University of Pittsburgh

8-Substituted Pyrido[3,4-d]pyrimidin-4(3H)-one Derivatives As Potent, Cell Permeable, KDM4 (JMJD2) and KDM5 (JARID1) Histone Lysine Demethylase Inhibitors

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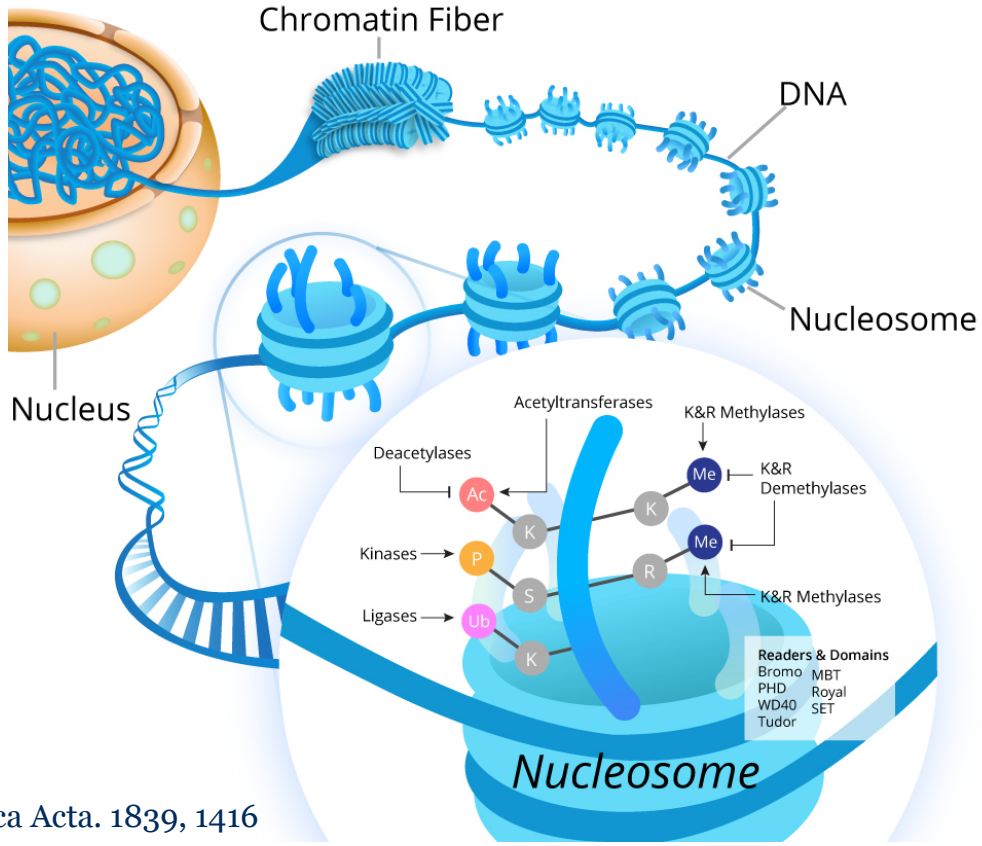
Current Literature: Marina Kovaliov

Histone modification

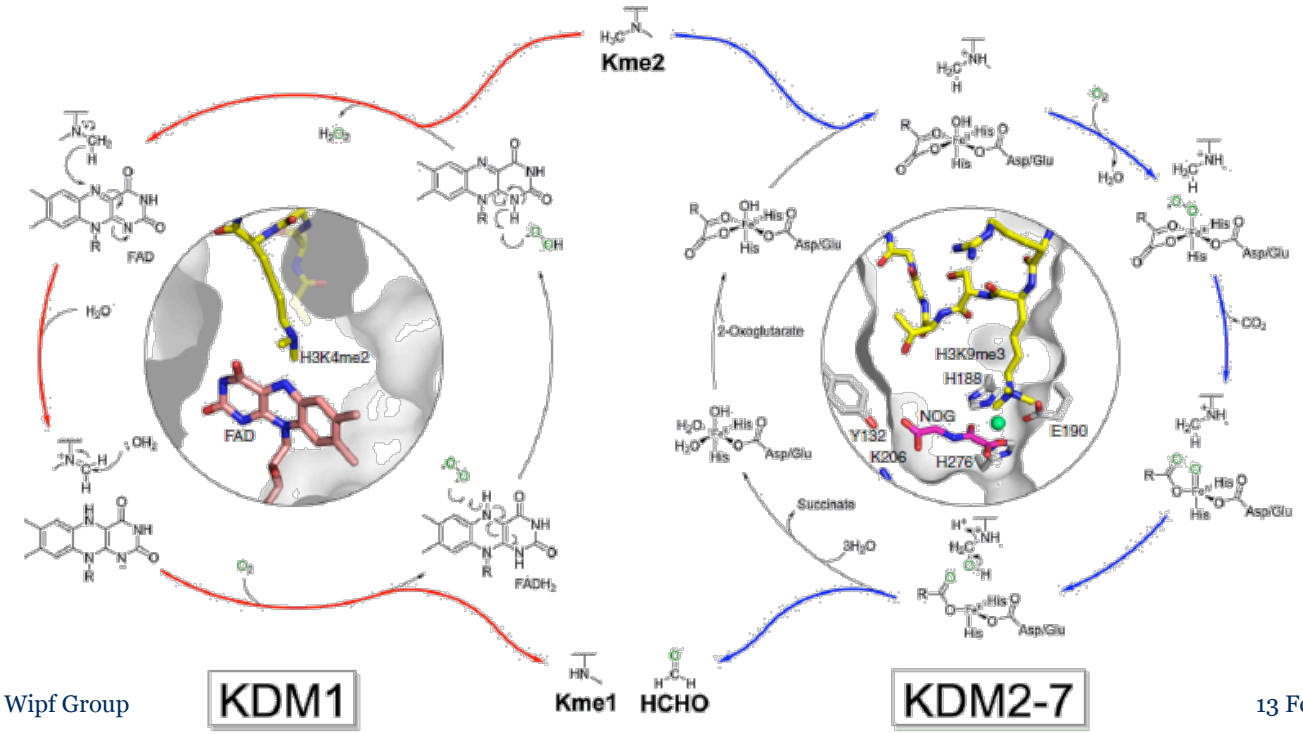
- Post-translational histone modifications, which include lysine or arginine methylation, play a essential role in maintaining chromatin structure and regulating transcription by mediating chromatin binding interactions
- Enzymes that catalyze those modification involve in wide range of epigenetic processes



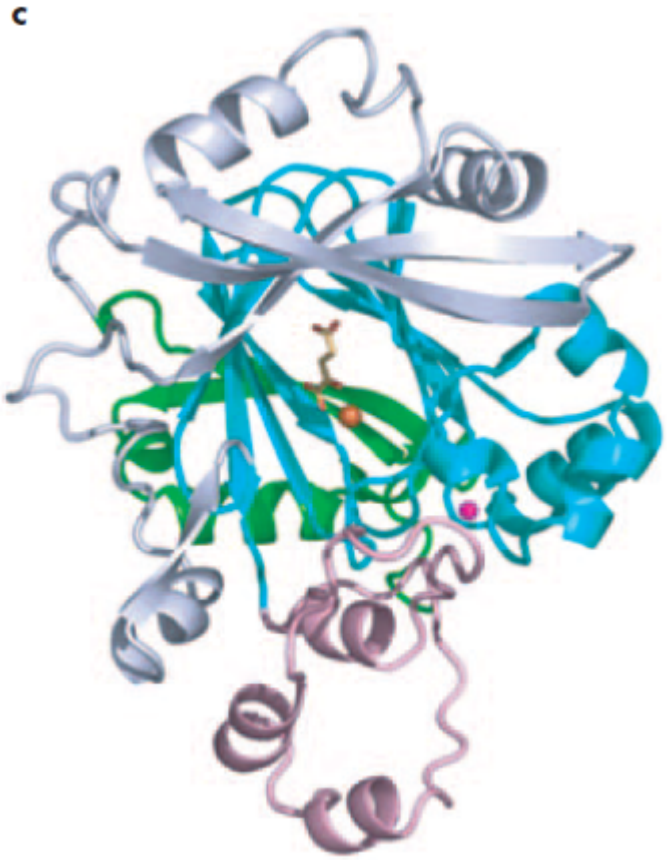
Richard J. Hopkinson et al, (**2014**) Biochimica et Biophysica Acta. 1839, 1416
Robert J. Klose et al, (**2007**) Molecular Cell Biology. 8, 307
Cell (**2002**) 111, 285

