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Discovery of new potent protein arginine methyltransferase 5 (PRMT5) inhibitors by assembly of key pharmacophores from known inhibitors

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ABSTRACT

Protein arginine methyltransferase 5 (PRMT5) is an epigenetics related enzyme that has been validated as a promising therapeutic target for human cancer. Up to now, two small molecule PRMT5 inhibitors has been put into phase I clinical trial. In the present study, a series of candidate molecules were designed by combining key pharmacophores of formerly reported PRMT5 inhibitors. The *in vitro* PRMT5 inhibitory testing of compound **4b14** revealed an IC_{50} of 2.71 μM, exhibiting high selectivity over PRMT1 and PRMT4 (> 70-fold selective). As expected, **4b14** exhibited potent anti-proliferative activity against a panel of leukemia and lymphoma cells, including MV4-11, Pfeiffer, SU-DHL-4 and KARPAS-422. Besides, **4b14** showed significant cell cycle arrest and apoptosis-inducing effects, as well as reduced the cellular symmetric arginine dimethylation level of SmD3 protein. Finally, affinity profiling analysis indicated that hydrophobic interactions, π-π stacking and cation-π actions made the major contributions to the overall binding affinity. This scaffold provides a new chemical template for further development of better lead compounds targeting PRMT5.

Protein methylation catalyzed by protein arginine methyltransferases (PRMTs) on arginine residues could influence many key biological processes.[1] As a member of the PRMT family, PRMT5 modulates the biological functions of its substrate proteins by symmetrically transferring up to two methyls to arginine residues.[2] Methylation mediated by PRMT5 is essential to maintain homeostasis in normal cells. However, increasing studies indicate that PRMT5 is also involved in cell growth and survival pathways that promote tumorigenesis and cancer development.[3–14] Specifically, PRMT5 is frequently overexpressed in diverse human cancers and its increased activity has been evidenced to promote cell transformation.[15] Besides, PRMT5 was reported to be a critical *in vitro* and *in vivo* regulator of breast cancer stem cell proliferation and self-renewal.[4] Also, it was demonstrated to control melanoma growth mediated by SHARPIN, the latter being an adaptor for the linear ubiquitin chain assembly complex.[5] In multiple myeloma (MM), PRMT5 has been reported to be a druggable target, as it is overexpressed in patient MM cells and has a close relationship with prognostic relevance.[6] All of these studies indicate that PRMT5 is a promising target for anticancer drug development.

Like most promising therapeutic targets, great efforts have been made to develop inhibitors against PRMT5.[9,16–27] The previously reported PRMT5 inhibitors, as summarized in Fig. 1, can be classified into two classes – SAM analogues and non-SAM analogues. Researchers have made their focus on the development of non-SAM analogues. As can be seen from the fact that except **Sinefungin**,[20] **DS-437**,[21] **A9145C**,[20] compound **1**,[22] **LLY-283**[25] and **JNJ-64619178**,[26] all the reported PRMT5 inhibitors belong to non-SAM analogues. Among them, **GSK-3326595** exhibited the most potent inhibitory activity at enzymatic level, with an IC_{50} of 6.2 nM.[28] In phase I clinical trial, dose escalation study of this compound in solid tumors and non-Hodgkin's lymphoma is undergoing. Its analogue **EPZ015666**[5] displayed effective anti-tumor activity in mantle cell lymphoma (MCL) xenograft models and has been used as a tool compound to investigate the oncogenic role of PRMT5. The Co-crystal structure of **EPZ015666** with PRMT5:MEP50 complex (PDB code: 4X61)[9] indicated that this series of compounds occupied the substrate binding pocket of PRMT5.

