Supplementary Information

Exploiting the co-reliance of tumours upon transport of amino acids and lactate: Gln and Tyr conjugates of MCT1 inhibitors

Reji N. Nair,[a†] Jitendra K. Mishra,[a†] Fangzheng Li,[a] Mariola Tortosa,[a] Chunying Yang,[b] Joanne R. Doherty,[c] Michael Cameron,[d] John L. Cleveland,[b] William R. Roush,[a] and Thomas D. Bannister*[a]

a. Department of Chemistry, The Scripps Research Institute, 110 Scripps Way, Jupiter, FL 33458, USA
b. Department of Tumor Biology, Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, Tampa, FL 33612, USA
c. Department of Cancer Biology, The Scripps Research Institute, 110 Scripps Way, Jupiter, FL 33458, USA
d. Department of Molecular Therapeutics, The Scripps Research Institute, 110 Scripps Way, Jupiter, FL 33458, USA
† RNN (current location – Pacific Northwest National Laboratory, Richland, WA) and JM (current location – St. Jude's Children's Research Hospital, Memphis,TN) were equal primary contributors to this work

*Corresponding author - tbannist@scripps.edu; phone 561-228-2206

General Chemistry Experimental:

All reagents and solvents were obtained from commercial suppliers and were used as is without further purification. NMR spectra were recorded on a Brüker 400 MHz spectrometer (400 MHz ^{1}H, 100 MHz ^{13}C) at 25 °C. Chemical shifts are reported in ppm (δ) referenced to the NMR solvent, and coupling constants (*J*) are in hertz. Flash column chromatography was performed to purify compounds as indicated using *RediSep* columns (60 Å mesh) on a Combiflash R_f® or Combiflash Companion® systems from *Teledyne Isco*. All reactions were monitored using TLC and LCMS (conducted using an ion-trap mass spectrometer system coupled with a liquid chromatography system from Thermo-Fisher). Wherever necessary, reactions were carried out under argon atmosphere. LDA was freshly prepared before use each time. Reaction products were routinely analyzed by analytical HPLC using an Agilent 1100 system equipped with a diode array detector, simultaneously monitoring multiple wavelengths (typically 215 nm, 230 nm, 254 nm, and 280 nm). Compounds were required to display >95% purity at all LC wavelengths monitored.

MTT assay protocols.

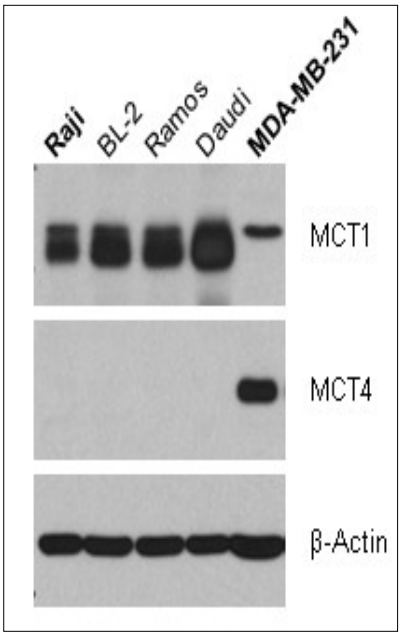
The EC_{50} values reported in Table 2 were obtained using a standard 4 day MTT cell growth assay, as described in reference 20.

DMPK methods.

The tissue distribution data reported in Table 1 was generated using standard LC-MS/MS bioanalytical methods for the test compounds, performed in the M. Cameron laboratory at Scripps FL. The appropriate parent and daughter ion pairs were determined for each molecule using direct infusion. *In vivo* PK was assessed using Sprague Dawley rats. All procedures are IACUC-approved and the Scripps FL vivarium is fully AAALAC-accredited. Tissue levels: Compounds were dosed at 20mg/kg IP, using solutions of 1 mg/mL in a vehicle of 10:10:80 DMSO:Tween80:water. At the predetermined time points, animals were humanely sacrificed and the indicated tissues were collected, mixed with organic solvent, and a probe tip sonicator was used to break up the tissue. Samples were analyzed by LC-MS/MS. Plasma drug levels are determined against standards made in plasma; tissue samples are compared to standards made in blank tissue matrix.

