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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Identification of protein arginine methyltransferase 7 (PRMT7) inhibitor by virtual screening and biological evaluation in vitro

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Abstract

Although protein arginine methyltransferase 7 (PRMT7) is an important mediator in various biological processes, its specific inhibitors remain to be identified. To identify novel inhibitors of PRMT7, we utilized high-throughput virtual screening of the ChemDiv database for novel PRMT7 inhibitors. Eight compounds were identified, and among them, compound V009-0749 exhibited potent anticancer activity against the HepG2 hepatocellular carcinoma cell line in a dose-dependent manner. It inhibited the activity of PRMT7 by decreasing the histone H4 Arg 3 symmetric dimethylation (H4R3me2s) modification level in the HepG2 and Hep3B carcinoma cell lines. Furthermore, compound V009-0749 led to HepG2 and Hep3B cell cycle G1 phase arrest and cell apoptosis. Molecular modeling studies also suggested that compound V009-0749 had strong affinity for the binding site of PRMT7 by forming six hydrogen bonds and significant hydrophobic interactions. Compound V009-0749 could serve as a lead compound targeting PRMT7 activity, and lay the foundation for investigating the role of PRMT7 in hepatocellular carcinoma and other diseases.

Keywords Virtual screening · PRMT7 · Proliferation · Hepatocellular carcinoma

Introduction

Epigenetic regulation, including DNA methylation, histone methylation, histone acetylation, and phosphorylation, is closely associated with tumorigenesis (Jaenisch and Bird 2003). The protein arginine methyltransferase PRMT7 is a type II methyltransferase that is capable of mediating symmetric dimethyl-arginine modification (Lee et al. 2005). It has been reported that PRMT7 participates in multiple physiological or pathological events. For example, PRMT7 regulates DNA damage by methylating histones H2A and H4 (Karkhanis et al. 2012). In cellular differentiation, PRMT7 inhibits MLL4 target genes by upregulating the symmetrical dimethylation of H4R3 (H4R3me2s) modification levels (Dhar et al. 2012).

Recently, it has been reported that PRMT7 promotes cancer metastasis by inhibiting E-cadherin transcription through a cascade of epigenetic mechanisms (Yao et al. 2014). PRMT7 has been reported to also methylate the splicing of associated proteins, regulate DNA repair-associated genes, and play a role in breast cancer metastasis. Therefore, in view of these PRMT7 effects in the prevention of cancers and aging diseases, it is reasonable to hypothesize that the development of PRMT7 inhibitors might present efficient and novel therapeutic remedies for cancers and aging diseases.

Due to the beneficial effects of PRMT7 inhibitors on human diseases, there has been great interest regarding the discovery and in developing molecules able to regulate PRMT7 activity. Recently, Smil et al. designed DS-437 (Fig. S1), a protein arginine methyltransferase 5 (PRMT5)

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inhibitor with a half maximal inhibitory concentration (IC50) value of 6 μM against PRMT5 and PRMT7 that is the first dual PRMT5-PRMT7 inhibitor that reduces symmetrical dimethylation of PRMT5 substrates (Smil et al. 2015). Another PRMT inhibitor, EPZ011652 (Fig. S1), was closely associated with metabolic clearance in rat hepatocytes (Rioux et al. 2015). Subsequently, selective small molecule inhibitors of type I PRMTs (PRMT-1, -3, -4, -6, and -8) have been pursued as chemical tools for testing the therapeutic effect, and MS023 and MS094 (Fig. S1) could be used as chemical tools for studying the role of type I PRMTs in disease (Eram et al. 2016). In recent years, type I PRMT inhibitors Compound 28d and AMI-1 (Fig. S1) had been reported to significantly decrease cell proliferation in leukemia cells and gastric cancer cells (Wang et al. 2017, Zhang et al. 2017). Therefore, it is urgent to pursue and develop novel small molecules targeting PRMT7.

