# Small-molecule inhibitors of the m$^7$G-RNA writer METTL1

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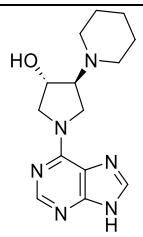
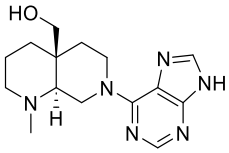
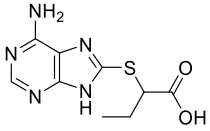
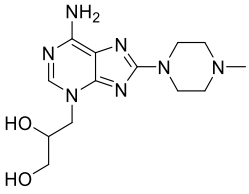
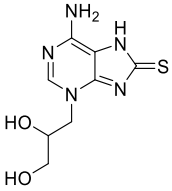
## Supporting information

| Compound number | 2D structure | SEED energy (rank) | Residual signal at 1 mM compound concentration (%) | IC50 M1-WDR4 (uM) | HillSlope |
|---|---|---|---|---|---|
| |  | -17.43 (7) | 98 | | |
| |  | -25.32 (1) | 76 | | |
| |  | -17.19 (8) [S] | 80 | | |
| |  | -16.04 (25) [S] | 89 | | |
| **1** |  | -18.10 (5) [R] | 50 | 144 | -0.97 |

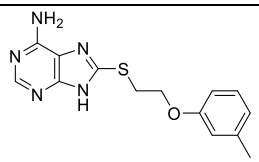
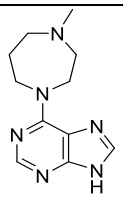
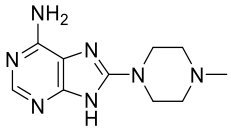
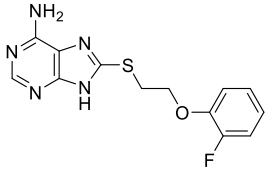
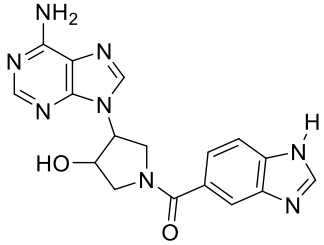
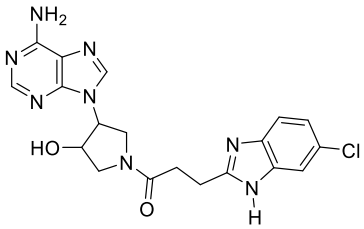
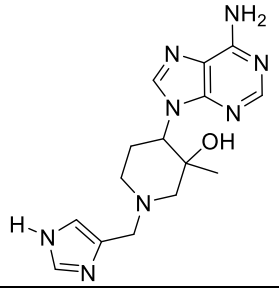
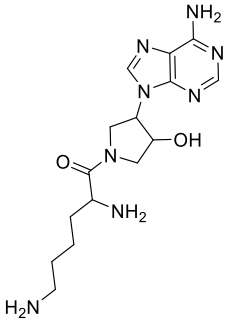
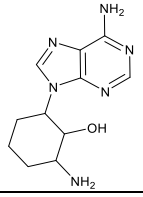
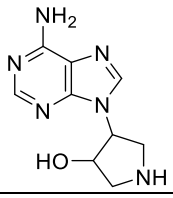
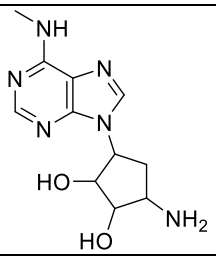
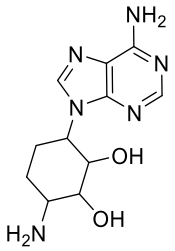
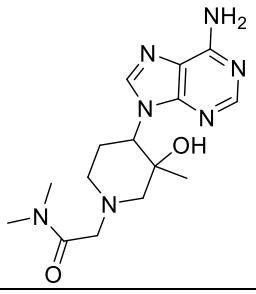
| | | | | | |
|---|---|---|---|---|---|
| |  | -16.65 (17) | 102 | | |
| |  | -18.67 (3) | 82 | | |
| **2** |  | -17.56 (6) | 46 | 187 | -0.91 |
| |  | -17.16 (10) | 94 | | |

*Table S1*. *In vitro* characterization of the nine adenine derivatives identified by the docking campaign on METTL1. The SEED energy is the predicted binding energy in kcal/mol. Where present, the IC50 value is the average of two or more biological replicates, and each biological replicate is the average of two technical replicates. In case of enantiomeric compounds, the enantiomer to which the SEED energy and rank are referred to is specified in square parenthesis.

| Compound number | ASINEX code | Structure | Residual signal at 2.5 mM compound concentration (%) | IC50 M1-WDR4 (µM) | HillSlope |
|---|---|---|---|---|---|
| | LAS51494822 |  | 58 | | |
| | LAS 40775464 |  | 40 | >1000 | -2.00 |

| | | | | | |
|---|---|---|---|---|---|
| | BDG34071792 |  | 20 | 478<br>549 | -1.35<br>-1.34 |
| | BDH34001749 |  | 28 | 960<br>586 | -1.26<br>-1.40 |
| | BDH33909967 |  | 10 | 473<br>555 | -1.09<br>-1.11 |
| **5** | LAS33909600 |  | 1 | 277 | -1.45 |
| | BDG33904788 |  | 21 | 783<br>634 | -1.91<br>-1.91 |
| **4** | BDH33920757 |  | 8 | 212 | -1.11 |
| | BDG34073059 |  | 68 | | |

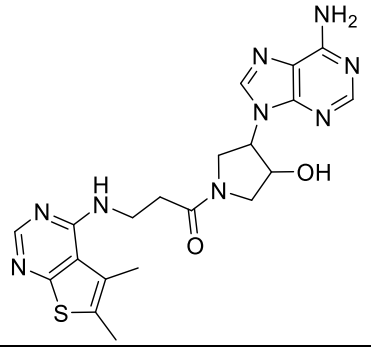
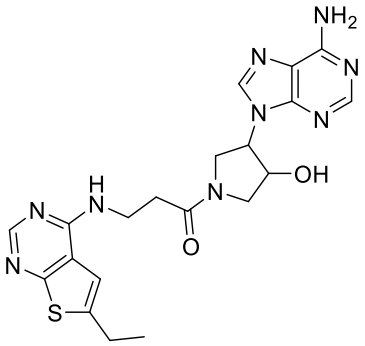
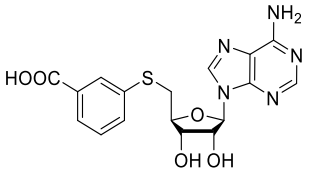
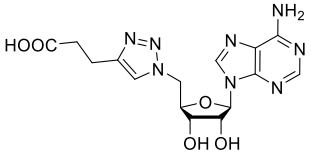
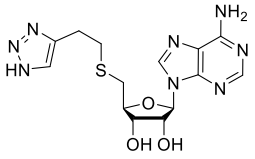
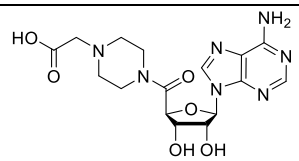
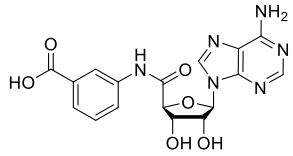
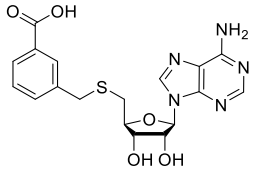
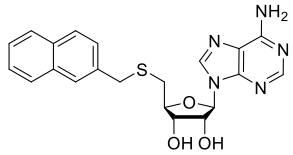
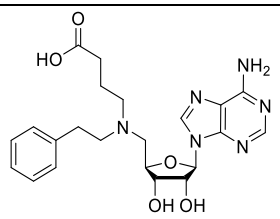
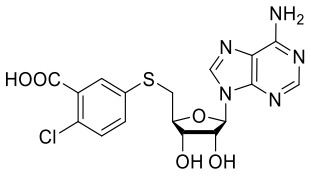
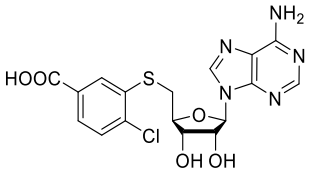
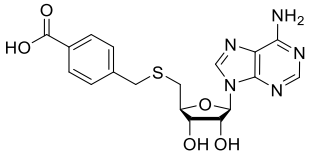
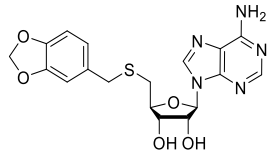
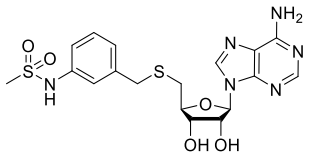
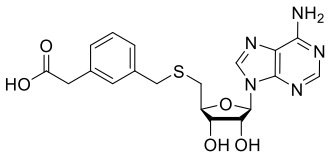
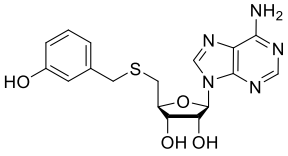
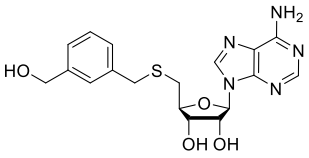
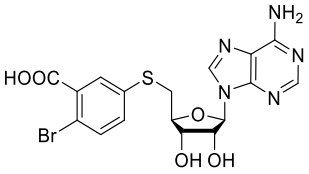
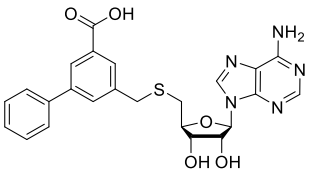
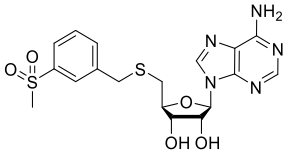
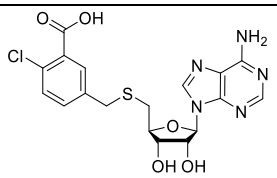
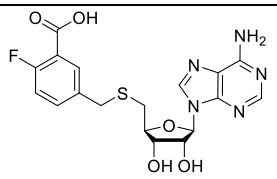
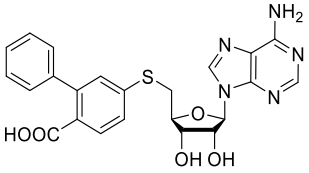
| | | | | | |
|---|---|---|---|---|---|
| | LAS34159697 |  | 7 | 291<br>271 | -1.40<br>-1.26 |
| **3** | BDG34159698 |  | -1 | 164 | -1.24 |