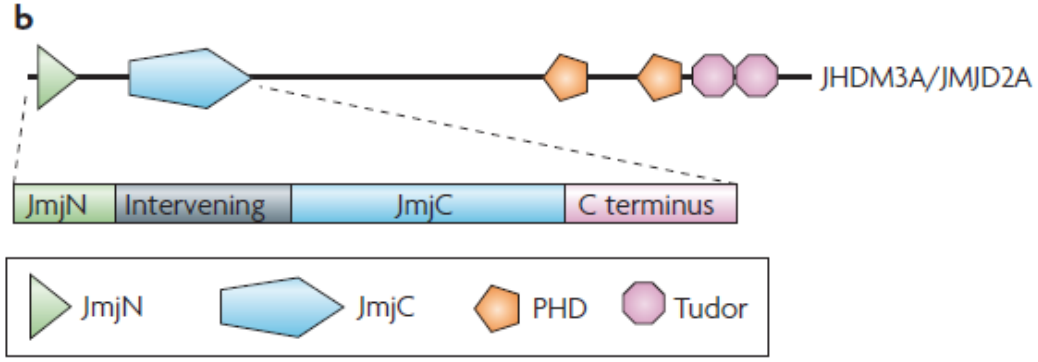
Histone lysine demethylases (KDMs)

- Two classes of KDMs: LSD/KDM1 the flavin-dependent lysine specific demethylase, and the larger class of Fe(II) and 2-oxoglutarate (2OG)-dependent
- The 2OG-dependent KDMs belong to the Jumonji C (JmjC) domain containing subfamily.



Human JmjC histone demethylases





- **KDM4A**/JHDM3A/JMJD2A bound to **Fe(II)**, 2-oxoglutarate and **zinc**.
- The catalytic site composed of eight **β-strands** forms the core of the **JmjC** domain and houses the 2-oxoglutarate and **Fe(II)** cofactors.
- The **JmjN domain** associates with the **JmjC** domain on the opposite face to the catalytic site.
- The **C-terminal** region and part of the **JmjC** domain coordinate the **zinc** ion.

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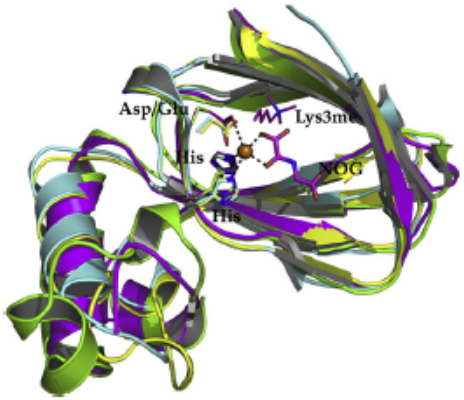
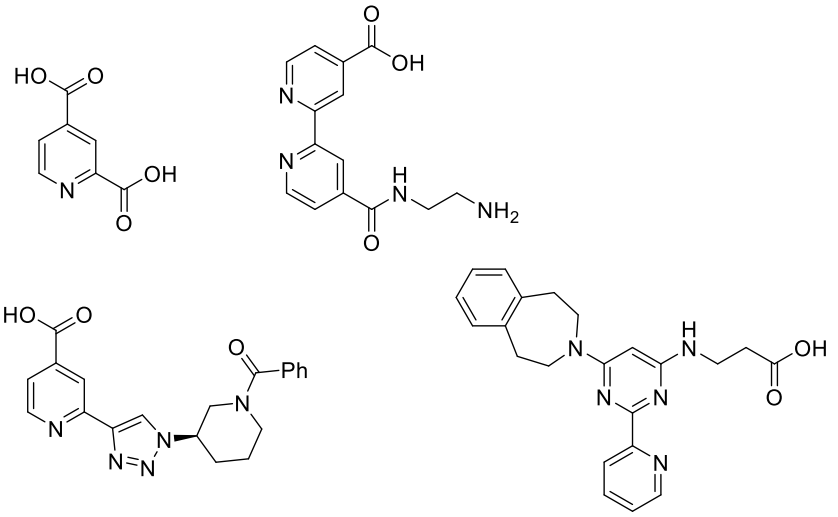
KDM4 & KDM5 subfamilies & cancer

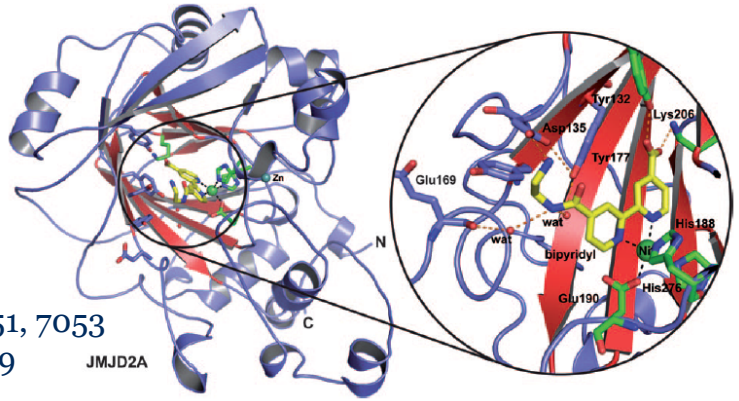
- KDM4 subfamily members play an important role in cancer initiation and progression various studies have shown overexpression of KDM4A–C in a range of human malignancies.
- KDM4B and KDM4C are reported to be overexpressed in breast cancer.
- KDM4B knockdown in human gastric cancer cells suppressed tumor growth.
- The KDM5 subfamily are histone (H3K4) demethylases and have been implicated in cancer progression in several tumor types.
- KDM5B and KDM5C have been shown to play a role in breast and prostate cancer, respectively.

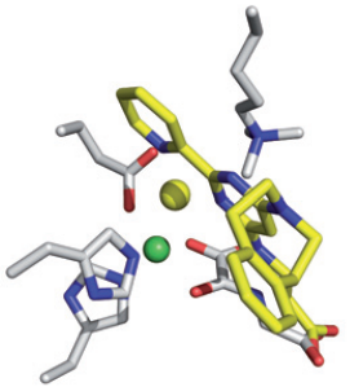


Inhibitors of JmjC KDMs

- Pyridine-2,4-dicarboxylic acid potent JMJD2E (KDM4D) competitive inhibition with 2-OG (IC50 = 1.4 µM)
- The bipyridyl ethylenediamine inhibits JMJD2E (KDM4D) with an IC50 = 180 nm
- Triazolopyridine KDM2A pIC50 = 7.2 µM) with excellent selectivity over representatives from other KDM subfamilies
- JMJD3 (KDM6B) inhibitor GSK-J1 binds competitively with 2-OG and repositions the metal cofactor in the active site.







