

Supplementary Information

Table S1:

Sinefungin Analogues, MS data and their IC₅₀ values against EHMT1/2 at 100 μM screening concentration

Analogues	Mass calculated	MS mass	IC ₅₀ (μM) EHMT1	IC ₅₀ (μM) EHMT2
3a	279,13	280,2	>100	>100
3b	307,16	308,26	86,5	103,2
3c	307,16	308,24	>100	>100
3d	351,15	352,2	>100	>100
3e	322,14	323,09	>100	>100
3f	355,16	356,28	>100	>100
3g	341,15	342,19	>100	>100
3h	359,14	360,2	>100	>100
3i	359,14	360,21	>100	>100
3j	348,1	349,2	>100	>100
3k	369,18	370,19	>100	>100
3l	366,14	367,2	>100	>100
4a	322,18	323,2	>100	>100
4b	348,19	349,3	>100	>100
4c	362,21	363,3	>100	>100
4d	376,22	377,3	1,46	1,6
4e	430,2	431,3	>100	>100
4f	398,21	399,3	>100	>100

Chemistry:

NMR spectra were recorded on Varian Mercury 300 or when indicated on a 600 MHz Bruker Avance III HD equipped with 5-mm DCH cryoprobe. Chemical shifts were reported in parts per million (ppm), with the solvent resonance as the internal standard (CD₃OD 3.31 ppm, DMSO-*d*₆ 2.50 ppm for ¹H NMR). Low resolution mass spectral data (electrospray ionization) were acquired on a API2000 mass spectrometer. LC-MS (System B) was performed using a Agilent 1100 HPLC systems with a XBridge 3.5 μm C-18 column (100 x 4.60 mm) using gradient elution from buffer A (H₂O:CH₃CN:HCOOH, 95:5:0.1) to buffer B

(H₂O:CH₃CN: HCOOH, 5:95:0.05) over 8 min flow rate: 0.5 mL/min, coupled to an Hewlett Packard 1100 series mass spectrometer with an electrospray ionization source. All compounds were more than 95 % pure according to LC-MS. Compounds **4a-f** are 1:1 mixtures of two diastereomers.

2',3'-O-isopropylideneadenosine (6):

p-Toluenesulfonic acid monohydrate (31.25g, 0.164 mol) and triethyl orthoformate (39 mL, 0.234 mol) were subsequently added into the solution of adenosine (**5**) (10g, 0.037 mol) in acetone (1.5 L). The resulted mixture was stirred at room temperature overnight. After neutralization with saturated solution of sodium carbonate, the solution was evaporated until small volume and cooled to crystallize. After filtration, 2',3'-*O*-isopropylideneadenosine (**6**) was obtained as white solid (11.4 g, yield: 100 %).

¹H NMR(DMSO-*d*₆): δ 8.35 (s, 1H), 8.17 (s, 1H), 7.36 (s, NH₂), 6.13 (d, *J* = 3.0 Hz, 1H), 5.35 (dd, *J* = 3.0 Hz, *J* = 6.1 Hz, 1H), 4.97 (dd, *J* = 2.4 Hz, *J* = 6.1 Hz, 1H), 4.22 (d, *J* = 2.4 Hz, 1H), 3.56 (m, 2H), 1.56 (s, 3H), 1.34 (s, 3H).

N⁶-benzoyl-2',3'-O-isopropylideneadenosine (7):

Benzoyl chloride (62 mL, 0.54 mol) was added to the solution of 2',3'-*O*-isopropylideneadenosine (**6**) (10.32g, 0.034 mol) in pyridine (103 mL) and the resulted mixture was stirred at 40 °C overnight. The reaction was stopped by adding iced water carefully. The mixture was extracted with chloroform (3x25 mL), washed subsequently with saturated solution of sodium carbonate (25 mL) and water (25 mL). The organic phase was then dried over sodium sulphate and filtered. The solvent was removed by evaporation and the residue was then redissolved in dioxane (100 mL). The solution was adjusted to pH 9-10 by 2N solution of sodium hydroxide and stirred for 1 hour. After neutralization by acetic acid, the mixture was extracted with chloroform (3x25 mL) and washed with water. After evaporation, the residue was purified on silica gel column eluting with ethyl acetate and 5% methanol/dichloromethane separately to give N⁶-benzoyl-2',3'-*O*-isopropylideneadenosine (**7**) as yellow solid (8.2 g, yield: 58 %).

¹H NMR(DMSO-*d*₆): δ 8.78 (s, 1H), 8.68 (s, 1H), 8.06 (d, *J* = 7.4 Hz, 2H), 7.66 (t, *J* = 7.5 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 2H), 6.28 (d, *J* = 2.6 Hz, 1H), 5.45 (dd, *J* = 2.64 Hz, *J* = 6.08 Hz, 1H), 5.15 (brs, OH), 5.01 (dd, *J* = 2.4 Hz, *J* = 6.1 Hz, 1H), 4.28 (m, 1H), 3.57 (d, *J* = 4.3 Hz, 2H), 1.58 (s, 3H), 1.36 (s, 3H).

