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Discovery of KDM5A Inhibitors: Homology Modeling, Virtual Screening and Structure-Activity Relationship Analysis

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ABSTRACT

Herein we report the discovery of a series of new KDM5A inhibitors. A three-dimensional (3D) structure model of KDM5A jumonji domain was firstly established based on homology modeling. Molecular docking-based virtual screening was then performed against commercial chemical databases. A number of hit compounds were retrieved. Further structural optimization and structure-activity relationship (SAR) analysis were carried out to the most active hit compound, **9** (IC_{50}: 2.3 μM), which led to the discovery of several new KDM5A inhibitors. Among them, compound **15e** is the most potent one with an IC_{50} value of 0.22 μM against KDM5A. This compound showed good selectivity for KDM5A and considerable ability to suppress the demethylation of H3K4me3 in intact cells. Compound **15e** could be taken as a good lead compound for further studies.

Keywords

KDM5A, histone lysine demethylation, structure-activity relationship, epigenetics, small molecule inhibitor

Histone lysine demethylation is one of the important histone modifications and plays a key role in epigenetic regulation.[1] Histone lysine demethylation is catalyzed by the histone lysine demethylases (KDMs). A number of KDMs have been identified at present.[2,3] These KDMs are categorized into two distinct classes based on their structure and mechanism, namely flavin-dependent histone lysine demethylases LSD1 and LSD2, and the 2-oxoglutarate-dependent, Jumonji domain-containing histone demethylases.[4]

Lysine demethylase 5A (KDM5A), also known as RBP2 or JARID1A, is a typical Jumonji domain-containing demethylase.[5] KDM5A can function as a transcriptional repressor through the demethylation of tri- and dimethylated histone lysines, such as H3 at lysine 4 (H3K4). A number of studies have shown that dysregulation of KDM5A is associated with the initiation and development of several human tumors, including breast cancer, prostate cancer, lung cancer, gastric cancer, and acute myeloid leukemia (AML).[6-8] Recent studies also demonstrated that KDM5A was involved in drug-resistance of anti-cancer drugs.[9-11] KDM5A has thus been thought as a potential anti-cancer drug target, and discovery of KDM5A inhibitors has attracted much attention in recent years. Unfortunately, just very limited number of KDM5A inhibitors have been reported at this moment.[3, 12, 13]

In this account, we report the discovery of a number of new KDM5A inhibitors with the aid of a molecule docking based virtual screening and structure-activity relationship analysis. For the most active compound, we further studied its selectivity against other KDMs and demethylation activity in intact cells.

Construction of a three-dimensional (3D) structure model of KDM5A with homology modeling. Since there is no 3D structure of KDM5A reported at present, we established a 3D structure model of the active site of KDM5A, namely jumonji

domain (JMJD),[14,15] with homology modeling in advance; MODELLER[16] in the Discovery Studio (Accelrys, San Diego, CA) (DS) was used for the homology modeling. The procedure for the model development is briefly described as follows.

Firstly, the amino acid sequence of human KDM5A jumonji domain, which contains 167 amino acids, was retrieved from UniProt database (http://www.uniprot.org) (UniProt entry P29375). PSI-BLAST program in DS was used for the template search. PSI-BLAST searched a number of templates, and four of them (PDB entries: 3DXT, 2W2I, 2OX0, and 2XML) possessed the minimum E-values (see Supplementary Table S1), which were used for the subsequent model construction.

Secondly, "Align Sequence to Templates" protocol in DS was used to align the target sequence to templates. Then the MODELLER program generated five structure models (see Supplementary Table S2). The model numbered KDM5A JMJD.M0002 was selected as an initial model for the succeeding optimization because it possesses the lowest values of PDF Total Energy and PDF Physical Energy.

Thirdly, the generated model was optimized. Here the "*standard dynamic cascade*" protocol in DS was used for the model optimization, and the CHARMM force field[17,18] was employed for the dynamic simulation. Various optimization methods, which differ in the used implicit solvent model and energy minimization algorithm, led to the generation of a total of 30 models (MS1-MS30, Supplementary Table S3).