In this study, we designed and screened existing drugs against PRMT7 and confirmed that compound V009-0749, which specifically targets PRMT7, inhibited the activity of PRMT7 by decreasing the H4R3me2s modification level. Furthermore, we found that compound V009-0749 reduced hepatocellular carcinoma (HCC) cell viabilities in a dose-dependent manner. Compound V009-0749 also led to HCC cell cycle G1 phase arrest and cell apoptosis. We showed that small molecule inhibitor V009-0749 is a promising candidate for further development as an anti-tumor agent for the treatment of HCC.

Materials and methods

High-throughput virtual screening

Because the crystal structure of human PRMT7 has not yet been resolved, a homologous model of human PRMT7 generated by SWISS-MODEL workspace (Arnold et al. 2006, Benkert et al. 2011, Biasini et al. 2014) (https://swissmodel.expasy.org/repository/uniprot//Q9NVM4) was used. This model was constructed based on the crystal structure of mouse PRMT7 (PDB ID: 4c4a), of which 85% of its amino acid sequences are identical to human PRMT7. The small molecule database used was the ChemDiv database, which is commercially available from TopScience Co. (Shanghai, China). The Surflex molecular docking module in Sybyl-X2.1 (Sybyl-X2.1 is available from Tripos Associates Inc., S. H. R., St. Louis, MO 631444, USA.) was used for high-throughput virtual screening. During the preparation of the receptor, the space where ligand S-adenosyl-L-homocysteine (SAH) was placed was selected as the binding site. To accelerate the virtual screening, a high-speed screening was carried out by decreasing the maximum quantity of conformations and rotatable bonds from 20 to 10, and from 100 to 50, respectively. Then, the molecules with a docking score within the top 1% were screened once more using the default docking parameters. The molecules with favorable characteristics were commercially purchased for the following in vitro biological activity assay.

Cell culture and western blot

The HepG2 human liver carcinoma cell line was purchased from the American Type Culture Collection (ATCC). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37°C with 5% CO2. Western blot was carried out as previously reported (Yao et al. 2014). The H4R3me2s (Epigentek) antibody was used against H3 (Proteintech).

Cell viability assay

Cell viability was measured with a Cell Counting Kit-8 (CCK-8) kit according to the manufacturer's procedure (Beyotime, China). Briefly, indicated cells were seeded at 1×10^4/well in 100 μl medium treated with different concentrations of compounds to be tested in a 96-well plate. After 48 h, 5 μl CCK-8 was added to each well, and the plate was incubated for 3 h at 37 °C. Then, the absorbance was read at 450 nm.

Cell cycle and apoptosis assay

Cell cycle was determined by flow cytometry using propidium iodide (PI) staining (Yao et al. 2017). The indicated cells treated by compound V009-0749 were washed and fixed by ethanol. Then, the cells were resuspended in phosphate-buffered saline (PBS) containing PI and RNase A for 30 min of incubation. The DNA content was analyzed with a FACSCalibur Flow Cytometer. The apoptosis assay was performed with an Annexin-V–FITC/PI staining kit according to the standard protocol (KeyGEN, China).

Statistical analysis

The data were analyzed by GraphPad Prism 5.0 software (GraphPad Software, Inc, USA). The data are presented as the mean ± SD as indicated. Student's *t*-test was used to determine the significance between groups. $P < 0.05$ was considered statistically significant.

