Electronic Supplementary Material (ESI) for ChemComm.


# Supplementary Information

# The small molecule JIB-04 disrupts $O_2$ binding in the Fe-dependent histone demethylase KDM4A/JMJD2A

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## I. Dioxygen Consumption Assay

KDM4A was tested for inhibition by the small molecule JIB-04 using the $O_2$ consumption assay. The reaction solution containing 50 mM HEPES (pH 7.5), 300 μM αKG (pH 7.5), 500 μM sodium L-ascorbate, 100 μM $H3_{7\text{-}14}K9me_3$ , and ± JIB-04 (in DMSO) was equilibrated 2 – 5 min under an atmosphere of varying $O_2$ concentration. Following equilibration, the electrode was lowered into the reaction solution, and a four minute background $O_2$ consumption reading was allowed. Separately, purified KDM4A (2 μM) was reconstituted with $Fe^{II}(NH_4)_2SO_4{\cdot}6H_2O$ (2 μM) and, if a pre-incubation trial, to the holoenzyme was added JIB-04 in DMSO (0 – 20 μM) for a pre-incubation time of approximately one minute. In pre-incubation trials, the volume of DMSO added to the KDM4A/Fe aliquot was 1 μL (less than 20% of the HDM/Fe aliquot volume, and 0.1% of the reaction volume). The reaction was started by the addition of KDM4A/Fe/± JIB-04 to the $O_2$ electrode chamber. All final rates were calculated by subtracting background oxygen consumption due to ascorbate and the holoenzyme in the absence of peptide substrate. Data were plotted as percentage of inhibition versus inhibitor concentration and were fit to $y = 100 / (1 + ([I]/IC_{50}))$ using Kaleidagraph (Synergy Software), where $IC_{50}$ is the inhibitor concentration at 50% activity.

