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Supporting Information

Small-Molecule Inhibitors of METTL3, the Major Human Epitranscriptomic Writer

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Supporting Information

Material and methods

Expression and purification of recombinant proteins

Recombinant METTL3/METTL14 complex constructs for crystallization and for the use in enzymatic activity assay were expressed using the baculovirus/Sf9 insect cell expression system as described previously.[1] Recombinant YTH domain of YTHDC1 protein comprising residues 345-509 ($YTHDC1_{345-509}$) was expressed in *E. coli* and purified as reported previously.[2]

Reader-based HTRF assay

Compound potencies were evaluated by using a previously reported METTL3 inhibition assay. Briefly, the level of m^6A in the oligoribonucleotide substrate after the reaction catalyzed by METTL3-METTL14 was quantified by measuring specific binding of modified oligoribonucleotide to the m^6A reader $YTHDC1_{345-509}$ by homogeneous time-resolved fluorescence (HTRF). Tested compounds that inhibit METTL3 decrease the m^6A level and thus reduce the HTRF signal. The biochemical assay was performed as described previously.[2] The IC_{50} values derived from fitting a dose-response curve to the data using nonlinear regression. The IC_{50} values are given as an average of at least two independent measurements for each compound in the main text.

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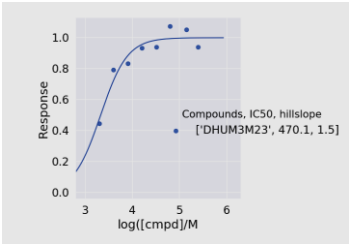
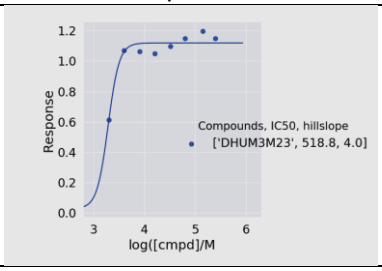
<tr><td> 
</td><td> 
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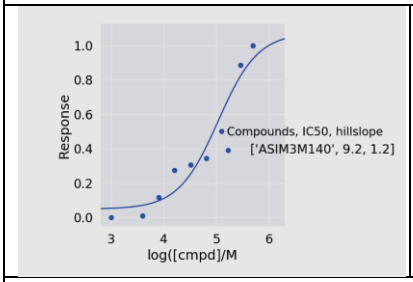
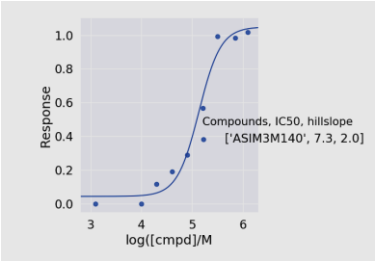
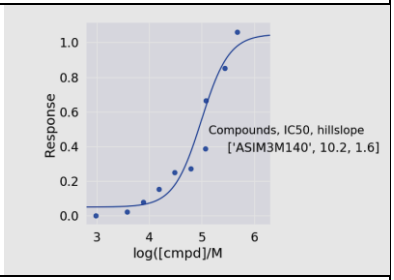
<tr><td> 
</td><td> 
</td><td> 
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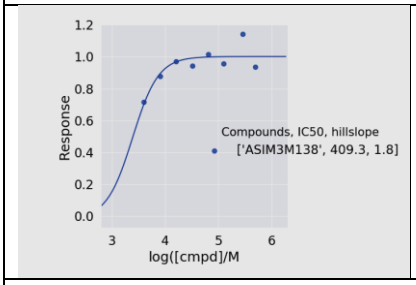
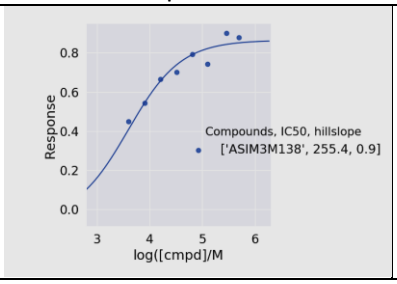
<tr><td> 
</td><td> 
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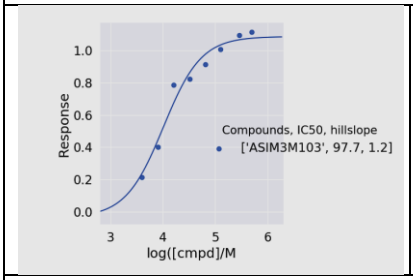
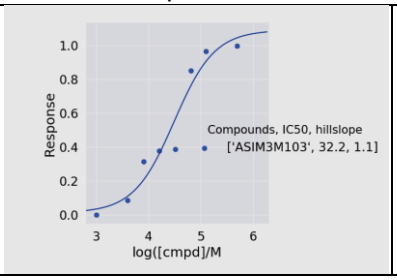
<tr><td> 
</td><td> 
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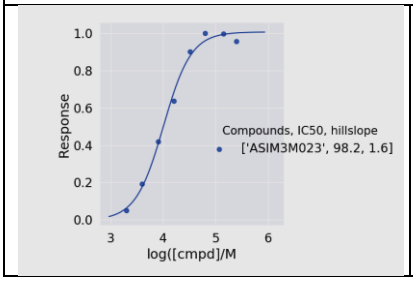
<tr><td> 
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Fig S1: Dose-response curves for compounds **1** – **7** as measured in the HTRF METTL3 inhibition assays.