Results and discussion

High-throughput virtual screening

Eight commercially available compounds were selected from the two rounds of high-throughput virtual

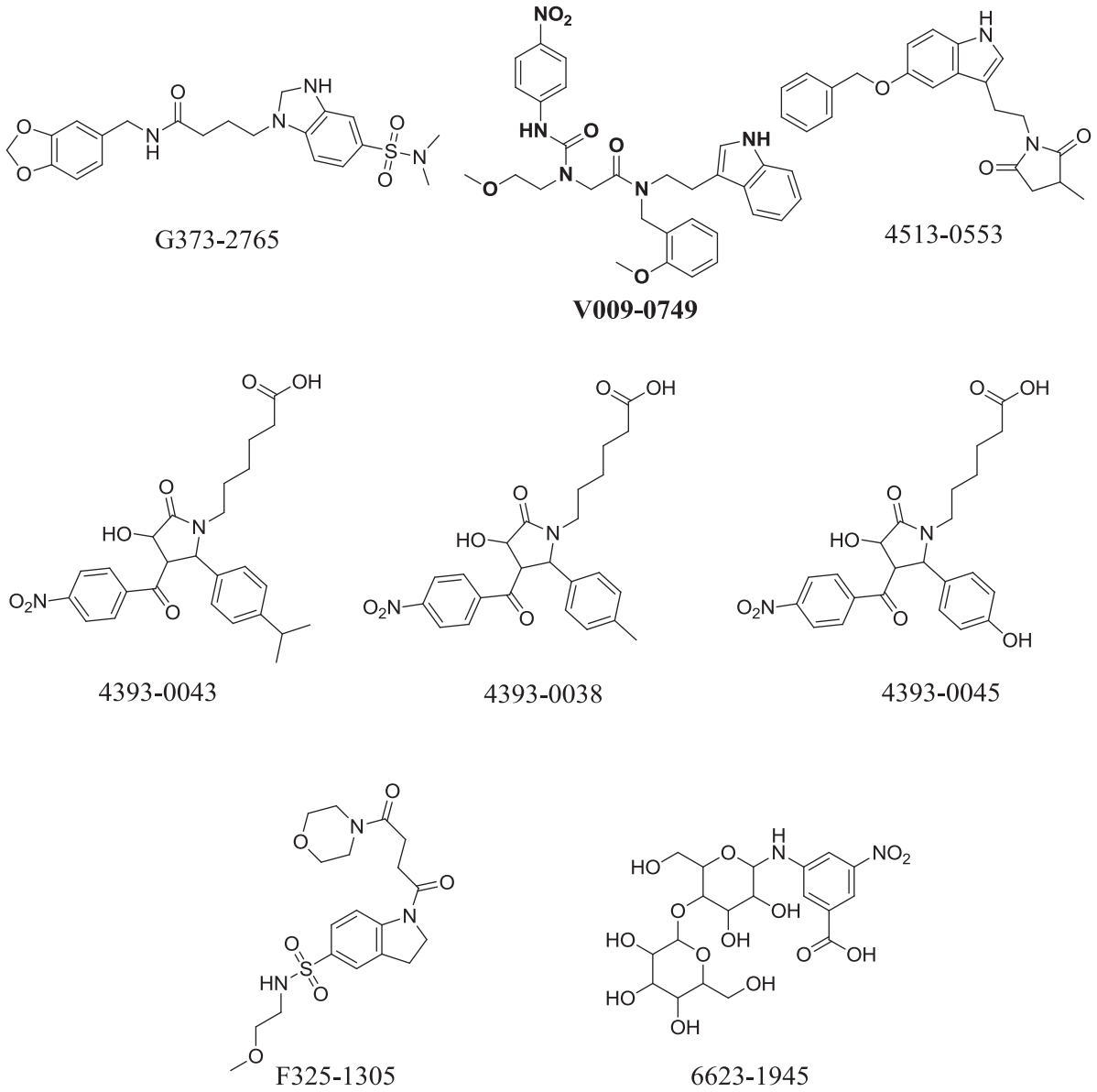

Fig. 1 Chemical structures of eight hits obtained through high-throughput virtual screening of the ChemDiv database

screenings by ranking the docking scores and clustering analysis. The docking scores of eight hit compounds including G373-2765, V009-0749, 4513-0553, 4393-0043, 4393-0038, 4393-0045, F325-1305, and 6623-1945 (commercially available from TopScience Co. (Shanghai, China)) were 11.0, 10.7, 10.7, 10.1, 10.1, 9.7, 9.7, and 9.6, respectively (Fig. 1 and Table 1). Because potent PRMT7 inhibitors should possess appropriate anti-proliferative activity in cancer cells, the hits from the virtual screening were firstly evaluated in vitro by cancer cell growth inhibition prior to PRMT7 enzyme activity inhibition.

