Supporting information

Title: Non-Substrate Based, Small Molecule Inhibitors of the Human Isoprenylcysteine Carboxyl Methyltransferase

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Materials:

AFC was purchased from Enzo Life Sciences, Inc. (Farmingdale, NY). [^{14}C]-SAM was purchased from Perkin-Elmer (Waltham, MA). The anti-myc monoclonal antibody and goat anti-rabbit IgG were purchased from Invitrogen. Cell lines were purchased from ATCC. All other reagents and chemicals were purchased from either Sigma-Aldrich (St. Louis, MO) or Invitrogen (Carlsbad, CA). Chemicals were purchased from Sigma-Aldrich or Fisher Scientific.

Chemical synthesis and characterization:

Analytical chemistry procedures

Mass spectra (MS) data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Nuclear Magnetic Resonance (NMR) spectra were recorded with a Varian Mercury spectrometer with 400 MHz for proton (^{1}HNMR) and 100 MHz for carbon (^{13}C NMR); chemical shifts are reported in ppm (δ). Preparative HPLC was performed on Agilent Prep 1200 series with UV detector set to 254 nm. Samples were injected onto a Phenomenex Luna 75 x 30 mm, 5 μM, C18 column at room temperature with a flow rate of 30 mL/min. A linear gradient was used with 10% of MeOH (A) in 0.1 % TFA in H2O (B) to 100% of MeOH (A). Analytical HPLC spectra for all compounds were acquired using an Agilent 6110 Series system with UV detector set to 254 nm. Analytical HPLC was used to establish the purity of target compounds, all compounds had > 95% purity using the analytical HPLC methods described above. Analytical HPLC spectra were acquired with an Agilent 6110 system with UV detector set to 254 nm. Samples were injected onto an Agilent Eclipse Plus 4.6 mm X 50 mm C18 column. Separation was performed using a linear gradient of 10% to 100% B for 5 minutes, followed by 100% B for 2 minutes, with a flow rate of 1 mL/min. Solvent B: 0.1% AcOH in MeOH. Solvent A: 0.1% AcOH in water.