*Table S2. In vitro* characterization of the 11 compounds selected after the initial METTL1-WDR4 enzymatic assay screening of the library of adenosine mimics. When present, the IC50 values are from the average of two technical duplicates, except for the compounds reported in the main text (numbered) for which we report the average of at least two biological replicates, and each biological replicate is the average of two technical replicates.

| Compound number | Structure | Residual signal at 1 mM compound concentration (%) | IC50 M1-WDR4 (µM) | HillSlope |
|---|---|---|---|---|
| |  | 47 | | |
| |  | 323 | | |
| |  | 82 | | |

| | | | | |
|---|---|---|---|---|
| **11** |  | 13 | 178 | -1.01 |
| |  | 38 | >1000 | -1.60 |
| |  | 55 | | |
| |  | 14 | | |
| |  | 28 | >1000 | -1.06 |
| |  | 30 | 608<br>579 | -1.56<br>-0.98 |
| |  | 26 | 419<br>311 | -1.04<br>-1.21 |
| |  | 28 | 393<br>562 | -0.98<br>-1.33 |
| **8** |  | 16 | 52 | -1.22 |
| |  | 4 | Interferent: reduces the maximal signal to 44% at 400 µM | |

| | | | | |
|---|---|---|---|---|
| |  | 28 | 423<br>413 | -1.21<br>-1.92 |
| |  | 51 | | |
| |  | 6 | Interferent: reduces the maximal signal to 69% at 100 µM | |
| |  | 30 | | |
| |  | 18 | Interferent: reduces the maximal signal to 50% at 20 µM | |
| **9** |  | 16 | 61 | -1.03 |
| |  | 24 | | |
| |  | 32 | 312<br>507 | -1.19<br>-1.02 |
| |  | 9 | 280<br>319 | -1.16<br>-0.81 |

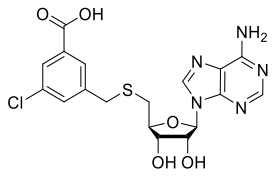
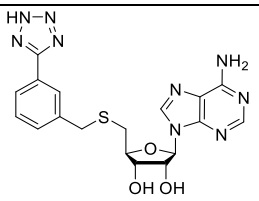
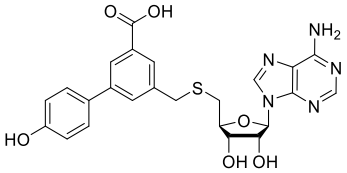
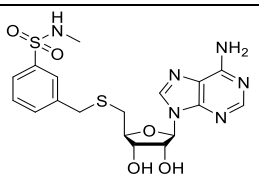
| | | | | |
|---|---|---|---|---|
| |  | 8 | Interferent: reduces the maximal signal to 65% at 200 µM | |
| **10** |  | 11 | 78 | -1.09 |
| **6** |  | 6 | 41 | -1.01 |
| **7** |  | 12 | 47 | -1.23 |

*Table S3*. Same as Table S2 for the 26 adenosine derivatives screened using the METTL1-WDR4 enzymatic assay. For the compounds with residual signal at 1 mM compound concentration (%) in red the IC50 value could not be calculated.

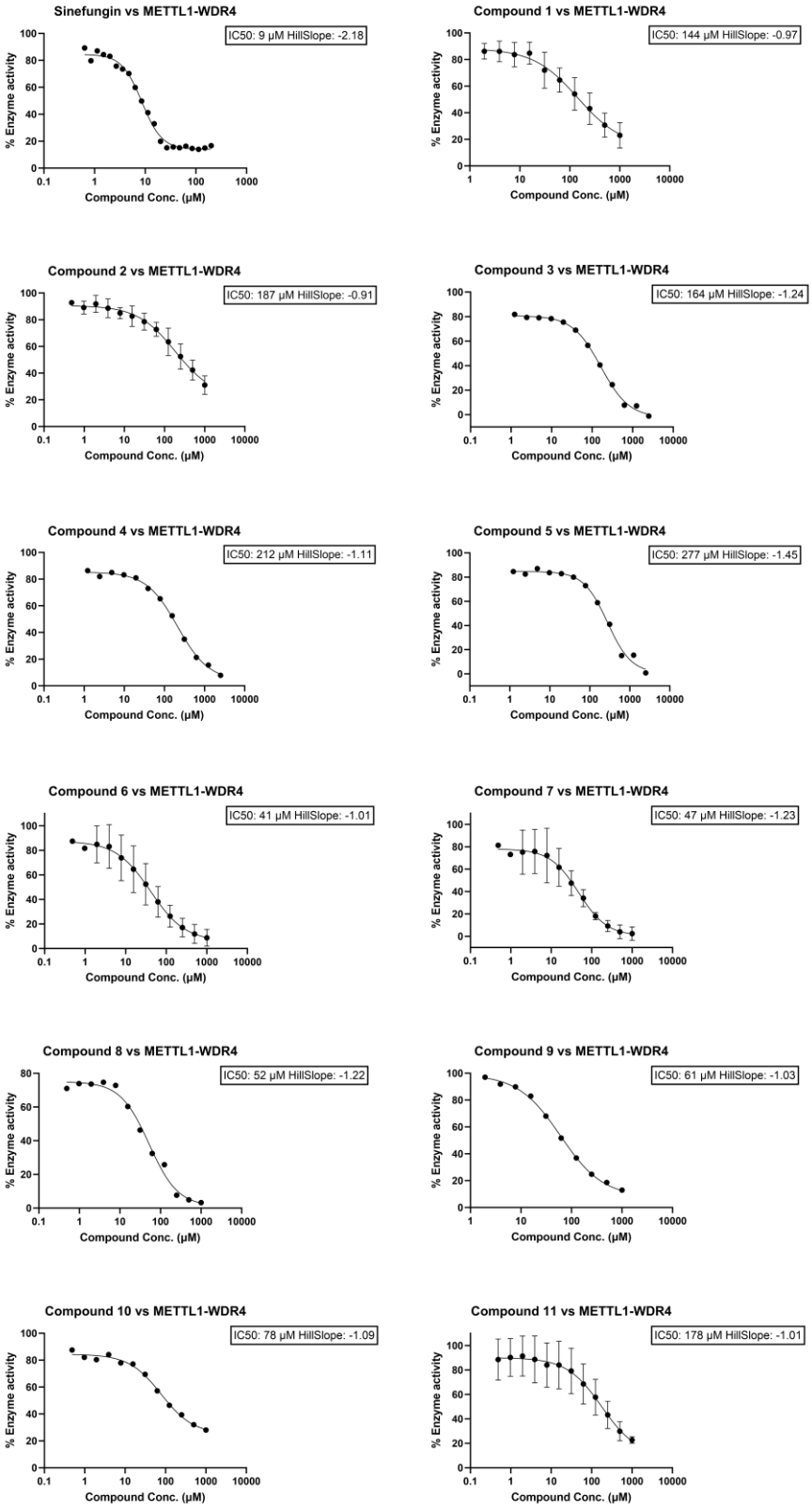



*Figure S1*. Dose-response curves for METTL1-WDR4 and the 11 compounds presented in Table 1. The curves come from the average of two or more biological replicates, and each biological replicate is the average of two technical replicates. The error bars represent the standard deviation for the biological triplicates.