Christopher J. Schofield et al, (**2008**) J. Med. Chem. 51, 7053
Paul E. Brennan et al, (**2014**) MedChemComm, 5, 1879

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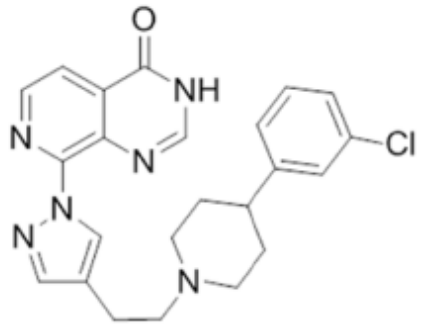
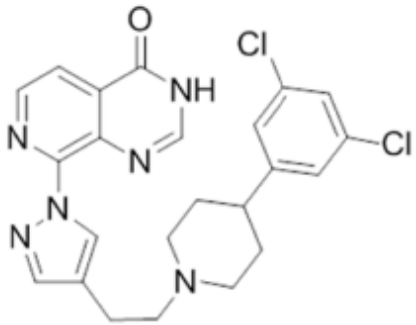
Pyrido[3,4-d]pyrimidin-4(3H)-one Derivatives

- Extensive interest in the KDM4/5 subfamilies in the progression of human cancers and well-defined catalytic mechanism for JmjC KDMs, lead to initiation of a program to identify histone KDM inhibitors.



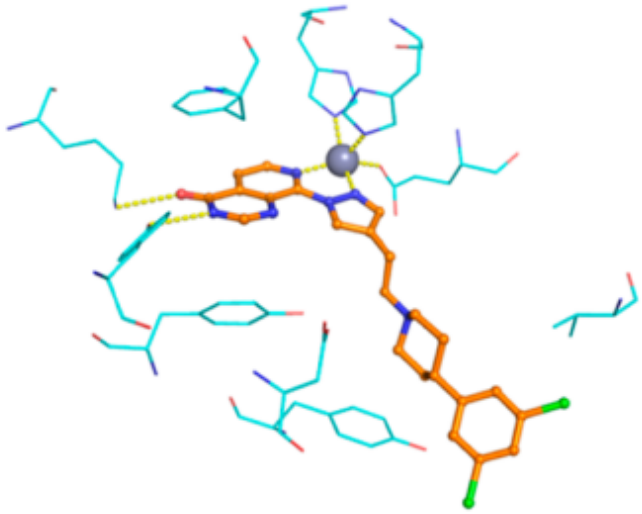
54k

KDM4B IC_{50} = 0.031μM
KDM5B IC_{50} = 0.023 μM



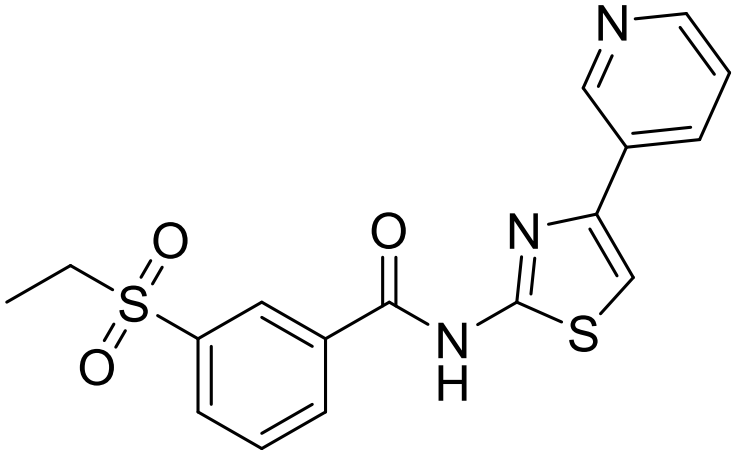
54j

KDM4B IC_{50} = 0.017μM
KDM5B IC_{50} = 0.014 μM



HTS campaign

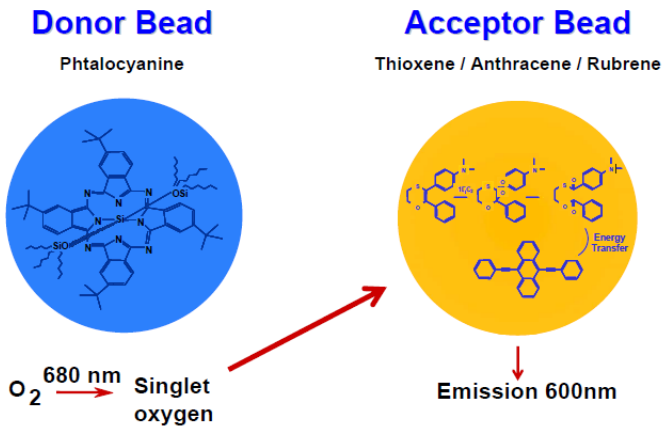
- HTS of a 150k compound collection tested at 30 μM versus human recombinant KDM4B
- Promising hit - N-substituted 4-(pyridin-2-yl)thiazole-2-amine **7c**

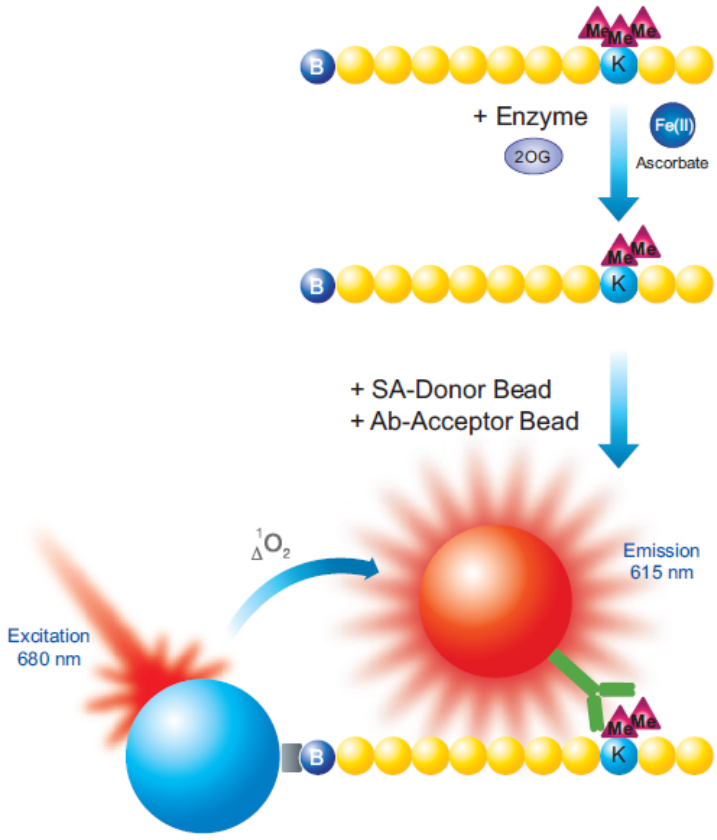


7c IC50 = 13.2 μM

AlphaScreen Histone demethylase assay (KDMS4 & KDMS5)

- Enzyme activity was measured using an AlphaScreen assay that monitored the demethylation of a biotinylated trimethylated H3K9 peptide using a H3K9 dimethyl specific antibody and appropriate donor and acceptors beads





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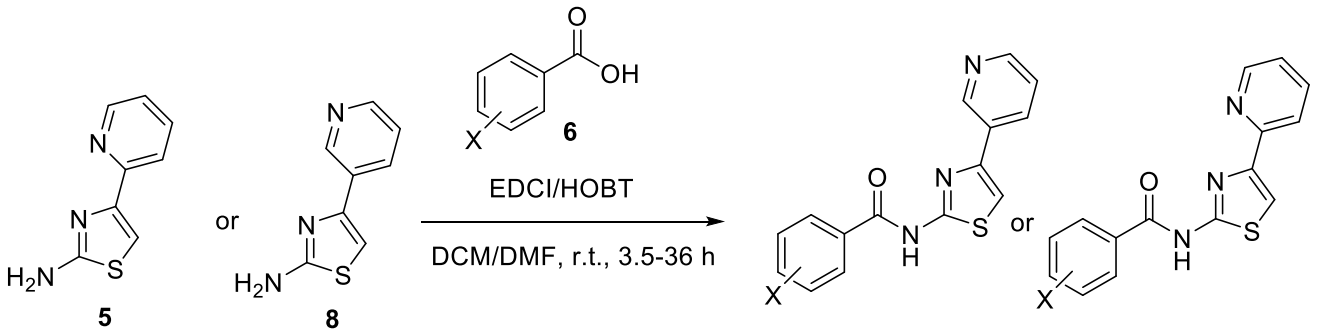
N-(4-(pyridin-2-yl)thiazol-2-yl) benzamides analogs

