hindrance from *N*,*N*-dimethyl group. We also introduced piperidine to the phenyl ring. The resulting compound **40** was found with moderate inhibitory activity and compound **39** with weaker activity (Table 5 and Fig. 3).

We selected compounds **34** and **40** to dock into menin utilizing the software GLIDE. The amino groups of compound **34** in the side chain formed electrostatic interactions with Asp285, with a distance of 2.63 Å between N and O. The amino group of compound **40** extended to the opposite direction of Asp285, forming electrostatic interactions with Glu363. These predicted binding mode highlighted that the positively charged groups in ligands can contribute new interactions, which may provide a new direction for the design of new inhibitors targeting the menin-MLL1 interaction.

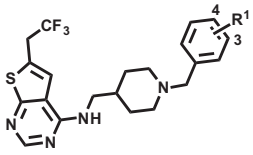
Based on the previous studies, human leukemia cells expressing MLL-AF4 were sensitive to menin inhibitors, such as MV-4-11 cell line.[19] However, K562 cell, another kind of chronic myelogenous leukemia (CML) cell, is independent of the MLL1-menin proteins and should not be sensitive to menin inhibitors. Disruption of the menin–MLL1 fusion protein interaction is expected to result in growth arrest of MLL leukemia cells. Therefore, we selected several compounds to test their activity in MV-4-11 cells and K562 cells (see the Supporting information for the experimental details). As expected, strong and dose-dependent inhibition of cell proliferation was observed for compounds **5**, **8**, **26** and **35** in MV-4-11 cells,

Table 1
Menin inhibitory activity of compounds **9**–**11**



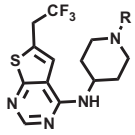
Compd	N	x	Menin IC_{50} (μM)
9	3	N	1.54 ± 0.20
10	2	O	>20
11	2	N	>20

Table 2
Menin inhibitory activity of compounds **12**–**16**



Compd	R	Menin IC_{50} (μM)	Compd	R	Menin IC_{50} (μM)
12	3-Me	3.80 ± 0.41	**15**	4-CF_3	18.5%@20 μM
13	4-OMe	1.07 ± 0.12	**16**	4-F	1.17 ± 0.00
14	4-CN	33.4%@20 μM			

Table 3
Menin inhibitory activity of compounds **17**–**27**



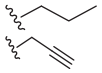
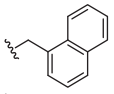
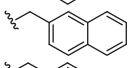
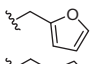
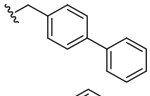
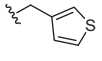
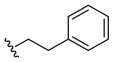
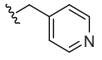
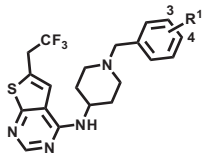
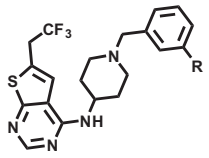
Compd	R	Menin IC_{50} (μM)	Compd	R	Menin IC_{50} (μM)
17	H	32.81 ± 6.77	**23**		5.67 ± 0.56
18		2.4%@20 μM	**24**		31.3%@20 μM
19		15.6%@20 μM	**25**		4.69 ± 0.11
20		4.6%@20 μM	**26**		0.22 ± 0.12
21		1.80 ± 1.39	**27**		2.70 ± 0.42
22		0.77 ± 0.03			

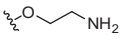
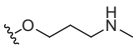
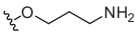
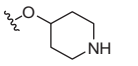
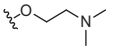
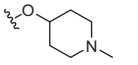
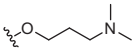
Table 4
Menin inhibitory activity of compounds **28**–**33**



Compd	R	Menin IC_{50} (μM)	Compd	R	Menin IC_{50} (μM)
28	4-OMe	0.64 ± 0.10	**31**	4-F	2.00 ± 1.02
29	4-CF_3	−2.5%@20 μM	**32**	3-NH_2	1.26 ± 0.21
30	4-CN	25%@20 μM	**33**	3-NO_2	43%@20 μM

Table 5
Menin inhibitory activity of compounds **34**–**40**



Compd	R	Menin IC_{50} (μM)	Compd	R	Menin IC_{50} (μM)
34		1.22 ± 0.14	**38**		1.12 ± 0.08
35		0.67 ± 0.17	**39**		13.39 ± 11.12
36		0.78 ± 0.12	**40**		2.35 ± 1.18
37		7.35 ± 5.36			

with $IC_{50} \leqslant 1$ μM, which is consistent with its higher binding activity against menin. From the Table 6, these compounds have a very limited effect on the proliferation of K562 cells, demonstrating preferred selectivity toward MLL1 fusion transformed cells.