Given the therapeutic potential of PRMT5 in cancer treatment and its limited inhibitors, there is an urgent need to discover more inhibitory molecules. In this study, we reported the design, synthesis and biological evaluation of a new potent PRMT5 inhibitor (**4b14**) with high selectivity over other two key members of PRMTs (PRMT1 and PRMT4). A panel of leukemia and lymphoma cells, including MV4-11, Pfeiffer, SU-DHL-4 and KARPAS-422, were found to be sensitive to this

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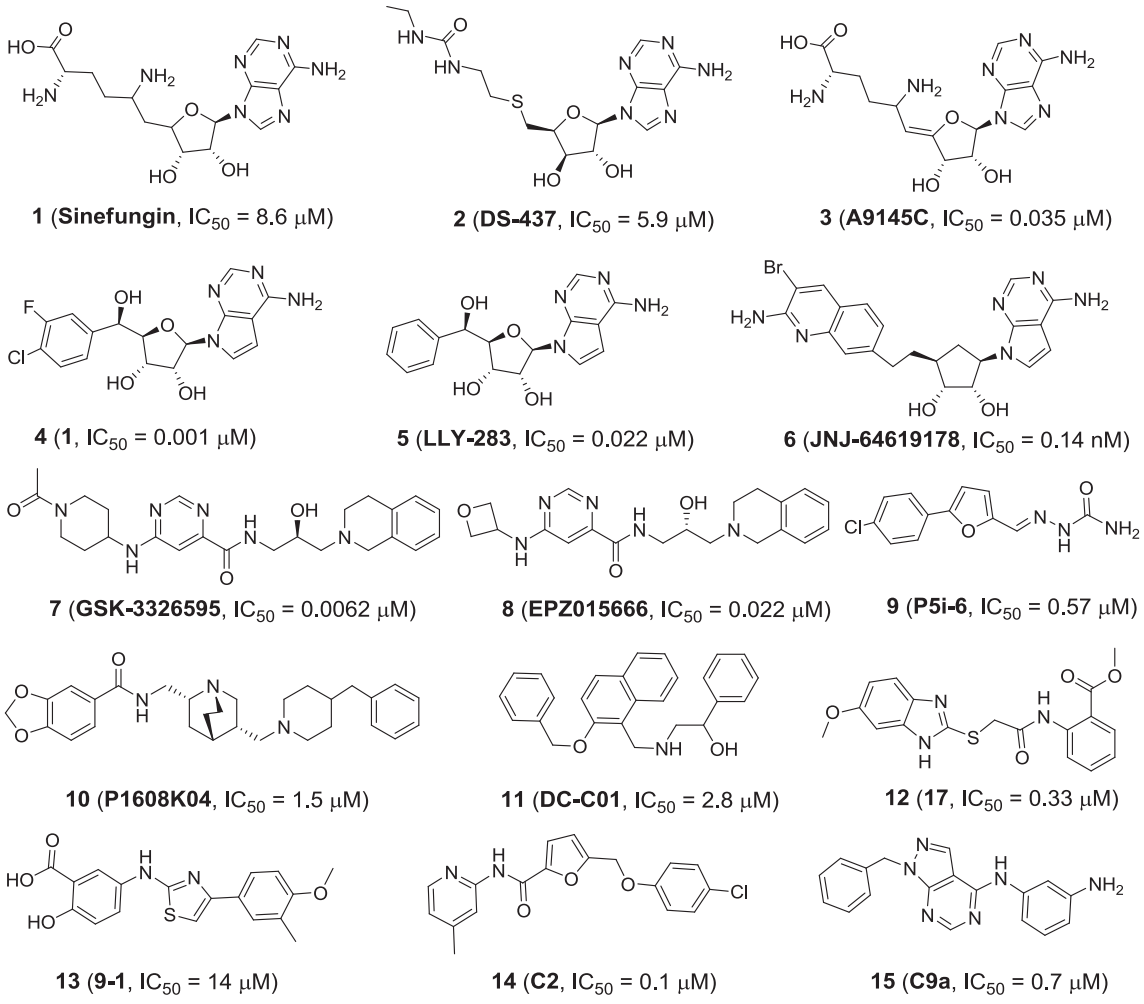


Fig. 1. Reported PRMT5 inhibitors.