N⁶-benzoyl-2',3'-O-isopropylidene-5'-(1'', 3''-diphenylimidazolide-2'')-adenosine (8):

Dichloroacetic acid (0.5 mL, 6.08 mmol) was added into the solution of N⁶-benzoyl-2',3'-*O*-isopropylideneadenosine (**7**) (5g, 12.15 mmol) and DCC (7.5 g, 36.46 mmol) in DMSO (27 mL), and the mixture was stirred at room temperature for 1.5 hours. It was terminated with 2 mL of the solution of oxalic acid in methanol (3.06 g, 24.3 mmol), and stirred for another 30 minutes. White solid was precipitated. It

was filtered and washed with methanol (5 mL). Diphenylethylenediamine (3g, 13.97 mmol) was added to the combined solution and stirred for 1 hour. After the addition of water, the mixture was extracted with chloroform (3x15 mL) and washed with water (3x15 mL) and dried with sodium sulphate. After filtration, the solvent was removed by evaporation in vacuo and the residue was crystallized with ethanol to give white solid (2.6 g, yield: 35 %).

$^1\text{H NMR}$ (DMSO- d_6): δ 8.24 (s, 1H), 1.46 (s, 3H), 3.31 (m, 2H), 3.44 (m, 2H), 3.58 (m, 1H), 4.03 (d, $J = 7.2$ Hz, 1H), 4.53 (brs, 1H), 5.94 (brs, 1H), 6.22 (d, $J = 3.0$ Hz, 1H), 6.70 (t, $J = 7.5$ Hz, 1H), 6.76 (t, $J = 8.7$ Hz, 2H), 6.88 (d, $J = 8.7$ Hz, 2H), 6.92 (d, $J = 8.1$ Hz, 2H), 7.16 (t, $J = 8.7$ Hz, 2H), 7.25 (t, $J = 7.8$ Hz, 2H), 7.55 (t, $J = 6.9$ Hz, 2H), 7.61 (t, $J = 7.5$ Hz, 1H), 8.05 (d, $J = 7.2$ Hz, 2H), 8.08 (d, $J = 5.4$ Hz, 1H), 1.29 (s, 3H).

Representative procedure for **3c** via **11c**:

***N*⁶-benzoyl-2',3'-*O*-isopropylidene-5'-desoxy-5'-(isopropylmethylene)-adenosine (11c):**

Dowex 50 (2.45 g) was added into the solution of *N*⁶-benzoyl-2',3'-*O*-isopropylidene-5'-(1",3"-diphenylimidazolidene-2")-adenosine (**8**) (1.6 g, 2.7 mmol) dissolved in 40 mL mixture of tetrahydrofuran and water (1:1). The mixture was stirred at room temperature for 1 hour. After filtration, the solution was evaporated until small volume. The white solid was precipitated and filtered to give *N*⁶-benzoyl-2',3'-*O*-isopropylideneadenosine-5'-aldehyde hydrate (0.8 g, 70.6 %).

The aldehyde derivative (0.2 g, 0.46 mmol) was dissolved in benzene (35 mL) for 1 hour and evaporated to dryness. In another flask 1.6 N *n*-butyllithium (0.6 mL, 0.92 mmol) was dropped slowly into the solution of isobutyltriphenylphosphonium bromide (0.35 g, 0.88 mmol) in tetrahydrofuran (2 mL) at 0°C under nitrogen. After addition, the mixture was stirred for another 10 minutes. After cooling to -78°C, the aldehyde in tetrahydrofuran (2 mL) was added and stirred at this temperature for 3 hours, 0.5 hour at -20°C, and 1 hour at 0 °C. The reaction was stopped with 50% Acetic acid (2 mL). The mixture was extracted with chloroform (3x10 mL), washed with water (3x10 mL) and dried over sodium sulphate. After filtration, the solution was evaporated in vacuo and purified on the silica gel column to give the product **11c** (0.10 g, yield: 50 %).

ESI: 449.5

$^1\text{H NMR}$ (DMSO- d_6): δ 8.70 (s, 1H), 8.56 (s, 1H), 8.05 (d, $J = 7.2$ Hz, 2H), 7.56 (m, 3H), 6.27 (s, 1H), 5.51 (d, $J = 7.5$ Hz, 1H), 5.38 (m, 2H), 4.96 (m, 1H), 4.89 (m, 1H), 1.87 (m, 1H), 1.57 (s, 3H), 1.34 (s, 3H), 0.91 (d, $J = 6.6$ Hz, 3H), 0.90 (d, $J = 6.3$ Hz, 3H).