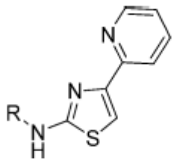
- Synthesis of the HTS-derived series of N-(4-(pyridin-2-yl)thiazol-2-yl) benzamides

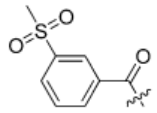
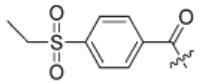
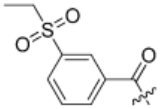
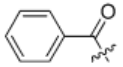


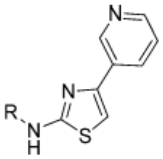
7a, X = *m*-SO_2Me (33%)
7b, X = *p*-SO_2Et (25%)
7c, X = *m*-SO_2Et (40%)
7d, X = H (50%)

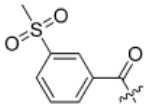
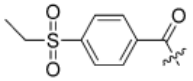
9a, X = *m*-SO_2Me (28%)
9b, X = *p*-SO_2Et (16%)

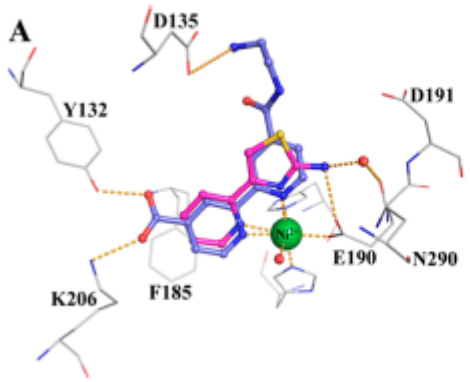
KDM4-5 inhibitory activity

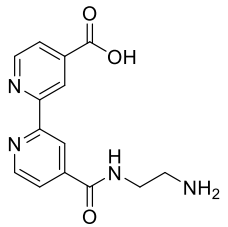


Compound	R	KDM4A IC_{50} (μM)	KDM4B IC_{50} (μM)	KDM5B IC_{50} (μM)	KDM5C IC_{50} (μM)
7a		16.7±3.9	11.3±1.4	29.7	71.9
7b		10.9±4.3	6.4±0.8	22.7	52.8
7c		13.2	10.9±0.3	52.6[a]	65% inh at 100 μM[a]
7d		20.9	12.1	51.2[a]	62% inh at 100 μM[a]
5	H	6% inh at 100 μM	30% inh at 100 μM	Not active at100 μM	Not active at 100 μM



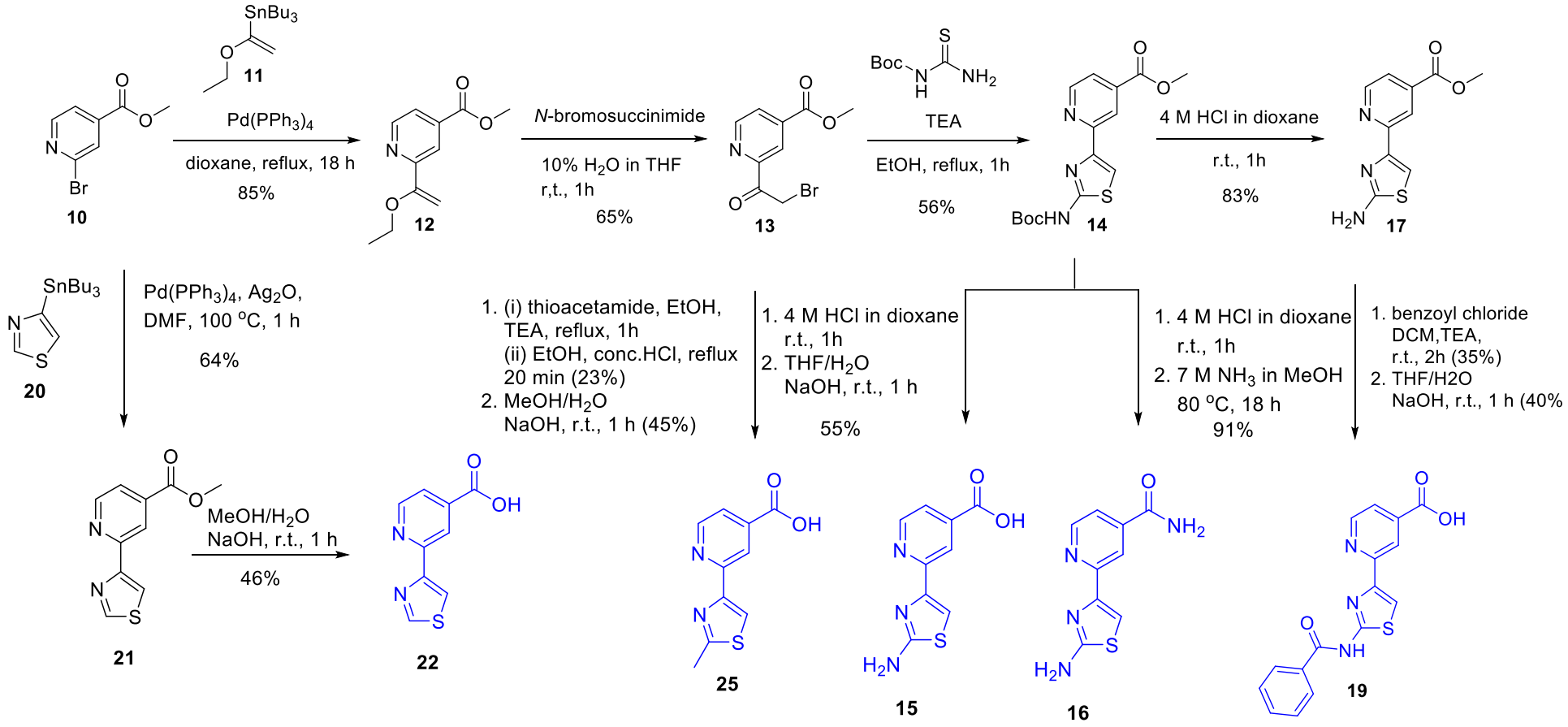
Compound	R	KDM4A, inhibition at 100 μM	KDM4B, inhibition at 100 μM	KDM5B, inhibition at 100 μM	KDM5C, inhibition at 100 μM
8	H	Not active	10%	17%	8%
9a		Not active	Not active	36%	14%
9b		Not active	Not active	43%	21%



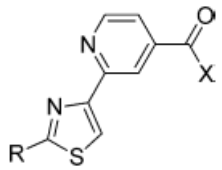


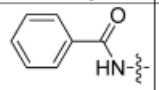
Christopher J. Schofield et al, (**2008**) J. Med. Chem. 51, 7053