All the generated models were finally evaluated by PROCHECK verification, Profile 3D evaluation, and Ramachandran Plot analysis. The detailed results are presented in Supplementary data (Supplementary Figure S1-S3). We finally chose MS28 as the 3D structure of KDM5A jumonji domain because it has the best

comprehensive performance in all the evaluation tests.

To mimic the active site of KDM5A, Fe(II) was also introduced manually into the jumonji domain of KDM5A according to the coordination models of similar KDMs with Fe(II) (For example, KDM4A, PDB entry 2OX0). The finally obtained structure model is shown in Figure 1.

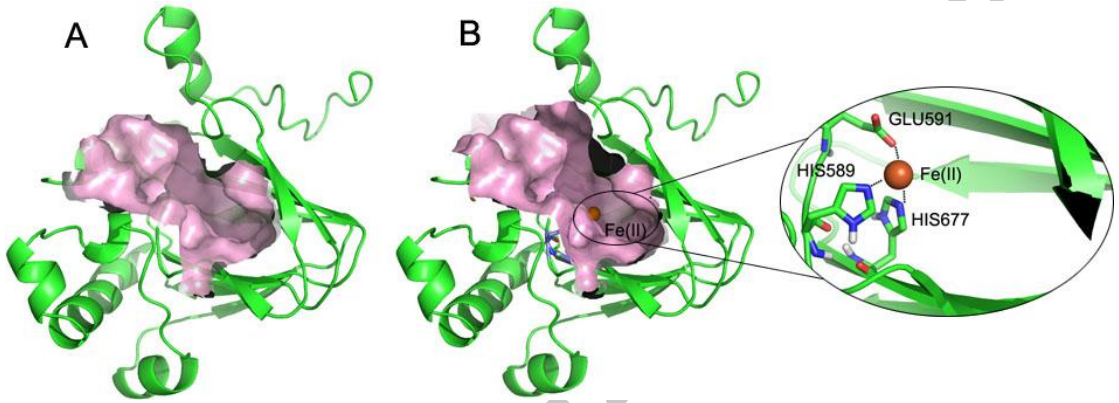


Figure 1. (A) The KDM5A jumonji domain homology model (MS28). The surface of 2-oxoglutarate (2OG)-binding site is colored in pink, while the cartoon defining the protein backbone is colored in green. (B) Fe(II)-containing KDM5A jumonji domain homology model. Fe(II) is colored in orange. Three key residues (Glu591, His589, His677) in the 2-oxoglutarate (2OG)-binding site are colored in green.

Virtual screening to retrieve new KDM5A inhibitors. Virtual screening based on molecular docking was carried out using the developed 3D structure of KDM5A jumonji domain. Chemical libraries used in the virtual screening include Specs (Specs, Inc. Zoetermeer, The Netherlands), Enamine (Enamine Ltd, Kiev, Ukraine), and ChemDiv (ChemDiv, Inc. San Diego, CA, USA), which contain about 1.6 million compounds. All the docking calculations were carried out with the GOLD program.[19] Goldscore[20] was chosen as the scoring function. From the top ranking compounds, forty-five compounds were selected for bioactivity validation. Twelve compounds

showed IC_{50} values less than 100 μM against KDM5A (see Figure 2).