HOXA9 is a part of the homeobox gene family, involved in setting the body plans of animals.[15] It is likely that HOXA9 would display increased expression in cells with higher differentiation potentials.[17] HOXA9 knockout mice have been shown to develop a reduction in the number of circulating common myeloid progenitor cells, which differentiate into erythroid progenitor cells.[20] The menin–MLL1 fusion protein interaction is required for the maintenance of high expression level of HOXA9 in MLL leukemia cells and for leukemic transformation by MLL fusions. And as described by Jizhou et al., the excision of menin can reduce about 70–80%

Table 6
The in vitro anti-proliferation activities of 14 compounds in MV-4-11 and K562 cells

Compd	MV-4-11 IC_{50} (μM)	K562 IC_{50} (μM)	Compd	MV-4-11 IC_{50} (μM)	K562 IC_{50} (μM)
5	1.02 ± 0.37	9.12 ± 5.63	**7**	2.33 ± 0.30	>20
9	2.69 ± 0.12	>20	**13**	1.28 ± 0.09	>20
22	6.49 ± 1.47	>20	**16**	2.98 ± 0.20	>20
26	0.79 ± 0.46	>20	**34**	4.15 ± 0.95	>20
8	1.02 ± 0.04	>20	**35**	0.39 ± 0.07	>20
28	2.42 ± 0.28	>20	**36**	3.49 ± 1.15	>20
31	6.32 ± 1.25	>20	**38**	1.65 ± 0.26	9.51 ± 7.83
			MI-2-2	0.64 ± 0.11	>20

Notes: Cell proliferation assay was done according to the method mentioned in the Materials and methods section, where cell were treated with drugs for 7 days. The IC_{50} values represent the mean ± SD of triplicate determinations.

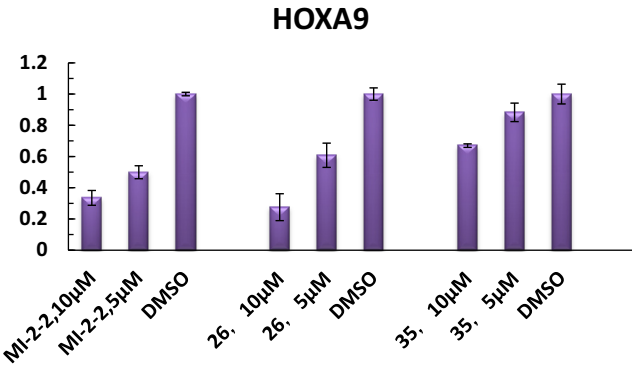


Figure 4. Quantitative real-time PCR analysis of HOXA9 expression in MV-4-11 cells following 6 day incubation with compounds **26**, **35** and MI-2-2. Expression of HOXA9 has been normalized to â-actin and is referenced to the DMSO-treated cells. Data represent the mean values for triplicates ± SD. The experiment was performed 3 times.

expression of HOXA 9 in two cell lines transformed by transduction of MLL-AF9 fusion genes.[21] To assess whether our compounds affect the expression level of HOXA9, we performed quantitative real-time PCR (qRT-PCR) experiments in human MLL leukemia cell MV-4-11 (see the Supporting information for the experimental details). Indeed, treatment with compound **26** or **35** resulted in a strong and dose dependent reduction in the expression level of Hoxa9 as compared to the DMSO control, with about 70% decrease in Hoxa9 level upon treatment with 10 μM of **26**. The effect of compound **26** on expression level of target genes is slightly more potent than the control MI-2-2 (Fig. 4).

In summary, based on the analysis of currently available inhibitors, including skeleton structure, drug-likeness and binding mode, we selected MI-2-2 (**1**) and made SAR study on the piperazinyl-dihydrothiazole moiety. The piperazine-dihydrothiazole group

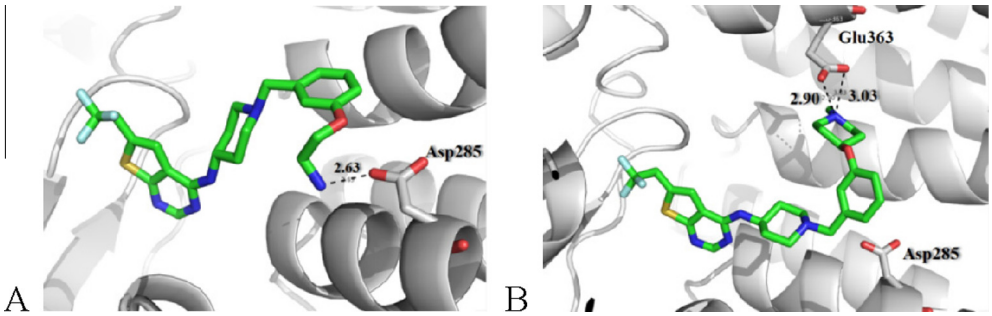


Figure 3. Docking study on compounds **34** (A) and **40** (B) in menin binding site. The menin protein is shown in cartoon style, while the ligands **34** and **40** are shown in stick model. The residues forming important charged interactions are illustrated in stick model.

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was replaced into benzylpiperidine and resulted in **8** with potent binding activity (IC_{50} = 0.47 μM). Medicinal chemistry optimization of the hit **8** led to the discovery of a series of compounds with potent inhibitory activity against menin and several of them showed good activities in in vitro anti-proliferation assay and cell-based functional studies on the HOXA9 gene regulation. Molecular docking analysis of compounds **34** and **40** revealed that the amino group may interact with a less explored subpocket and form salt bridged interactions with surrounding negatively charged residues (Asp285 or Glu363). Together with the newly identified menin–MLL1 inhibitors, it may provide the researchers new direction for designing novel chemotypes of menin–MLL1 inhibitors.

Acknowledgements

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Supplementary data

Supplementary data (schemes and procedures of chemical syntheses, conditions and protocols of the biological assays) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.07.074.

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