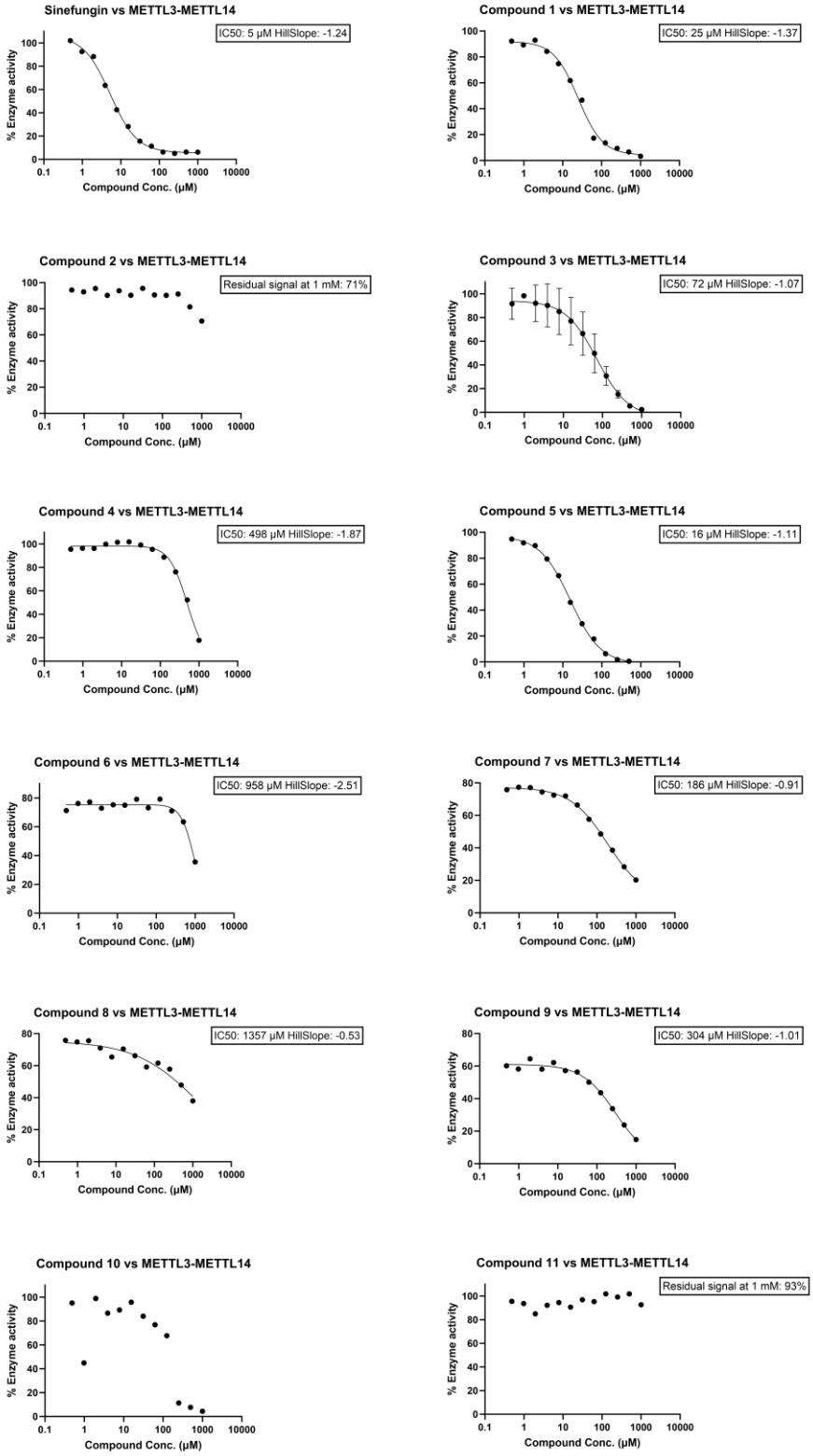



*Figure S2.* Selectivity testings against METTL3-METTL14 for the 11 compounds presented in Table 1. The curves come from the average of two or more biological replicates, and each biological replicate is the average of two technical replicates. The error bars represent the standard deviation for the biological triplicates.

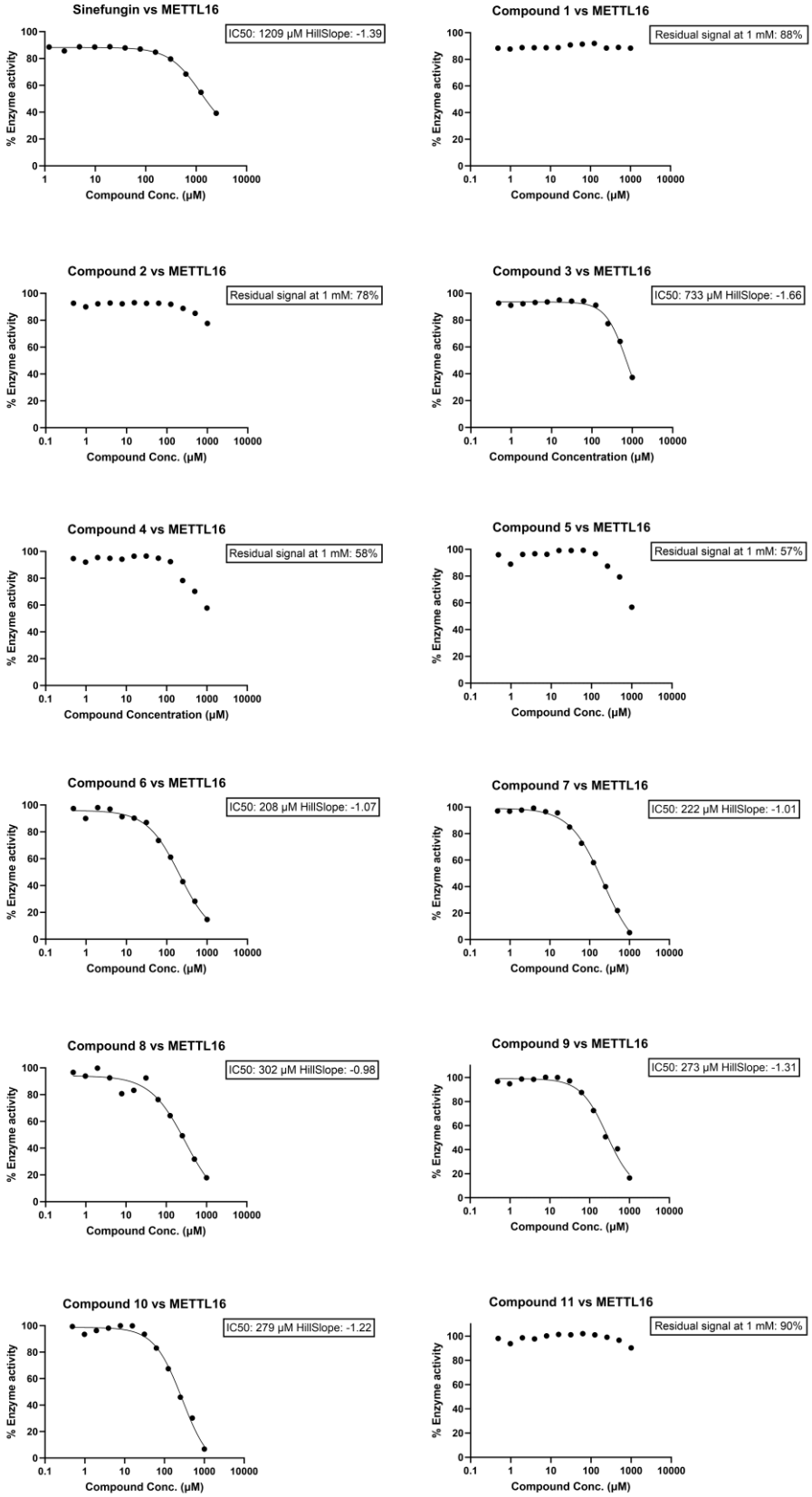



*Figure S3.* Selectivity testings against METTL16 for the 11 compounds presented in Table 1. The curves come from the average of two biological replicates, and each biological replicate is the average of two technical replicates.