Crystallization

The protein crystals of $METTL3_{354-580}$-$METTL14_{106-396}$ were obtained as previously described.[1] The soaking experiment was carried out by transferring crystals to a 1 µL drop containing 200 mM compound directly dissolved in the buffer containing 100mM TRIS at pH 8.0, 30% PEG 3350 and 200 mM Mg acetate. After 16 h incubation at 22°C, the crystals were harvested and flash-frozen in liquid nitrogen.

Data collection and structure solution

Diffraction data were collected at the PXIII beamline at the Swiss Light Source (SLS) of the Paul Scherrer Institute (PSI, Villigen, Switzerland) and processed using XDS.[3] The crystal structures were solved by molecular replacement techniques using 5L6D structure as the search model with the Phaser program[4] from the Phenix package. In the crystals not subjected to soaking, clear electron density for product cofactor S-adenosyl-homocysteine (SAH) is visible. Therefore, in this soaking experiment setup test compounds competed with SAH for the S-adenosyl methionine (SAM) binding site. In the crystal structures of adenosine analogues that were able to replace SAH in the binding site, the electron density due to the homocysteine part of SAH was no longer visible. All of the crystallographic models were constructed through iterative cycles of manual model building with COOT[5] and refinement with phenix.refine.[6] Default XYZ (reciprocal-space), XYZ (real-space), individual B-factors and occupancies refinement parameters appropriate for the resolution range were utilized. During the first run of the refinement update water was used in phenix.refine followed by addition of the missing water molecules manually.

PDB ID	6TTP	6TTT	6TTV
Compound	**1**	**2**	**3**
Data Collection			
Space group	$P3_221$	$P3_221$	$P3_221$
Cell dimension a, b, c (Å)	64.25, 64.25, 226.32	64.05, 64.05, 225.67	64.15, 64.15, 225.40
Cell dimension α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	44.8 (2.0-2.13)	49.78 (2.30-2.44)	44.67 (2.14-2.27)
Unique reflections*	36716 (5758)	24646 (3894)	30741 (4796)
Completeness*	97.7 (96.7)	99.1 (99.5)	99.9 (99.5)
Redundancy*	8.5 (8.7)	6.5 (6.7)	8.2 (8.5)
R_{merge}*	5.3 (78.5)	8.2 (90.5)	10.7 (117.5)
CC (1/2)	100 (83.8)	99.9 (68.8)	99.9 (75.6)
I/σI	24.79 (2.64)	17.80 (2.03)	14.53 (1.58)
Refinement			
R_{work}/R_{free}	0.1932/0.2274	0.1901/0.2381	0.1976/0.2439
RMSD bond (Å)	0.008	0.008	0.008
RMSD angle (°)	0.958	0.932	0.933
B-factor (Å^2) **	44.24/43.78/46.78	50/51.5/50.5	45.82/47.02/48.04
Ramanchandran Favored	96.92	96.20	97.39
Ramanchandran allowed	2.84	3.56	2.38
Ramanchandran Disallowed	0.24	0.24	0.24
Number of water molecules	213	165	163

PDB ID	6TTW	6TTX	6TU1
Compound	**4**	**5**	**8**
Data Collection			
Space group	$P3_221$	$P3_221$	$P3_221$
Cell dimension a, b, c (Å)	63.88, 63.80, 225.63	64.21, 64.21, 226.38	64.21, 64.21, 226.57
Cell dimension α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	49.67 (2.2-2.34)	44.77 (2.00-2.12)	44.78 (2.31-2.44)
Unique reflections*	26290 (4206)	37538 (5913)	24823 (3873)
Completeness*	93.6 (95.2)	99.8 (99.5)	99.5 (98.7)
Redundancy*	6.9 (7.0)	6.5 (6.5)	6.5 (6.6)
R_{merge}*	13.9 (99.6)	10.9 (109.9)	16.3 (126.3)
CC (1/2)	99.7 (69.1)	99.9 (67.4)	99.5 (53.7)
I/σI	11.67 (1.96)	13.35 (1.69)	7.98 (1.45)
Refinement			
R_{work}/R_{free}	0.1845/0.2285	0.1914/0.2173	0.1949/0.2339
RMSD bond (Å)	0.009	0.009	0.008
RMSD angle (°)	1.065	0.949	0.969
B-factor (Å^2) **	39.07/36.79/42.98	36.28/40.19/42.77	46.30/46.45/48.14
Ramanchandran Favored	96.91	97.83	97.12
Ramanchandran allowed	2.61	2.17	2.64
Ramanchandran Disallowed	0.48	0	0.24
Number of water molecules	237	321	177

*Statistics for the highest resolution shell is shown in parentheses.