5'-desoxy-5'-(3"-methyl-propyl-1")-adenosine (3c):

10 % palladium /carbon (0.10 g) was added into the solution *N*⁶-benzoyl-2',3'-*O*-isopropylidene-5'-desoxy-5'-(isobutylmethylene)-adenosine (**11c**) (0.1g, 0.2 mmol) in methanol (100 mL). The mixture was hydrogenated under the pressure of 3 kg at 30 °C for 5-6 hours. After filtration, the solution was evaporated in vacuo and the resulting residue **12c** was reacted in the next step directly. *N*⁶-benzoyl-2',3'-*O*-

isopropylidene-5'-(3"-methyl-butyl-1")-adenosine (**12c**) was dissolved in the mixture of tetrahydrofuran (4 mL) and trifluoroacetic acid/water (9:1) (2 mL). The solution was stirred at room temperature overnight. After evaporation in vacuo, the residue was purified on the preparative TLC to give the product (28 mg, yield: 41.2% in two steps).

^1H NMR (600 MHz, methanol- d_4): δ 8.23 (s, 1H), 8.20 (s, 1H), 5.95 (d, $J=5.1$ Hz, 1H), 4.74 (t, $J=5.3$ Hz, 1H), 4.15 (t, $J=5.2$ Hz, 1H), 3.97 (dt, $J=7.6, 5.1$ Hz, 1H), 1.73-1.81 (m, 1H), 1.54-1.62 (m, 1H), 1.30-1.41 (m, 3H), 0.91 (d, $J=6.6$ Hz, 3H), 0.90 (d, $J=6.6$ Hz, 3H).

^{13}C NMR (150 MHz, methanol- d_4): δ 157.3, 153.9, 150.7, 141.3, 120.6, 90.0, 86.2, 75.1, 75.0, 36.1, 32.6, 29.1, 23.0, 22.9.

5'-desoxy-5'-ethyladenosine 3a:

^1H NMR (600 MHz, methanol- d_4): δ 8.25 (s, 1H), 8.21 (s, 1H), 5.97 (d, $J=5.1$ Hz, 1H), 4.71 (t, $J=5.2$ Hz, 1H), 4.15 (t, $J=5.1$ Hz, 1H), 3.97 (dt, $J=4.8, 6.6$ Hz, 1H), 1.74 (m, 2H), 1.42-1.55 (m, 2H), 0.97 (t, $J=7.4$ Hz, 3H).

^{13}C NMR (150 MHz, methanol- d_4): δ 157.2, 153.6, 150.6, 141.3, 120.5, 89.0, 85.6, 75.1, 75.0, 36.8, 20.1, 14.3.

5'-desoxy-5'-butyladenosine 3b:

^1H NMR (600 MHz, methanol- d_4): δ 8.23 (s, 1H), 8.20 (s, 1H), 5.96 (d, $J=5.0$ Hz, 1H), 4.73 (t, $J=8.6, 5.3$ Hz, 1H), 4.14 (t, $J=5.0$ Hz, 1H), 4.06 (m, 1H), 1.87 (m, 1H), 1.75 (m, 1H), 1.54-1.32 (m, 6H), 0.91 (t, $J=7.0$ Hz, 3H).

^{13}C NMR (150 MHz, methanol- d_4): δ 157.3, 153.9, 150.7, 141.3, 120.5, 89.9, 85.9/85.6, 75.1, 75.1/75.0, 34.7/34.6, 32.8/32.0, 26.6, 23.6, 14.3.

5'-desoxy-5'-(2-ethoxycarbonylethyl)adenosine 3d:

^1H NMR (DMSO- d_6): δ 8.34 (s, 1H), 8.14 (s, 1H), 5.90 (d, 1H), 5.82 (d, 1H), 4.78 (m, 2H), 4.63 (m, 1H), 4.25-3.91 (m, 4H), 1.85 (s, 2H), 1.19 (t, 3H).

5'-desoxy-5'-(2-aminocarbonylethyl)adenosine 3e:

^1H NMR (DMSO- d_6): δ 8.31 (s, 1H), 8.12 (s, 1H), 5.96 (d, 1H), 5.82 (d, 1H), 4.86 (m, 1H), 4.31-3.98 (m, 4H), 1.87 (m, 1H), 1.64 (s, 2H), 1.38 (s, 2H), 1.01 (s, 2H)

5'-desoxy-5'-(2-phenylethyl)adenosine 3f:

^1H NMR (600 MHz, methanol- d_4): δ 8.18 (s, 2H), 7.23 (MM' , 2H), 7.16 (AA' , 2H), 7.13 (t, $J=7.5$ Hz, 1H), 5.91 (d, $J=5.0$ Hz, 1H), 4.70 (t, $J=5.2$ Hz, 1H), 4.15 (t, $J=5.2$ Hz, 1H), 3.97 (dt, $J=7.2, 5.2$ Hz, 1H), 1.70-1.80 (m, 4H).

^{13}C NMR (150 MHz, methanol- d_4): δ 157.3, 153.9, 150.6, 143.7, 141.3, 129.4, 129.3, 120.6, 90.0, 85.7, 75.1,

75.0, 36.6, 34.1, 28.8.