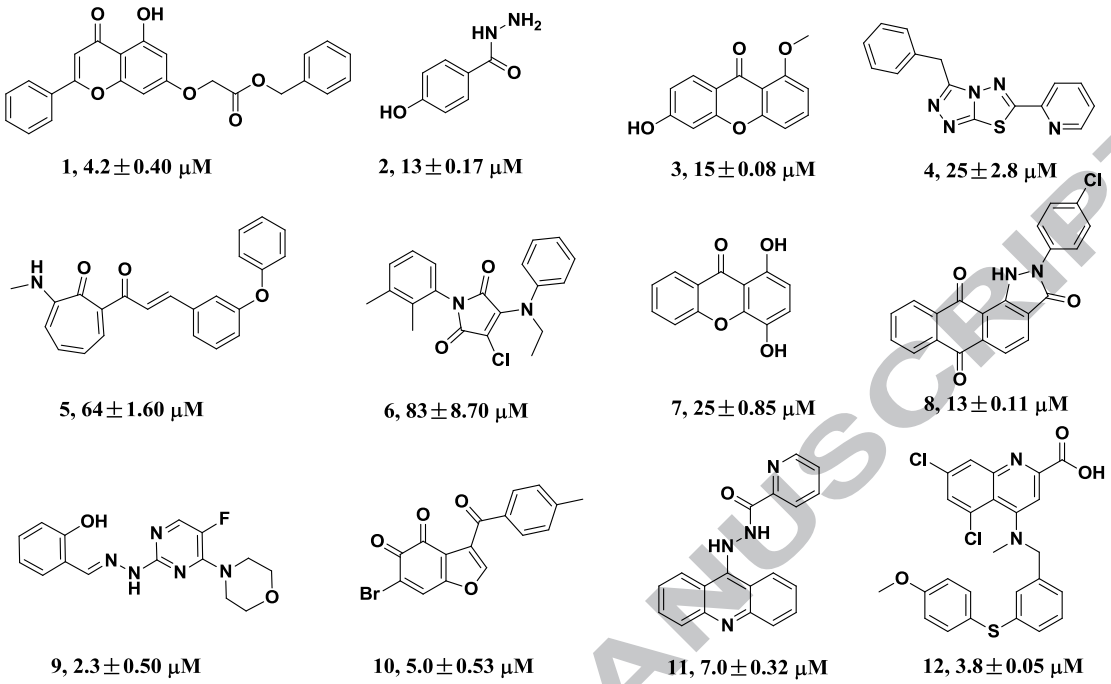


Figure 2. Chemical structures together with bioactivities of retrieved compounds.

Among the twelve compounds, compound **9** is the most active one. We next performed structure optimization and SAR analysis to this compound.

Structural Optimization and SAR analysis. To improve the potency of compound **9**, structure optimization was carried out. We firstly fixed the 2-(iminomethyl) phenol moiety of compound **9** (left) and changed the C-4 (R^2) and C-5 (R^1) positions of pyrimidine (right) with different substituents (see Figure 3). A total of six compounds were synthesized. The synthetic routes for these compounds are depicted in Scheme 1. Intermediates **15** and **15a-e** were first prepared through nucleophilic substitution reactions between self-prepared intermediate (**14**) or commercially available regents (**14a-e**) and hydrazine hydrate. Condensation reactions between these intermediates and 2-hydroxybenzaldehyde gave target compounds **16a-f.**

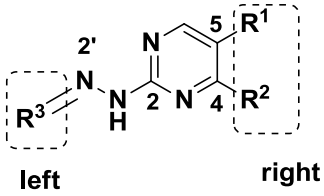
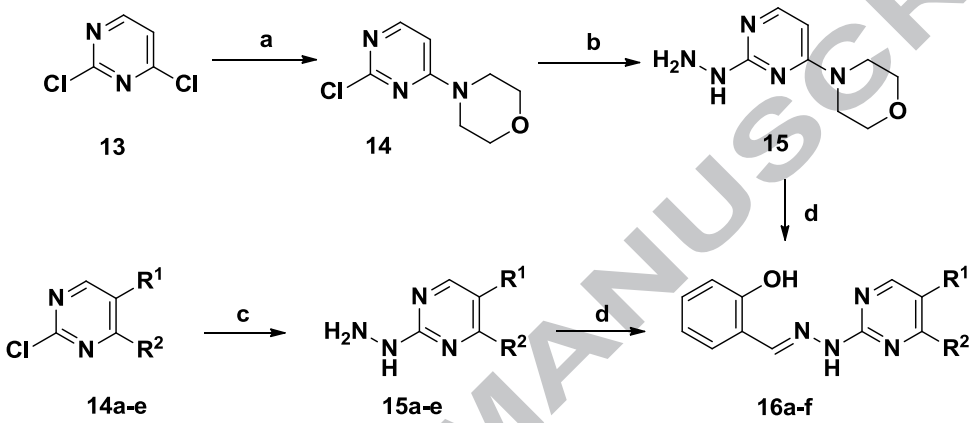


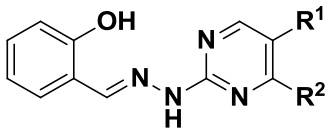
Figure 3. Schematic showing the focus of structural modifications.