Table 1 In vitro viability of cultured cells treated with eight hits against the HepG2 cell line

Compounds	Survival rate at 10 μM (%)	Survival rate at 30 μM (%)
G373-2765	86.5 ± 2.97	80.8 ± 2.74
V009-0749	**68.5 ± 2.87**	**56.4 ± 3.33**
4513-0553	71.9 ± 2.70	69.7 ± 1.82
4393-0043	98.9 ± 1.43	93.7 ± 2.58
4393-0038	91.7 ± 1.42	90.4 ± 0.94
4393-0045	93.2 ± 3.06	89.5 ± 5.36
F325-1305	70.6 ± 1.24	68.5 ± 1.71
6623-1945	96.6 ± 1.27	83.5 ± 4.50

V009-0749 is the only one compound which had potent activity in this study compared to the other compounds

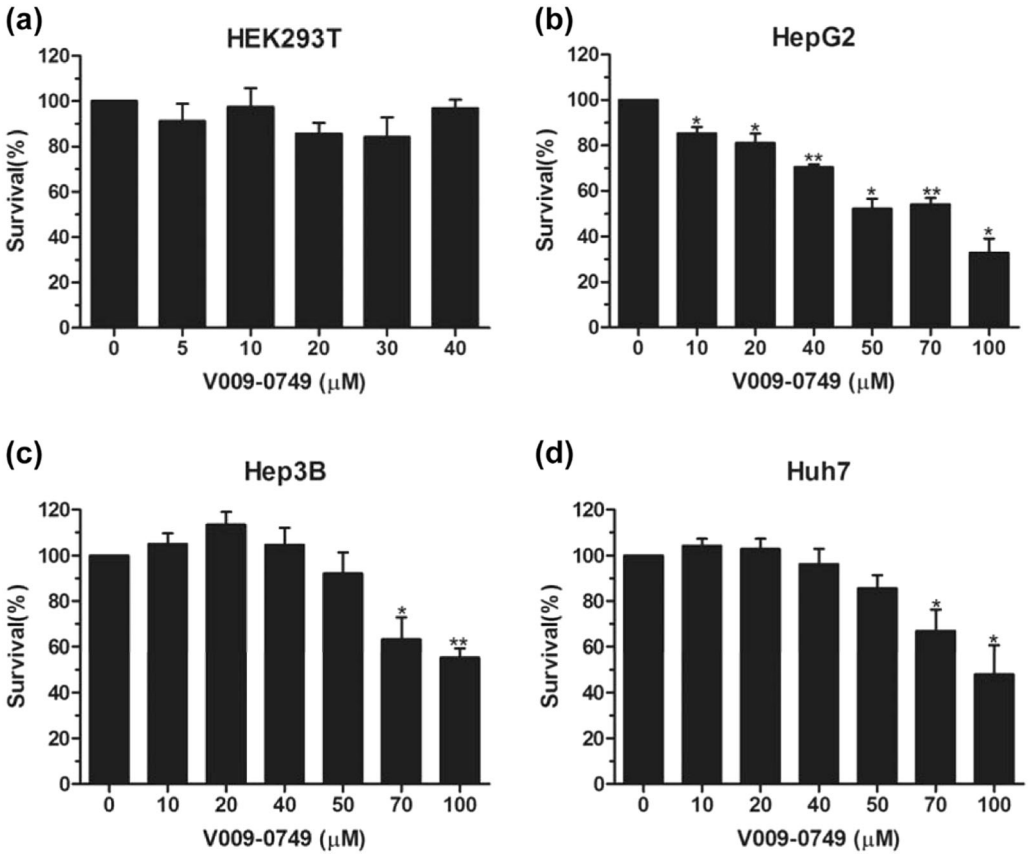

Fig. 2 Viability of cultured cells treated with increasing concentrations (0-50 μM) of compound V009-0749 for 48 h was assessed by the CCK-8 assay in **a** HEK293T, **b** HepG2, **c** Hep3B, and **d** Huh7 cell lines. All experiments were independently repeated at least three times. Error bars, mean ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$

In vitro tumor cell killing activity of eight hits

To determine the cytotoxic potential of the eight hits using the human HCC cell line, the CCK-8 assay was used with the HepG2 cell line. As shown in Table 1, the eight hits exhibited a low in vitro inhibition rate against HepG2 cells at concentrations of 10 or 30 μM, except for compound V009-0749, which moderately inhibited the growth of HepG2 cells at 30 μM. Firstly, we analyzed the effect of compound V009-0749 on normal human embryonic kidney HEK293T cells. As shown in Fig. 2a, compound V009-0749 did not decrease the viability of HEK293T cells. Further studies showed that compound V009-0749 treatment inhibited HepG2 cell proliferation