** P/L/W indicate protein, ligand/ion and water molecules, respectively.

Table S1: PDB codes, X-ray data collection and refinement statistics for structures of ternary complexes of METTL3, METTL14 and inhibitors.

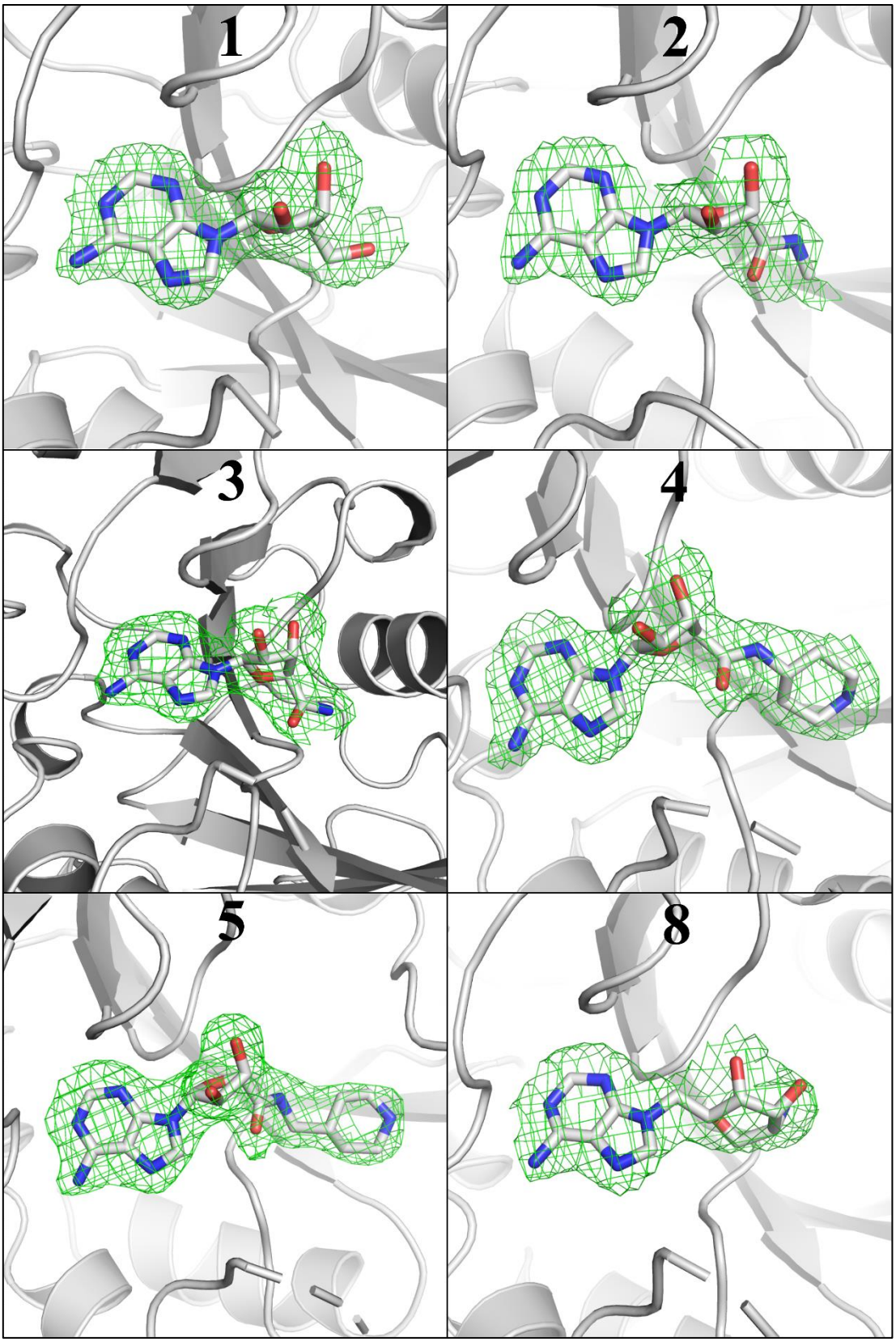

Fig S2: F_o-F_c electron density maps of compounds **1**, **2**, **3**, **4**, **5** and **8** bound to METTL3 at a contour level of 2.0 sigma.

References

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[6] P. V. Afonine, R. W. Grosse-Kunstleve, N. Echols, J. J. Headd, N. W. Moriarty, M. Mustyakimov, T. C. Terwilliger, A. Urzhumtsev, P. H. Zwart, P. D. Adams, *Acta Crystallogr D Biol Crystallogr* **2012**, *68*, 352-367.