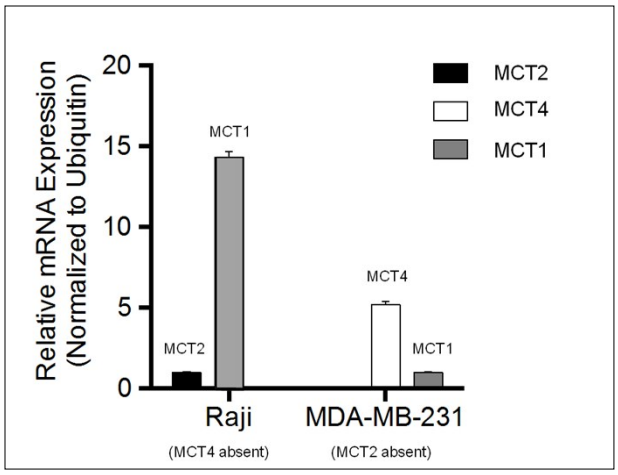
Expression profiling analysis.

Protein expression levels of the MCT transporter isoforms were analyzed, as described in reference 20. As shown in supplementary Figure A, by Western blot Raji cells (left lane) were found to abundantly express MCT1 but not MCT4. MDA-MB-231 cells (right lane) were found to abundantly express MCT4, with diminished levels of MCT1.



Supplementary Figure A

As shown in supplementary Figure B, by qRT-PCR, Raji cells show high MCT1 expression, low MCT2 expression, and undetectable MCT4 expression. MDA-MB-231 cells show MCT4 expression, little MCT1 expression, and no detectable MCT2 expression.

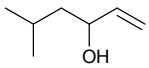


Supplementary Figure B

Scheme 1 chemistry methods.

Compounds in Scheme 1 (**19-27**) were prepared as per AstraZeneca's methods,[19] with modifications previously published from our labs.[25-26] Intermediate halides required in the alkylation step were synthesized in-house by the published synthetic procedures.[26]

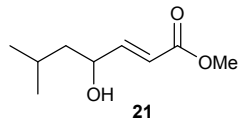
Data for alcohol 20:



20

^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.90-5.81 (m, 1H), 5.22 (td, J = 17.2, 1.4 Hz, 1H), 5.08 (td, J = 10.4, 1.3 Hz, 1H), 4.19-4.14 (br m, 1H), 1.79-1.69 (m, 1H), 1.64 (br s, 1H), 1.49-1.43 (m, 1H), 1.35-1.28 (m, 1H), 0.92 ppm (dd, J = 6.6, 2.8 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 141.6, 114.3, 71.5, 46.2, 24.5, 23.0, 22.3 ppm; FT-IR (neat, cm^{-1}) 3338.9, 2955.7, 2925.8, 2870.8, 1644.9, 1468.8, 1423.8, 1384.8, 1367.8, 1308.9, 1152.8, 1091.8, 1055.8, 1016.8, 988.6, 918.6, 841.8, 661.8 cm^{-1}. Note: alcohol **20** is appreciably volatile, so care must be taken while evaporating the solvents after workup.

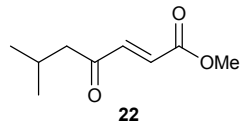
Data for alcohol 21:



21

^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.96 (dd, *J* = 15.7, 5.0 Hz, 1H), 6.04 (dd, *J* = 15.6, 1.6 Hz, 1H), 4.39-4.34 (br m, 1H), 3.75 (s, 3H), 1.79 (obscured d, 2H), 1.77 (d, *J* = 5.2 Hz, 1H), 1.54-1.46 (m, 1H), 1.41-1.34 (m, 1H), 0.95 ppm (d, *J* = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.0, 150.9, 119.4, 69.4, 51.7, 45.7, 24.5, 23.1, 22.1 ppm; FT-IR (neat, cm^{-1}) 3441.9, 2955.7, 2870.8, 1724.6, 1704.5, 1658.7, 1467.8, 1436.7, 1385.8, 1367.8, 1304.6, 1276.6, 1194.7, 1168.5, 1078.7, 1035.7, 983.6, 926.8, 863.8, 840.8, 714.8 cm^{-1}.