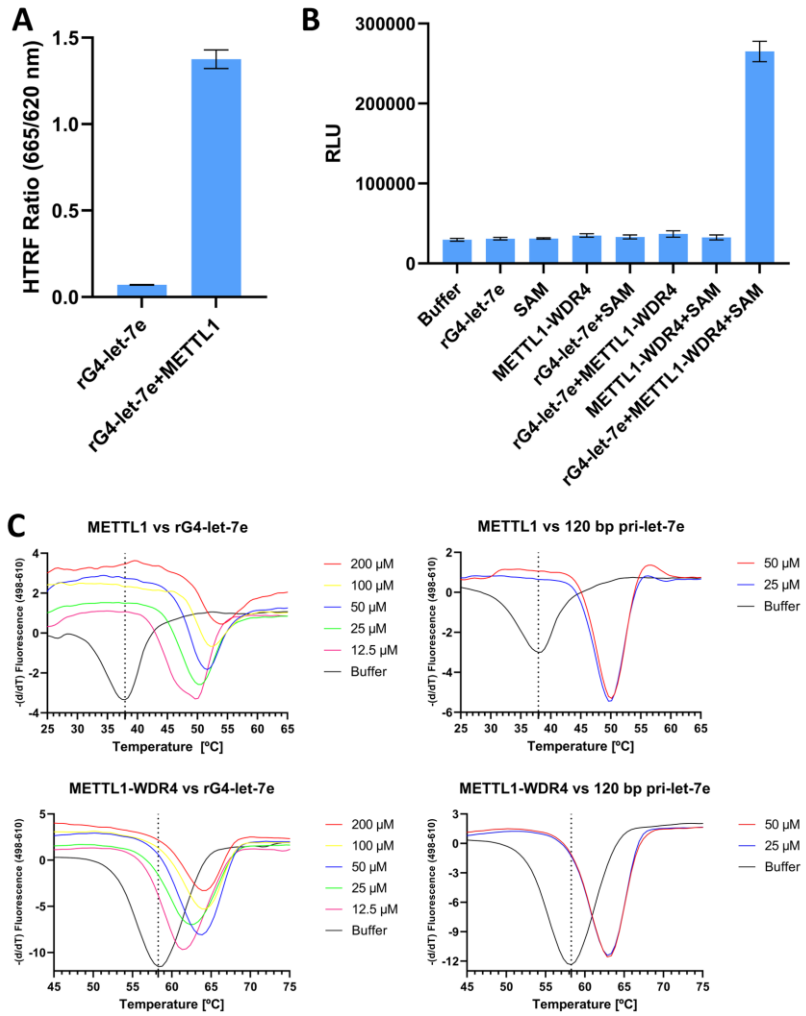

A
HTRF Ratio (665/620 nm)
1.5
1.0
0.5
0.0
rG4-let-7e
rG4-let-7e+METTL1
B
RLU
300000
200000
100000
0
Buffer
rG4-let-7e
SAM
METTL1-WDR4
rG4-let-7e+SAM
rG4-let-7e+METTL1-WDR4
METTL1-WDR4+SAM
rG4-let-7e+METTL1-WDR4+SAM
C
METTL1 vs rG4-let-7e
METTL1 vs 120 bp pri-let-7e
METTL1-WDR4 vs rG4-let-7e
METTL1-WDR4 vs 120 bp pri-let-7e
-(d/dT) Fluorescence (498-610)
Temperature [°C]
200 µM
100 µM
50 µM
25 µM
12.5 µM
Buffer
D
280 nM UV absorbance
Injection
Fraction number
E
280 nM UV absorbance
Injection
Fraction number

*Figure S4.* pri-let-7e and METTL1/METTL1-WDR4 complex formation. (A) FRET emission in presence of rG4-let-7e or rG4-let-7e+METTL1. (B) Luminescence emission from the METTL1 enzymatic assay in presence of different combinations of the components. A significant luminescence emission can only be observed when both METTL1-WDR4 and the rG4-let-7e are added to the reaction. (C) Thermal shift of METTL1 and METTL1-WDR4 in presence of rG4-let-7e or 120bp pri-let-7e. (D) Size exclusion profile of METTL1-WDR4 in presence of 120 bp pri-let-7e on a Superdex 200 10/300 GL column (Cytiva). The elution volumes of the first peak correspond to the molecular weight of the pri-let-7e-METTL1-WDR4 complex while the ones of the second and third peaks correspond to the molecular weight of METTL1-WDR4 and pri-let-7e, respectively. (E) Size exclusion profile after reinjection of the first peak on a Superdex 200 10/300 GL column (Cytiva), the tripartite complex does not dissociate.

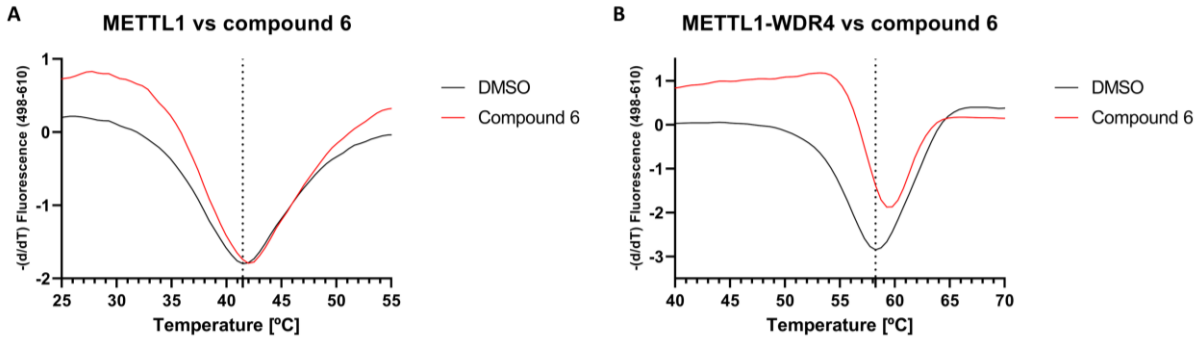



*Figure S5.* Thermal shift of METTL1 (A) and METTL1-WDR4 (B) in the presence of DMSO control (black) or compound **6** (red) which causes thermal stabilization at 1 mM concentration.

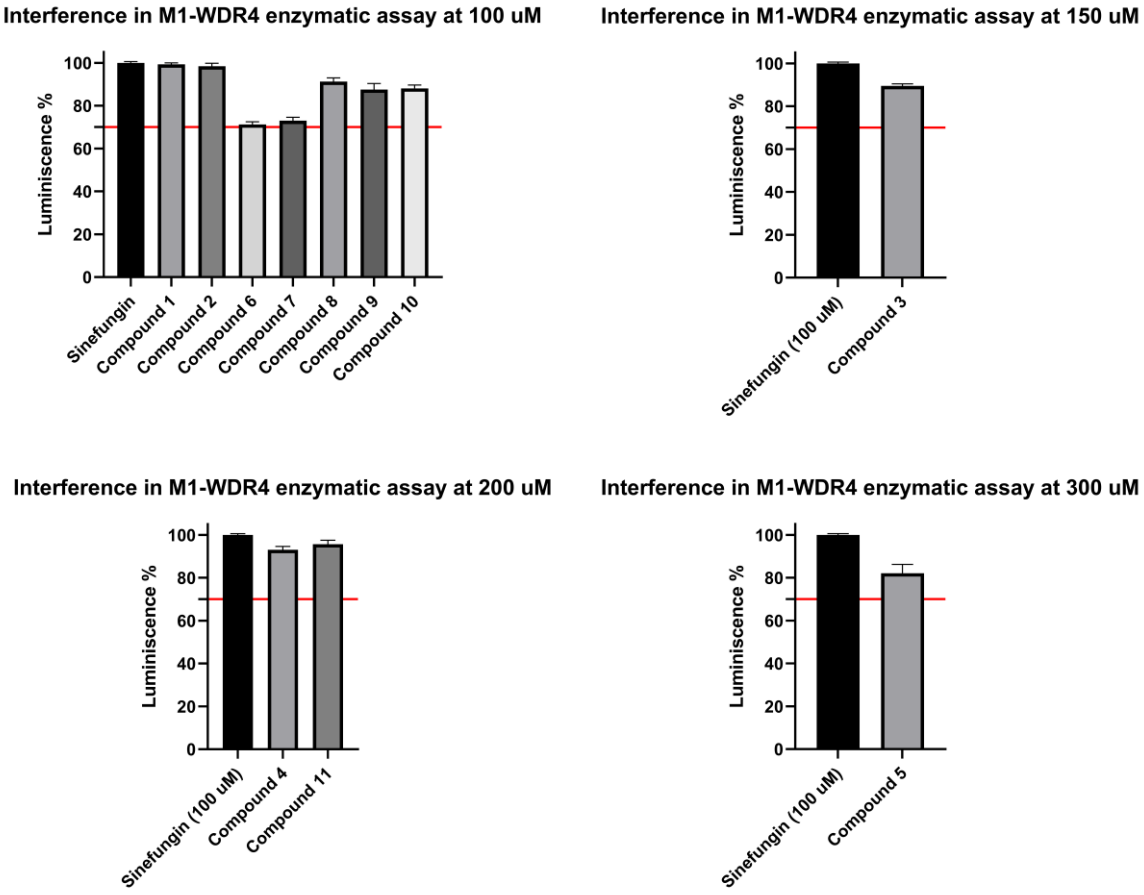



*Figure S6.* Interference screening of the compounds. The compounds that decreased by more than 30% the maximal emission (calculated in presence of 100 µM of sinefungin, which does not interfere with the assay) were excluded. The threshold at 70% is indicated as a red line. The interfering compounds are indicated in Table S3.