Synthetic Schemes

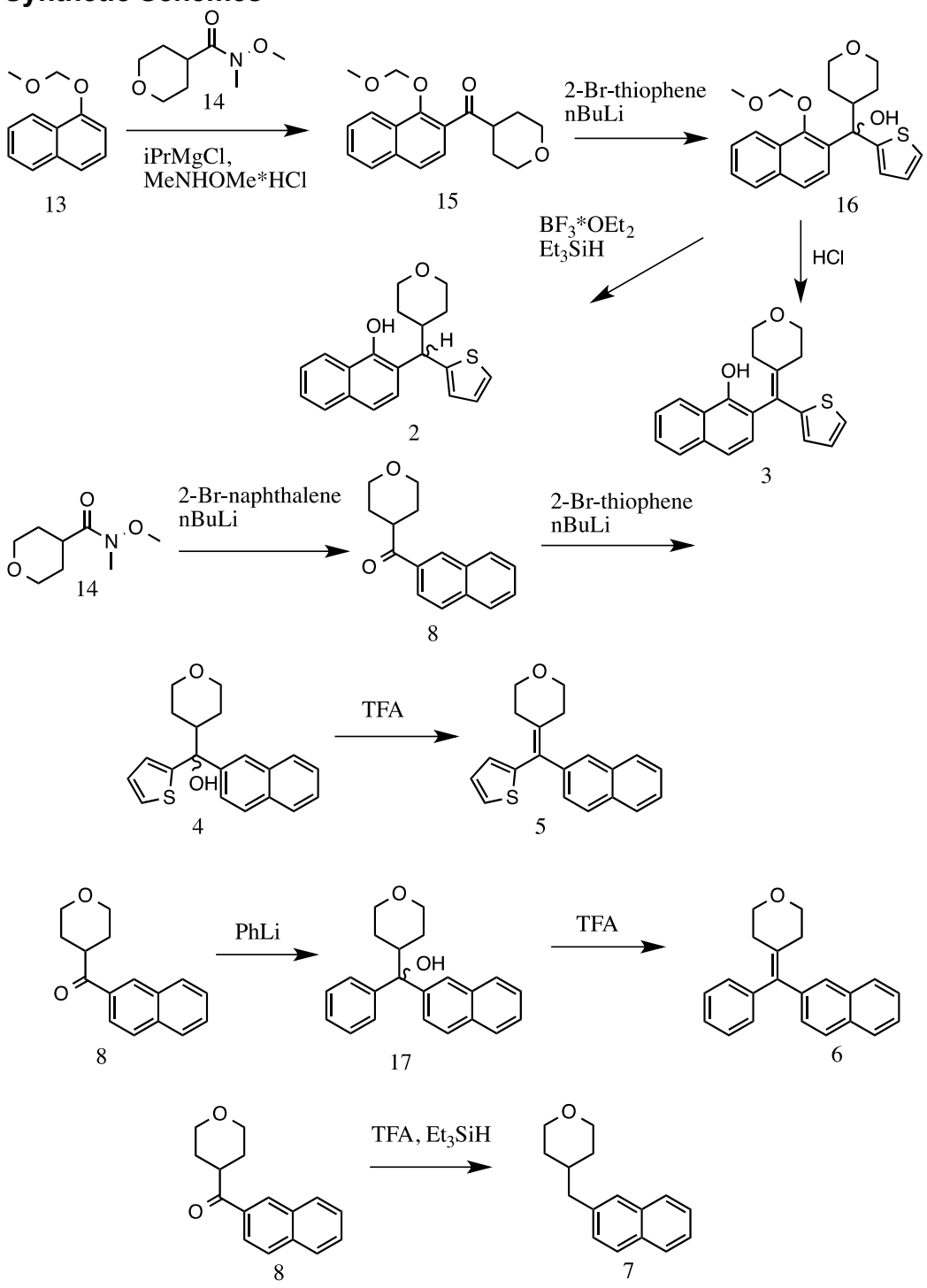

Figure S1: Synthesis of compounds **2**-**8**. See synthetic methods section for details

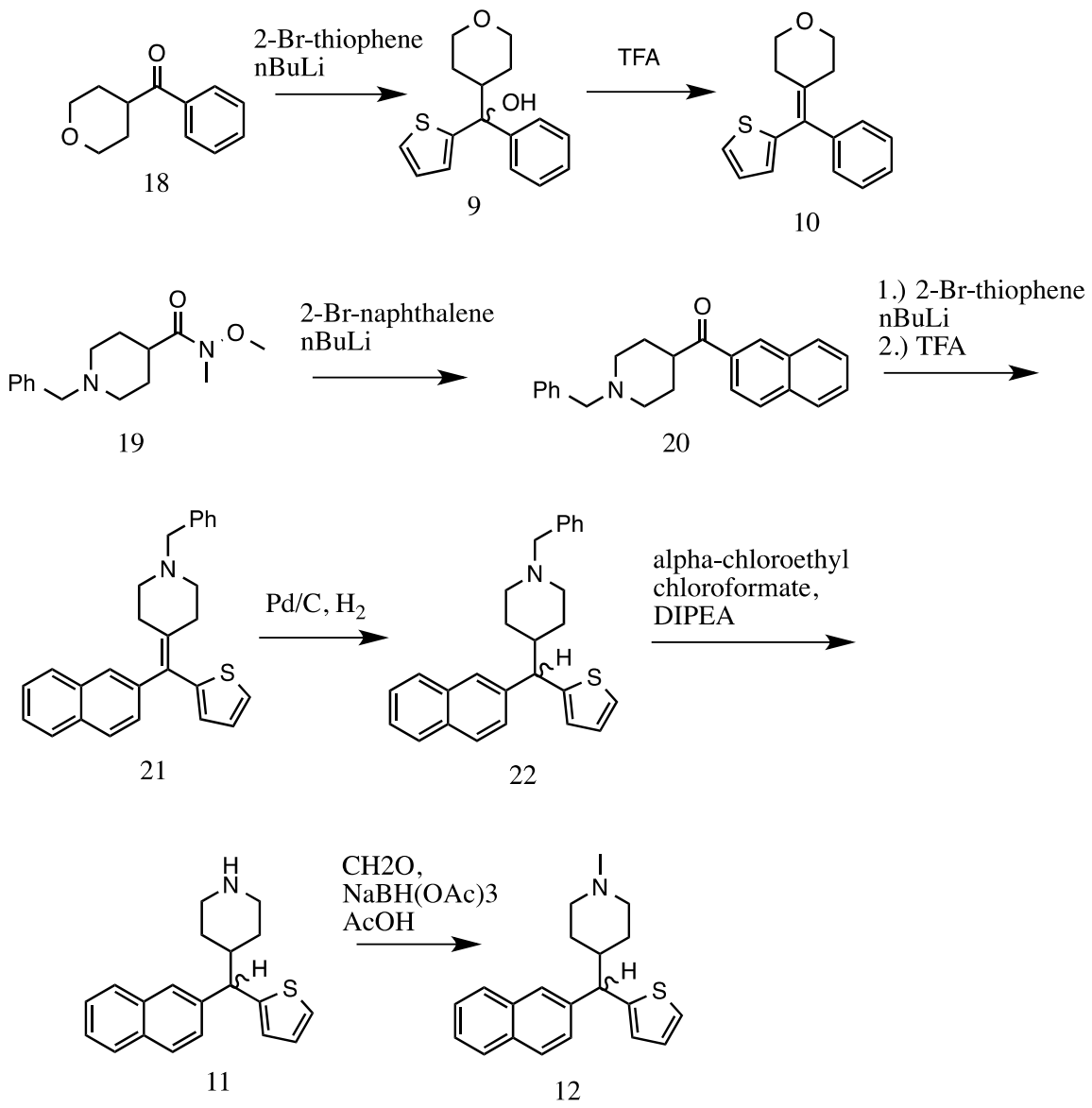

Figure S2: Synthesis of compounds **9**-**12**. See synthetic methods section for details