in a dose-dependent manner, with an IC50 of 36 μM (Fig. 2b). However, Hep3B and Huh7 cells treated with compound V009-0749 were inhibited only at high concentrations (Fig. 2c, d). Compound V009-0749 demonstrated excellent anti-tumor activity compared with the effect on normal cells. This is the first report describing the biological function of small molecule V009-0749 in HCC, which increases the knowledge of HCC progression. Decreasing cell viabilities were closely associated with cell cycle arrest and apoptosis (Vermeulen et al. 2005, Lu et al. 2016). Thus, it was unknown whether compound V009-0749 participated in cell apoptosis and cell cycle arrest in HCC cell lines.

Effect of compound V009-0749 on the cell cycle progression and apoptosis of HCC cells

Because compound V009-0749 inhibited HCC cell proliferation, and cell survival was closely correlated with cell cycle, flow cytometry analysis was employed to examine the effect of compound V009-0749 on cell cycle progression. Our preliminary results showed that low concentration of compound V009-0749 had no influence on cell cycle (data not shown). Incubation of HepG2 cells with 40 μM compound V009-0749 resulted in the accumulation of cells in the G1 phase (Fig. 3a). Similarly, compound V009-0749 also induced Hep3B cell cycle G1 phase arrest at a concentration of 40 μM (Fig. 3b). Subsequently, we detected the effect of compound V009-0749 on cell death. HepG2 cells treated with compound V009-0749 (40 μM) for 48 h were subjected to Annexin V-FITC and PI staining followed by flow cytometric analysis. As shown in Fig. 3c, d, HepG2 and Hep3B cells treated with V009-0749,