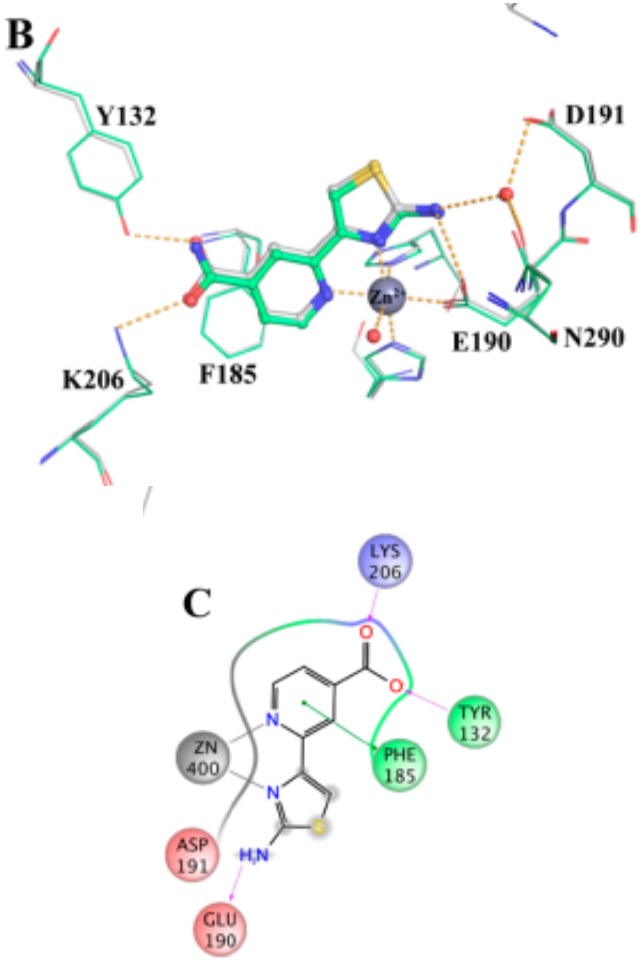
Carboxylate analogs



2-(2-Aminothiazol-4-yl)isonicotinic Acid Derivatives



Compound	R	X	KDM4A IC_{50} (μM)	KDM4B IC_{50} (μM)	KDM5B IC_{50} (μM)	KDM5C IC_{50} (μM)
15	NH_2	OH	0.200	0.083±0.005	0.012[a]	0.012[a]
16	NH_2	NH_2	104.2	32.7	2.2[a]	6.8[a]
22	H	OH	0.90	0.35±0.14	0.098	0.122
25	CH_3	OH	16.5	2.3	1.3	1.7
19		OH	30.6	17.0	0.59	2.0



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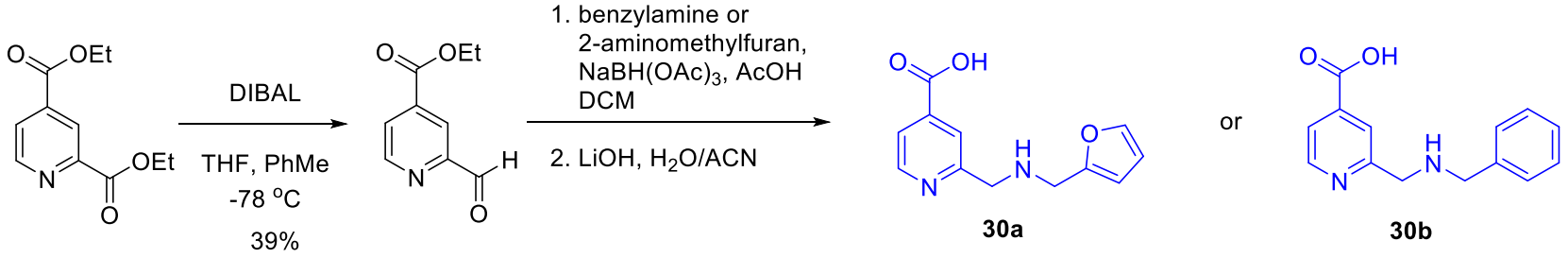
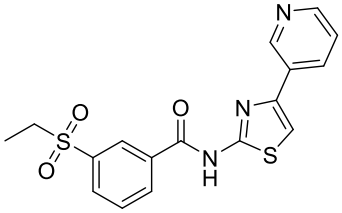
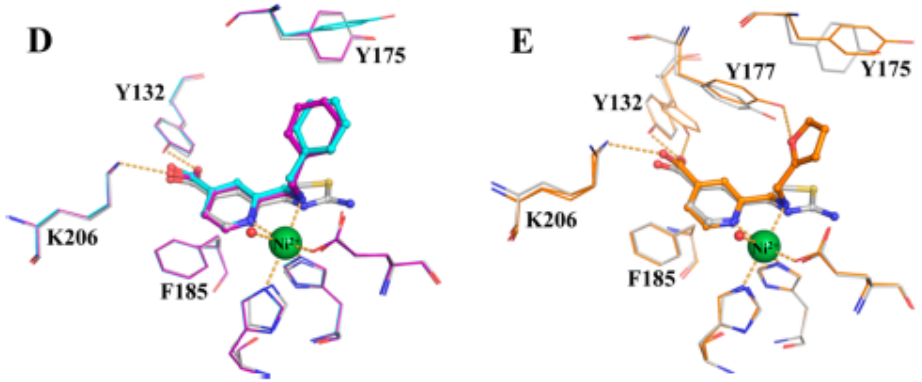
2-(Aminomethyl)pyridine-4-carboxylate Derivatives

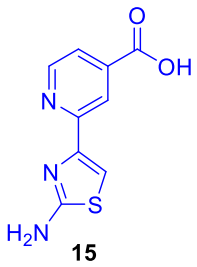


Compound	KDM4A IC_{50} (μM)	KDM4B IC_{50} (μM)	KDM5B IC_{50} (μM)	KDM5C IC_{50} (μM)
30a	0.30[a]	0.17[a]	0.04[a]	0.03[a]
30b	0.95[a]	1.2[a]	0.13[a]	0.06



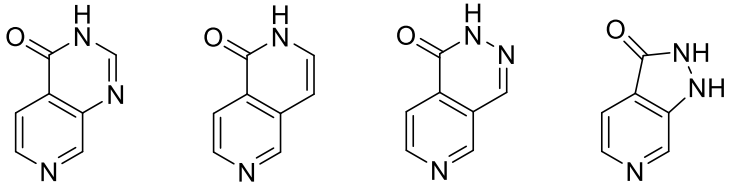


Pyridine carboxylic acid isosters



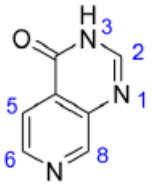
15

58



KDM4C pIC_{50}	5.7	<4.0	<4.0	<4.0

- Poor cellular permeability for **15** (Caco-2 A to B flux <0.8 × 10−6 cm/s).
- **58:** KDM4B IC50 = 1.0 μM; KDM5B IC50 = 1.3 μM; KDM3A (IC50 = 26.2 μM); KDM2A (29% inhibition at 100 μM); KDM6B (28% inhibition at 100 μM).
- High cellular permeability (Caco-2 A to B flux = 42.61 × 10−6 cm/s) & pKa for the pyridopyrimidinone amide moiety of 8.23