Chemical synthesis and characterization

2-((Tetrahydro-2*H*-pyran-4-yl)(thiophen-2-yl)methyl)naphthalen-1-ol (2)
(1-(Methoxymethoxy)naphthalen-2-yl)(tetrahydro-2*H*-pyran-4-yl)(thiophen-2-yl)methanol (**16**) (50 mg, 0.13 mmol), dichloromethane (DCM) (1 ml), and triethylsilane (84 ul, 0.52 mmol) were placed in a flask under Ar and cooled to -78°C. The mixture was treated with boron trifluoride diethyl etherate (64 ul, 0.52 mmol) dropwise, and was stirred for 1h, then allowed to warm to room temperature (RT). The mixture was stirred for 2h at RT, quenched with water, and the product was extracted with ethyl acetate (EtOAc). The product was purified by HPLC. Yield 18 mg, 0.056 mmol, 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ

7.97 (dd, 1H), 7.78 (dd, 1H), 7.50-7.42 (m, 4H), 7.15 (dd, 1H), 7.01 (dd, 1H), 6.92 (dd, 1H), 5.30 (br, 1H), 4.49 (d, 1H), 3.95 (ddm, 2H), 3.41 (dtd, 2H), 2.42 (qt, 1H), 1.47 (m, 2H), 1.34 (m, 2H). MS (ESI): $[M + H]^+$ 325.2.

2-((Tetrahydro-4*H*-pyran-4-ylidene)(thiophen-2-yl)methyl)naphthalen-1-ol (3)
(1-(Methoxymethoxy)naphthalen-2-yl)(tetrahydro-2*H*-pyran-4-yl)(thiophen-2-yl)methanol (**16**) (50 mg, 0.13 mmol) was refluxed for 2 h in a 1:4 mixture of conc. aq. HCl and ethanol (3 ml). The reaction mixture was concentrated and purified by HPLC. Yield 17 mg, 0.052 mmol, 41%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (m, 1H), 7.78 (m, 1H), 7.49 (m, 2H), 7.40 (d, 1H), 7.27 (dd, 1H), 7.14 (d, 1H), 6.97 (dd, 1H), 6.88 (dd, 1H), 5.66 (s, 1H), 3.84 (t, 2H), 3.72 (m, 2H), 2.85 (m, 2H), 2.29 (t, 2H). MS (ESI): $[M + H]^+$ 323.1.

Naphthalen-2-yl(tetrahydro-2*H*-pyran-4-yl)(thiophen-2-yl)methanol (4)
The product was prepared from naphthalen-2-yl(tetrahydro-2*H*-pyran-4-yl)methanone (**8**) using the method described for **16**. Yield: 200 mg 0.61 mmol, 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.86 (m, 1H), 7.81 (d, 2H), 7.55 (dd, 1H), 7.49 (m, 2H), 6.90 (d, 1H), 6.81 (d, 1H), 4.00 (ddd, 2H), 3.43 (dt, 2H), 2.56 (tt, 1H), 1.73 (d, 1H), 1.54 (m, 2H), 1.28 (d, 1H). MS (ESI): $[M + Na]^+$ 347.200.

4-(Naphthalen-2-yl(thiophen-2-yl)methylene)tetrahydro-2*H*-pyran (5)
Naphthalen-2-yl(tetrahydro-2*H*-pyran-4-yl)(thiophen-2-yl)methanol (50 mg, 0.15 mmol) was treated with DCM (2.1 ml) and trifluoroacetic acid (TFA) (0.7 ml), and stirred at RT overnight. The reaction mixture was concentrated and purified by MPLC (silica, 0 to 50% EtOAc in hexane) then HPLC. Yield: 14 mg, 0.046 mmol, 31%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (M, 3H), 7.65 (s, 1H), 7.47 (m, 2H), 7.28 (dd, 1H), 7.24 (dd, 1H), 6.97 (dd, 1H), 6.83 (dd, 1H), 3.81 (t, 2H), 3.72 (t, 2H), 2.69 (t, 2H), 2.39 (t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ^{13}C NMR (101 MHz, $cdcl_3$) δ 143.90, 139.38, 136.84, 133.21, 132.33, 129.16, 127.94, 127.93, 127.83, 127.80, 127.63, 127.09, 126.60, 126.08, 125.87, 125.35, 69.19, 69.11, 33.39, 33.17. MS (ESI): $[M + H]^+$ 307.0.

4-(Naphthalen-2-yl(phenyl)methylene)tetrahydro-2*H*-pyran (6)
The product was prepared from naphthalen-2-yl(phenyl)(tetrahydro-2*H*-pyran-4-yl)methanol (**17**) in a manner analogous to that described for **5**. Yield: 25 mg, 35%. ^{1}H NMR (400 MHz, CDCl3) δ 7.80 (m, 2H), 7.77 (d, 1H), 7.60 (m, 1H), 7.45 (m, 2H), 7.29 (m, 2H), 7.23 (m, 2H), 7.17 (m, 2H), 3.76 (m, 4H), 2.46 (m, 4H). MS (ESI): $[M + H]^+$ 301.1.