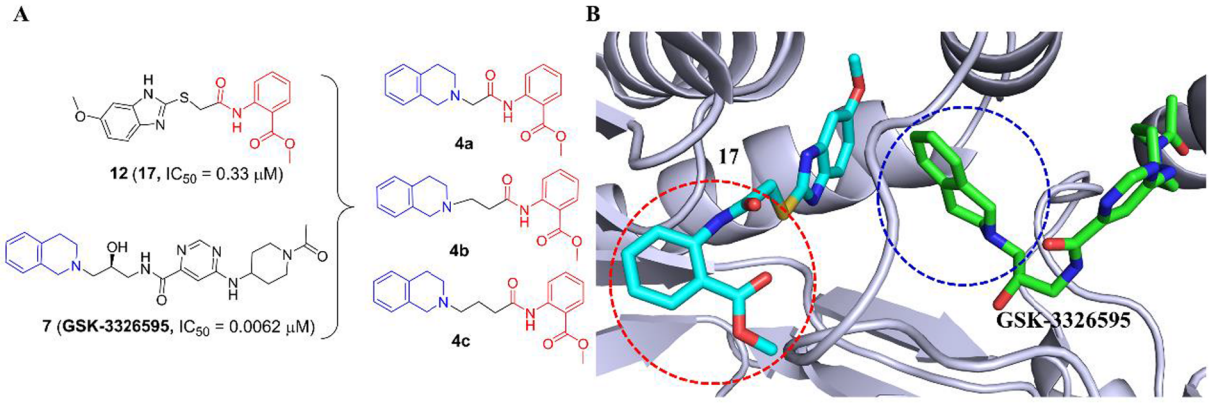
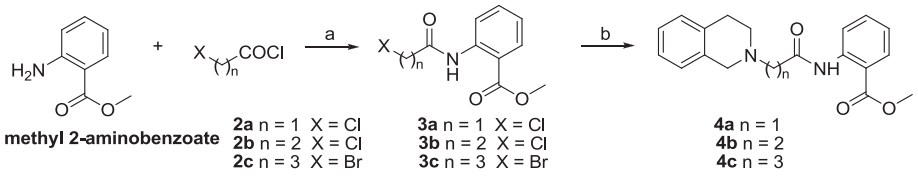
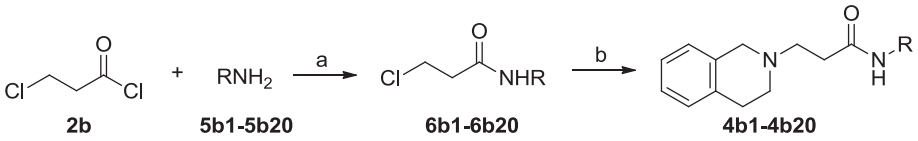


Fig. 2. Design strategy of compound **4a, 4b, and 4c**. (A) Chemical structures of **17** and **GSK-3326595**. (B) Superimposition of binding modes of **17** and **GSK-3326595.**

compound with EC_{50} values lower than 16.0 μM. Flow cytometric analysis demonstrated that **4b14** showed obvious cell cycle arrest and apoptosis-inducing effects. In SU-DHL-4 and KARPAS-422 cells, **4b14** reduced the cellular symmetric arginine dimethylation levels of SmD3, a protein that has been popularly used to characterize the cellular biochemical activity of PRMT5.[29,30] The binding mode of **4b14** with PRMT5 was probed by molecular docking and further validated by molecular dynamics simulation. Affinity binding profiling analysis indicated that hydrophobic interactions, π-π stacking and cation-π actions made the major contributions to the overall binding affinity.



Scheme 1. Synthesis of **4a–4c**. Reagents and conditions: a) triethylamine, dichloromethane, 0 °C to r.t., overnight, 62–84%; b) 1,2,3,4-tetrahydroisoquinoline, triethylamine, acetonitrile, microwave heating 100 °C, 10 min, 66%-83%.



