Supporting Information for:

Inhibitors of nicotinamide N-methyltransferase designed to mimic the methylation reaction transition state

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NMR spectra

1-Ethyl-3-carbamoyl-pyridin-1-ium iodide (2a)
1-Propyl-3-carbamoyl-pyridin-1-ium bromide (2b)
1-Butyl-3-carbamoyl-pyridin-1-ium bromide (2c)
1-Allyl-3-carbamoyl-pyridin-1-ium bromide (2d)
1-Isopropyl-3-carbamoyl-pyridin-1-ium iodide (2e)
1-Cyclopropylmethyl-3-carbamoyl-pyridin-1-ium bromide (2f)
1-Cyclobutylmethyl-3-carbamoyl-pyridin-1-ium bromide (2g)
1-Cyclohexylmethyl-3-carbamoyl-pyridin-1-ium bromide (2h)
1-Benzyl-3-carbamoyl-pyridin-1-ium bromide (2i)
3-((((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-di-hydroxytetrahydrofuran-2-yl)methyl)amino) methyl) benzamide (9)
3-(((2S,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl(thio)methyl)benzamide (10)
3-(((2S,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-di-hydroxytetrahydrofuran-2-yl)methoxy)methylbenzamide (11)
5-(3-Carbamoylphenyl)pent-4-ynoic acid (12)
5-(3-Carboxamidophenyl)pent-4-ynoic amide (13)
6-(3-Carbamoylphenyl)hex-5-ynoic acid (14)
6-(3-Carbamoylphenyl)hex-5-ynoic amide (15)
7-(3-Carbamoylphenyl)hept-6-ynoic acid (16)
7-(3-Carbamoylphenyl)hept-6-ynoic amide (17)
5-(3-Carbamoylphenyl)pentanoic acid (18)
5-(3-Carbamoylphenyl)pentanoic amide (19)
6-(3-Carbamoylphenyl)hexanoic acid (20)
6-(3-Carbamoylphenyl)hexanoic amide (21)
7-(3-Carbamoylphenyl)heptanoic acid (22)
7-(3-Carbamoylphenyl)heptanoic amide (23)
(Z)-6-(3-Carbamoylphenyl)hex-5-enoic acid (24)
(E)-6-(3-Carbamoylphenyl)hex-5-enoic acid (25)
(S)-2-Amino-6-(3-carbamoylphenyl)hex-5-ynoic acid (27)
(S)-2-Amino-6-(3-carbamoylphenyl)hexanoic acid (28)
(S)-2-Amino-4-((3-carbamoylbenzyl)amino)butanoic acid (35)
(S)-2-Amino-4-((3-carbamoylbenzyl)thio)butanoic acid (41)
(S)-2-Amino-4-(((2R,3S,4R,5R)-5-(6-aminopurin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(3-carbamoylbenzyl)amino)butanoic acid (45)
HPLC Traces

3-(((2R,3S,4R,5R)-5-(6-Amino-9H-purin-9-yl)-3,4-di-hydroxytetrahydrofuran-2-yl)methyl)amino)methyl) benzamide (9)

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Supplemental Figure 1. SDS-PAGE gel showing the purity of wild type human nicotinamide N-methyltransferase (wt-hNNMT). Proteins were stained with Coomassie Brilliant Blue. Lane 1 contains a molecular weight standard, lane 2 contains 10 µg of the crude product in an extract of IPTG-induced E.coli BL21 (DE3) harbouring pET-28a-wt-hNNMT, lane 3 contains 10 µg of the affinity purified recombinant wt-hNNMT, lane 4 contains 5 µg of the affinity purified recombinant wt-hNNMT and lane 5 contains 2 µg of the affinity purified recombinant wt-hNNMT.
### IC₅₀ Curves

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ and R²</th>
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<tbody>
<tr>
<td><strong>Sinefungin</strong></td>
<td>IC₅₀ = 17.0 ± 3.4 µM, R² = 0.899</td>
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<tr>
<td><strong>MNA</strong></td>
<td>IC₅₀ = 24.6 ± 3.2 µM, R² = 0.958</td>
</tr>
<tr>
<td><strong>AdoHcy</strong></td>
<td>IC₅₀ = 75.4 ± 6.3 µM, R² = 0.979</td>
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<tr>
<td><strong>4MeMNA</strong></td>
<td>IC₅₀ = 95.9 ± 14.1 µM, R² = 0.934</td>
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<tr>
<td><strong>Norharmane</strong></td>
<td>IC₅₀ = 115.3 ± 20.6 µM, R² = 0.909</td>
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<tr>
<td><strong>Compound 14</strong></td>
<td>IC₅₀ = 189.7 ± 30.0 µM, R² = 0.870</td>
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<tr>
<td>Compound 16</td>
<td>Compound 20</td>
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<tr>
<td><img src="image1" alt="Graph for Compound 16" /></td>
<td><img src="image2" alt="Graph for Compound 20" /></td>
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</table>
| IC₅₀ = 57.8 ± 4.2 µM  
R² = 0.983 | IC₅₀ = 69.0 ± 14.8 µM  
R² = 0.862 |
| Compound 22 | Compound 23 |
| ![Graph for Compound 22](image3) | ![Graph for Compound 23](image4) |
| IC₅₀ = 148.1 ± 36.3 µM  
R² = 0.644 | IC₅₀ = 30.8 ± 3.6 µM  
R² = 0.961 |
| Compound 45 |  |
| ![Graph for Compound 45](image5) |  |
| IC₅₀ = 29.2 ± 4.0 µM  
R² = 0.945 |