Supporting information

Structure-based design of a potent and selective YTHDC1 ligand

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Table of Contents

1. Figure S1: Inhibitory activity of compound 40 at 2 µM on a panel of 58 kinases

2. Figure S2: Dose-response curve for the antiproliferative effect of compound 40 against MOLM-13, NOMO-1 and HEK293T cell line.

3. Figure S3: Representative Western blot membranes for the data presented in Figure 4 (A) CETSA YTHDC1 (B) Cleaved PARP

4. Figure S4: HTRF dose-response curves of compound 40 against YTHDF1, YTHDF2, and YTHDF3, respectively.

5. Figure S5: Dose-response thermal shift of YTHDF1, YTHDF2, YTHDF3, and YTHDC2 in presence of compound 40

6. GST-YTHDC1 HTRF dose-response curves

7. Figure S6: Isothermal titration calorimetry curve of compound 3

8. NMR traces of final compounds

9. HPLC traces of final compounds
1. **Figure S1** - Inhibitory activity of compound 40 at 2 µM on a panel of 58 kinases

![Kinase Profiling](image)

2. **Figure S2**: Dose-response curve for the antiproliferative effect of compound 40 against (A) MOLM-13, NOMO-1 and (B) HEK293T cell lines.

![Dose-response curves](image)

<table>
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<th>Conc. [µM]</th>
<th>IC50</th>
<th>HillSlope</th>
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<td>MOLM-13</td>
<td>25</td>
<td>15.6</td>
<td>-2.7</td>
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<td>40</td>
<td>5.6</td>
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<tr>
<td>NOMO-1</td>
<td>25</td>
<td>61.6</td>
<td>-2.7</td>
</tr>
<tr>
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<td>8.2</td>
<td>-3.3</td>
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<tr>
<td>HEK293T</td>
<td>25</td>
<td>73.5</td>
<td>-3.3</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>14.3</td>
<td>-3.0</td>
</tr>
</tbody>
</table>
3. **Figure S3**: Representative Western blot membranes for the data presented in Figure 4.

(A) CETSA YTHDC1 (B) Cleaved PARP
4. **Figure S4:** HTRF dose-response curves of compound 40 against YTHDF1, YTHDF2, and YTHDF3, respectively.

5. **Figure S5:** Dose-response thermal shift of YTHDF1, YTHDF2, YTHDF3, and YTHDC2 in presence of compound 40.
6. **GST-YTHDC1 HTRF dose-response curves.** With the exceptions of compounds 2b, 4, 7, 8, 9, 15, 24, 29, 33, and 39 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 and quadruplicate. Compound 38 was only tested in single dose at 5 micromolar (103%).
7. Figure S6: Isothermal titration calorimetry curve of compound 3

- **N**: $1.05 \pm 0.00567$ Site
- **K**: $6.85E5 \pm 1.53E6$ M$^{-1}$
- **$\Delta H$**: $-1.26E4 \pm 130.7$ kcal/mol
- **$\Delta S$**: $-12.3$ cal/mol/deg
8. NMR traces of final compounds

5-chloro-N,3-dimethyl-1H-pyrazolo[4,3-d]pyrimidin-7-amine 3
2-chloro-N-methyl-9-phenyl-9H-purin-6-amine 4
9-benzyl-2-chloro-N-methyl-9H-purin-6-amine 5
2-chloro-N-methyl-9-(pyridin-4-ylmethyl)-9H-purin-6-amine 6
2-chloro-N-methyl-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine 7
3-(2-chloro-6-(methylamino)-9H-purin-9-yl)benzoic acid 10
2-(2-chloro-6-(methylamino)-9H-purin-9-yl)-N-phenylacetamide 11
9-(2-aminobenzyl)-2-chloro-N-methyl-9H-purin-6-amine 12
N-(2-((2-chloro-6-(methylamino)-9H-purin-9-yl) methyl)phenyl) methanesulfonamide 13
N-(2-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)phenyl)-4-methylbenzenesulfonamide
N-(2-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)phenyl)-2,2,2-trifluoroacetamide 15
2-chloro-9-(2-(difluoromethyl)benzyl)-N-methyl-9H-purin-6-amine 16
2-chloro-N-methyl-9-(2-(trifluoromethyl)benzyl)-9H-purin-6-amine 17
2-chloro-9-(2-methoxybenzyl)-N-methyl-9H-purin-6-amine 18
methyl 3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoate 19
3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoic acid 20
9-(3-(2H-tetrazol-5-yl)benzyl)-2-chloro-N-methyl-9H-purin-6-amine 21
3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzamide 22

![Chemical Structure]

**NMR Spectrum**

- **f1 (ppm)**
- **N**
- **N**
- **Cl**
- **N**
- **H**
- **C**
- **H**
- **O**

**Chemical Shifts**

- 2.53
- 2.00
- 2.99
- 2.00
- 0.91
- 1.85

**Assignments**

- 2.50
- DMSO-d6
- 2.91
- 2.93
- 5.39
- 7.39
- 7.40
- 7.41
- 7.41
- 7.41
- 7.43
- 7.45
- 7.75
- 7.76
- 7.76
- 7.78
- 7.78
- 7.79
- 7.79
- 7.80
- 7.80
- 7.98
- 8.24
- 8.25