Scheme 2. Synthesis of **4b1–4b20**. Reagents and conditions: a) triethylamine, dichloromethane, 0 °C to r.t., overnight, 57%–85%; b) 1,2,3,4-tetrahydroisoquinoline, triethylamine, acetonitrile, microwave heating 100 °C, 10 min, 42%–79%.

Design and synthesis PRMT5 inhibitors

Compound **17** and **GSK-3326595** were previously reported PRMT5 inhibitors. While **17** was identified and proposed to occupy the SAM binding site in our recent work,[16] **GSK-3326595** was confirmed as a substrate binding site inhibitor.[9] A detailed analysis of their binding modes allowed the identification of key pharmacophores in each compound, and the following anticipation of the merging of these fragments including tetrahydroiso-quinoline (blue color) and methyl 2-acetamidobenzoate (red color). In order to investigate the optimal distance between both fragments for desired activity, three alkyl linker (black color) was first rationally exploited to yield the new potential PRMT5 inhibitors **4a–4c** (Fig. 2).

The synthetic route for **4a–4c** was shown in Scheme 1. Briefly, the intermediates **3a–3c** were prepared with good yields of 62%–84% through acylation reaction of methyl 2-aminobenzoate with corresponding acyl chlorides **2a–2c**. Then **3a–3c** were reacted with 1,2,3,4-tetrahydroisoquinoline under microwave condition to give corresponding target compounds **4a–4c** in 66%–85% yields. The three compounds were then evaluated for PRMT5 inhibitory activity at enzymatic level. To investigate the substituent effect of phenylamine fragment, twenty analogues of **4b** were synthesized. Scheme 2 showed the preparation of analogues **4b1–4b20** (Table 1), from the staring materials **2b** and **5b1-5b20** in 33%-58% overall yields, which was achieved according to the same protocol as that of **4a–4c**.

PRMT5 inhibitory activity evaluation and structure-activity relationship analysis

Radiometric-based scintillation proximity assay (SPA) was used to detect the inhibitory activity of the synthesized **4a–4c** against PRMT5. The bioassay results showed that compound **4b** displayed better PRMT5 inhibitory activity (IC_{50} = 31.62 ± 2.15 μM) than **4a** and **4c**, indicating that propionyl group (−$(CH_2)_2$CO−) is the superior linker for the improved activity.

To expand the substituent/fragment diversity and investigate electronic or steric effect on improving the PRMT5 inhibitory activity, compound **4b** was next used as a template based on the above bioassay result. The introduction of different substituents into phenylamine moiety or replacement of phenylamine moiety by other fragments were carried out with chemical agents in hand, which resulted into the production of **4b1–4b20**. Among the compounds (**4b1–4b4**) with *ortho*-substituents, **4b4** with 2-CN showed slightly better activity (IC_{50} = 25.55 ± 1.67 μM) compared with **4b**. From the assay results of **4b6–4b13**, compound **4b9** with 3-Br showed the best activity (IC_{50} = 18.12 ± 0.71 μM), while others with more bulky or electron-withdrawing group at 3-position had decreased activity or no obvious inhibition on PRMT5. Since compounds **4b4** and **4b9** exhibited increased activity, analogue **4b14** with 3-Br and 2-CN was next prepared. As expected, **4b14** showed the most potent activity with an IC_{50} value of 2.71 ± 0.21 μM, being 11.7-fold as active as the initial hit **4b**. Further introduction of substituents on the 4-position of benzene (**4b15–4b17**) did not have positive effects on the PRMT5 inhibitory activity. In addition, replacement of the benzene ring with bulky naphthalene or quinolone moieties also led to the loss of activity.

Selectivity test for 4b14

In enzyme-inhibitory assays using PRMT1 and PRMT4, compound **4b14** was assessed for its activity against the two enzymes to confirm its selectivity. As shown in Fig. S1 (Supporting information), the IC_{50} values of **4b14** against PRMT1 and PRMT4 were both above 200 μM, which indicated that **4b14** was a selective inhibitor of PRMT5 over PRMT1 and PRMT4.