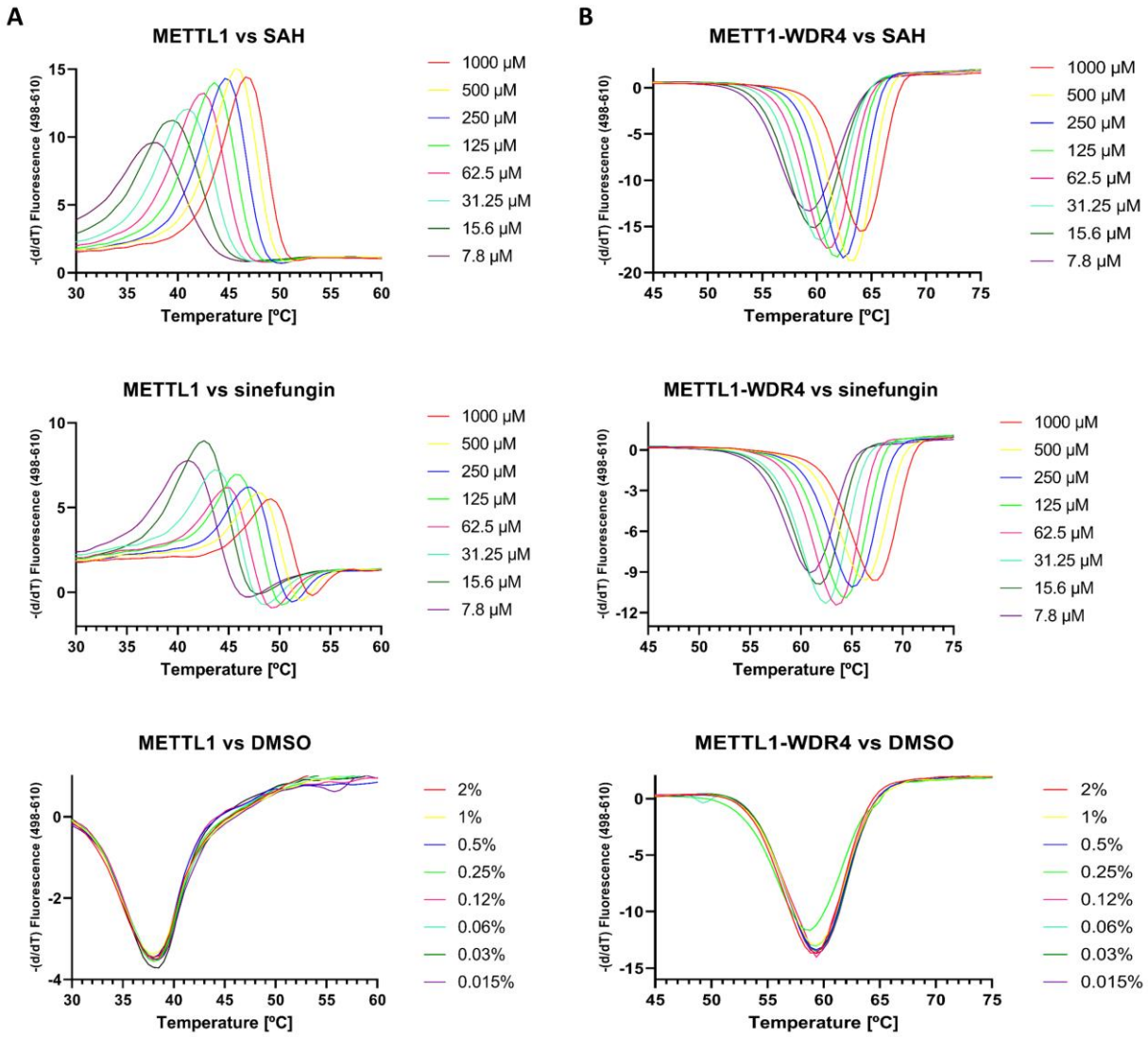



*Figure S7.* Dose-response thermal shift of METTL1 (A) and METTL1-WDR4 (B) in the presence of SAH, sinefungin, and DMSO.

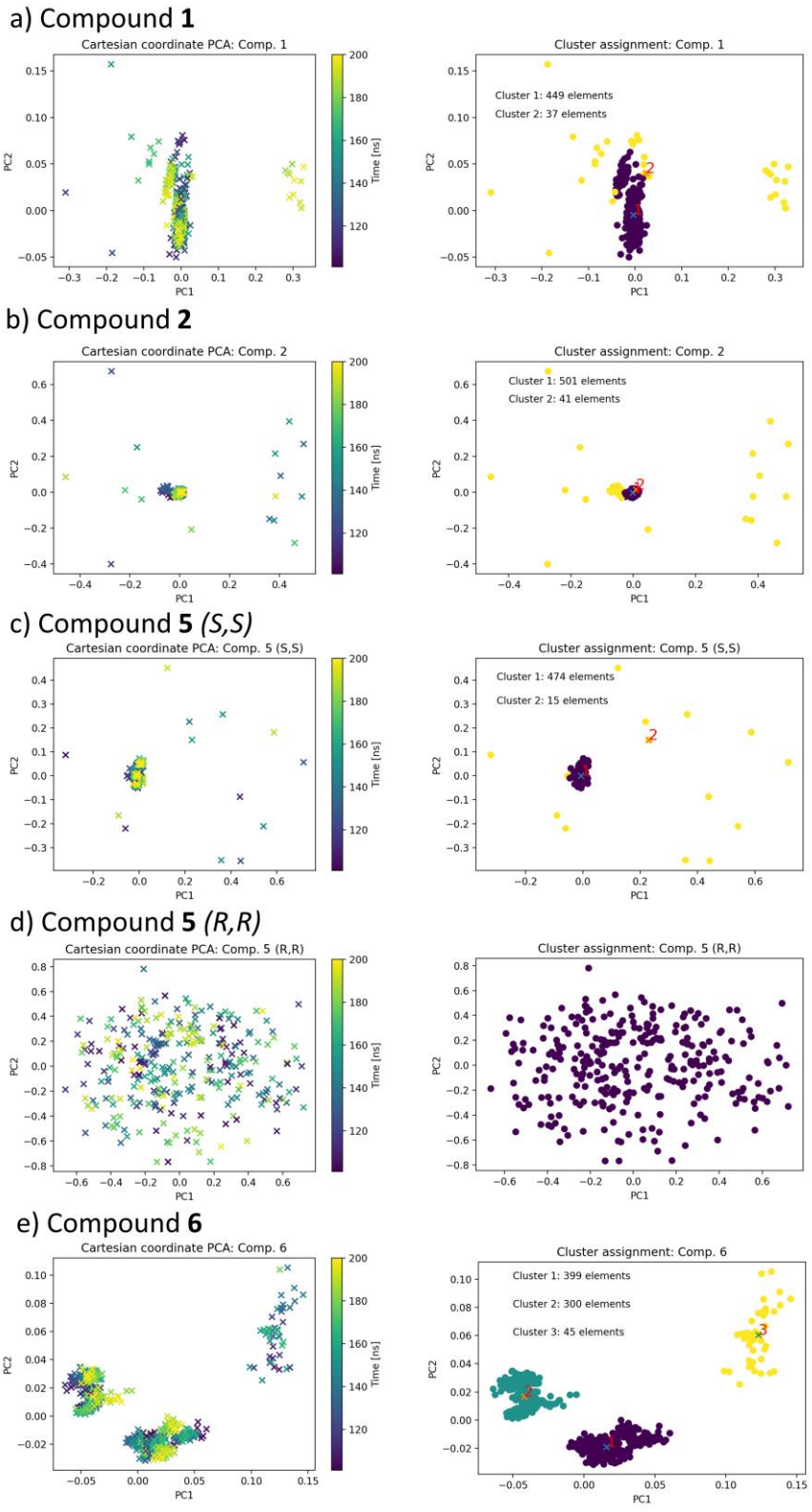



*Figure S8.* Calculation of representative binding poses for the last 100 ns of each independent simulation. (*Left*) Cartesian coordinates of the heavy atoms for each compound were projected on reduced (principal component) space. Each data point is colored according to time of simulation. (*Right*) Gaussian mixture model cluster assignments per frame (colored by cluster). The centroid point for each cluster (highlighted and numbered in red) was considered as representative pose.