4-(Naphthalen-2-ylmethyl)tetrahydro-2*H*-pyran (7)
Naphthalen-2-yl(tetrahydro-2*H*-pyran-4-yl)methanone (**8**) (100 mg, 0.41 mmol) was treated with TFA (0.31 ml) and triethylsilane (0.17 ml) and stirred at 0°C for 2h. The reaction mixture was concentrated and the product was purified by

MPLC (silica, 0 to 10% EtOAc in hexane). Yield: 48.1 mg, 0.21 mmol, 52%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (dd, 1H), 7.78 (m, 2H), 7.59 (m, 1H), 7.45 (m, 2H), 7.30 (dd, 1H), 4.06 (dd, 2H), 3.44 (td, 2H), 2.73 (d, 2H), 1.90 (ttt, 1H), 1.64 (m, 2H), 1.43 (dtd, 2H). MS (ESI): $[M + H]^+$ 227.1.

Naphthalen-2-yl(tetrahydro-2*H*-pyran-4-yl)methanone (8)
2-Bromonaphthalene (600 mg, 2.90 mmol) in tetrahydrofuran (THF) (10 ml) was cooled to -78^{o}C and treated with nBuLi (1.6 M, 1.63 ml) dropwise. This was stirred 20 min followed by addition of methyl tetrahydro-2*H*-pyran-4-carboxylate (**14**) (250 mg, 1.45 mmol, as a solution in 1ml THF). This was stirred 2h at -78^{o}C. The reaction was quenched with sat. aq. NH_4Cl and the product was extracted with EtOAc. Purification by MPLC (silica, 0 to 100% EtOAc in hexane) gave the product. Yield: 203 mg, 0.85 mmol, 29%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 7.99 (dd, 1H), 7.96 (dd, 1H), 7.90 (d, 1H), 7.88 (d, 1H), 7.60 (td, 1H), 7.55 (td, 1H), 4.08 (dt, 2H), 3.61 (td, 2H), 1.93 (td, 2H), 1.83 (dm, 2H). MS (ESI): $[M + H]^+$ 241.0.

Phenyl(tetrahydro-2*H*-pyran-4-yl)(thiophen-2-yl)methanol (9)
The product was prepared from phenyl(tetrahydro-2*H*-pyran-4-yl)methanone (**18**) in a manner analogous to that described for **16**. Yield: 10 mg, 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (dm, 2H), 7.36-7.30 (m, 2H), 7.25-7.17 (m, 2H), 7.00 (dd, 1H), 6.96 (dd, 1H), 3.98 (ddd, 2H), 3.72 (dt, 1H), 3.42 (dtd, 2H), 2.50 (tt, 1H),1.65 (dm, 1H), 1.53 (dtd, 2H), 1.29 (dm, 1H). MS (ESI): $[M + H]^+$ 257.1.

4-(Phenyl(thiophen-2-yl)methylene)tetrahydro-2*H*-pyran(10)
The product was prepared from phenyl(tetrahydro-2*H*-pyran-4-yl)(thiophen-2-yl)methanol (**9**) in a manner analogous to that described for **5**. Yield: 34.21 mg, 47%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 2H), 7.24 (m, 2H), 7.17 (m, 2H), 6.95 (dd, 1H), 6.79 (dd, 1H), 3.77 (t, 2H), 3.70 (t, 2H), 2.63 (t, 2H), 2.34 (t, 2H). MS (ESI): $[M + H]^+$ 257.1.

4-(Naphthalen-2-yl(thiophen-2-yl)methyl)piperidine (11)
1-Benzyl-4-(naphthalen-2-yl(thiophen-2-yl)methyl)piperidine (**22**) (142 mg, 0.38 mmol) was treated with DCM (2 ml) then diisopropylethylamine (DIPEA) (62 ul, 0.38 mmol), and cooled to 0^{o}C. The reaction was treated with alpha-chloroethyl chloroformate (82 ul, 0.76 mmol) and stirred at RT for 3h. The mixture was evaporated to dryness and stirred in MeOH (5 ml) overnight. The mixture was concentrated and purified by HPLC. Yield: 21.8 mg, 0.07 mmol, 18%. ^{1}H NMR (400 MHz, MeOD) δ 7.79-7.75 (m, 3H), 7.70 (s, 1H), 7.46-7.39 (m, 3H), 7.13 (d, 1H), 6.93 (m, 1H), 6.88 (m, 1H), 4.00 (d, 1H), 3.32 (dd, 2H), 2.30 (m, 1H), 1.94 (d, 1H), 1.53 (t, 2H), 1.47 (m, 1H). MS (ESI): $[M + H]^+$ 308.0.

1-Methyl-4-(naphthalen-2-yl(thiophen-2-yl)methyl)piperidine (12)
4-(Naphthalen-2-yl(thiophen-2-yl)methyl)piperidine (**11**) (60 mg, 0.14 mmol) was dissolved in dichloroethane (DCE) (2 ml) and treated with $NaBH(OAc)_3$ (47 mg, 0.22 mmol), acetic acid (AcOH) (16 ul, 0.28 mmol), and formaldehyde (37% in

H_2O, 17 ul, 0.21 mmol). This was stirred for 2h. The reaction was quenched with saturated aqueous $NaHCO_3$, and the product was extracted with EtOAc. Following aqueous workup, the product was purified by HPLC. Yield: 36.4 mg, 0.11 mmol, 80%. ^{1}H NMR (400 MHz, MeOD) δ 7.80-7.73 (m, 3H), 7.70 (m, 1H), 7.46-7.37 (m, 3H), 7.12 (dd, 1H), 6.93 (dd, 1H), 6.87 (dd, 1H), 4.02 (d, 1H), 3.54 (m, 1H), 2.69 (s, 3H), 2.56 (m, 2H), 1.91 (dt, 1H), 1.70 (dtd, 2H), 1.57 (dt, 1H) MS (ESI): $[M + H]^+$ 322.1.