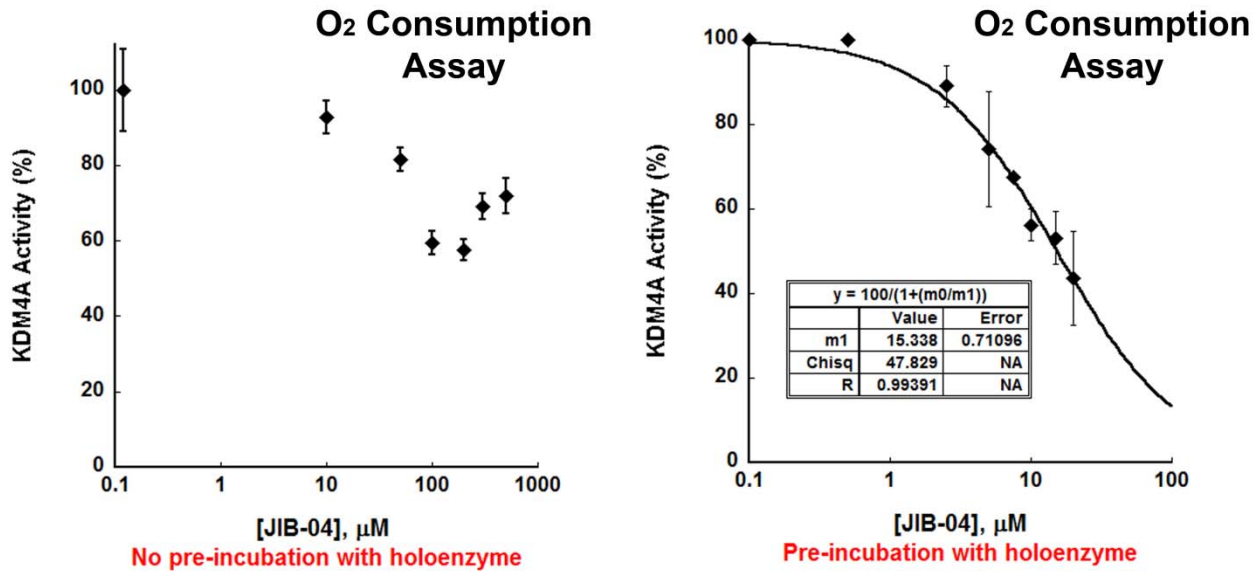
## II. MALDI-TOF MS Assay

KDM4A was tested for inhibition by the small molecule JIB-04 using a discontinuous MALDI-TOF MS assay. Purified KDM4A (8 μM) was incubated with 8 μM $Fe^{II}(NH_4)_2SO_4{\cdot}6H_2O$ and, if a pre-incubation trial, 0-500 μM JIB-04 in DMSO. Pre-incubation trials allowed for five minutes of pre-incubation on ice before the reaction was started by the addition of a solution containing 80 μM $H3_{(7\text{-}14)}K9me_3$ in 50 mM HEPES (pH 7.5), 500 μM sodium L-ascorbate, and 300 μM αKG. The reaction was allowed to proceed at 37 °C for 20 minutes. The enzymatic demethylation was stopped by addition of MeOH and TFA, the precipitated protein removed via centrifugation, and the resulting supernatant collected. An aliquot of the reaction solution was added to 10 mg/mL α-cyano-4-hydroxycinnamic acid MALDI matrix in 50% acetonitrile/0.1% TFA and spotted directly onto the MALDI plate. For analysis, an ABI DE-STR MALDI-TOF instrument was used.

Percent demethylation values were determined using relative intensities of the mono-, di-, and tri-methylated $H3_{(7\text{-}14)}K9$ peaks in the following equation:

$$\%\ \text{demethylation} = \frac{(\%\ \mathrm{NHme_2} + 2{\cdot}\%\mathrm{NH_2me_1})}{(\%\ \mathrm{Nme_3} + \%\ \mathrm{NHme_2} + 2{\cdot}\%\mathrm{NH_2me_1})}$$

Data were plotted as percent demethylation versus inhibitor concentration and were fit to y = 100 / (1 + ([I]/$IC_{50}$)) using Kaleidagraph (Synergy Software), where $IC_{50}$ is the inhibitor concentration at 50% activity.




**MALDI-TOF MS Assay**

| [JIB-04], µM | | Percent Demethylation |
|---|---|---|
| *without pre-incubation* | 0 | 95 |
| | 500 | 80 |
| *with pre-incubation* | 0 | 85 |
| | 500 | 12.5 |

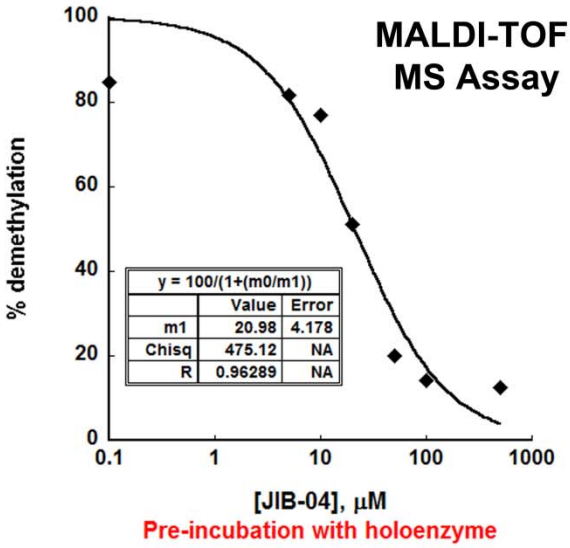



**Figure S1**. Effects of pre-incubation of JIB-04 with the KDM4A/Fe(II) holoenzyme, as seen in two assays. (A) Mild inhibition ($IC_{50}$ > 1 mM) of KDM4A results when JIB-04 is added to the reaction solution in the $O_2$ consumption assay. (B) Increased inhibition ($IC_{50}$ = 15 ± 1 µM) of KDM4A when JIB-04 is added to the holoenzyme for one minute before the start of the reaction in the $O_2$ consumption assay. (C) and (D) Pre-incubation is necessary for marked inhibition of KDM4A in the MALDI-TOF MS assay ($IC_{50}$ = 20 ± 4 µM with pre-incubation).

## III. Molecular Docking

(*E*)-JIB-04 was generated using ChemDraw 3D and was energy minimized in Autodock Vina (ver. 1.1.2). Twenty low-energy poses of (*E*)-JIB-04 were modeled in five JmjC-HDM complexes (with crystallographic ligands removed) using grid boxes of 36 x 38 x 32 Å and the level of exhaustiveness set to 40.

**Table S1. Relative binding affinities of (*E*)-JIB-04 in JmjC-HDM crystal structures**

| PDB ID (Resolution) | JmjC-HDM Complex | Lowest-energy pose (kcal/mol) |
|---|---|---|
| 5TVS (2.75 Å) | KDM4A·Ni(II) | -9.8 |
| 5A1F (2.1 Å) | JARID1B·Mn(II)·NOG | -9.7 |
| 5TVR (2.1 Å) | KDM4A·Ni(II)·αKG | -7.9 |
| 2Q8C (2.05 Å) | KDM4A·Ni(II)·αKG·H3K9me3 | -7.4 |
| 2OQ7 (2.15 Å) | KDM4A·Ni(II)·NOG | -7.2 |