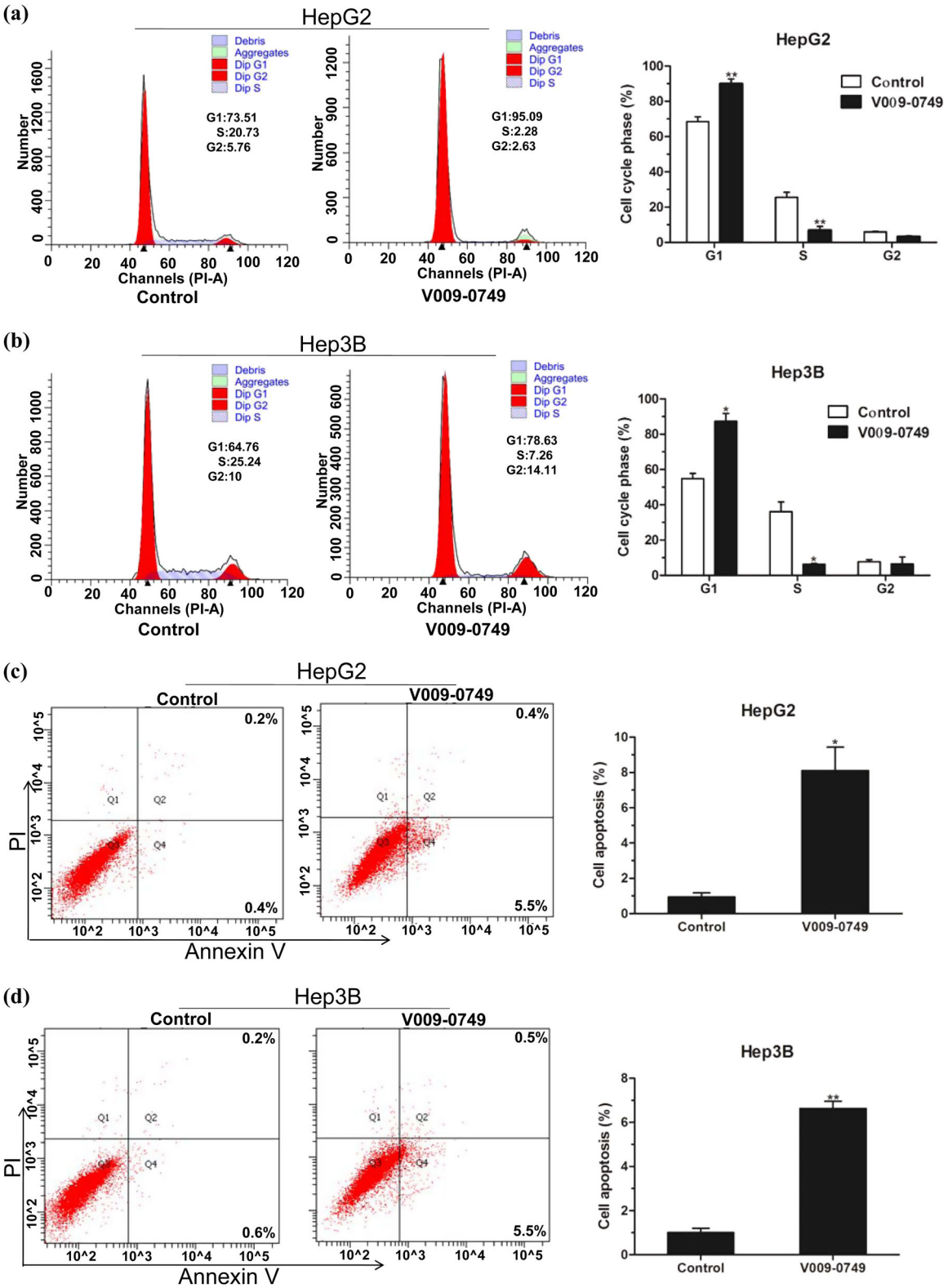

Fig. 3 Effect of compound V009-0749 on cell cycle progression and apoptosis. **a**, **b** Cell cycle analysis of HepG2 and Hep3B cell lines treated with Control and V009-0749 for 48 h. **c**, **d** HepG2 and Hep3B cells were treated with 40 μM V009-0749 for 48 h, and then, flow cytometric analysis of cell apoptosis was performed using Annexin V-FITC and PI. The error bars indicate the mean ± SD. $*p < 0.05$, $**p < 0.01$

respectively, become Annexin V-positive, suggesting a rapid onset of apoptosis. Cell cycle arrest and cell apoptosis could be the mechanisms that compound V009-0749 uses to mediate lower HCC cell viabilities.