1-(Methoxymethoxy)naphthalene (13)
1-Naphthol (1.44 g, 10 mmol), DCM (15 ml), DIPEA (2.62 ml, 15 mmol), and methylchloromethyl ether (0.84 ml, 11 mmol) were stirred at RT overnight. The reaction mixture was concentrated, taken up in EtOAc, and washed with sat. aq. NH_4Cl and brine. The organic layer was dried (Na_2SO_4) filtered and concentrated. Yield: 1.148 g, 6.10 mmol, 61%. Purification by MPLC (0 to 100% EtOAc in hexane) gave the product.^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (m, 1H), 7.62 (m, 1H), 7.50 (m, 3H), 7.39 (t, 1H), 7.11 (d, 1H), 5.41 (s, 2H), 3.57 (s, 3H). MS (ESI): $[M + H]^+$ 189.1.

***N*-Methoxy-*N*-methyltetrahydro-2*H*-pyran-4-carboxamide (14)**
To THF (20 mL), methyl tetrahydro-2*H*-pyran-4-carboxylate (1.0 g, 6.94 mmol), and N,O-dimethylhydroxylamine hydrochloride (0.846 g, 8.68 mmol) at -10^{o}C was added iPrMgCl (2M, 8.68 ml) dropwise. This was stirred for 1h at -10^{o}C. Saturated aq. NH_4Cl (20 ml) was added and the reaction was extracted with EtOAc. Purification by MPLC (0 to 100% EtOAc in hexane gave the product. Yield: 600mg, 3.46 mmol, 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 (ddd, 2H), 3.71 (s, 3H), 3.46 (td, 2H), 3.19 (s, 3H), 2.92 (tt, 1H), 1.85 (qd, 2H), 1.65 (dm, 2H).

(1-(Methoxymethoxy)naphthalen-2-yl)(tetrahydro-2*H*-pyran-4-yl)methanone (15)
1-(Methoxymethoxy)naphthalene (**13**) (2.66 mmol) in THF (8 ml) and TMEDA (2 ml) at 0^{o}C was treated with nBuLi (1.6 M, 2.1 ml) dropwise. This was stirred 30 min at 0^{o}C, *N*-Methoxy-*N*-methyltetrahydro-2*H*-pyran-4-carboxamide was added and this was stirred for 1h at RT. The reaction was quenched with sat. aq. NH_4Cl and the product was extracted with EtOAc. Purified by MPLC (0 to 100% EtOAc in hexane). Yield: 375 mg, 1.25 mmol, 47%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (dt, *J* = 7.0, 3.5 Hz, 1H), 7.88 (dt, *J* = 7.1, 3.5 Hz, 1H), 7.69 (d, *J* = 8.5 Hz, 1H), 7.60 (dd, *J* = 6.4, 3.2 Hz, 2H), 7.51 (dd, *J* = 8.6, 1.1 Hz, 1H), 5.17 (d, *J* = 1.1 Hz, 2H), 4.03 (dt, *J* = 11.7, 3.4 Hz, 2H), 3.63 - 3.56 (m, 1H), 3.56 - 3.53 (m, 3H), 3.53 - 3.47 (m, 2H), 1.88 - 1.75 (m, 4H). MS (ESI): $[M + Na]^+$ 323.1.

(1-(Methoxymethoxy)naphthalen-2-yl)(tetrahydro-2*H*-pyran-4-yl)(thiophen-2-yl)methanol(16)
A dry flask under Ar was treated with THF (3 ml) and 2-bromo-thiophene (78ul, 0.8 mmol) and cooled to -78^{o}C. This was treated with nBuLi (1.6 M, 0.50 ml) drop wise and stirred 30 min at -78^{o}C. Next, (1-(methoxymethoxy)naphthalen-2-

yl)(tetrahydro-2*H*-pyran-4-yl)methanone (**15**) (200 mg, 0.67 mmol, as a solution in 0.5 ml THF) was added slowly. This was stirred for 1h at -78°C then allowed to warm to RT overnight. The reaction was quenched with sat. aq. NH_4Cl, and the product was extracted with EtOAc. The combined organic layers were washed with brine and purified on MPLC (0 to 40% EtOAc in hexane). Yield: 163 mg, 0.42 mmol, 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (m, 1H), 7.83 (m, 1H), 7.66 (dd, 2H), 7.51 (M, 2H), 7.16 (T, 1H), 6.91 (M, 1H), 6.47 (S, 1H), 5.29 (S, 1H), 4.01 (DD, 2H), 3.69 (S, 3H), 3.41 (Q, 2H), 2.54 (M, 1H), (2.04 (S, 2H), 1.87 (M, 2H), 1.42 (DD, 2H). MS (ESI): $[M + H]^+$ 407.2.