Mechanism of action and binding mode analysis

The direct binding of compound **4b14** to PRMT5 was confirmed by surface plasmon resonance (SPR) assay with a K_D (equilibrium dissociation constant) value of 10.8 ± 3.2 μM (Fig. 3A). The mechanism of action (MOA) of **4b14** was assessed by determining the IC_{50} values in the presence of various concentrations of SAM and peptide. As shown in Fig. 3B, **4b14** displayed competitive inhibition with peptide while noncompetitive with SAM. This results indicated that **4b14** was a substrate binding site inhibitor of PRMT5. To probe the probable binding mode of **4b14** with PRMT5, molecular docking and molecular dynamics simulation were performed. The putative binding mode and the detailed interactions between **4b14** and PRMT5 were shown in Fig. 3C and D, respectively. As indicated in Fig. 3D, although there are a few polar interactions, the most are from hydrophobic, π-π stacking and cation-π interactions as expected.

Based on this binding mode, 100 ns molecular dynamics (MD) simulation were performed to further validate the putative binding mode and calculate the binding free energy. As shown in Fig. S2 (Supporting information), the interactions between **4b14** and PRMT5 were found to be stable during the MD simulation, by analyzing the root mean square deviation (RMSD) values of PRMT5 (heavy atoms Cα, C, N) and **4b14**, respectively. Then the binding free energy was obtained by MM/PBSA method to be −36.38 kcal/mol, which was well in accordance with its enzymatic inhibition activity.

Table 1
Inhibition of PRMT5 by series of compounds **4**.

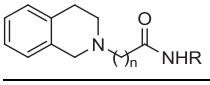
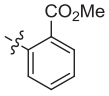
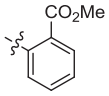
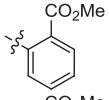
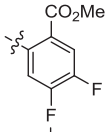
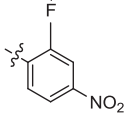
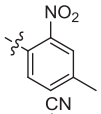
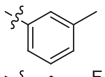
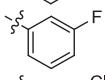
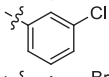
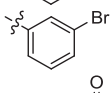
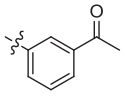
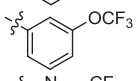
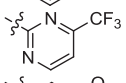
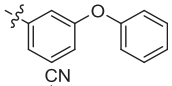
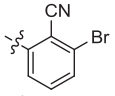
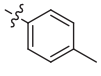
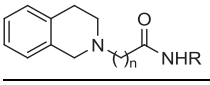
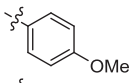
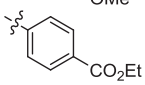
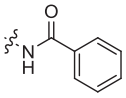
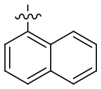
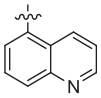
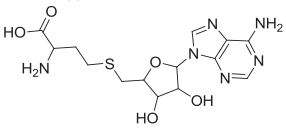
Compound	 n	R	IC_{50}[a] ± SD[b] (μM)
4a	1		60.02 ± 1.44
4b	2		31.62 ± 2.15
4c	3		87.89 ± 4.27
4b1	2		> 100
4b2	2		> 100
4b3	2		> 100
4b4	2		25.55 ± 1.67
4b5	2		> 100
4b6	2		> 100
4b7	2		45.87 ± 3.14
4b8	2		42.13 ± 2.97
4b9	2		18.12 ± 0.71
4b10	2		51.95 ± 2.55
4b11	2		62.95 ± 2.55
4b12	2		69.87 ± 2.54
4b13	2		> 100
4b14	2		2.71 ± 0.21
4b15	2		> 100

Table 1 (*continued*)

Compound	 n	R	IC_{50}[a] ± SD[b] (μM)
4b16	2		> 100
4b17	2		> 100
4b18	2		72.65 ± 3.21
4b19	2		> 100
4b20	2		> 100
Control (SAH)			0.56 ± 0.03
8 (EPZ015666)			0.047 ± 0.009

[a] IC_{50}: half maximal inhibitory concentration.
[b] SD: standard deviation.