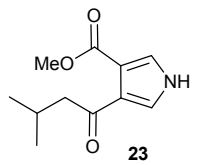
Data for ketone 22:



22

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (d, *J* = 16.0 Hz, 1H), 6.66 (d, *J* = 16.0 Hz, 1H), 3.81 (s, 3H), 2.50 (d, *J* = 7.0 Hz, 2H), 2.24-2.14 (m, 1H), 0.94 ppm (d, *J* = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.5, 166.1, 139.8, 130.2, 52.3, 50.5, 24.7, 22.5 ppm; FT-IR (neat, cm^{-1}) 2957.7, 2873.8, 1729.5, 1698.5, 1466.8, 1436.7, 1368.7, 1298.5, 1272.5, 1196.6, 1171.5, 1113.8, 1062.7, 1027.7, 979.6, 857.8, 702.8 cm^{-1}.

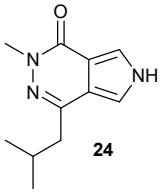
Data for pyrrole 23:



23

^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.14 (br s, 1H), 7.39 (s, 1H), 7.29 (s, 1H), 3.84 (s, 3H), 2.79 (d, J = 7.0 Hz, 2H), 2.28 – 2.20 (m, 1H), 0.97 ppm (d, J = 7.0 Hz, 6H).

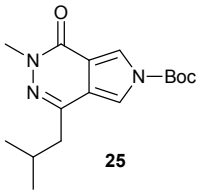
Data for pyrrole 24:



24

^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.25 (br s, 1H), 7.55 (s, 1H), 7.27 (merged with $CDCl_3$ signal, s, 1H), 3.79 (s, 3H), 2.63 (d, J = 7.4 Hz, 2H), 2.23-2.13 (septet, 1H), 0.98 ppm (d, J = 6.6 Hz, 6H); ^{13}C NMR (C*D*Cl_3, 100 MHz) δ 159.5, 145.6, 120.2, 116.5, 115.6, 42.5, 38.0, 28.0, 22.7 ppm; FT-IR (neat, cm^{-1}) 3164.9 (br), 2956.8, 2865.9, 1777.9, 1729.7, 1630.7, 1582.7, 1523.8, 1464.8, 1367.7, 1250.8, 1167.8, 1094.8, 1069.8, 880.9, 763.7, 698.8 cm^{-1}; HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{11}H_{16}N_3O$: 206.1293, Found: 206.1286.

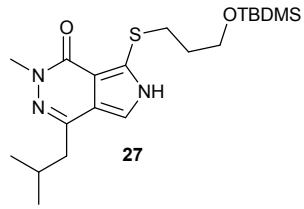
Data for Boc-protected pyrrole 25:



25

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.00 (d, J = 2.0 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 3.70 (s, 3H), 2.56 (d, J = 7.0 Hz, 2H), 2.20-2.11 (septet, 1H), 0.97 (d, J = 6.6 Hz, 6H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.9, 148.0, 144.2, 121.3, 118.6, 117.7, 114.3, 86.6, 42.2, 37.8, 27.8, 22.6 ppm; FT-IR (neat, cm^{-1}) 3411.9, 2963.8, 2870.9, 1746.6, 1659.6, 1595.8, 1522.8, 1476.8, 1459.8, 1389.7, 1369.7, 1335.8, 1351.8, 1319.8, 1290.7, 1273.5, 1257.6, 1144.5, 1101.6, 1081.7, 986.6, 840.7, 768.6 cm^{-1}; HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{16}H_{24}N_3O_3$: 306.1814, Found: 306.1818.