V009-0749 inhibited PRMT7-mediated H4R3me2s activity

As an epigenetic modification enzyme, PRMT7's regulation of gene transcription mainly relies on the modification of H4R3me2s (Jelinic et al. 2006, Dhar et al. 2012, Karkhanis et al. 2012, Yao et al. 2014, Ying et al. 2015, Blanc et al. 2016). We investigated whether compound V009-0749 inhibited the enzyme activity of PRMT7. HepG2 cells were treated with compound V009-0749 for 48 h, and nuclear proteins were extracted. The Western blot results indicated that compound V009-0749 significantly decreased the H4R3me2s modification level compared with the DMSO control group (Fig. 4a), with histone H3 used as an internal control. We also found that the H4R3me2s level was decreased in Hep3B cells following treatment with 40 μM V009-0749 (Fig. 4b). Next, we would purify the PRMT7 protein and conducted in vitro methylation assays to further verify the impact of compound V009-0749 on the PRMT7-mediated H4R3me2s modification level. Collectively, our preliminary study confirmed that compound V009-0749 likely represented a potential scaffold for the further development of PRMT7 inhibitors to treat HCC.

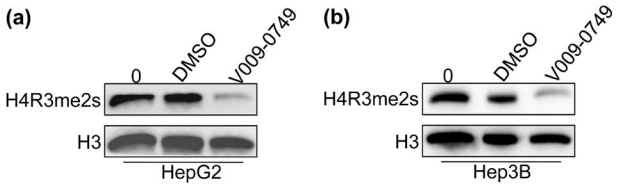

Fig. 4 Compound V009-0749 inhibited PRMT7 activity. **a** HepG2 and **b** Hep3B cell lines were seeded in 6-well plates at the appropriate density and treated with V009-0749 for 48 h. Nuclear proteins were then extracted, and Western blots were used to analyze the H4R3me2s levels; histone H3 was used as an internal control

Binding of the PRMT7 complex with compound V009-0749 from molecular docking and molecular dynamics simulation studies

The docked conformation of the PRMT7/V009-0749 complex obtained from the molecular docking-based virtual screening is shown in Fig. 5, illustrating successful docking into the binding site of PRMT7 (Fig. 5a). In detail, the nitryl of compound V009-0749 formed three hydrogen bonds with residues Arg44, Thr75, and Leu78. At the same time, three additional hydrogen bonds formed between compound V009-0749 and residues Glu144, Tyr35, and Glu153. The hydrogen-bonded network may greatly facilitate the binding affinity of compound V009-0749 to PRMT7. Additionally, the 2-methoxyphenyl group of compound V009-0749 was inserted into a hydrophobic pocket formed by residues Tyr35, Ser33, Met99, Glu29, and Phe96. The indole group of the inhibitor also had strong hydrophobic interactions with residues Val95, His122, Ser123, Glu153, Ser158, Leu145, and Ile71 (Fig. 5b).