Anti-proliferative activity and cellular symmetric dimethylation effects of 4b14

One leukemia (MV4-11) and three lymphoma (Pfeiffer, SU-DHL-4 and KARPAS-422) cell lines were used to measure the anti-proliferative activity of **4b14**. As shown in Fig. 4A, 4b14 showed a dose-dependent inhibition against the four types of cells with 4-day IC_{50} values ranging from 9.14 μM to 16.42 μM.

The effects of **4b14** and **EPZ015666** (positive control) on cellular symmetric arginine dimethylation in KARPAS-422 cells (Fig. 4B and Fig. S3) were also tested by immunoblot using symmetric dimethyl arginine (SDMA) antibody. In agreement with **EPZ015666**, treatment of **4b14** on KARPAS-422 cells led to a concentration-dependent decrease in the level of symmetric arginine dimethylation of PRMT5 substrate SmD3, while in SU-DHL-4 cells this effect was not so obvious. The assay results demonstrated that **4b14** could target PRMT5 in a cellular context.

Inhibition of cell cycle progression and induction of cell apoptosis

PRMT5 inhibitors could result in the inhibition of cell proliferation and/or induction of apoptosis.[31] Thus, we investigated whether **4b14** could affect the progression of human lymphoma cancer KARPAS-422 cells through the cell cycle. As shown in Fig. 5A, the control cells displayed a characteristic distribution of cells in different phases of the cell cycle. In contrast, compound **4b14** arrested the cell cycle in the G1 phase in a concentration-dependent manner, which was indicative of the cellular engagement of **4b14** on targeting PRMT5.

To confirm the capacity of **4b14** to induce apoptosis, KARPAS-422 cells were treated with the compound for 48 h. As can be seen in

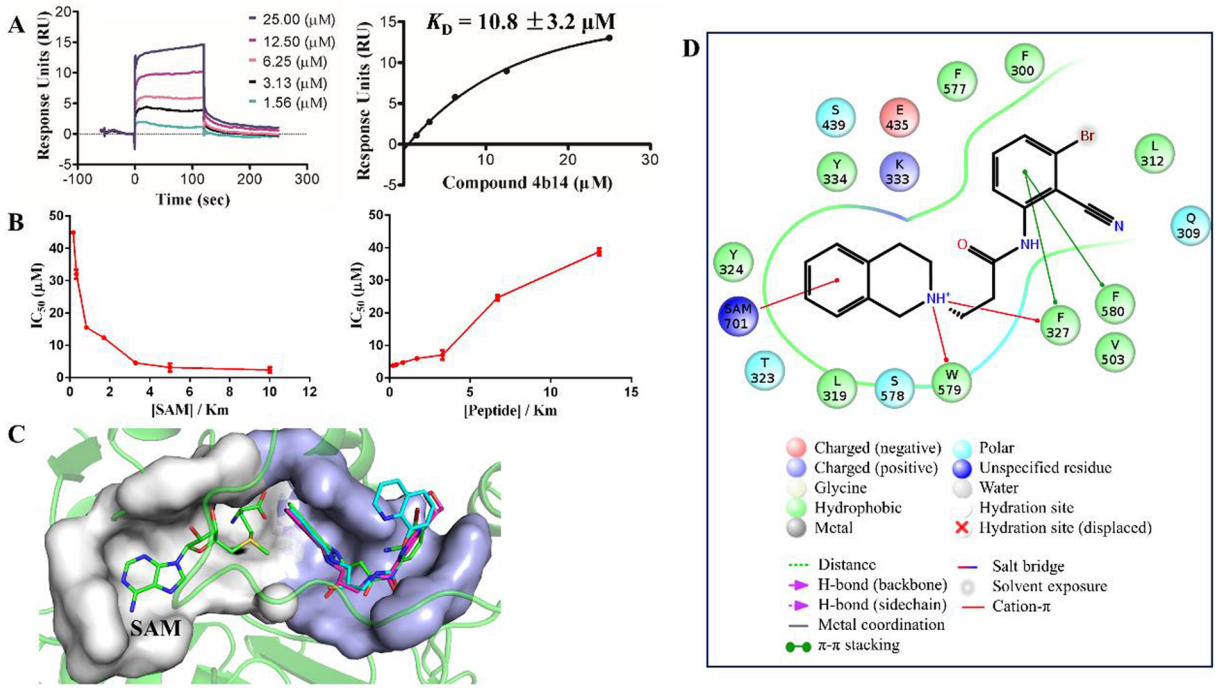
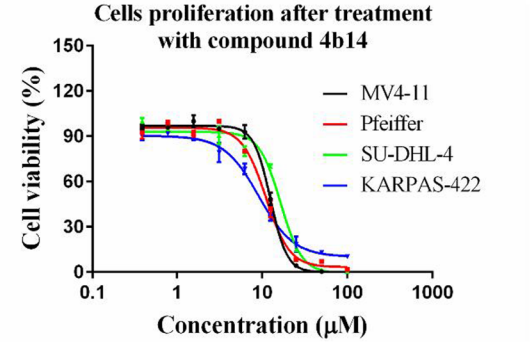