5'-desoxy-5'-benzyladenosine 3g:

¹HNMR (DMSO-*d*₆): δ 8.340 (s, 1H), 8.150 (s, 1H), 7.094-7.420 (m, 5H), 5.849-5.866 (d, *J*=5.1 Hz, 1H), 4.707 (m, 1H), 4.078 (m, 1H), 3.821 (m, 1H), 2.597-2.657 (m, 2H), 1.946 (m, 2H)

5'-desoxy-5'-(2-fluorobenzyl)adenosine 3h:

¹HNMR (DMSO-*d*₆): δ 8.31 (s, 1H), 8.12 (s, 1H), 5.96 (d, 1H), 5.82 (d, 1H), 4.86 (m, 1H), 4.31-3.98 (m, 4H), 1.87 (m, 1H), 1.64 (s, 2H), 1.38 (s, 2H), 1.01 (s, 2H).

5'-desoxy-5'-(4-fluorobenzyl)adenosine 3i:

¹HNMR (DMSO-*d*₆): δ 8.326 (s, 1H), 8.141 (s, 1H), 7.213-7.243 (m, 2H), 7.041-7.100 (t, *J*=17.7 Hz, 2H), 5.843-5.860 (d, *J*=5.1 Hz, 1H), 4.683-4.718 (t, *J*=10.5 Hz, 1H), 4.053-4.085 (t, *J*=9.6 Hz, 1H), 3.771-3.852 (m, 1H), 2.620-2.716 (m, 2H), 1.891-1.981 (m, 2H).

5'-desoxy-5'-(1-thiazolylmethyl)adenosine 3j:

¹HNMR (DMSO-*d*₆): δ 8.34 (s, 1H), 8.12 (s, 1H), 7.67 (s, 1H), 7.55 (s, 1H), 5.85 (d, 1H), 5.56 (s, 1H), 5.35 (s, 1H), 4.69 (s, 1H), 4.12 (s, 1H), 3.90 (s, 1H), 3.14-3.03 (t, 2H), 2.10 (d, 2H), 1.74 (s, 2H).

5'-desoxy-5'-(3-phenylpropyl)adenosine 3k:

¹HNMR (600 MHz, methanol-*d*₄)^c: δ 8.18 (s, 2H), 7.23 (*MM'*, 2H), 7.16 (*AA'*, 2H), 7.13 (t, *J*=7.5 Hz, 1H), 5.91 (d, *J*=5.0 Hz, 1H), 4.70 (t, *J*=5.2 Hz, 1H), 4.15 (t, *J*=5.2 Hz, 1H), 3.97 (dt, *J*=7.2, 5.2 Hz, 1H), 1.70-1.80 (m, 4H).

¹³CNMR (150 MHz, methanol-*d*₄)^c: δ 157.3, 153.9, 150.6, 143.7, 141.3, 129.4, 129.3, 120.6, 90.0, 85.7, 75.1, 75.0, 36.6, 34.1, 28.8.

5'-desoxy-5'-(2-cyanobenzyl)adenosine 3l:

¹HNMR (DMSO-*d*₆): δ 8.33 (s, 1H), 8.13 (s, 1H), 7.77-7.28 (m, 4H), 5.88 (d, 1H), 5.54 (d, 1H), 5.34 (m, 1H), 4.69-3.85 (m, 4H), 2.89-2.82 (m, 3H).

Representative procedure for **4c** via **21c** and **17c**:

Methyl-5-desoxy-5-(cyclohexylidene)cyanomethyl-2,3-O-isopropyliden-β-D-ribofuranosid (17c):

Sodium hydride (4 eq) was added into the solution of Methyl-5-deoxy-5-(diethoxyphosphinyl)cyanomethyl-2,3-*O*-isopropyliden-β-D-ribofuranosid (**16**) (1 eq) in tetrahydrofuran (5 mL) under nitrogen and mixture was stirred for 10 minutes followed by the addition of cyclohexone (4 eq). The reaction was stirred at room temperature for 3 hours. It was terminated by addition of the solution of 10% oxalic acid/methanol (2 mL). The resulted solution was evaporated in vacuo and the residue was extracted with dichloromethane (3x10 mL), washed with water (3x10 mL), and dried over sodium sulphate. After filtration, the solvent was removed by evaporation in vacuo and the residue was purified on silica gel column eluting with 5%, 10%

ethyl acetate/petroleum separately to give the product as oil.

¹HNMR (CDCl₃): δ 1.32 (s, 3H), 1.48 (s, 3H), 1.64 (m, 4H), 1.85 (m, 2H), 2.33 (m, 4H), 2.52 (m, 2H), 3.37 (s, OCH₃), 4.32 (t, *J* = 8.1Hz, 1H), 4.63 (m, 2H), 4.97 (s, 1H).