**Chemical Bonds**

- H-C-N
- C-N
- N-H-C
- N-H
- O

**Additional Data**

- **f1 (ppm)**
- **N**
- **N**
- **Cl**
- **N**
- **H**
- **C**
- **H**
- **O**
3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)-N-methylbenzamide 23
9-(3-bromobenzyl)-2-chloro-N-methyl-9H-purin-6-amine 24
$^1$H spectrum of 2-chloro-9-(3-chlorobenzyl)-N-methyl-9H-purin-6-amine 25
3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzonitrile 26
2-chloro-9-(3-methoxybenzyl)-N-methyl-9H-purin-6-amine 27
methyl 4-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoate 28
3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoic acid 29
(4-((2,6-dichloro-9H-purin-9-yl)methyl)phenyl)methanol 30
N-(4-chloro-2-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)phenyl) methanesulfonamide 31
3-chloro-5-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoic acid 32
methyl 3-chloro-5-((2,6-dichloro-9H-purin-9-yl)methyl)benzoate 33

\[
\begin{align*}
\text{\text{CH}_3} & \quad \text{Cl} \\
\text{Cl} & \quad \text{N} \\
\text{N} & \quad \text{H} \\
\text{O} & \\
\text{C} & \quad \text{H}_3 \\
\end{align*}
\]
2-chloro-9-(3-chloro-4-methoxybenzyl)-N-methyl-9H-purin-6-amine 34
2-chloro-9-(3,5-dichloro-4-methoxybenzyl)-N-methyl-9H-purin-6-amine 35
9-(3-chlorobenzyl)-2-fluoro-N-methyl-9H-purin-6-amine 36
2-chloro-9-(3-chlorobenzyl)-N-cyclopropyl-9H-purin-6-amine 37
2,6-dichloro-9-(3-chlorobenzyl)-9H-purine 38

![Chemical Structure](image)

**1H NMR (CDCl3):**
- δ 7.29 (m, 1H)
- δ 7.20 (s, 2H)
- δ 3.06 (s, 3H)
- Δ 77.16 (s, 126.23)
6-chloro-9-(3-chlorobenzyl)-N-methyl-9H-purin-2-amine 39
9. HPLC traces of final compounds

2-chloro-N-methyl-9-phenyl-9H-purin-6-amine 4

9-benzyl-2-chloro-N-methyl-9H-purin-6-amine 5

2-chloro-N-methyl-9-(pyridin-4-ylmethyl)-9H-purin-6-amine 6
2-chloro-N-methyl-9-(tetrahydro-2H-pyran-2-yl)-9H-purin-6-amine

Retention time (min)

0.005 100.00%

N
N
N
N
Cl
N
H
O
C
H
3

2-chloro-9-(cyclohexylmethyl)-N-methyl-9H-purin-6-amine

Retention time (min)

2.693 100.00%

N
N
N
N
Cl
N
H
C
H
3

2-chloro-N-methyl-9-((3-(trifluoromethyl)cyclohexyl)methyl)-9H-purin-6-amine

Retention time (min)

2.747 100.00%

N
N
N
N
Cl
N
H
C
H
3
F
F
3-(2-chloro-6-(methylamino)-9H-purin-9-yl)benzoic acid 10

2-(2-chloro-6-(methylamino)-9H-purin-9-yl)-N-phenylacetamide 11

9-(2-aminobenzyl)-2-chloro-N-methyl-9H-purin-6-amine 12
N-(2-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)phenyl) methanesulfonamide 13

N-(2-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)phenyl)-4-methylbenzene sulfonamide 14

N-(2-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)phenyl)-2,2,2-trifluoroacetamide 15
2-chloro-9-(2-(difluoromethyl)benzyl)-N-methyl-9H-purin-6-amine 16

2-chloro-N-methyl-9-(2-(trifluoromethyl)benzyl)-9H-purin-6-amine 17

2-chloro-9-(2-methoxybenzyl)-N-methyl-9H-purin-6-amine 18
methyl 3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoate 19

3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoic acid 20

3-(2H-tetrazol-5-yl)benzyl)-2-chloro-N-methyl-9H-purin-6-amine 21
3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzamide 22

3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)-N-methyl benzamide 23

9-(3-bromobenzyl)-2-chloro-N-methyl-9H-purin-6-amine 24
2-chloro-9-(3-chlorobenzyl)-N-methyl-9H-purin-6-amine 25

3-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzonitrile 26

2-chloro-9-(3-methoxybenzyl)-N-methyl-9H-purin-6-amine 27
N-(4-chloro-2-((2-chloro-6-(methylamino)-9H-purin-9-yl) methyl)phenyl) methanesulfonamide 31

3-chloro-5-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoic acid 32

methyl 3-chloro-5-((2-chloro-6-(methylamino)-9H-purin-9-yl)methyl)benzoate 33
2-chloro-9-(3-chloro-4-methoxybenzyl)-N-methyl-9H-purin-6-amine 34

2-chloro-9-(3,5-dichloro-4-methoxybenzyl)-N-methyl-9H-purin-6-amine 35

9-(3-chlorobenzyl)-2-fluoro-N-methyl-9H-purin-6-amine 36
2-chloro-9-(3-chlorobenzyl)-N-cyclopropyl-9H-purin-6-amine 37

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

2,6-dichloro-9-(3-chlorobenzyl)-9H-purine 38

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

6-chloro-9-(3-chlorobenzyl)-N-methyl-9H-purin-2-amine 39

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0

Retention time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
5-chloro-3-(3-chlorobenzyl)-N-methyl-2H-pyrazolo[4,3-d]pyrimidin-7-amine 40