Data for pyrrole 27:



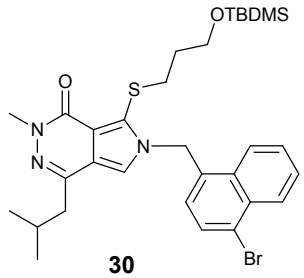
27

^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.45 (br s, 1H), 7.05 (d, J = 2.8 Hz, 1H), 3.79 (t, J = 5.8 Hz, 1H), 3.71 (s, 3H), 3.13 (t, J = 7.3 Hz, 2H), 2.55 (d, J = 7.4 Hz, 2H), 2.18-2.08 (septet, 1H), 1.80 (pentet, 2H), 0.97 (d, J = 6.6 Hz, 6H), 0.90 (s, 9H), 0.08 ppm (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.4, 144.0, 123.2, 121.8, 115.8, 112.6, 61.1, 42.1, 37.7, 32.9, 32.4, 27.9, 25.9, 22.7, 18.3, -5.2 ppm; FT-IR (neat, cm^{-1}) 3128.8, 2953.7, 2928.7, 2856.7, 1621.5, 1579.6, 1502.7, 1462.7, 1439.8, 1342.6, 1253.7, 1096.5, 1062.7, 1005.7, 939.7, 832.4, 772.4, 697.6 cm^{-1}; HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{20}H_{36}N_3O_2SSi$: 410.2297, Found: 410.2281.

Scheme 2 chemistry methods.

Bromide **29** was prepared following the methods shown in reference 21.

Compound **30**.

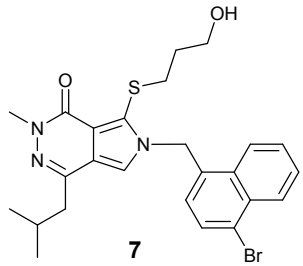


30

Alkylation to give compound **30** followed the AstraZeneca method (see reference 19). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.38 (br. d, J = 8.4 Hz, 1H), 7.92 (br. d, J = 8.4 Hz, 1H), 7.63-7.74 (m, 3H), 7.02 (s, 1H, pyrrole CH), 6.65 (d, J = 7.6 Hz, 1H), 5.94 (s, 2H), 3.77 (s, 3H), 3.63 (t, J = 6.4 Hz, 2H), 3.16 (t, J = 6.4 Hz, 2H), 2.50 (d, J = 7.2 Hz, 2H), 2.04-2.11 (m, 1H), 1.73-1.81 (m, 2H), 0.94 (d, J = 6.4

Hz, 6H), 0.85 (s, 9H), 0.00 ppm (s, 6H). m/z: [M + H]+ Calc'd for $C_{31}H_{43}BrN_3O_2SSi$: 630.2 and 628.2, Found: 630.2 and 628.2.