## IV. X-Ray Crystallography

**Table S2. Summary of crystallographic statistics for KDM4A/JMJD2A**

| Crystal | JMJD2A•Ni(II)•αKG | JMJD2A•Ni(II) |
|---|---|---|
| Space group | $P22_12_1$ | $P22_12_1$ |
| Cell dimensions | *a*=57.1 Å, *b*=101.1 Å, *c*= 149.0 Å | *a*=56.7 Å, *b*=101.1 Å, *c*=148.9 Å |
| Data Collection | | |
| Wavelength (Å) | 0.979 | 0.979 |
| Resolution range (Å) (highest shell) | 35.15-2.09 (2.17-2.09) | 41.8-2.75 (2.84-2.75) |
| Reflections (total/unique) | 50,379 / 4,412 | 23,006 / 2,190 |
| Completeness (highest shell) | 99.8% (99.8%) | 100.0% (100.0%) |
| <I/□> (highest shell) | 23.7 (2.9) | 35.3 (2.8) |
| $R_{sym}$[a] (highest shell) | 7.7% (79.4%) | 9.6% (75.9%) |
| Refinement | | |
| $R_{cryst}$[b] / $R_{free}$[c] | 18.1% / 22.4% | 20.6% / 26.6% |
| No. of protein atoms | 5,735 | 5,750 |
| No. of waters | 366 | - |
| No. of ligand atoms | 24 | 2 |
| R.m.s. deviation, bond lengths (Å) | 0.005 | 0.020 |
| R.m.s. deviation, bond angles (°) | 0.93 | 1.54 |
| Avg. B-factor (Å$^2$): protein, water,ligand | 34.7, 39.2, 31.5 | 66.9, - , 54.3 |
| Stereochemistry: most favored, allowed, outliers | 98.0, 2.0, 0.0 | 94.0, 5.0. 0.7 |

[a]$R_{sym} = \Sigma|I_h - \langle I_h \rangle|/\Sigma I_h$, where $\langle I_h \rangle$ is the average intensity over symmetry.

[b]$R_{cryst} = \Sigma|F_o - \langle F_c \rangle|/\Sigma F_o$, where summation is over the data used for refinement.

[c]$R_{free}$ is defined the same as $R_{cryst}$, but was calculated using 5% of data excluded from refinement.

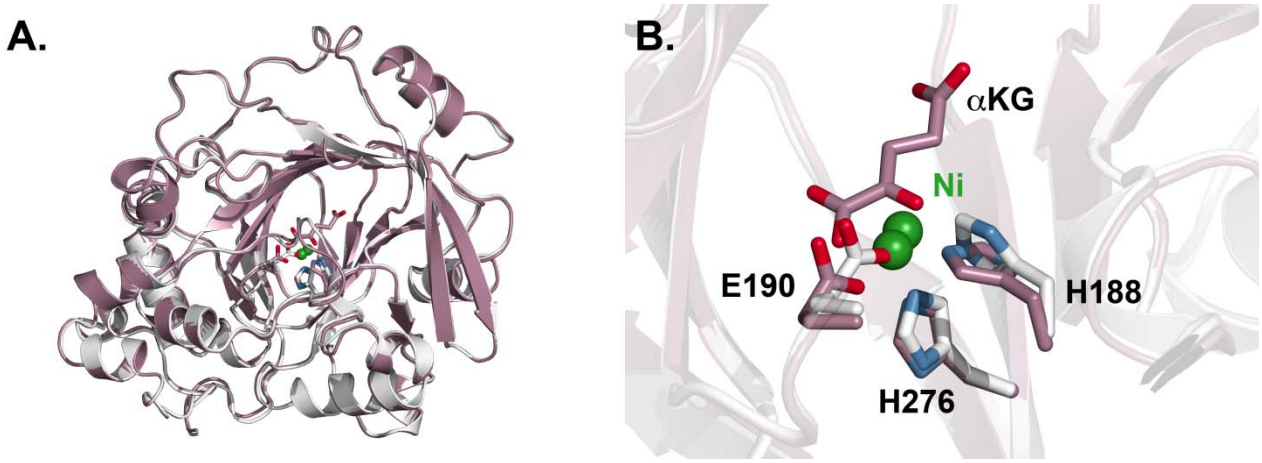



**Figure S2**. Structure comparison of KDM4A·Ni(II)·αKG (PDB 5TVR, in rose) and KDM4A·Ni(II) (PDB 5TVS, in white). (A) Overlay of the two structures shows a high degree of structural similarity. (B) Metal center and metal-coordinating residue displacements in the two active sites.