Naphthalen-2-yl(phenyl)(tetrahydro-2*H*-pyran-4-yl)methanol(17)
The product was prepared from naphthalen-2-yl(tetrahydro-2*H*-pyran-4-yl)methanone (**8**) in a manner analogous to that described for **18**. Yield: 88mg, 38%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (m, 1H), 7.84 (m, 1H), 7.76 (m, 2H), 7.53 (m, 3H, 7.45 (m, 2H), 7.31 (m, 2H), 7.20 (m, 1H), 4.00 (m, 2H), 3.47 (tdt, 2H), 2.80 (tt, 1H), 1.60 (m, 2H), 1.45 (dd, 2H). MS (ESI): $[M + H]^+$ 341.0.

Phenyl(tetrahydro-2*H*-pyran-4-yl)methanone (18)
Methyl tetrahydro-2*H*-pyran-4-carboxylate (250 mg, 1.45 mmol) in THF (10 ml) was cooled to -78°C. Phenyllithium (1.8 M, 1.61 ml) was added drop wise with stirring. The mixture was stirred 2h at -78°C, and quenched with sat aq NH_4Cl. The product was extracted with EtOAc. Following aqueous workup, the product was purified by MPLC (silica, 0 to 30% EtOAc in hexane). Yield: 204 mg, 1.07 mmol, 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 - 7.90 (m, 2H), 7.61 - 7.53 (m, 1H), 7.48 (t, *J* = 7.6 Hz, 2H), 4.11 - 4.00 (m, 2H), 3.57 (td, *J* = 11.6, 2.3 Hz, 3H), 3.51 (m, 1H), 1.95 - 1.83 (m, 2H), 1.83 - 1.73 (m, 2H). MS (ESI): $[M + H]^+$ 191.1.

1-Benzyl-*N*-methoxy-*N*-methylpiperidine-4-carboxamide (19)
The product was prepared from methyl 1-benzylpiperidine-4-carboxylate in a manner analogous to that described for **14**. Yield: 2.22 g, 8.47 mmol, 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.24 (m, 4H), 7.23 (m, 1H), 3.67 (s, 3H), 3.49 (s, 2H), 3.15 (s, 3H), 2.92 (dt, 2H), 2.63 (m, 1H), 2.00 (td, 2H), 1.82 (dtd, 2H), 1.69 (m, 2H). MS (ESI): $[M + H]^+$ 263.1.

(1-Benzylpiperidin-4-yl)(naphthalen-2-yl)methanone (20)
The product was prepared from 1-benzyl-*N*-methoxy-*N*-methylpiperidine-4-carboxamide (**19**) in a manner analogous to that described for **16**. Yield: 1.778 g, 5.40 mmol, 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (d, *J* = 1.7 Hz, 1H), 8.03 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.99 (d, *J* = 8.1 Hz, 1H), 7.91 (dd, *J* = 14.2, 8.3 Hz, 2H), 7.62 (dd, *J* = 8.0, 6.7 Hz, 1H), 7.58 (dd, *J* = 8.2, 6.8 Hz, 1H), 7.42 - 7.31 (m, 4H), 7.31 - 7.28 (m, 1H), 3.61 (s, 2H), 3.45 (q, *J* = 7.4 Hz, 1H), 3.04 (d, *J* = 11.9, 2H), 2.24 (m, 2H), 1.95 (m 4H), 1.82 (m, 2H). MS (ESI): $[M + H]^+$ 330.1.

1-Benzyl-4-(naphthalen-2-yl(thiophen-2-yl)methylene)piperidine (21)
(1-Benzylpiperidin-4-yl)(naphthalen-2-yl)(thiophen-2-yl)methanol was prepared from (1-benzylpiperidin-4-yl)(naphthalen-2-yl)methanone (**20**) in a manner

analogous to that described for **16**. This intermediate ((1-benzylpiperidin-4-yl)(naphthalen-2-yl)(thiophen-2-yl)methanol) could not be fully purified so it was converted to **21** by treating with DCM (2.1 ml) and TFA (0.7 ml), and stirring at RT overnight. The reaction mixture was concentrated and purified by MPLC (silica, 0 to 1000% EtOAc in hexane). Yield: 620 mg, 1.57 mmol, 52%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (m, 2H), 7.76 (d, 1H), 7.63 (m, 1H), 7.44 (m, 2H, 7.36 (m, 1H), 7.29 (m, 3H), 7.23 (m, 3H), 6.94 (dd, 1H), 6.81 (dd, 1H), 3.53 (s, 2H), 2.67 (t, 2H), 2.56 (t, 2H), 2.47 (t, 2H), 2.37 (t, 2H). MS (ESI): $[M + H]^+$ 396.0.

1-Benzyl-4-(naphthalen-2-yl(thiophen-2-yl)methyl)piperidine (22)
1-Benzyl-4-(naphthalen-2-yl(thiophen-2-yl)methylene)piperidine (**21**) (500 mg, 1.20 mmol) was treated with MeOH (10 ml) then Pd/C (10%) (225 mg) and ammonium formate (378 mg, 6 mmol). This was refluxed for 8 h. The mixture was filtered through celite, concentrated, and purified by MPLC (silica, 10 to 100% EtOAc in hexane). Yield: 332 mg, 0.83 mmol, 70%. ^{1}H NMR (400 MHz, MeOD) δ 7.75 (m, 3H), 7.68 (m, 1H), 7.45-7.39 (m, 3H), 7.38-7.32 (m, 5H), 7.10 (dd, 1H), 6.91 (m, 1H), 6.86 (dd, 1H), 4.02 (s, 2H), 3.33 (m, 2H), 2.45 (dtd, 2H), 2.15 (m, 1H), 1.86 (m, 1H), 1.67 (m, 2H), 1.55 (m, 1H). MS (ESI): $[M + H]^+$ 398.0.