Scheme 1. Synthetic routes for compounds **16a-f.** Reagents and conditions: (a) Morpholine, K_2CO_3, EtOH, rt, 6h; (b) $N_2H_4 \cdot H_2O$, DIEA, EtOH, 90°C, sealed-tube, over night; (c) $N_2H_4 \cdot H_2O$, EtOH, rt-60°C, over night; (d) 2-hydroxybenzaldehyde, MeOH, 40°C, overnight.

Bioactivities of the synthesized compounds are displayed in Table 1; the bioactivities used in the SAR analysis were the inhibition rates at the fixed concentration of 10 μM of compounds. From Table 1, we can see that compound **16f**, which contains a carboxyl group at the R^2 position and a hydrogen atom at the R^1 position, is the most active one. Here one may notice that compound **16b** has an inhibition rate of -13%. This type of minus inhibition rate in a single concentration does not mean that this compound is an activator. It could be due to the experimental error since all compounds with minus inhibition rates did not show activating effect on the KDM5A enzyme at western blot assays (data not shown).

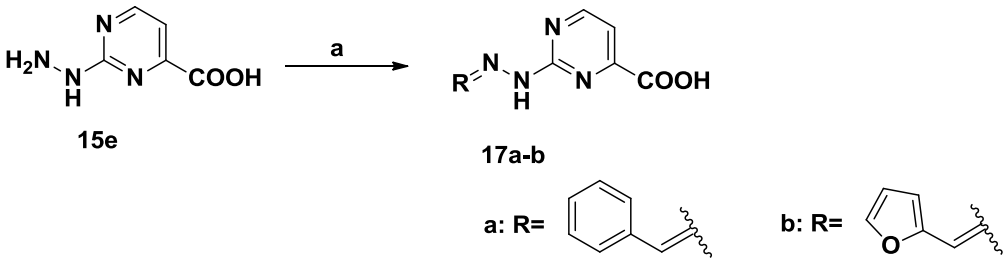
Table 1. Bioactivities of compounds **9** and **16a-f**



Compound	R^1	R^2	Inhibition rate@10μM[a]
9	F	morpholyl	58 ± 6.4
16a	H	morpholyl	42 ± 8.7
16b	F	H	-13 ± 3.5
16c	Cl	H	19 ± 1.3
16d	H	H	32 ± 3.1
16e	H	CH_3	44 ± 3.4
16f	H	COOH	88 ± 1.3

[a]All assays were conducted in duplicate.

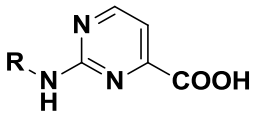
We then fixed R^1 as hydrogen and R^2 as a carboxyl group, and varied the substitution group of 2-amino. Two new compounds were prepared (see Scheme 2). These compounds were easily prepared through a condensation between **15e** and aldehydes. Table 2 lists the bioactivities of the selected compounds. Obviously, compound **15e**, which bears a hydrazine group in 2-position of pyrimidine, displayed more potent activity compared with **16f**.

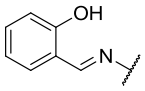
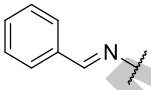
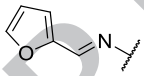


Scheme 2: The synthetic route for compounds **17a** and **17b.** Reagents and conditions:

(a) Benzaldehyde (or furan-2-carbaldehyde) , MeOH, 40°C, over night.