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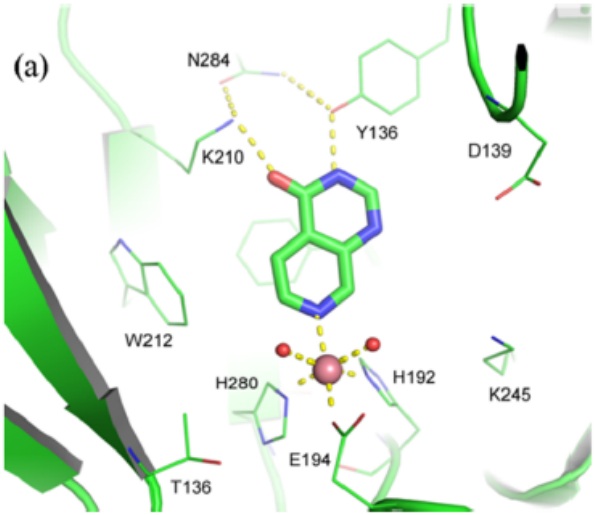
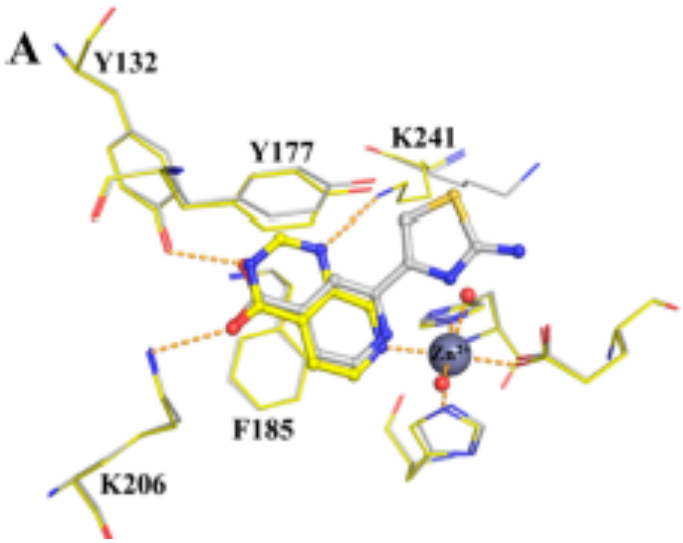
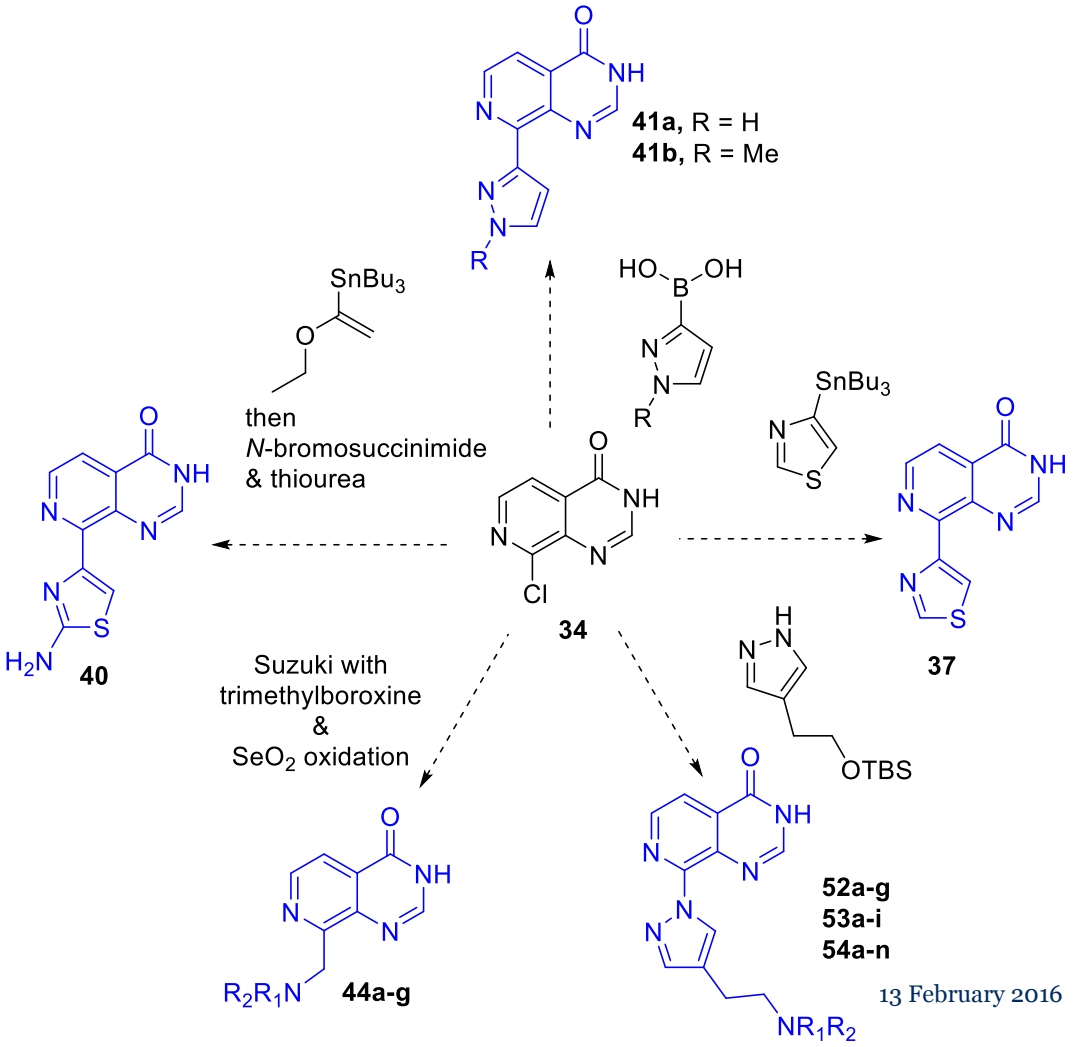


Rab K. Prinjha et al, (**2008**) J. Med. Chem. DOI: 10.1021/acs.jmedchem.5b01538

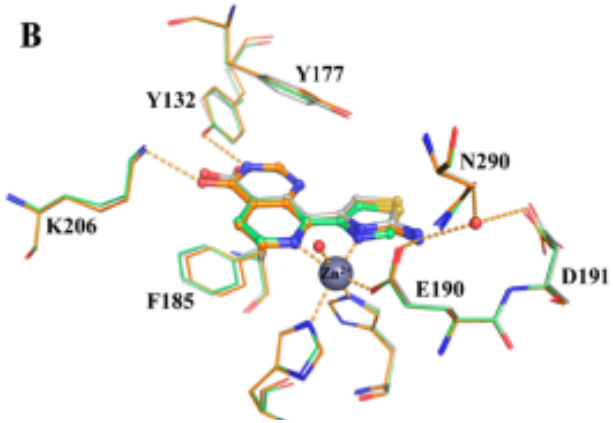
Pyridopyrimidinone analogs

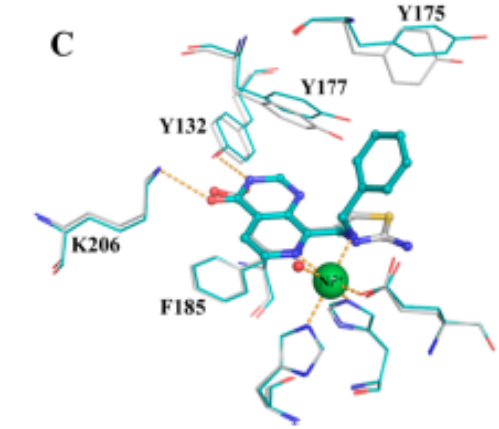


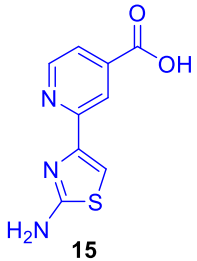


C8-Pyrido[3,4-d] pyrimidin-4(3H)-one analogs

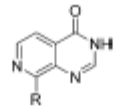
Comparison of binding modes of 15, 37, 40, and 58 to 44a

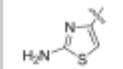
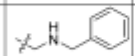
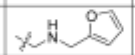
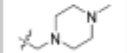
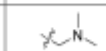
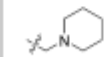
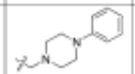
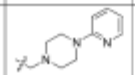