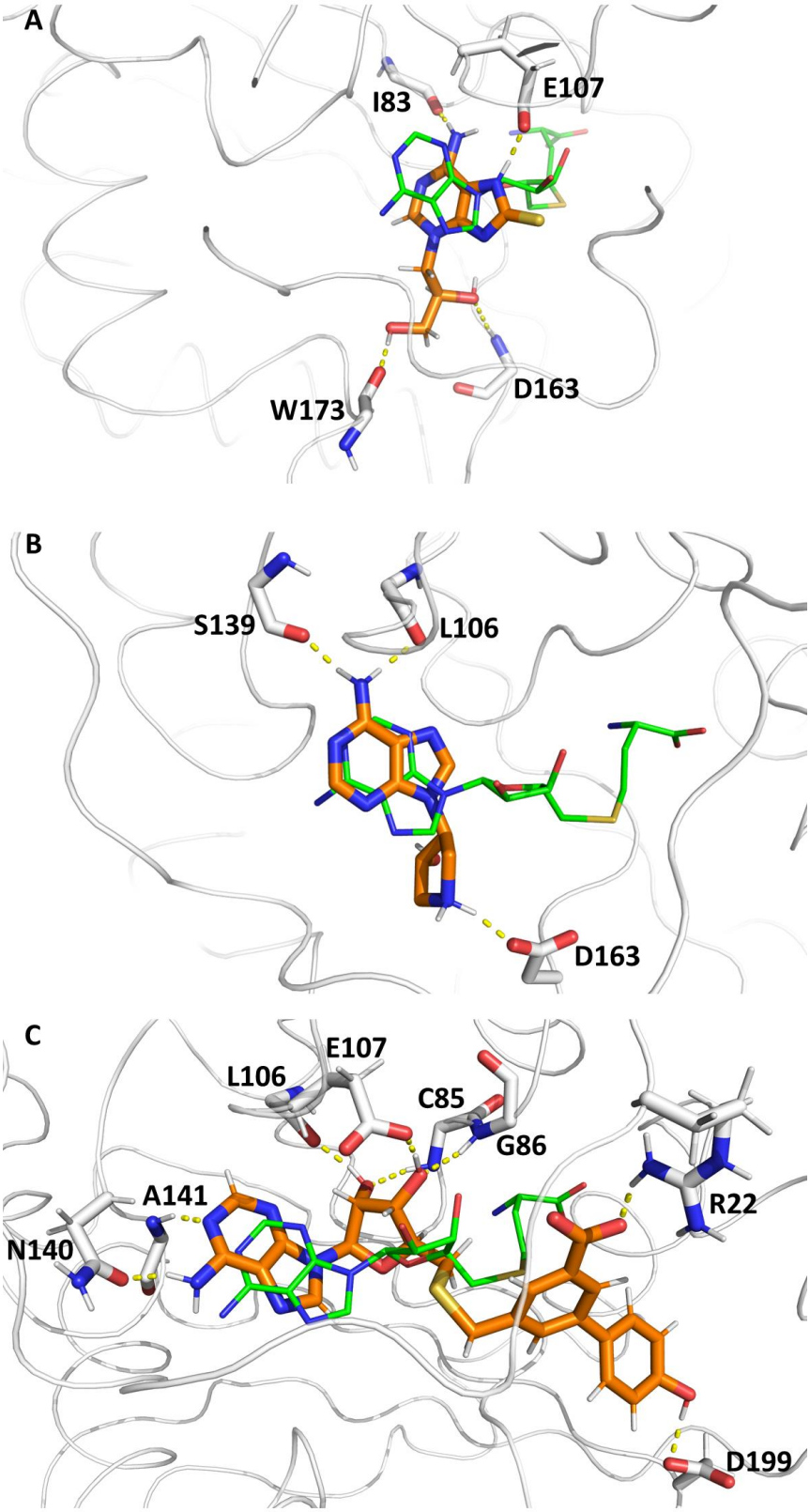
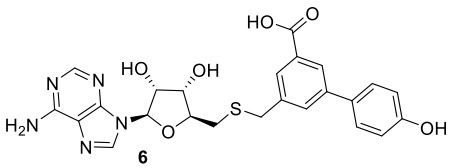



*Figure S9.* Secondary binding modes of **1** (A), **5**(S,S) (B), and **6** (C) as observed in the MD simulations. The carbon atoms of the binders are in orange and those of the protein in white, hydrogen bonds, and salt bridges are represented as yellow dashed lines. The binding mode of SAH (carbon atoms in green) from the crystal structure of the METTL1-SAH complex (PDB code: 7OGJ) is shown for comparison.

| | 7OGJ | 7PL1 |
|---|---|---|
| Wavelength | 1 | 1 |
| Resolution range | 46.92 - 1.59 (1.647 - 1.59) | 40.72 - 1.85 (1.916 - 1.85) |
| Space group | P 21 21 21 | P 43 21 2 |
| Unit cell | 63.57 80.46 138.23 90 90 90 | 128.76 128.76 39.43 90 90 90 |
| Total reflections | 781500 (77137) | 456918 (41897) |
| Unique reflections | 95912 (9442) | 28797 (2783) |
| Multiplicity | 8.1 (8.1) | 15.9 (15.0) |
| Completeness (%) | 99.82 (99.40) | 99.37 (98.13) |
| Mean I/sigma(I) | 18.42 (1.20) | 29.99 (3.13) |
| Wilson B-factor | 24.83 | 28.46 |
| R-merge | 0.0616 (1.509) | 0.06876 (0.9389) |
| R-meas | 0.0658 (1.611) | 0.07105 (0.972) |
| R-pim | 0.02279 (0.5571) | 0.01767 (0.2487) |
| CC1/2 | 0.999 (0.608) | 1 (0.835) |
| CC* | 1 (0.87) | 1 (0.954) |
| Reflections used in refinement | 95783 (9438) | 28787 (2783) |
| Reflections used for R-free | 4791 (473) | 1441 (139) |
| R-work | 0.1930 (0.3745) | 0.1900 (0.2436) |
| R-free | 0.2150 (0.3955) | 0.2120 (0.2877) |
| CC(work) | 0.953 (0.625) | 0.953 (0.855) |
| CC(free) | 0.937 (0.490) | 0.945 (0.755) |
| Number of non-hydrogen atoms | 4092 | 1897 |
| macromolecules | 3425 | 1647 |
| ligands | 86 | 66 |
| solvent | 581 | 184 |
| Protein residues | 425 | 210 |
| RMS(bonds) | 0.007 | 0.008 |
| RMS(angles) | 1.01 | 0.96 |
| Ramachandran favored (%) | 98.8 | 98.53 |
| Ramachandran allowed (%) | 1.2 | 1.47 |
| Ramachandran outliers (%) | 0 | 0 |
| Rotamer outliers (%) | 0.28 | 0.61 |
| Clashscore | 6.24 | 7.69 |
| Average B-factor | 31.22 | 31.31 |
| macromolecules | 29.42 | 29.99 |
| ligands | 42.68 | 37.26 |
| solvent | 40.1 | 40.99 |

*Table S4*. Data collection and refinement statistics for METTL1 in complex with SAH (PDB code: 7OGJ) and METTL1 in complex with sinefungin (PDB code: 7PL1) as generated by phenix.table one. Statistics for the highest-resolution shell are shown in parentheses.

# HPLC traces of compounds 6-11




**6**

Empower 2 software

## Default Individual Report

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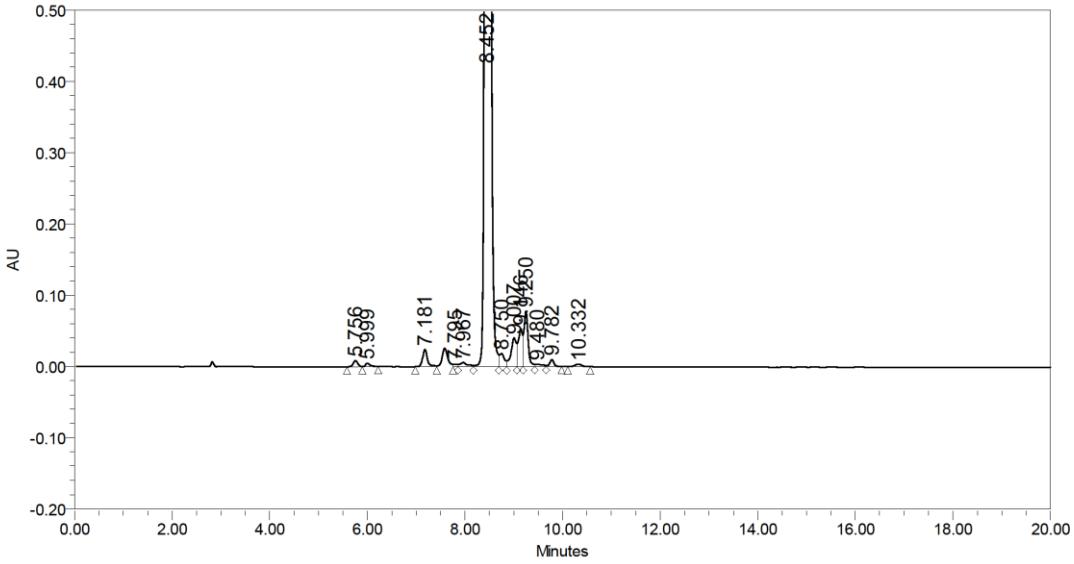
| Sample Type: | Unknown | Sample Set Name: | Olga_20210518 |
| Vial: | 2 | Acq. Method Set: | MeCN_0_100% |
| Injection #: | 1 | Processing Method: | Processing |
| Injection Volume: | 20.00 ul | Channel Name: | W2489 ChB |
| Run Time: | 20.0 Minutes | Proc. Chnl. Descr.: | W2489 ChB 254nm |
| Date Acquired: | 2021.05.18. 17:38:52 EET | | |
| Date Processed: | 2021.05.19. 11:34:00 EET | | |