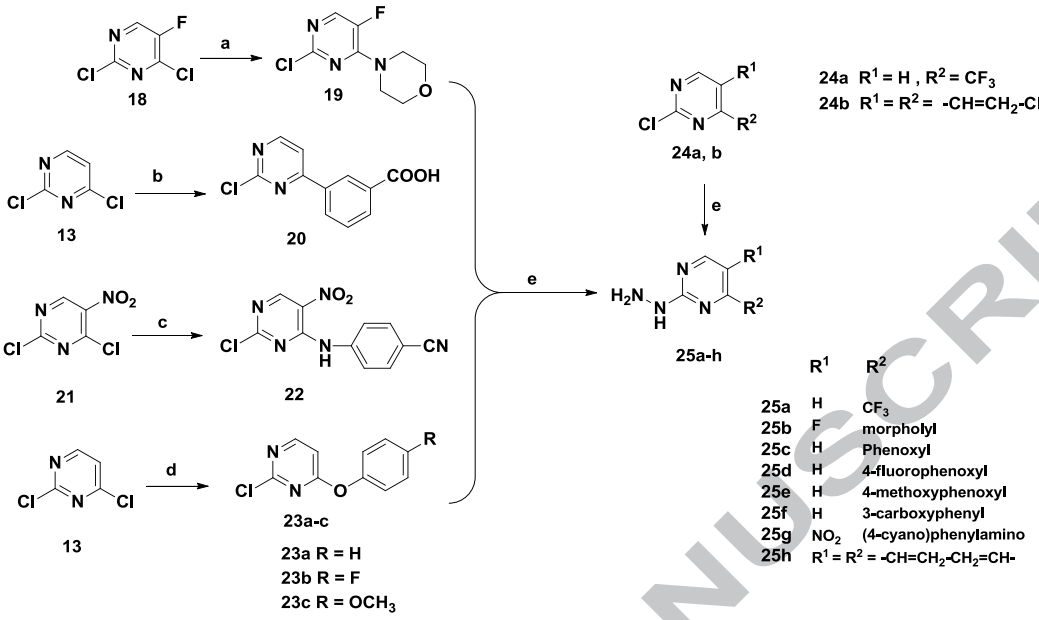
Table 2. Bioactivities of compounds **16f**, **15e**, **17a-b**.



Compound	R	Inhibition rate@10μM[a]
16f		88 ± 1.3
15e	NH_2	99 ± 0.1 (IC_{50} = 0.22 ± 0.01 μM)
17a		0 ± 0.4
17b		3 ± 1.3

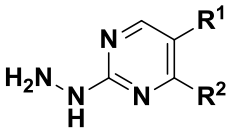
[a]All assays were conducted in duplicate.

We finally fixed the left part as the optimal amino group and again changed the C-4 (R^2) and C-5 (R^1) positions of pyrimidine (right) with different substituents. Eight compounds were synthesized. As illustrated in Scheme 3, compounds **19**, **22** and **23a-c** were synthesized through nucleophilic substitution reactions between commercially available materials and several desired nucleophilic reagents. Intermediate 3-(2-chloropyrimidin-4-yl) benzoic acid (compound **20**) was prepared based on a palladium-catalyzed coupling between 2,4-dichloro-pyrimidine and 3-boronobenzoic acid (Buchwald conditions).[21] Target compounds **25a-h** were synthesized following the procedure for compounds **15a-e** which was presented above. Bioactivities of these compounds are presented in Table 3. From Table 3, we can see that the newly synthesized compounds did not surpass **15e** in terms of the bioactivity.

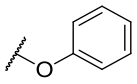
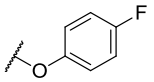
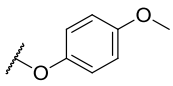
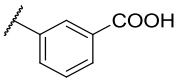
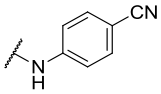


Scheme 3. Synthetic routes for compounds **25a-h.** Reagents and conditions: (a) Morpholine, K_2CO_3, EtOH, 0°C, 4 h; (b) 3-boronobenzoic acid, $NaHCO_3$, $Pd(PPh_3)_4$, H_2O, $(CH_2OMe)_2$, 7 h, 80°C, overnight; (c) 4-aminobenzonitrile, K_2CO_3, EtOH, 0°C-rt, 4 h; (d) substituted-phenol, DIEA, acetone, 60°C, overnight; (e) $N_2H_4 \cdot H_2O$, EtOH, 60°C, overnight.