15



Compound	R	KDM4A IC_{50} (μM)	KDM4B IC_{50} (μM)	KDM5B IC_{50} (μM)	KDM5C IC_{50} (μM)
58	H	1.7±0.6	1.0±0.4	1.3[a]	2.0[a]
40		11.5	5.0±1.2	0.124[a]	0.331[a]
37		8.0±3.6	3.1±1.1	0.31	0.83
34	Cl	No activity at 100μM[a]	30% at 200μM[a]	32% at 100μM[a]	22% at 100μM[a]
41a		6.9	2.8	4.1	7.3
41b		42.8	9.2	21.4	103.4
56		16.7[a]	4.6	7.0[a]	9.4[a]
44a		15.6	6.5	0.69	0.44
44b		6.1	3.1	0.63	0.73
44c		13.1	8.5	1.4	1.3
44d		9.8[a]	7.8[a]	7.0	8.4
44e		>100	>100	66.0	111.0
44f		>100	81.5	3.9	>100
44g		58.6	24.6	2.0	88.0[a]

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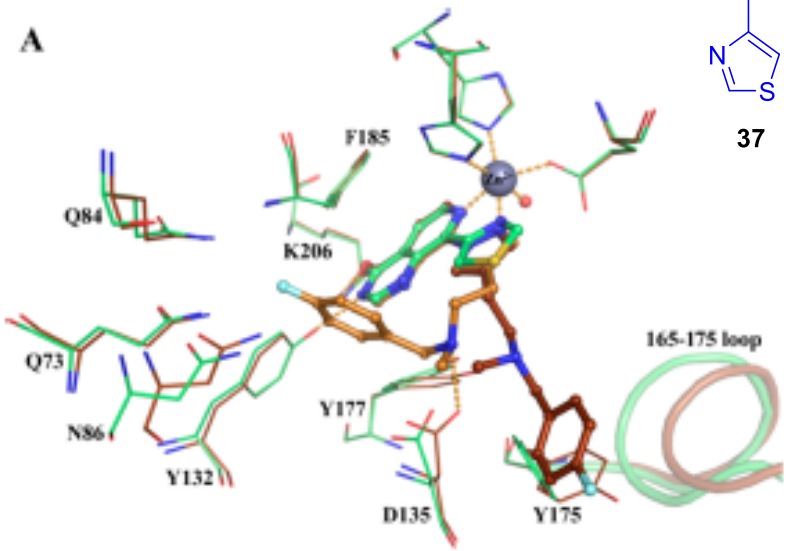
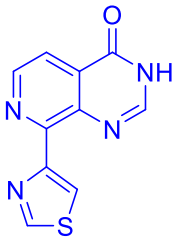
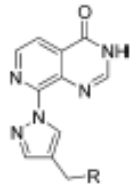
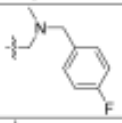
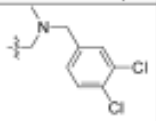
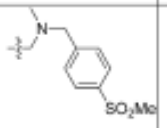
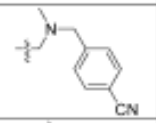
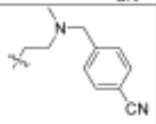
8-(1H-Pyrazol-1-yl)pyrido[3,4-d] pyrimidin-4(3H)-one analogs





37



Compound	R	KDM4A IC_{50} (μM)	KDM4B IC_{50} (μM)	KDM5B IC_{50} (μM)	KDM5C IC_{50} (μM)	Caco-2 ($x10^{-6}$ cm/s)
57		21.8	8.8	4.6[a]	8.4[a]	0.76
52a		4.1±3.7	2.3	0.35	0.34	0.76
52b		1.4	0.75	0.071	0.150	3.08
52c		1.8	0.65	0.199	0.342	1.64
52d		0.90±0.59	0.39±0.12	0.042	0.078	9.98
52e		0.39	0.165	0.048	0.080	21.21
52f		0.66	0.40	0.072	0.136	0.76
52g		0.60	0.34	0.048	0.076	n.d.
59		0.88	0.26	0.048	0.064	n.d.

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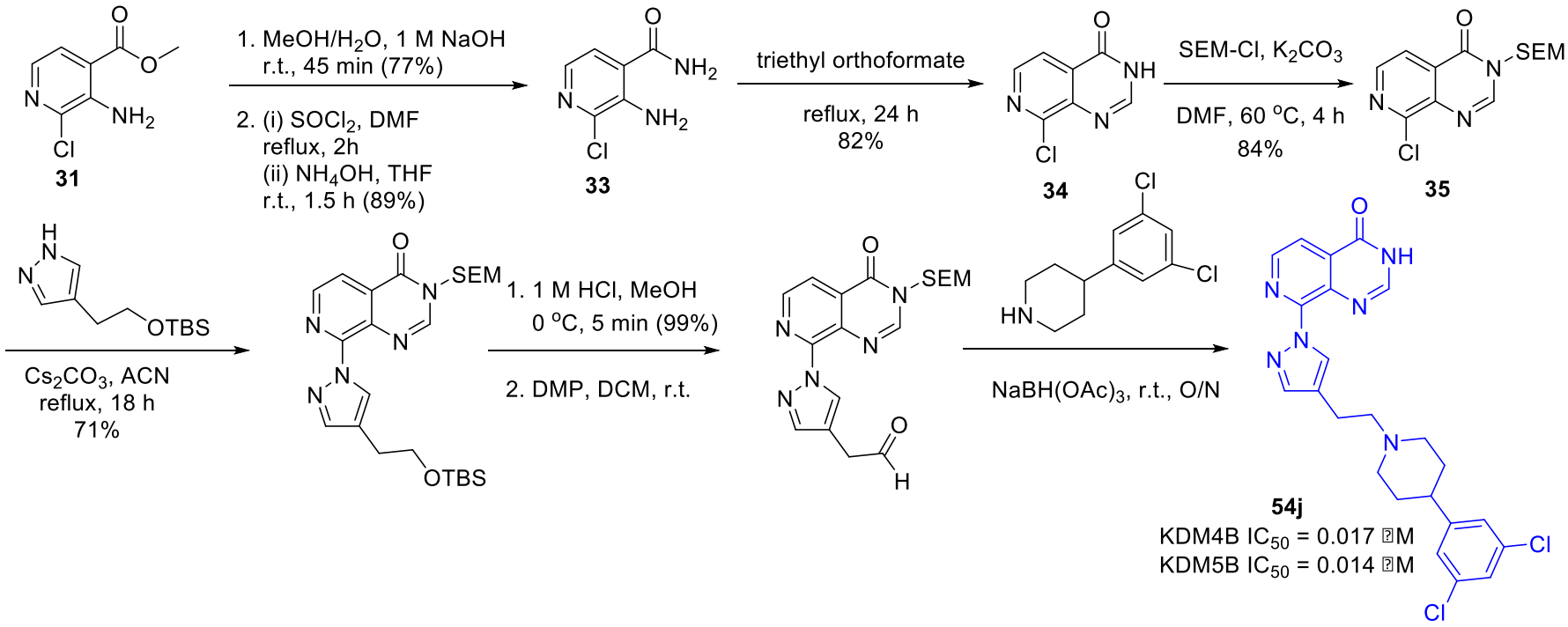
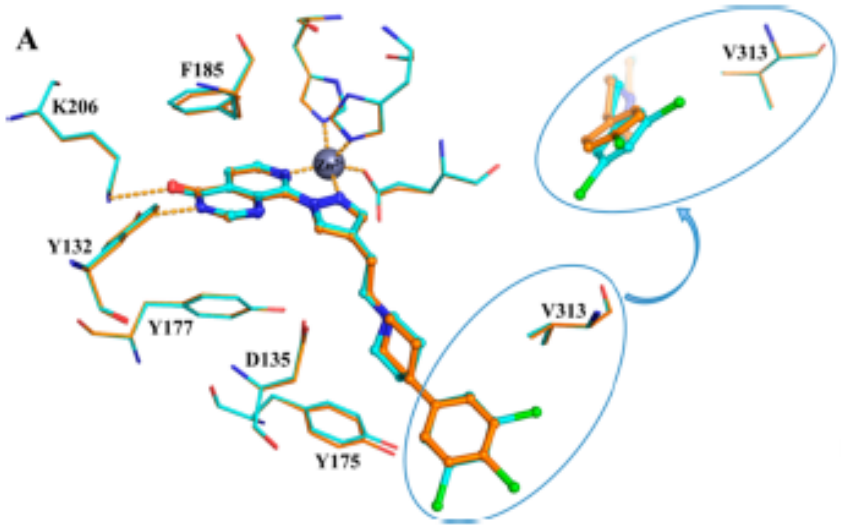
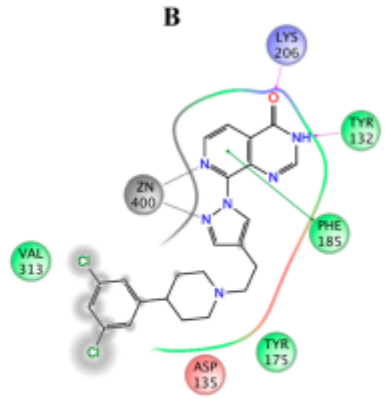
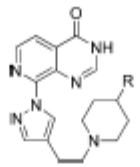
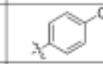
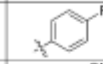
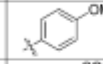
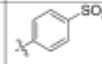
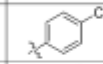
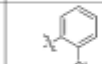
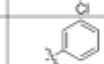
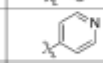
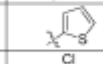
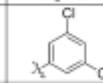
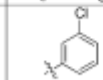
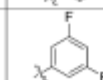
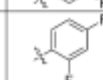
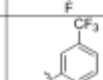
Synthesis of 54J