Fig. 3. Mechanism of action and binding mode prediction of **4b14**. (A) Binding of **4b14** to PRMT5 was confirmed by SPR. (B) Mechanism of action (MOA) of **4b14**. MOA was assessed by determining the IC_{50} values in the presence of various concentrations of peptide and SAM. Experiments were performed in triplicate. (C) The proposed binding mode of **4b14** with PRMT5. (D) The detailed interactions between **4b14** and PRMT5.

A

Compounds	IC_{50} (μM) MV4-11	Pfeiffer	SU-DHL-4	KARPAS-422
4b14	12.5±0.7	11.1±1.5	16.4±2.8	9.1±1.5
8 (control)	1.8±0.8	1.1±0.5	2.6±0.6	0.96±0.3



B

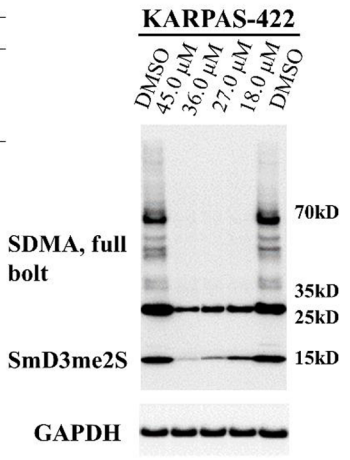


Fig. 4. Antiproliferative effect and arginine methylation alterations upon treatment with **4b14** in cell-based assays. (A) Treatment of MV4-11, Pfeiffer, SU-DHL-4 and KARPAS-422 cells with **4b14** inhibited cell proliferation. Results shown are mean ± SD of three replicates. (B) Treatment with **4b14** for 72 h dose-dependently inhibited the methyltransferase activity of PRMT5 in KARPAS-422 cells. SDMA levels were detected by Western blotting.

Fig. 5B, compared to the control group, **4b14** was capable of inducing apoptosis in a dose-dependent manner. After 48 h, the maximum apoptotic rate of ~20% was achieved at the concentration of 16.0 μM.

In conclusion, new PRMT5 inhibitors were successfully discovered in the current study by combination of key pharmacophores from the reported PRMT5 inhibitors. Among all the analogues, compound **4b14** showed the most potent PRMT5 inhibitory activity with an IC_{50} value of 2.71 ± 0.21 μM. Besides, this compound also displayed moderate antiproliferative activity against a panel of leukemia (MV4-11) and lymphoma (Pfeiffer, SU-DHL-4 and KARPAS-422) cells, with IC_{50} values all below 16.0 μM. Flow cytometric analysis demonstrated that **4b14** exhibited obvious cell cycle arrest and apoptosis-inducing effects. In SU-DHL-4 and KARPAS-422 cells, **4b14** reduced the cellular symmetric arginine dimethylation levels of SmD3, a protein used to characterize the cellular biochemical activity of PRMT5. Besides, the binding mode of **4b14** with PRMT5 was probed by molecular docking and further validated by molecular dynamics simulation. Affinity binding profiling analysis indicated that hydrophobic interaction contributed mainly to the overall binding affinity. This compound provides a new scaffold for further PRMT5 inhibitor development.