Table 3. Bioactivities of compounds **15**, **15a-e** and **25a-h.**



Compound	R^1	R^2	Inhibition rate@10μM[a]
15	H	morpholyl	-45 ± 9.90
15a	F	H	10 ± 4.70
15b	Cl	H	-18 ± 8.30
15c	H	H	8 ± 0.77
15d	H	CH_3	-6 ± 0.22
15e	H	COOH	99 ± 0.12

			(IC_{50} = 0.22 ± 0.01 μM)
25a	H	CF_3	-35 ± 4.30
25b	F	morpholyl	0 ± 6.02
25c	H		-72 ± 0.27
25d	H		-50 ± 18.03
25e	H		-121 ± 14.83
25f	H		26 ± 2.40
25g	NO_2		14 ± 0.50
25h	-CH=CH_2-CH_2=CH-		-42 ± 1.41

[a]All assays were conducted in duplicate.

Selectivity of compound 15e. The selectivity of compound **15e** for KDM5A against other KDMs was evaluated. In this test, KDM3A, KDM6B, KDM4E, KDM3C, and KDM5B were included. The IC_{50} values of compound **15e** against the selected KDMs are shown in Table 4. Compound **15e** displayed moderate inhibitory activity against KDM3C and KDM4E with IC_{50} values of 1.9 ± 0.32 μM and 13 ± 1.50 μM, respectively, and very weak activity against KDM3A (IC_{50} = 43 ± 2.50 μM). Compound **15e** displayed almost no activity against KDM6B and KDM5B (IC_{50} > 100 μM). These results indicate that compound **15e** has a good selectivity for KDM5a against other selected KDMs.

Table 4. Inhibitory activities of compound **15e** against selected KDMs.

Compound	IC_{50} (μM)[a]					
	KDM3A	KDM6B	KDM4E	KDM3C	KDM5B	KDM5A

15e	43 ± 2.50	> 100	13 ± 1.50	1.9 ± 0.32	> 100	0.22 ± 0.01

[a] All assays were conducted in duplicate.

Predicted interaction model between compound 15e and KDM5A. Molecular docking was adopted to predict the binding model of the most active compound **15e** in the active pocket of KDM5A. Again, GOLD was used for the docking study. The predicted interaction model between compound **15e** and KDM5A is depicted in Figure 4. From Figure 4, we can see that there is a strong electrostatic interaction between the carboxyl group of **15e** and Fe(II). One of the carboxyl oxygen atoms also forms a hydrogen bond with His589. In addition, a pyrimidine nitrogen atom (1-N) forms a hydrogen bond with Lys607.

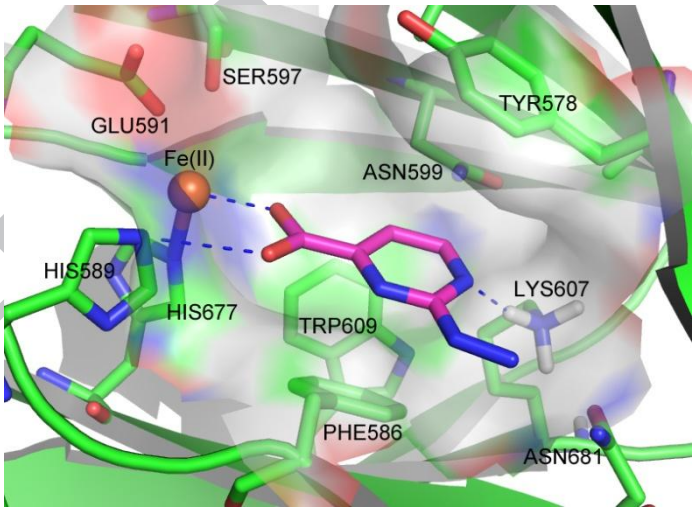


Figure 4. Predicted binding model of compound **15e** in the active pocket of KDM5A. Compound **15e** is colored in pink, Fe(II) is in orange and residues of **KDM5A** are in green. Hydrogen bonds are shown in blue dashed line.