Compound	R	KDM4A IC_{50} (μM)	KDM4B IC_{50} (μM)	KDM5B IC_{50} (μM)	KDM5C IC_{50} (μM)	Caco-2 ($\times 10^{-6}$ cm/s)
54a		0.138	0.086±0.019	0.030[a]	0.067[a]	2.66
54b		0.175	0.061	0.018	0.052	14.33
54c		0.123	0.039±0.002	0.010[a]	0.034[a]	5.77
54d		0.109	0.069	0.010	0.032	n.d.
54e		0.118	0.069	0.007[a]	0.024[a]	1.84
54f		0.411	0.179	0.056	0.119	n.d.
54g		0.456	0.274	0.058[a]	0.142[a]	8.05
54h		0.145	0.055	0.015	0.050	1.14
54i		0.143	0.029	0.026	0.046	17.83
54j		0.080±0.042	0.017±0.002	0.014	0.051	6.34
54k		0.102±0.058	0.031±0.012	0.023	0.065	11.80
54l		0.138	0.041	0.043[a]	0.126[a]	21.21
54m		0.110	0.036	0.011[a]	0.054[a]	15.74
54n		0.128	0.039	0.018[a]	0.070[a]	15.31

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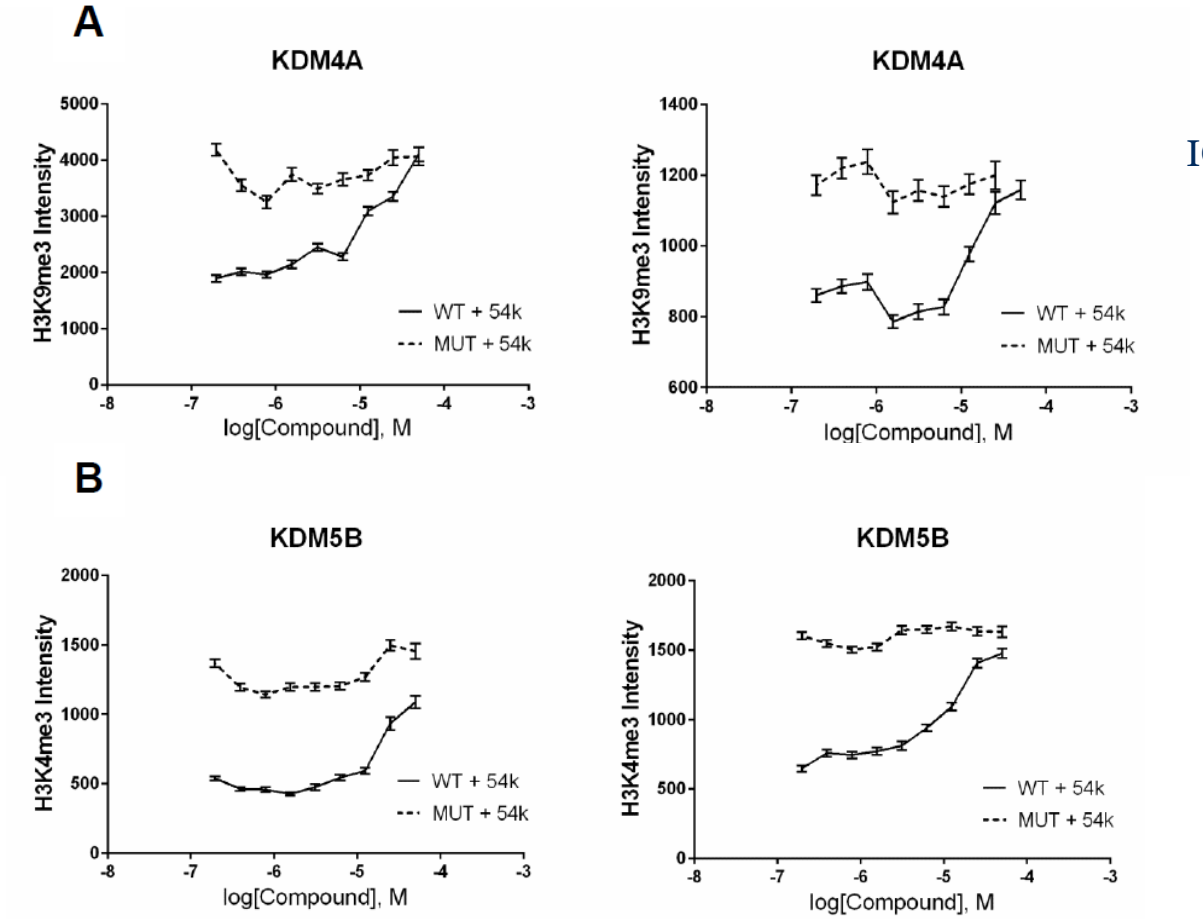
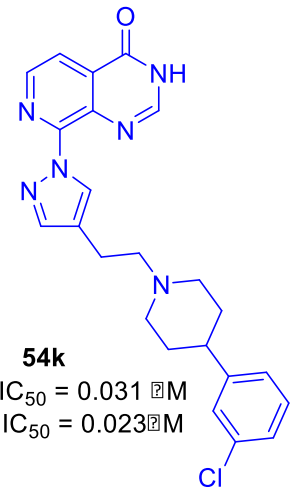
Cellular activity of 54k



IC_{50} ~10-32 μM



54k

KDM4B IC_{50} = 0.031 μM

KDM5B IC_{50} = 0.023 μM

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13 February 2016

Conclusions

- An HTS resulted discovery of ***N*-substituted 4-(pyridin-2-yl)thiazole-2-amine** derivatives and their subsequent optimization
- A structure-based design gave **8-(1H-pyrazol-3-yl)pyrido[3,4-d] pyrimidin-4(3H)-ones**, a series of potent JmjC histone KDM inhibitors which bind to Fe(II) in the active site.
- Substitution from C4 of the pyrazole moiety allows access to the histone peptide substrate binding site.
- Incorporation of a conformationally constrained 4-phenylpiperidine linker gives derivatives such as **54j** and **54k** which demonstrate equipotent activity versus the KDM4 and KDM5 subfamily demethylases, cellular permeability in the Caco-2 assay, and inhibition of H3K9Me3 and H3K4Me3 demethylation in a cell-based assay.