Screening and enzymatic assays

Crude Membrane Preparation

Crude membrane extracts were prepared as previously described from $His_{10}myc_3$N-Icmt (His-Icmt) yeast strains.[1] Briefly, yeast cells were cultured in SC-URA medium to an OD_{600} of 4.0-5.0. The cells were then harvested by centrifugation and the resulting pellet was stored at -80 °C until use. Upon thawing, the pellet was resuspended in lysis buffer (0.3 M sorbitol, 10 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 5 mM $MgCl_2$, 1% aprotinin, and 2 mM AEBSF) and allowed to swell on ice for 15 min. The cells were rapidly frozen and thawed twice by submersion into liquid N_2 and room temperature respectively. The cells were then lysed by two passes through a French press at 18,000 p.s.i. The lysate was then centrifuged at 500xg to removed cell debris. The supernatant was then ultracentrifuged at 100,000xg for 1 h at 4 °C. The supernatant was then removed and the pellet was resuspended in 10 mM Tris-HCl, pH 7.5. The crude membrane extracts were separated into aliquots, flash frozen in liquid N_2 and stored at -80 °C. Total protein concentration was determined using Coomassie Plus protein reagent (Pierce).

384-well Plate Screen, IC_{50}, and Substrate Methyltransferase Assays

The methyltransferase vapor diffusion assays were conducted as previously described with minor modifications.[2,3] Crude membrane extracts overexpressing hIcmt (5 µg) in the presence of a solution of Tris-HCl buffer (100 mM, pH 7.4), AFC (10 µM) and [^{14}C]-SAM (60 µM) were added to a 384-well plate containing 1 µL of inhibitor to make the final concentration of inhibitor 167

μM in a final volume of 60 μL. After incubating the plate at 37 °C for 30 min, the reaction was stopped by the addition of 50 μl of 1 M NaOH/1% SDS. The reaction mixture was spotted on to filter paper. The filter paper was lodged into the neck of a scintillation tube filled with 10 mL of scintillation fluid and capped. The filter papers were removed after 2.5 h and the radioactivity was quantified using a Packard 1600CA Liquid Scintillation Analyzer. IC_{50} values were obtained using the same procedure, except in microcentrifuge tubes with increasing concentrations of compound. hIcmt specific activity (pmol/mg/min) was plotted against compound concentration and IC_{50} values were calculated using GraphPad Prism 4.0. Assays were conducted three separate times in duplicate. DMSO concentrations were kept constant at 5% for both assays.

Kinetic Competition Assay

The procedure for the methyltransferase activity assay was carried out as described above with minor modifications.[4] For AFC competition, the concentration of AFC was varied while the concentration of inhibitor and SAM (5 μM) was held constant. This was repeated for increasing concentrations of inhibitor and plotted on a double reciprocal plot. The same was done for SAM competition by varying SAM concentration and keeping AFC (25 μM) and inhibitor concentration constant. GraphPad Prism 4.0 was used to generate α and K_I values.

Cell Killing Assay

The ability of an inhibitor to kill cancer cells was analyzed using the colorimetric MTT assay.[5] 96-well plates were plated with MIAPaCa-2 or Panc-1 cells (1,000 cells per well) in 100 μL per well and incubated at 37 °C one day before the assay. On the day of the assay, the media was replaced with 100 μL media containing increasing concentrations of inhibitor. The DMSO concentration was kept constant at 0.5%. Cell were incubated at 37 °C for 5 days. Then 20 μL of a 5 mg/mL solution of MTT was added to each well and incubated an additional 4 h at 37 °C. The media was aspirated and the formazan crystals were solubilized in 100 μL DMSO. The absorbance was read at 590 nm and the average absorbance for each concentration of dimer was plotted using GraphPad Prism 4.0. IC_{50} values represent the concentration of dimer leading to 50% cell viability ± SD.