Demethylation activity of compound 15e in intact cells. Western blot assays were used to detect the demethylation activity of compound **15e** in intact cells. In

these assays, breast cancer cell line ZR-75-1 was chosen because ZR-75-1 expresses a high level of KDM5A.[22] The results (see Figure 5) showed that the expression level of H3K4me3 increases with the increase of the concentration of compound **15e**, implying that the demethylation activity of KDM5A was suppressed dose dependently.

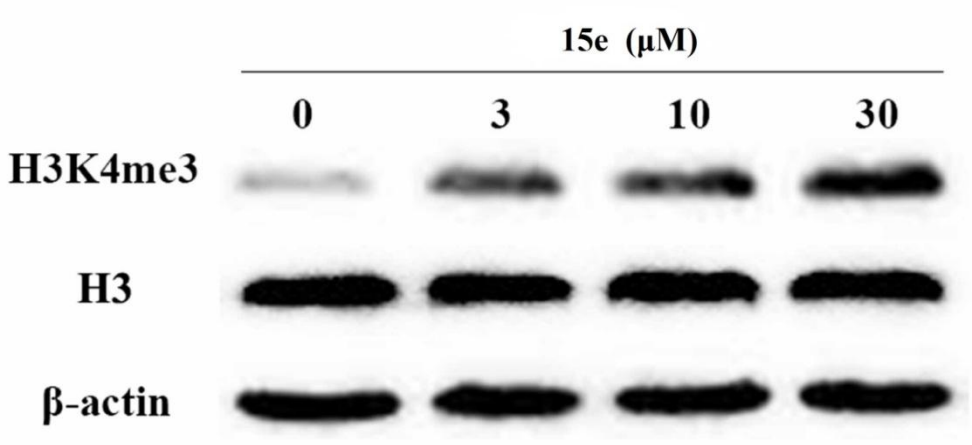


Figure 5. Western blot analysis showing the ability of **15e** to inhibit KDM5A in ZR-75-1 cells

In summary, virtual screening together with bioactivity validation led to the retrieval of a number of hit compounds against KDM5A. The most active hit compound, compound **9**, was chosen for structural optimization to improve its potency. A series of compounds containing pyrimidin-2-amine were synthesized. The SAR analysis led to the discovery of a potent KDM5A inhibitor, namely compound **15e**. This compound showed a good inhibitory activity and selectivity for KDM5A, and considerable ability to inhibit the demethylation of H3K4me3 in intact cancer cells. Collectively, compound **15e** could be a good lead compound, and deserves further studies.

Acknowledgments

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Supporting Information: Supplementary data associated with this article can be found in the online version.

REFERENCES

1. Mair, B.; Kubicek, S.; Nijman, S. M.; *Trends Pharmacol Sci*. **2014**, *35*, 136.
2. Suzuki, T.; Miyata, N. *J. Med. Chem.* **2011**,*54*, 8236.
3. Pachaiyappan, B.; Woster, P. M. *Bioorg. Med. Chem.* **2014**, *24*, 21.
4. Suzuki, T.; Ozasa, H.; Itoh, Y.; Zhan, P.; Sawada, H.; Mino, K.; Walport, L.; Ohkubo, R.; Kawamura, A.; Yonezawa, M.; Tsukada, Y.; Tumber, A.; Nakagawa, H.; Hasegawa, M.; Sasaki, R.; Mizukami, T.; Schofield, C. J.; Miyata, N. *J. Med. Chem.* **2013**, *56*, 7222.
5. Blair, L. P.; Cao, J.; Zou, M. R.; Sayegh, J.; Yan, Q. *Cancers (Basel)* **2011**, *3*, 1383.
6. Beshiri, M. L.; Holmes, K. B.; Richter, W. F.; Hess, S.; Islam, A. B.; Yan, Q.; Plante, L.; Litovchick, L.; Gevry, N.; Lopez-Bigas, N.; Kaelin, W. G., Jr.; Benevolenskaya, E. V. *Proc. Natl. Acad. Sci U S A.* **2012**, *109*, 18499.
7. Chaturvedi, C. P.; Somasundaram, B.; Singh, K.; Carpenedo, R. L.; Stanford, W. L.; Dilworth, F. J.; Brand, M. *Proc. Natl. Acad. Sci U S A.* **2012**,*109*, 18845.
8. Ge, Z.; Li, W.; Wang, N.; Liu, C.; Zhu, Q.; Bjorkholm, M.; Gruber, A.; Xu, D. *FASEB J.* **2010**,*24*, 579.
9. Dannenberg, J. H.; Berns, A. *Cell* **2010**,*141*, 18.