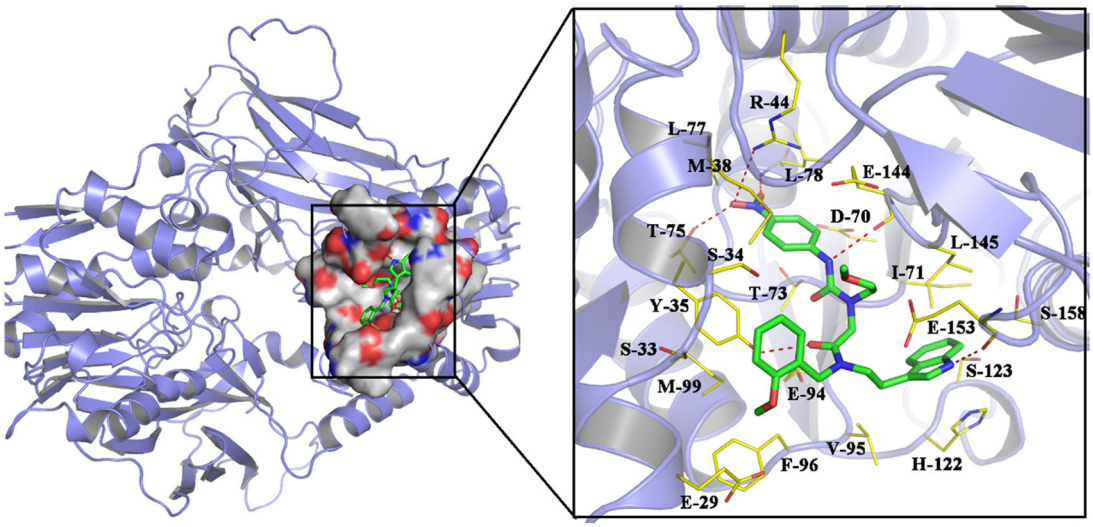

Fig. 5 Compound V009-0749 docked into the active site of PRMT7. PRMT7 residues within 4.5 Å distance from the ligand are shown with surface representation, and ligand V009-0749 is shown with green carbon atoms (left). A cartoon representation of the detailed binding of ligand V009-0749 to the active site of PRMT7 (right). The protein is colored in blue, with the key residues highlighted in yellow carbon atoms, red oxygen atoms, and blue nitrogen atoms. Ligand V009-0749 is shown as a stick representation. Hydrogen bonds are indicated by red dotted lines

Molecular dynamics simulation was also used to validate the obtained binding mode of PRMT7/V009-0749. To explore the dynamic stability of the complex, the root mean square deviation (RMSD) value of the protein (PRMT7) backbone atoms was calculated with the reference of the starting structure, and the results are shown in Fig. S2. It is likely that the conformation of the PRMT7/V009-0749 complex quickly reached equilibrium in 2 ns. The RMSD value of the complex fluctuated around 2.5 Å, which indicated that there was little conformational change of the complex in the last 8 ns of molecular dynamics simulation. The molecular mechanics/generalized Born surface area (MM/GBSA) binding free energy calculation showed that the binding affinity of the PRMT7/V009-0749 complex was -28.60 ± 2.82 kcal/mol, and the van der Waals (ΔE_{VDW}) and non-polar desolvation free energy (ΔG_{SA}) contributions were larger than those of the net electrostatic free energy ($\Delta E_{ELE} + \Delta G_{GB}$) (Table S1).

To obtain the quantitative contribution of each residue around the binding site, MM/GBSA free energy decomposition analysis was applied. The residue spectrograms of protein PRMT7 (Fig. S3) bound with compound V009-0749 were in good agreement with the molecular docking results. Compared to the qualitative molecular docking study, the molecular dynamics simulation combined with MM/GBSA free energy decomposition analysis suggested that Val95, His122, Glu144, Leu145, and Glu153 of protein PRMT7 were the key residues for inhibitor V009-0749's binding (the binding free energy of each residue was more than −1 kcal/mol). The binding modes of the remaining hits are depicted in Fig. S4.

Conclusions

Dysregulation of PRMTs has been linked to various tumors. PRMT7 is an important mediator of tumor progression and metastasis. However, to date, there are few known PRMT7 inhibitors. Herein, high-throughput virtual screening combined with biological activity assay was employed to screen the ChemDiv database for novel PRMT7 inhibitors. Compound V009-0749, obtained from virtual screening, exhibited characteristics of an excellent PRMT7 inhibitor that could decrease the H4R3me2s modification level in HCC cell lines. This compound exhibited potent anticancer activity against the HepG2 cell line in a dose-dependent manner. Furthermore, it led to HepG2 and Hep3B cell cycle G1 phase arrest and cell apoptosis. Our molecular simulations indicated that compound V009-0749 easily fit into the hydrophobic binding pocket of PRMT7 and formed six hydrogen bonds with the receptor. Compound V009-0749 may represent a valid scaffold for the further development of more potent PRMT7 inhibitors to treat HCC.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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