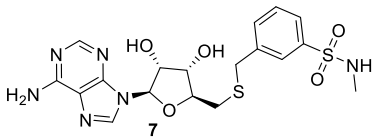



| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 5.756 | 55410 | 0.28 | 8608 |
| 2 | 5.999 | 30037 | 0.15 | 4638 |
| 3 | 7.181 | 168506 | 0.86 | 24393 |
| 4 | 7.795 | 20268 | 0.10 | 3492 |
| 5 | 7.967 | 65104 | 0.33 | 5825 |
| 6 | 8.452 | 18009393 | 91.40 | 2812469 |
| 7 | 8.750 | 131797 | 0.67 | 18340 |
| 8 | 9.007 | 306224 | 1.55 | 40336 |

Reported by User: Olita
Report Method: Default Individual Report
Report Method ID 5088
Page: 1 of 2

Project Name: Martins
Date Printed:
2021.05.19.
11:34:51 Europe/Riga

| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 9 | 9.146 | 330497 | 1.68 | 52883 |
| 10 | 9.250 | 457505 | 2.32 | 77597 |
| 11 | 9.480 | 36460 | 0.19 | 3414 |
| 12 | 9.782 | 59610 | 0.30 | 9893 |
| 13 | 10.332 | 33978 | 0.17 | 3275 |



7

Empower 2 software

# Default Individual Report

## SAMPLE INFORMATION

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| Sample Type: | Unknown | Sample Set Name: | Olga_20210608 |
| Vial: | 4 | Acq. Method Set: | MeCN_0_100% |
| Injection #: | 1 | Processing Method: | Processing |
| Injection Volume: | 20.00 ul | Channel Name: | W2489 ChB |
| Run Time: | 20.0 Minutes | Proc. Chnl. Descr.: | W2489 ChB 254nm |
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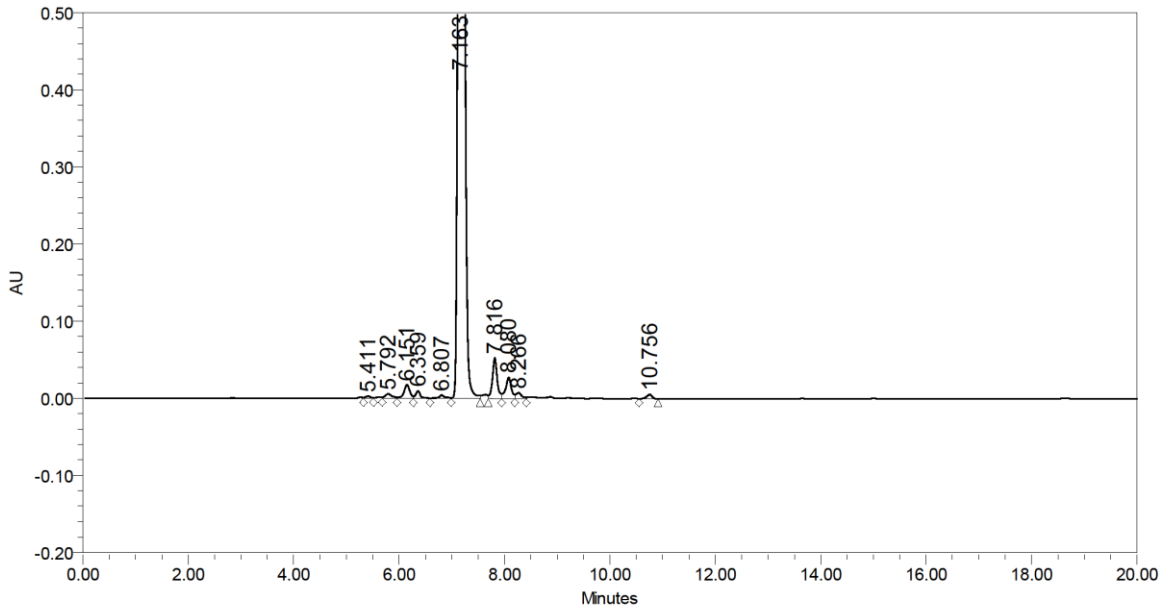
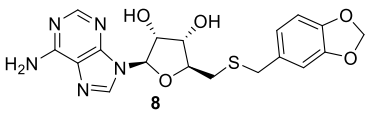
| Date Processed: | 2021.06.08. 9:53:31 EET | | |




| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 5.411 | 22095 | 0.15 | 3184 |
| 2 | 5.792 | 58362 | 0.39 | 6043 |
| 3 | 6.151 | 140283 | 0.95 | 17771 |
| 4 | 6.359 | 66047 | 0.45 | 9964 |
| 5 | 6.807 | 41802 | 0.28 | 4618 |
| 6 | 7.163 | 13847920 | 93.45 | 2140438 |
| 7 | 7.816 | 334560 | 2.26 | 52714 |
| 8 | 8.080 | 207821 | 1.40 | 27667 |

| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 9 | 8.266 | 59413 | 0.40 | 7663 |
| 10 | 10.756 | 40564 | 0.27 | 5616 |



8

Empower 2 software

# Default Individual Report

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| Injection #: | 1 | Processing Method: | Processing |
| Injection Volume: | 10.00 ul | Channel Name: | W2489 ChB |
| Run Time: | 20.0 Minutes | Proc. Chnl. Descr.: | W2489 ChB 254nm |
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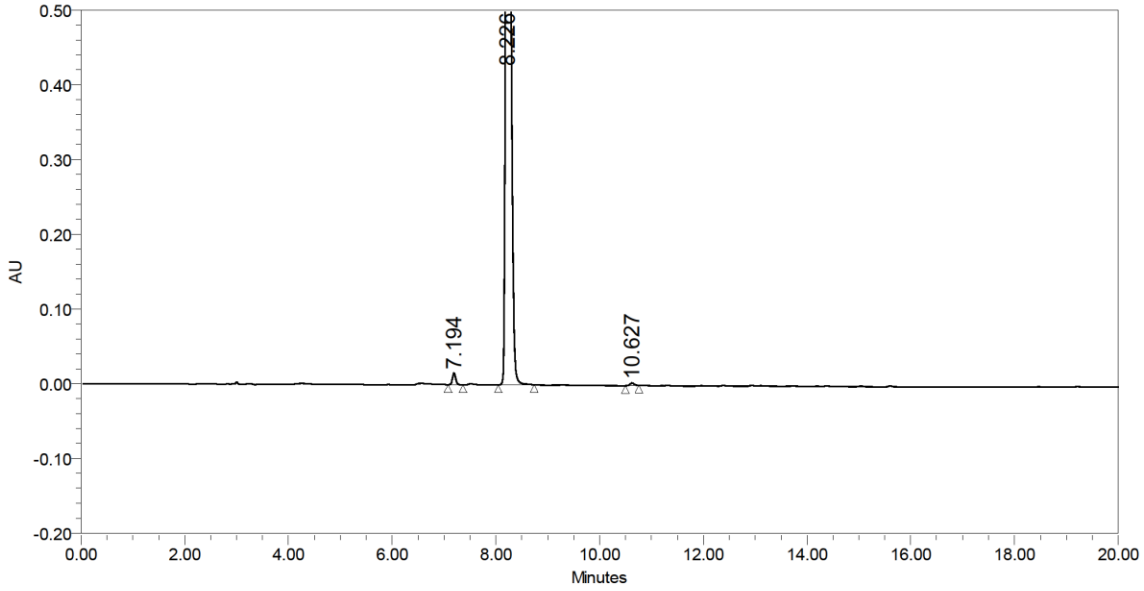
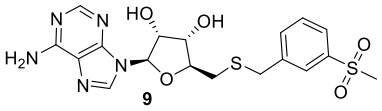



| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 7.194 | 67545 | 0.79 | 15574 |
| 2 | 8.226 | 8412372 | 98.99 | 1390846 |
| 3 | 10.627 | 18204 | 0.21 | 3379 |

Reported by User: Olita
Report Method: Default Individual Report
Report Method ID 5088
Page: 1 of 1

