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Discovery of pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile derivatives as a new class of histone lysine demethylase 4D (KDM4D) inhibitors

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ABSTRACT

Herein we report the discovery of a series of new small molecule inhibitors of histone lysine demethylase 4D (KDM4D). Molecular docking was first performed to screen for new KDM4D inhibitors from various chemical databases. Two hit compounds were retrieved. Further structural optimization and structure-activity relationship (SAR) analysis were carried out to the more selective one, compound **2**, which led to the discovery of several new KDM4D inhibitors. Among them, compound **10r** is the most potent one with an IC_{50} value of 0.41 ± 0.03 μM against KDM4D. Overall, compound **10r** could be taken as a good lead compound for further studies.

Histone lysine demethylase 4D (KDM4D), also known as JMJD2D, is a typical jumonji domain-containing demethylase, which can catalyze the removal of a methyl group from lysine 9 on histone 3 (H3K9) or H1.4K26.[1–4] Unlike other widely studied histone demethylase, the studies of KDM4D are just getting started.[5,6] Despite that biochemical functions of KDM4D have been known, its role in pathogenesis is far from clear,[7,8] and often opposite in different studies. For examples, a recent study by Bur et al. showed that KDM4D overexpression might lead to a higher risk for resistance to radiotherapy in Hodgkin lymphomas.[9] Kim et al. revealed that KDM4D was required for colon cancer cell proliferation and survival. However, evidence also showed that KDM4D was able to stimulate p53-dependent gene expression, implying a tumor suppressor gene.[10] The research revealing the net effect, if any, of KDM4D in tumorigenesis is complicated, and not the intention of this investigation. We know that small molecule inhibitors of KDM4D, particularly those with good selectivity, could be used as a tool to help researchers to unravel the action and mechanism.[11] Nevertheless, very few KDM4D inhibitors have been reported by now.[12–15] Our purpose here is to discover potent and selective KDM4D inhibitors. We hope the compounds discovered could enrich the arsenal of KDM4D inhibitors, and facilitate biofunctional and disease treatment studies related to KDM4D in the further.

To retrieve new KDM4D inhibitors, we firstly performed molecular docking-based virtual screening against various chemical libraries. All the calculations were carried out using the platform of Discovery Studio 3.1 (Accelrys Inc., San Diego, CA, USA). The receptor structure for molecular docking was prepared from the crystal structure of jmjC domain of KDM4D (PDB entry: 5FP4). The binding site was defined as a sphere containing the residues that stay within 8 Å from its original ligand, which is large enough to cover the catalytic site. The Charmm force field was assigned. Chemical libraries used in the virtual screening include SKLB (compounds synthesized by our laboratory), Specs (Specs, Inc. Zoetermeer, The Netherlands), and ChemDiv (ChemDiv, Inc., San Diego, CA, USA). All the docking calculations were carried out with the GOLD program.[16] Goldscore[17] was chosen as the scoring function. From the top ranking compounds, ID-Score[18] and RASA[19] were further used to rank the compounds and estimate their synthetic accessibility. Thirty compounds were finally selected for bioactivity validation. Two compounds (**1** and **2**, see Fig. 1) showed inhibition rate more than 50% against KDM4D at the concentration of 10 μM (see Table 1). We also measured the activities of the two hit compounds against other three KDMs, namely KDM2B, KDM3B,

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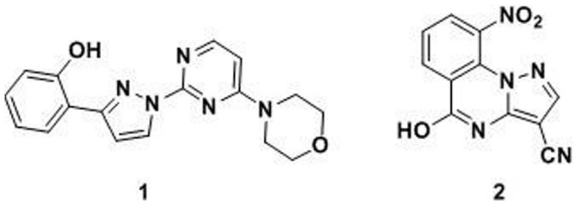


Fig. 1. Chemical structures of the hit compounds.

Table 1
Bioactivities of compounds **1** and **2**.

Compound	Inhibition rate @10μM[a]			
	KDM4D	KDM2B	KDM3B	KDM5A
1	99.67 ± 3.96	84.12 ± 8.21	90.65 ± 9.16	73.62 ± 5.83
2	73.33 ± 19.14	−4.85 ± 4.72	−2.18 ± 3.19	23.09 ± 7.51

[a] All assays were conducted in triplicate.

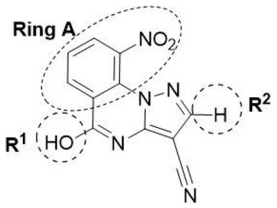


Fig. 2. Schematic showing the focus of structural modifications.

and KDM5A to examine the selectivity roughly; here just the three KDMs are selected because they are available for us at the moment. It was found that only compound **2** showed a better selectivity against KDM4D.