***N*⁶-benzoyl-2',3'-*O*-diacetyl-5'-desoxy-5'-(cyclohexyl-(carbamoyl)-methyl)-adenosine (21c):**

10 % palladium /carbon (0.1 g) was added into the solution of **17c** (0.1 g) in methanol (100 mL). The mixture was hydrogenated under the pressure of 3 kg at 30 °C for 5-6 hours. After filtration, the solution was evaporated in vacuo and the resulted residue methyl-5-deoxy-5-(1-cyclohexyl)cyanomethyl-2,3-*O*-isopropyliden-β-D-ribofuranosid (**18c**) was reacted in the next step directly. Methyl-5-deoxy-5-(1-cyclohexyl)cyanomethyl-2,3-*O*-isopropyliden-β-D-ribofuranosid (**18c**) (1.21 mmol) was dissolved in the mixture of DMSO (0.106 mL), 30% hydroperoxide (0.57 mL), 2N solution of sodium hydroxide (9.07 mL, 18 mmol), and methanol (30 mL). The mixture was refluxed for 1 hour and evaporated in vacuo. The residue was extracted with dichloromethane (3x15 mL), washed with water, dried over sodium sulphate. After filtration, the solution was evaporated in vacuo to obtain methyl-5-deoxy-5-(cyclohexyl)-(carbamoyl)-methyl-2,3-*O*-isopropyliden-β-D-ribofuranosid (**19c**) as white solid, which was reacted directly in the next step (Yield: 83 %). 0.2 N sulphuric acid (1.75 mL) was added into a solution of methyl-5-deoxy-5-(cyclohexyl)-(carbamoyl)-methyl-2,3-*O*-isopropyliden-β-D-ribofuranosid (**19c**) (2 mmol) in the mixture of water and dioxane (1:1, 140 mL) and the resulted solution was refluxed overnight. After neutralization, the solvent was removed by evaporation in vacuo. The residue was redissolved in pyridine (14 mL) and acetic anhydride (7 mL) and the solution was stirred at room temperature overnight. The solvent was removed by evaporation in vacuo and resulted was reacted directly in the next step without further purification. A mixture of adenine (35 mg, 0.15 mmol) in acetonitrile (2 mL) and *N,O*-Bis(trimethylsilyl)acetamide (2 mL) was refluxed for 2 hours. After evaporation in vacuo, the residue was dissolved in dichloroethane (3 mL) and was followed by the addition of the solution of acetyl-5-deoxy-5-(cyclohexyl)-(carbamoyl)-methyl-2,3-*O*-diacetyl-β-D-ribofuranosid (**20c**) (1 mmol) in dichloroethane (2 mL) and trifluoromethanesulfonic acid trimethylsilylester (12 d). The mixture was reacted in the microwave oven at 90°C for 20 minutes. After neutralization, the mixture was extracted with dichloromethane (3x15 mL), washed with water, dried over sodium sulphate. After filtration, the solution was evaporated in vacuo and the residue was purified on the preparative TLC to give the product as yellow oil (Yield: 41 %).

¹HNMR (CDCl₃): δ 1.23 (m, 6H), 1.74 (m, 7H), 2.08 (m, 7H), 4.22 (m, 1H), 5.46 (m, 2H), 6.13 (s, 1H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.63 (d, *J* = 5.7 Hz, 1H), 8.10 (m, 3H), 8.82 (d, *J* = 2.7 Hz, 1H).

5'-desoxy-5'-(1''-cyclohexyl-1''aminomethyl)-adenosine (4c):

7 N ammonia in methanol (1.2 mL) was added into the solution *N*⁶-benzoyl-2',3'-*O*-diacetyl-5'-desoxy-5'-(cyclohexyl-(carbamoyl)-methyl)-adenosine (**21c**) (0.186 mmol) in the methanol (30 mL) and the resulted

solution was stirred at 40-50°C overnight. After evaporation, the residue was dissolved in acetone (30 mL) and followed by the addition of trifluoromethanesulfonic acid trimethylsilylester (0.12 mL) in dichloroethane (0.36 mL). After stirring for 1 hour, the mixture was evaporated in vacuo to give **22c** without further purification. [Bis(trifluoroacetoxy)iodo]benzene (42mg, 0.093 mmol) was added into the solution of 2',3'-*O*-isopropylidene-5'-desoxy-5'-(cyclohexyl-(carbamoyl)-methyl)-adenosine (**22c**) (0.062 mmol) in DMF (1 mL) and water (1 mL). After stirring at the room temperature for 15 min, Py (9.5×10^{-3} mL) was added and the solution was stirred for another 0.5 hour. The process was detected by TLC. After the reaction was completed, the solution was evaporated in vacuo and the residue was redissolved in the mixture (2 ml) of dioxane/water/TEA (2:1:1) followed by the addition of Boc₂O (15.8 mg, 0.0724 mmol) and the resulted solution was stirred at the room temperature for another 1 hour. After evaporation, the residue was extracted with dichloromethane (3x15 mL), washed with water (3x15 mL) and dried over Na₂SO₄. After filtration, the solvent was removed by evaporation and the residue 2',3'-*O*-isopropylidene-5'-desoxy-5'-(*N*-*tert*-butoxycarbonyl-2"-cyclohexyl-1"aminoethyl)-adenosine **23c** was purified on the preparative TLC. 2',3'-*O*-isopropylidene-5'-desoxy-5'-(*N*-*tert*-butoxycarbonyl-2"-cyclohexyl-1"aminoethyl)-adenosine (**23c**) (0.06 mmol) was dissolved in the mixture of trifluoroacetic acid (0.5 mL) and water (0.5 mL). After stirring for 10 minutes, the solvent was removed by evaporation and product **4c** was obtained as its trifluoroacetate.