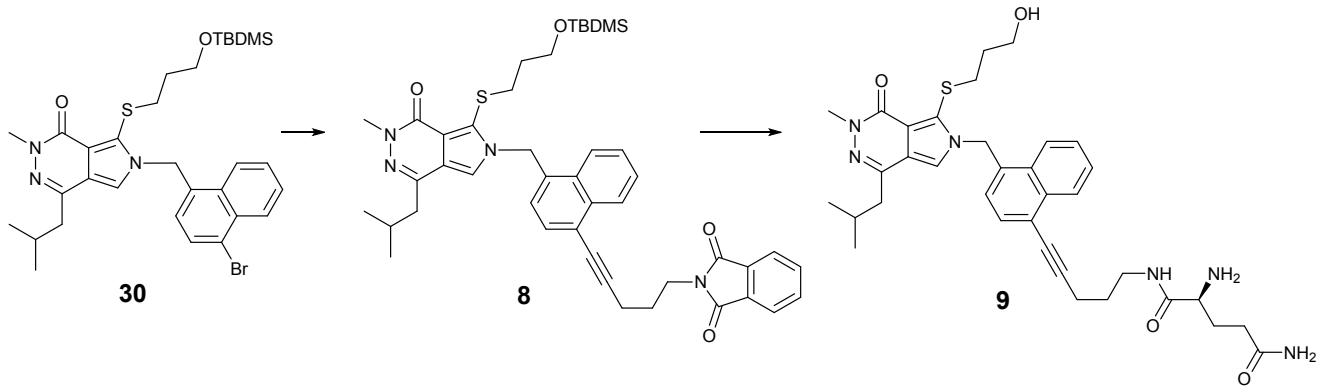
Compound **7.**



7

Compound **30** was desilylated under standard conditions (1.2 eq. TBAF, THF, 88%) to give compound **7**, which was purified by column chromatography on silica gel using an appropriate EtOAc:hexanes gradient elution. Compound **7** was isolated in 79% yield. m/z: [M + H]+ Calc'd for $C_{25}H_{29}BrN_3O_2S$: 516.1, 514.1, Found: 516.1 and 514.1.

Compound **9** (Gln conjugate):



30 **8** **9**

N-phthaloyl 1-amino-4-pentyne was prepared in nearly quantitative yield following the reference 29 procedure, except that 5-chloro-1-pentyne was used in place of 5-bromo-1-pentyne in a reaction with potassium phthalimide in DMF at 90 ^{o}C.

To N-phthaloyl 1-amino-4-pentyne (43 mg, 0.20 mmol) in a minimal amount (~1 mL) of degassed anhydrous pyrrolidine in a small pressure tube was added the bromide **30** (100 mg, 0.16 mmol), also

in a minimal amount of degassed anhydrous pyrrolidine. $Pd(Ph_3P)_4$ (0.15 eq) was then added. The tube was sealed and the solution was heated to 80 ^oC for 8 h, cooled to rt, and concentrated under vacuum to give the crude coupled material, which was purified by preparative HPLC to give 51 mg of analytically pure compound **8** (42% yield). m/z: [M + H]+ Calc'd for $C_{44}H_{53}N_4O_4SSi$: 761.3, Found: 761.2; HPLC purity >95%.

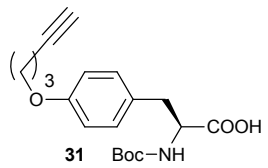
A sample of compound **8** (41 mg, 0.053 mmol) was dissolved in minimal ethanol and then hydrazine monohydrate (10 eq.) was added. The solution was stirred overnight at rt, filtered through a plug of cotton, and the solution was concentrated *in vacuo* to give the crude amine intermediate. m/z: [M + H]+ Calc'd for $C_{36}H_{51}N_4O_2SSi$: 631.3, Found: 631.2; HPLC purity >95%.

The entire sample of amine intermediate from the previous step was dissolved in a minimal amount of DMF. To this solution was added N-Boc-Gln (1.5 eq), HATU (1.2 eq), and $EtN(i\text{-}Pr)_2$ (2 eq). The mixture was stirred at rt for 12 hr, diluted with water, extracted with ethyl acetate, and the organic extracts were dried and concentrated *in vacuo*. The residue was purified by preparative HPLC to give the protected form of compound **9**, with an OTBDMS group and an N-Boc group present (9.6 mg, 21% yield for 2 steps). m/z: [M + H]+ Calc'd for $C_{46}H_{67}N_6O_6SSi$: 859.5, Found: 859.4; HPLC purity >95%.

A sample of this protected form of compound **9** (5.2 mg, 0.006 mmol) was dissolved in 1 mL of 4M HCl in dioxane. The mixture was stirred at rt for 6 h, diluted with water, extracted with ethyl acetate, and the organic layers were dried, filtered, and concentrated *in vacuo*. An analytically pure sample of compound **9** was thus obtained (3.8 mg, 97%). ^1H NMR (d^6-DMSO, 400 MHz) δ 8.43-8.47 (m, 1H), 8.27-8.31 (m, 1H), 8.16-8.19 (m, 1H), 8.05-8.09 (br. s, 2H, exchanges w. D_2O, Gln side chain amide NH_2), 7.77 (s, 1H, pyrrole CH), 7.63-7.68 (br. s, 1H, exchanges w. D_2O, internal amide NH), 7.46 (d, J = 7.6 Hz, 1H), 6.85-6.90 (m, 1H), 6.30 (d, J = 7.6 Hz, 1H), 5.97 (s, 2H), 3.51 (s, 3H), 3.46-3.50 (m, 2H), 3.24-3.29 (m, 5H, includes 1 exchangeable H: alcohol), 2.83-2.89 (m, 2H), 2.55-