10. Sharma, S. V.; Lee, D. Y.; Li, B.; Quinlan, M. P.; Takahashi, F.; Maheswaran, S.; McDermott, U.; Azizian, N.; Zou, L.; Fischbach, M. A.; Wong, K. K.; Brandstetter, K.; Wittner, B.; Ramaswamy, S.; Classon, M.; Settleman, J. *Cell* **2010**,*141*, 69.

11. Hou, J.; Wu, J.; Dombkowski, A.; Zhang, K.; Holowatyj, A.; Boerner, J. L.; Yang, Z. Q. *Am. J. Transl. Res.* **2012**,*4*, 247.

12. Chan, S. W.; Hong, W. *J. Biol. Chem.* **2001**,*276*, 28402.

13. van Zutven, L. J.; Onen, E.; Velthuizen, S. C.; van Drunen, E.; von Bergh, A. R.; van den Heuvel-Eibrink, M. M.; Veronese, A.; Mecucci, C.; Negrini, M.; de Greef, G. E.; Beverloo, H. B. *Genes Chromosomes Cancer* **2006**,*45*, 437.

14. Spannhoff, A.; Hauser, A. T.; Heinke, R.; Sippl, W.; Jung, M. *Chem.Med.Chem.* **2009**,*4*, 1568.

15. Colas, C.; Pajor, A. M.; Schlessinger, A. *Biochemistry.* **2015**, *54*, 4900.

16. Eswar, N.; Eramian, D.; Webb, B.; Shen, M. Y.; Sali, A. *Methods. Mol. Biol.* **2008**, *426*, 145.

17. Dwyer, P. J.; Vander Valk, R. J.; Caltaldo, V.; Demianicz, D.; Kelty, S. P. *J. Phys. Chem. A.* **2014**, *118*, 11583.

18. Patel, S.; Brooks, C. L., 3rd. *J. Comput. Chem.* **2004**,*25*, 1.

19. Bissantz, C.; Folkers, G.; Rognan, D. *J. Med. Chem.* **2000**, *43*, 4759.

20. Yamagishi, M. E.; Martins, N. F.; Neshich, G.; Cai, W.; Shao, X.; Beautrait, A.; Maigret, B. *J. Mo.l Model.* **2006**, *12*, 965.

21. William, A. D.; Lee, A. C. H.; Goh, K. C.; Blanchard, S.; Poulsen, A.; Teo, E. L.; Nagaraj, H.; Lee, C. P.; Wang, H.; Williams, M.; Sun, E. T.; Hu, C.; Jayaraman, R.; Pasha, M. K.; Ethirajulu, K.; Wood, J. M.; Dymock, B. W. *J. Med. Chem.* **2012**, *55*, 169.

22. Hou, J.; Wu, J.; Dombkowski, A.; Zhang, K.; Holowatyj, A., Boerner, J. L.; Yang,

Z.-Q. *Am. J. Transl. Res.* **2012**, *4*, 247.

Graphical abstract

Discovery of KDM5A Inhibitors: Homology Modeling, Virtual Screening and Structure-Activity Relationship Analysis

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9 KDM5A IC_{50} = 2.3±0.50 μM

15e KDM5A IC_{50} = 0.22±0.01 μM