¹H NMR (methanol-*d*₄): δ 8.22 (s, 1H), 7.97 (s, 1H), 6.0 (m, 1H), 4.22 (m, 2H), 3.96 (m, 1H), 2.99 (s, OH), 2.86 (s, OH), 2.05 (m, 1H), 1.74 (m, 11H), 1.25 (m, 2H).

5'-desoxy-5'-(1"-isopropyl-1"aminomethyl)-adenosine 4a:

¹H NMR (600 MHz, methanol-*d*₄): δ 8.27/8.22 (s, 1H), 8.22/8.21 (s, 1H), 6.00/5.98 (d, *J*=4.1/4.0 Hz, 1H), 4.74/4.67 (t, *J*=4.7/4.8 Hz, 1H), 4.23/4.21 (t, *J*=5.8/5.4 Hz, 1H), 4.18/4.13 (m, 1H), 2.86 (dt, *J*=9.0,4.4 Hz, 1H), 2.02/2.00 (m, 1H), 1.68-1.78 (m, 2H), 0.95/0.94 (d, *J*=6.6 Hz, 3H).

¹³C NMR (150 MHz, methanol-*d*₄): δ 156.1, 153.9, 150.5, 141.4, 120.6, 90.8/90.7, 84.7/83.4, 75.2, 74.9, 56.6/54.8, 37.8, 34.7/33.7, 19.0/18.8, 18.2/18.0.

5'-desoxy-5'-(2"-cyclopentyl-1"aminoethyl)-adenosine 4b:

¹H NMR (methanol-*d*₄): δ 8.22 (m, 1H), 7.96 (s, 1H), 6.00 (m, 1H), 4.10 (m, 2H), 3.61 (m, 1H), 3.35 (s, OH), 3.31 (s, OH), 2.80 (m, 1H), 1.68 (m, 7H), 1.21 (m, 4H).

5'-desoxy-5'-(2"-cyclohexyl-1"aminoethyl)-adenosine 4d:

¹H NMR (600 MHz, methanol-*d*₄): δ 8.28/8.26 (s, 1H), 8.25 (s, 1H), 6.00/5.96 (d, *J*=3.9/4.4 Hz, 1H), 4.76/4.68 (t, *J*=4.9/4.8 Hz, 1H), 4.34/4.33 (t, *J*=5.3/5.6 Hz, 1H), 4.23/4.17 (m, 1H), 1.40-1.80 (m, 16H).

¹³C NMR (150 MHz, methanol-*d*₄): δ 157.1/157.0, 153.5/153.4, 150.3, 142.2/142.0, 130.9/130.8, 91.3,

83.1/81.7, 75.1/74.6, 74.6/74.4, 49.7/49.6, 48.0, 36.1, 33.0, 27.4, 27.0, 23.7

5'-desoxy-5'-(2''-3,4-dimethoxyphenyl-1''aminoethyl)-adenosine 4e:

¹H NMR (methanol-*d*₄): δ 8.123-8.279 (m, 2H), 7.966 (s, NH₂), 6.770-6.910 (m, 3H), 5.988-6.001 (d, J=3.9Hz, 1H), 5.944-5.958 (d, J=4.2Hz, 1H), 4.527 (m, 2H, a, b), 4.274 (m, 2H, a, b), 3.779 (s, 3H), 3.603 (m, 2H, a, b), 3.342 (s, 3H), 2.885-3.082 (m, 3H), 1.956-2.192 (m, 2H).

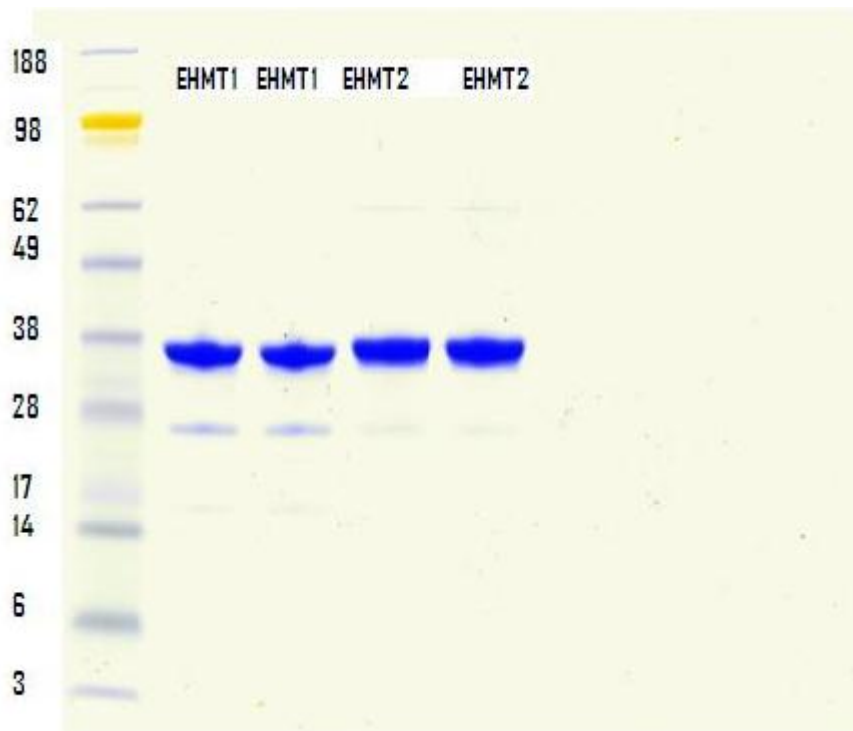
5'-desoxy-5'-(2''-4-ethylphenyl-1''aminoethyl)-adenosine 4f:

¹H NMR (methanol-*d*₄): δ 8.186-8.197 (m, 1H), 7.974 (s, 1H), 7.081-7.119 (m, 4H), 6.009 (m, 1H), 5.350-5.364 (m, 1H), 4.206-4.262 (m, 1H), 3.596-3.643 (m, 1H), 3.5 (m, 1H), 2.581-2.641 (m, 4H), 2.026-2.187 (m, 2H), 1.216-1.277 (m, 3H).

Protein Purification EHMT1/2

EHMT1 and EHMT2 constructs were obtained from NNF-Center for Protein Research.

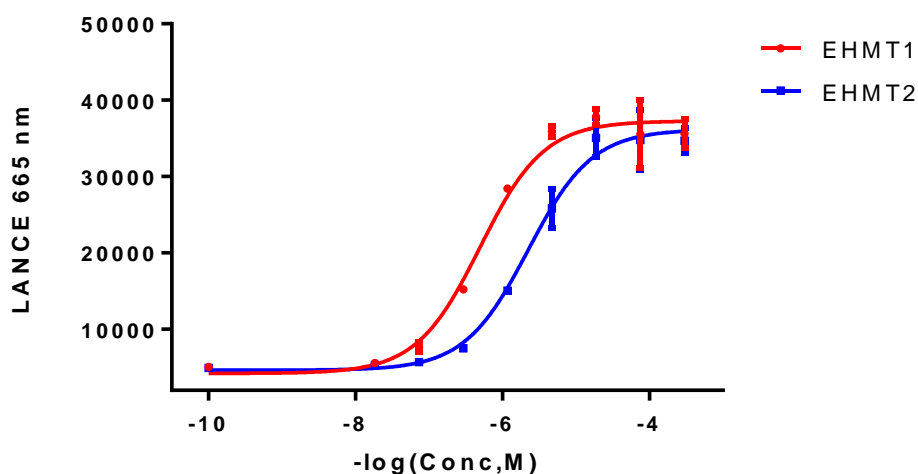
For intracellular protein expression, plasmid pNIC28-Bsa4 (GenBank: EF198106), containing N-terminal hexa-histidine tag followed by a TEV protease recognition site and two flanking *BsaI* restriction sites for ligation independent cloning (LIC) was used. DNA sequences of EHMT1 and EHMT2 catalytic domains with LIC sites were PCR amplified, T4 treated and cloned into expression vector- pNIC28-Bsa4. Positive colonies were screened on LB agar plates containing 50 μ g/ml kanamycin and 5% sucrose, purified and further transformed into expression strain Rosetta-gami 2 (DE3) [Merck, Germany]. Small-scale expression and purification analysis was performed to identify expressed PKMT targets that are soluble. Upon SDS page analysis of small scale purified targets and sequence verification, successful targets were taken for larger scale expression in *E.Coli*. Cell pellets were resuspended in lysis buffer (50 mM NaP, 300 mM NaCl, 10 mM imidazole, 10% glycerol, 0.5 mM tris(2-carboxyethyl)phosphine, pH 7.5 supplemented with 1x complete Mini EDTA free protease inhibitor and 50U/ml Benzonase; and lysed by passage through high pressure homogenizer. After centrifugation and filtration, proteins were purified on AKTA xpress system at 4°C using Immobilized Metal Ion Affinity Chromatography (IMAC) followed by Size Exclusion Chromatography (SEC). Eluted proteins were sequence and MS verified. The SDS gel image of purified protein has been shown in supplementary figure 1.