2.59 (m, 1H), 2.08-2.13 (m, 2H), 1.98-2.02 (m, 2H), 1.74-1.88 (m, 5H), 1.39-1.44 (m, 2H), 0.84 ppm (d, J = 6.4 Hz, 6H). m/z: [M + H]+ Calc'd for $C_{35}H_{45}N_6O_4S$: 645.3, Found: 645.2.

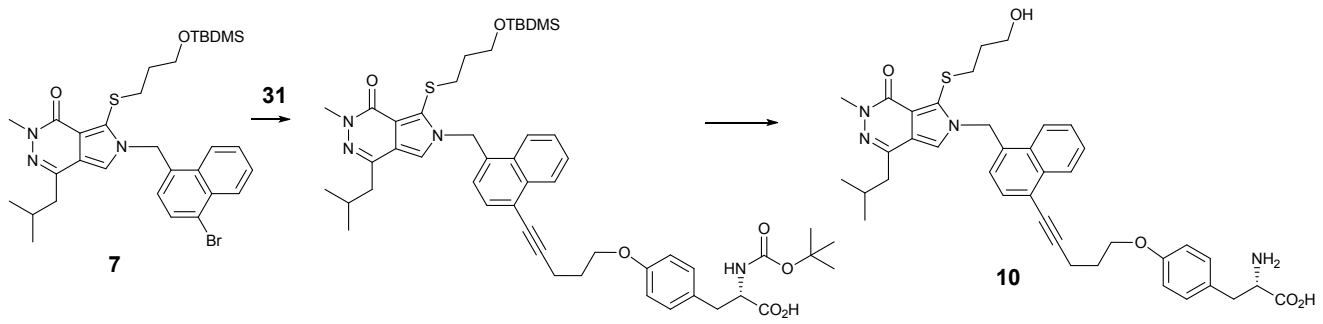
Compound **31**.



31

This compound was prepared by the method of ref 27 except that 5-chloro-1-pentyne was used in place of 5-bromo-1-pentyne in the phenol alkylation step.

Compound **10** (Tyr conjugate):



To alkyne **31** (83 mg, 0.24 mmol) in a minimal amount of degassed anhydrous pyrrolidine (~1 mL) in a small pressure tube was added bromide **7** (100 mg, 0.16 mmol), also in a minimal amount of degassed anhydrous pyrrolidine (~1 mL). $Pd(Ph_3P)_4$ (0.15 eq) was then added. The tube was sealed and the solution was heated to 80 ^oC for 6 h, cooled to rt, and concentrated under vacuum to give the crude coupled material, which was purified by preparative HPLC to give 55 mg of the coupled product, the protected form of compound **10** with an OTBDMS group and an N-Boc group present (39% yield). ^1H NMR ($CDCl_3$, 400 MHz) δ 8.43 (m, 1H), 7.92 (m, 1H), 7.60 (m, 2H), 7.51 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 8.4 Hz, 2H), 7.14 (s, 1H, pyrrole H), 6.88 (d, J = 8.4 Hz, 2H), 6.69 (d, J = 8.4 Hz, 1H), 5.95 (s, 2H), 4.19 (t, J = 6.0 Hz, 2H), 3.77 (s, 3H), 3.62 (t, J = 6.0 Hz, 2H), 3.14 (t, J = 6.0 Hz, 2H), 2.81 (t, J = 6.0 Hz, 2H), 2.50 (br. d, J = 7.2 Hz, 2H), 2.15-2.25 (br. m, 3H), 2.05-2.10 (br. m, 2H),

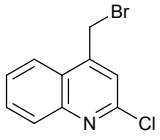
1.72-1.80 (br. m, 2H), 1.44 (s, 9H), 0.94 (d, J = 6.4 Hz, 6H, *i*-Bu methyls), 0.85 (s, 9H), 0.00 ppm (s, 6H). MS (EI) m/z: [M + H]+ Calc'd for $C_{50}H_{66}N_4O_7SSi$: 895.4 Found: 895.3, 795.3 (-t-Boc).