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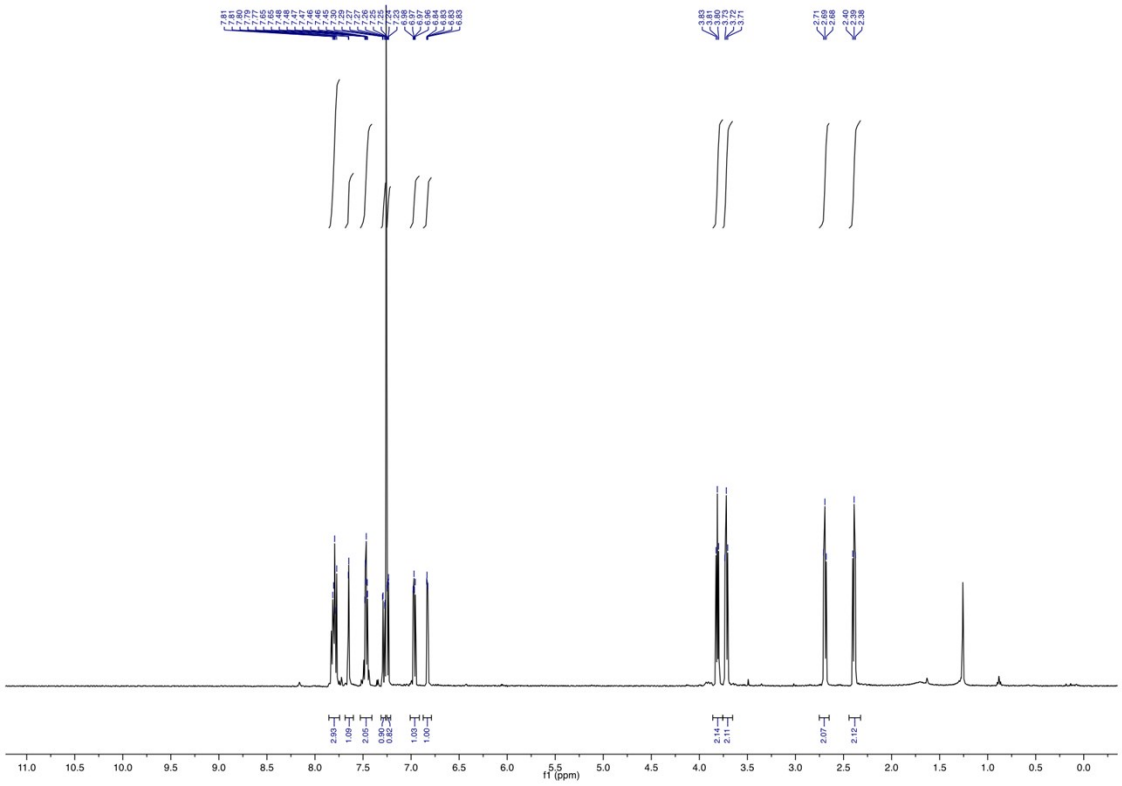
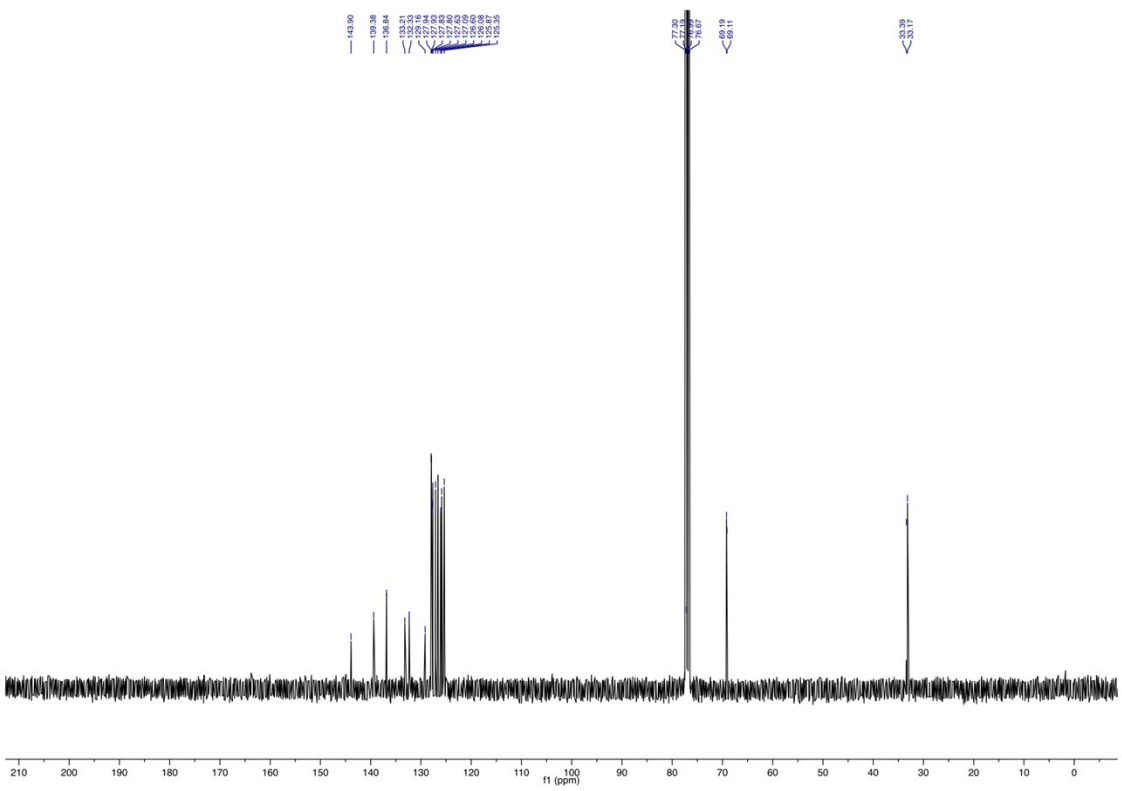


^{1}H NMR spectra of **5.**



^{13}C NMR spectra of **5.**