S2: SDS-gel image showing the bands of EHMT1 and EHMT2

Measuring activity of Proteins

Activities of protein of interest were tested using LANCE-Ultra Europium-anti-methyl-Histone H3 Lysine 9 assay kit. The final concentrations for screening were: 400 nM EHMT1, 80 nM EHMT2; 500 nM biotinylated peptide (H3, 1-24) and 150 μ M SAM. The detection mix was prepared by mixing Eu-Ab to 4 nM, *ULight*-Streptavidin to 100 nM and poly-L-lysine to 0.0002% in 1X LANCE Detection Buffer (final concentrations of 2 nM, 50 nM and 0.0001%, respectively, in 20 μ L total assay volume). Assay buffer used was 50 mM Tris-HCl, pH 9.0, 50 mM NaCl, 1mM DTT, 0.01% Tween-20. Once the activities were confirmed by using the LANCE Assay kit, DMSO tolerance tests and inhibition of proteins with known inhibitor BIX 01294 were performed under similar conditions as described above. Titration analysis of EHMT1 and EHMT2 with co factor SAM was also performed. (Supplementary figure 2)

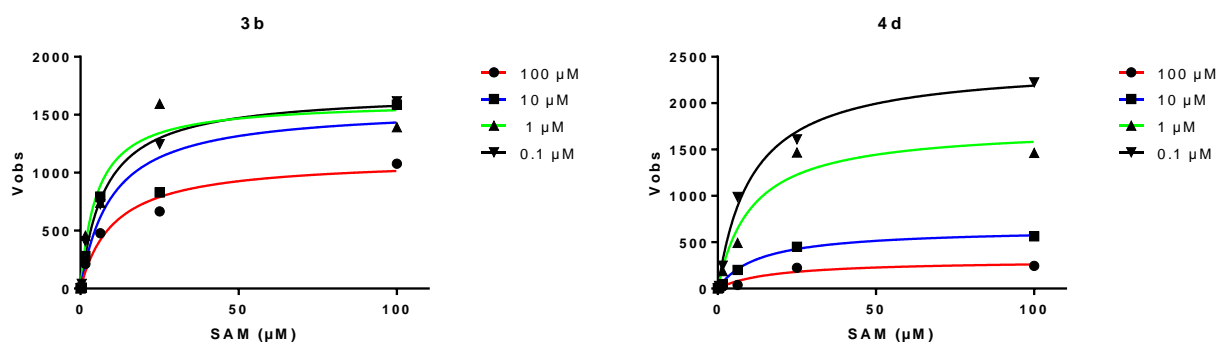


S3: Titration analysis of EHMT1 and EHMT2 with co-factor SAM

Initial inhibition screening was done by using 100 μ M compounds dissolved in DMSO (2% DMSO final conc.). Protein/peptide mix was incubated with inhibitors for 15 minutes prior to addition of SAM. Methylation reaction was allowed to proceed for 30 min. Then samples were incubated with detection buffer for 60 min. and fluorescent readings were taken in TR-FRET mode by exciting at 320 or 340 nm and emitting at 665 nm by using a Tecan Safire 2 microplate reader.

Analysis of data were done by using Graphpad Prism[®] software version 6.01. Inhibitor constants, K_i were calculated using the following formula; $K_i = IC_{50}$ for non- competitive inhibition, where IC_{50} is the half maximal inhibitory concentration, S is the substrate concentration and K_m is the Michaelis constant.

AdoMet Competitiveness Test



S4 : AdoMet competitiveness test for compounds 3b and 4d. As seen in the graph, for both 3b and 4d, the K_m remains the same while the V_{max} decreases with increasing Inhibitor concentration, displaying a pattern of AdoMet non-competitive inhibition.

S5: K_m and V_{max} values for varying inhibitor concentrations of 3b and 4d.

Conc (μM)	3b		4d	
	Vmax	Km	Vmax	Km
100	1121	10,5	306,3	17,44
10	1573	9,744	649,3	13,07
1	1618	5,154	1753	10,7
0,1	1698	7,699	2426	10,82

Test with other PMTs

S6: Dose response data for DNMT1, PRMT1 and SET 7/9 for compounds 3b and 4d^b

Conc.(M)	DNMT1		PRMT1		SET7/9	
	3b	4d	3b	4d	3b	4d
2,00E-04	231,76	31,79	91,89	72,30	86,36	94,11
5,00E-05	147,68	132,23	101,94	83,51	94,22	103,17
1,25E-05	93,38	102,17	106,79	97,03	95,54	99,54
3,13E-06	117,90	122,65	109,40	95,20	93,15	91,34
7,81E-07	99,16	104,19	96,48	96,30	95,79	92,08
1,95E-07	98,97	108,25	94,40	97,77	100,46	87,39
4,88E-08	92,65	106,40	96,76	98,91	101,83	94,87
1,22E-08	95,95	130,64	94,30	94,07	91,85	90,78
3,05E-09	168,26	118,87	104,61	97,53	101,34	98,37
7,63E-10	101,20	115,11	102,77	100,30	100,32	102,11
DMSO	97,36	105,03	100,69	102,42	99,88	99,25

^b Dose response data was collected for samples starting from 200 μM with a serial 4 fold dilution for compounds 3b and 4d. As seen, no significant inhibition was observed for any of the targets above by 3b and 4d. Only DNMT1 seems to be inhibited at 200 μM of 4d and the IC_{50} determination was very ambiguous (IC_{50} of 179 μM).