This protected intermediate material (50 mg, 0.056 mmol) was dissolved in 2 mL of 4M HCl in dioxane under argon. The mixture was stirred at rt for 3 hr, concentrated *in vacuo*, and the residue was purified by preparative HPLC to give compound **10** (29 mg, 76%). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.25 (br. d, J = 8.0, 1H), 8.16 (br. d, J = 8.0, 1H), 7.76 (s, 1H, pyrrole CH), 7.57-7.67 (m, 2H), 7.46 (d, J = 7.6 Hz, 1H), 7.10 (d, J = 8.8 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 6.29 (d, J = 7.6 Hz, 1H), 5.96 (s, 2H), 4.04-4.09 (br. t, J = 6.0 Hz, 2H), 3.51 (s, 3H), 3.41-3.46 (m, 1H), 3.22-3.28 (overlapping br. triplets, 4H), 2.96-3.01 (m, 1H), 2.86 (br. t, J = 7.2 Hz, 2H), 2.68-2.79 (m, 3H), 1.97-2.04 (m, 3H), 1.38-1.44 (m, 2H), 0.83 ppm (d, J = 6.4 Hz, 6H). MS (EI) m/z: [M + H]+ Calc'd for $C_{39}H_{45}N_4O_5S$: 681.3 Found: 681.2.

Scheme 3 chemistry methods.

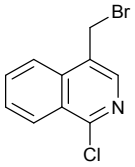
^{1}H NMR data for the bromo compounds used for alkylation of compound **27** to give compounds **32-35**, as shown in Scheme 3 (see ref 26 for their method of preparation).

4-(bromomethyl)-2-chloroquinoline:



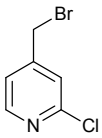
white solid (3.47 g, 60% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (d, *J* = 8.5 Hz, 2H), 7.74 (t, *J* = 8.3 Hz, 1H), 7.63 (t, *J* = 8.4 Hz, 1H), 7.39 (s, 1H), 4.74 ppm (s, 2H).

4-(bromomethyl)-1-chloroisoquinoline:



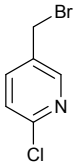
white solid (0.58 g, 80% yield). ^{1}H NMR (C*D*Cl$_3$, 400 MHz) δ 8.45 (d, *J* = 8.5 Hz, 1H), 8.37 (s, 1H), 8.18 (d, *J* = 8.5 Hz, 1H), 7.95 (t, *J* = 8.3 Hz, 1H), 7.80 (t, *J* = 8.2 Hz, 1H), 4.88 ppm (s, 2H).

4-(bromomethyl)-2-chloropyridine:



dark oil (2.2 g, 21% yield). ^{1}H NMR (C*D*Cl$_3$, 400 MHz) δ 8.34 (d, *J* = 5.1 Hz, 1H), 7.34 (s, 1H), 7.22 (d, *J* = 5.1 Hz, 1H), 4.35 ppm (s, 2H).

5-(bromomethyl)-2-chloropyridine:



buff solid (0.55 g, 76% yield). ^{1}H NMR (C*D*Cl$_3$, 400 MHz) δ 8.48 (d, *J* = 5.3 Hz, 1H), 7.47 (s, 1H), 7.24 (dd, *J* = 5.4, 2.0 Hz, 1H), 4.52 ppm (s, 2H).

Scheme 3, continued: General procedure for the heteroaryl methyl bromide alkylation reactions.