Project Name: Martins
Date Printed:
2020.12.23.
11:52:30 Europe/Riga



**9**

Empower 2 software

# Default Individual Report

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| Injection #: | 1 | Processing Method: | Processing |
| Injection Volume: | 20.00 ul | Channel Name: | W2489 ChB |
| Run Time: | 20.0 Minutes | Proc. Chnl. Descr.: | W2489 ChB 254nm |
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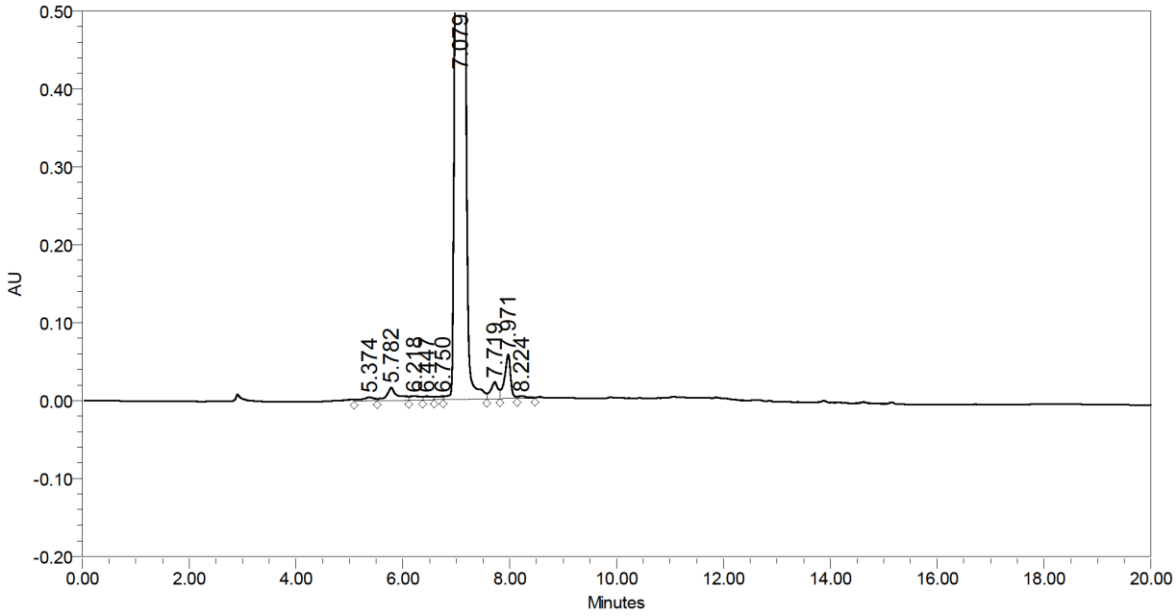
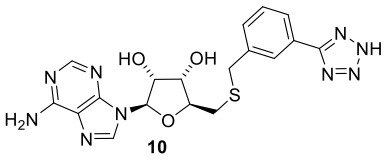



| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 5.374 | 76345 | 0.35 | 4586 |
| 2 | 5.782 | 242504 | 1.10 | 16618 |
| 3 | 6.218 | 73734 | 0.33 | 5129 |
| 4 | 6.447 | 55033 | 0.25 | 4462 |
| 5 | 6.750 | 40338 | 0.18 | 4161 |
| 6 | 7.079 | 20882114 | 94.69 | 2644567 |
| 7 | 7.719 | 202748 | 0.92 | 21484 |
| 8 | 7.971 | 442905 | 2.01 | 57032 |

| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 9 | 8.224 | 36468 | 0.17 | 3006 |




10

Empower 2 software

# Default Individual Report

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| Run Time: | 20.0 Minutes | Proc. Chnl. Descr.: | W2489 ChB 254nm |
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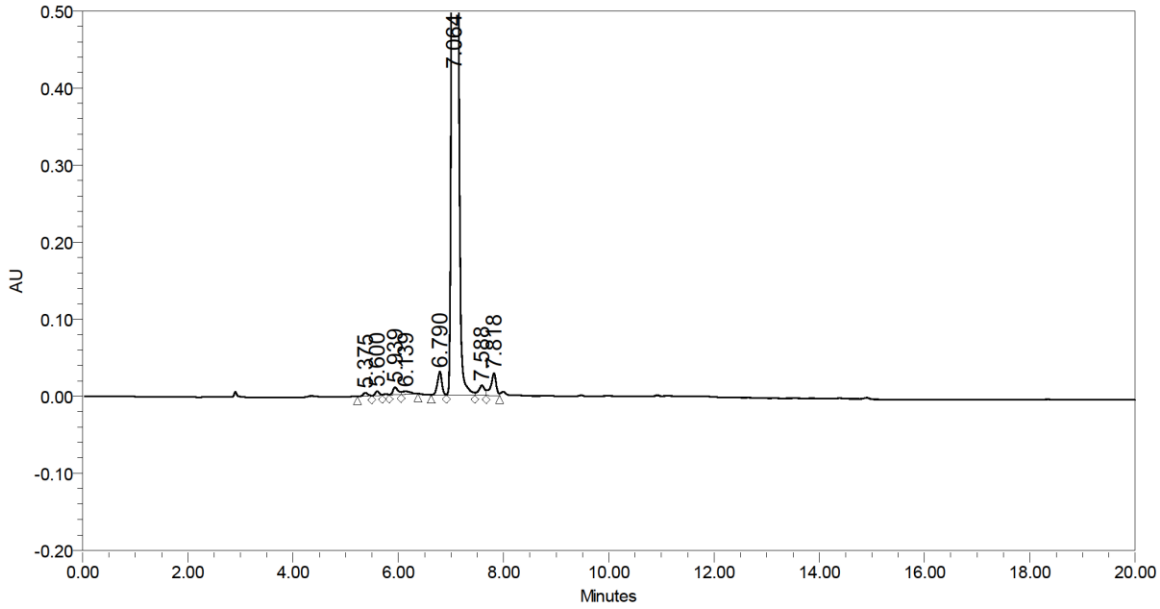
| Date Processed: | 2021.04.26. 11:02:03 EET | | |




| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 5.375 | 28336 | 0.16 | 4484 |
| 2 | 5.600 | 31678 | 0.17 | 5848 |
| 3 | 5.939 | 68638 | 0.38 | 9814 |
| 4 | 6.139 | 41866 | 0.23 | 3990 |
| 5 | 6.790 | 176140 | 0.97 | 30233 |
| 6 | 7.064 | 17539889 | 96.40 | 2864629 |
| 7 | 7.588 | 105890 | 0.58 | 13175 |
| 8 | 7.818 | 202515 | 1.11 | 29068 |

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Page: 1 of 1

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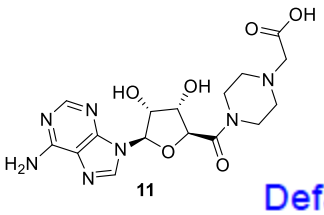


11

Empower software

# Default Individual Report

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Project Name: CLI

## SAMPLE INFORMATION

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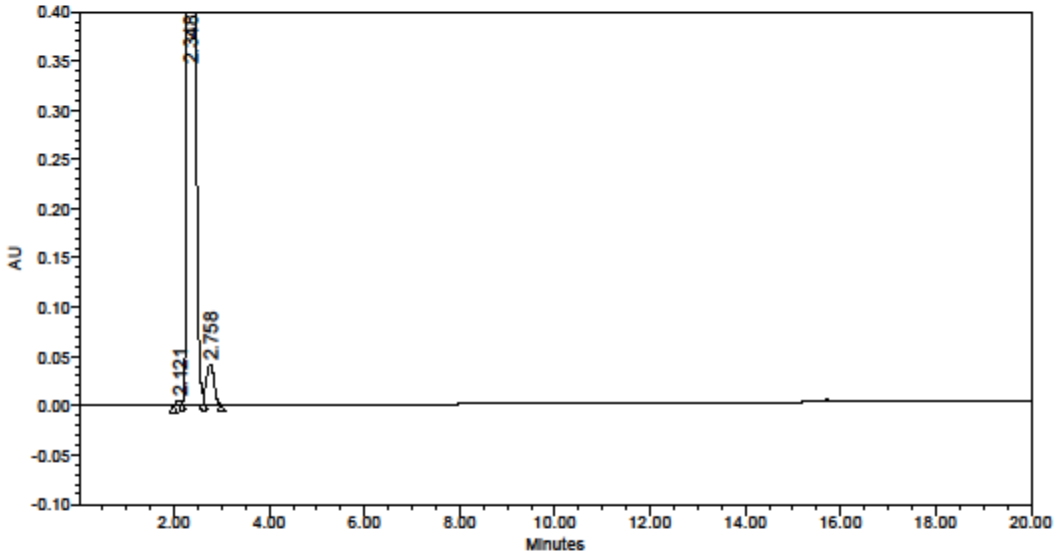
| Injection Volume: | 10.00 ul | Processing Method: | Certificate_100%_n |
| Run Time: | 20.0 Minutes | Channel Name: | 2487Channel 1 |
| Sample Set Name: | | Proc. Chnl. Descr.: | |




| | RT | Area | % Area | Height |
|---|---|---|---|---|
| 1 | 2.121 | 33872 | 0.18 | 4794 |
| 2 | 2.348 | 18763282 | 97.47 | 2313833 |
| 3 | 2.758 | 453372 | 2.36 | 40926 |