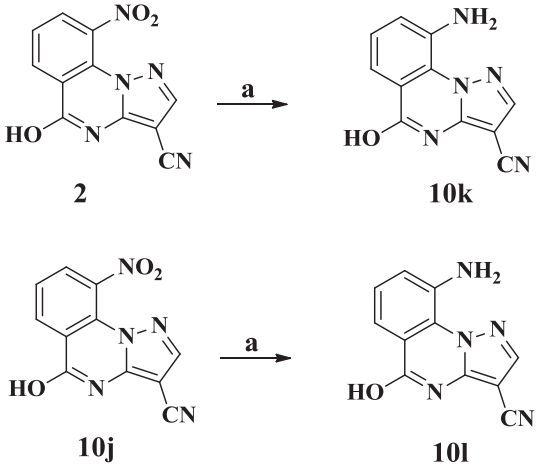
We then carried out a structural optimization to the selective compound, **2**. The structural modifications were focused on three regions (see Fig. 2): ring A (nitrobenzene), R^1 (the hydroxyl group), and R^2 (the hydrogen atom). Various substituents or groups were used to replace the ring A, R^1, and R^2, and a total of twenty compounds (**10a**–**t**) were synthesized.

The synthetic routes for compound **10a**–**j** are depicted in Scheme 1. Acid chloride intermediates **4a**–**j** were first prepared through chlorination of commercially available regents (**3a**–**j**) by thionyl chloride or oxalyl chloride. Nucleophilic substitution reaction between **4d** or **4e** and 3-amino-1*H*-pyrazole-4-carbonitrile (**6**) gave target compounds **10d** and **10e**. Reaction of **4a**–**c** or **4f**–**j** and **6** produced amide derivatives **5a**–**c** and **5f**–**j**, which then went through a ring close reaction to give target compounds **10a**–**c** and **10f**–**j**. Compounds **10k** and **10l** were prepared with **2** and **10j**, respectively, by Pd/C-catalyzed reduction reactions (see Scheme 2). Target compounds **10m**–**o** were synthesized through very similar reaction routes as those of compounds **10d**, **10e** (see Scheme 3).

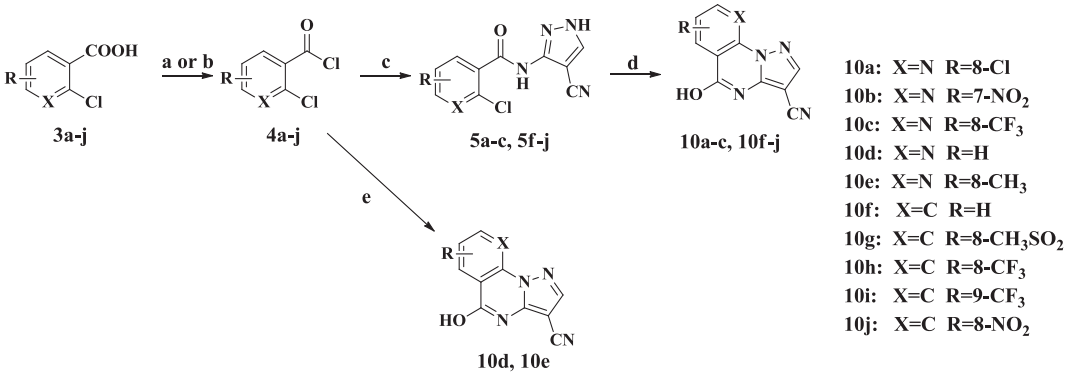
Compounds **10p** and **10q** were prepared through copper-catalyzed tandem reactions of 2-chloronicotinaldehyde (**3p**) or 1-(2-chloropyridin-3-yl)ethanone (**3q**) and 3-amino-1*H*-pyrazole-4-carbonitrile (**6**) (see Scheme 4).[20]

Reaction routes for compounds **10r**–**t** are shown in Scheme 5. Commercially available reagent malononitrile (**7**) reacted with triethyl orthoacetate derivatives through a Knoevenagel reaction to give intermediate **8a**–**c**, which then reacted with hydrazine hydrate to produce intermediates **9a**–**c**.[21] Treatment of **4a** with **9a**–**c** provided 2-chloro-*N*-(4-cyano-1*H*-pyrazol-3-yl)nicotinamide derivatives **5r**–**t**. A ring close reaction of **5r**–**t** was carried out to afford the desired compounds **10r**–**t**.

All the synthesized compounds were first tested for their inhibitory rates against KDM4D at the fixed concentration of 10 μM. Bioactivities for all the compounds are summarized in Table 2. From Table 2, we can see that there are three compounds (**10p**, **10r**, **10s**) that showed more potent activities than compound **2**. For these compounds whose inhibitory rates are higher than



Scheme 2. Synthetic routes for compounds **10k** and **10l**. Reagents and conditions: (a) Pd/C, methanol, H_2O, 60 °C.



Scheme 1. Synthetic routes for compounds **10a**–**j**. Reagents and conditions: (a) thionyl chloride, DMF, 80 °C; (b) oxalyl chloride, DMF, DCM, 0 °C-rt; (c) 3-amino-1*H*-pyrazole-4-carbonitrile (**6**), THF, 0 °C-rt; (d) K_2CO_3, DMF, Argon, 90 °C; (e) 3-amino-1*H*-pyrazole-4-carbonitrile (**6**), K_2CO_3, DMF, Argon, 0 °C-rt-140 °C.