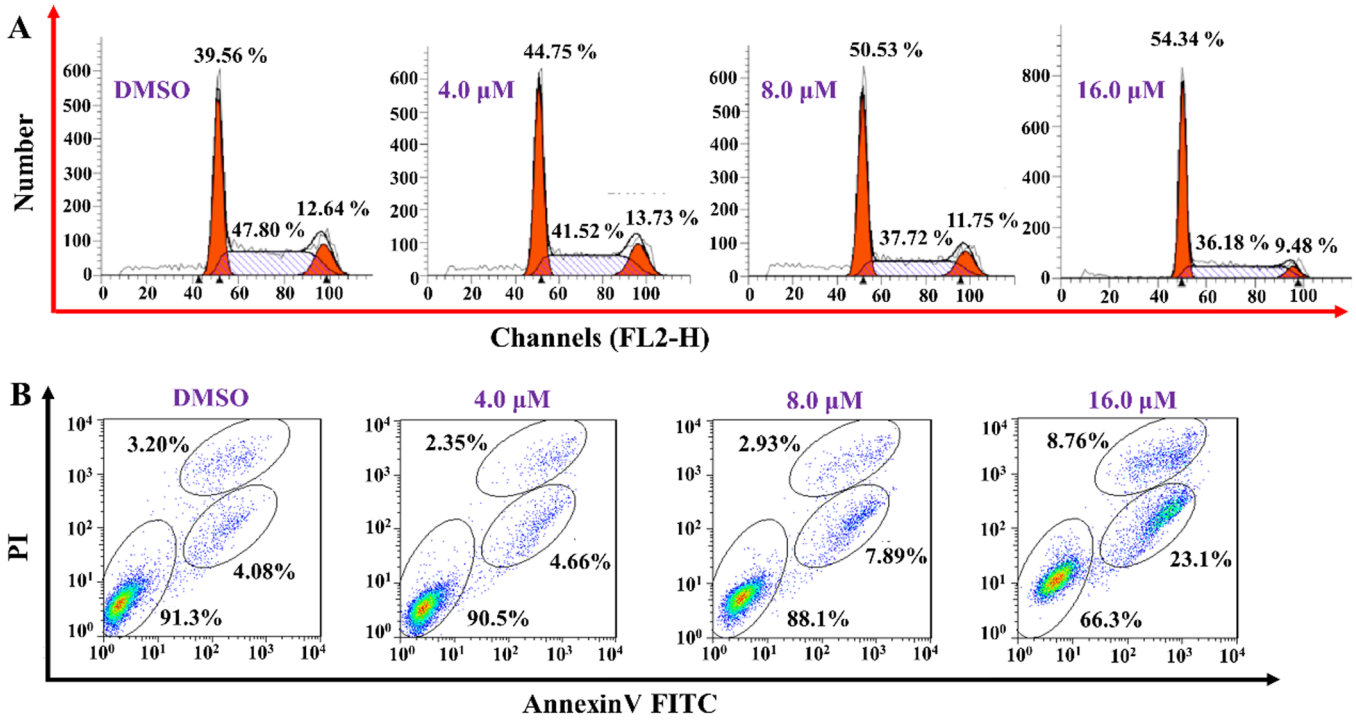


Fig. 5. Cell cycle progression and of cell apoptosis analysis. (A) Treatment with **4b14** arrested KARPAS-422 cells at G1 phase at 48 h. (B) Treatment with 4b14 induced apoptosis of KARPAS-422 cells at 48 h.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2018.10.026.

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