Alkylation step (a) - To an oven dried 100 mL round bottom flask equipped with a stir bar was added sodium hydride (1.2 eq) and anhydrous DMF (10 mL). The flask was flushed with argon for 5 min and simultaneously was cooled in an ice bath. To a scintillation vial was added **27** (1.0 eq) in DMF (5 mL). This solution was deoxygenated by bubbling with argon for 5 min, then it was added to the reaction mixture by cannula and the mixture was allowed to stir at 0 °C for 2h. In a separate vial,

additional halo heterocycle (1.1 eq) was dissolved in anhydrous DMF (5 mL), deoxygenated by bubbling argon for 5 min, and then added to the reaction mixture drop-wise. The resultant mixture was allowed to stir overnight, thereby warming to rt. After confirming the completion of the reaction by LCMS and TLC, it was quenched with aq. NH_4Cl, extracted with ethyl acetate, the organic layer washed with water and brine, dried over Na_2SO_4, filtered, and evaporated to give a crude solid. [A part of this crude solid (10%) was used for deprotection of TBDMS and the remaining part was (90%) was used for next step]. Yields for compounds **32-35** ranged from 68-81%.

General procedure for the Buchwald-Hartwig coupling step (c) – The crude solid (1.0 eq) obtained in step (a) above was dissolved in toluene (5 mL) in a sealable microwave reaction vial. To this mixture was added $Pd(OAc)_2$ (0.005 eq), BiNAP (0.01 eq), NaO*t*Bu (1.5 eq) and 1,5-diaminopentane (5 eq). The solution was degassed by bubbling with argon for 5 min, the vessel was sealed, and then it was subjected to microwave irradiation for 4 h at 100 °C. LCMS analysis confirmed the completion of the reaction. The reaction mixture was then filtered through a pad of Celite®, concentrated, and was purified using flash chromatography using a solvent system of 10% MeOH in dichloromethane, containing 0.1% NH_4OH each. In some cases, 20% MeOH was also required. After collecting the desired fractions, the pure product was isolated and analyzed by LCMS and ^1H NMR, after which the sample was divided appropriately to perform TBDMS deprotection reactions and coupling chemistry. Yields for compounds **40-43** ranged from 42-77%.

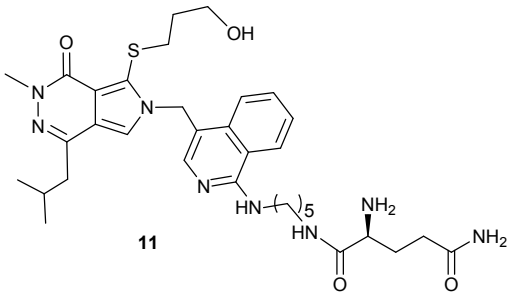
General procedure for the amide coupling step (d) – The pure compound obtained in step (c) (1 eq) was dissolved in DMF (5 mL) and to it was added coupling agent HATU (1.2 eq), diisopropylethyl amine (4 eq), Boc-Gln-OH (1.2 eq) and allowed to stir overnight. LCMS analysis confirmed desired mass of product and completion of the reaction, thus the mixture was quenched with sat. NH_4Cl solution. The mixture was extracted with ethyl acetate. The combined organic layers were then washed with water and brine, dried over sodium sulfate, filtered and evaporated to give a crude oil.

General procedure for silyl deprotection in Scheme 3, conversion (b) and the final reaction of conversion (d)

Small amounts (~10-20 mg) of compounds that were obtained in steps a, c and d above were then subjected to deprotection using HCl to give compounds **11-14**, and **15-19** shown in Scheme 3. In the general procedure, the small quantity of the TBDMS-protected and/or BOC-protected starting material was dissolved in THF (2 mL) and to this was added 4.0 M HCl in 1,4-dioxane, 3 mL. The reaction mixture was allowed to stir for 2-5 h, when LCMS analysis confirmed the completion of the reaction. Solvents were then evaporated and the crude material obtained was subjected to purification via preparative HPLC. The desired fractions corresponding to target compounds were isolated, lyophilized, analyzed and finally submitted for assays of MCT1 inhibition. The compounds obtained were white or off-white solids after lyophilization and they were obtained in 29-53% yield for the coupling and deprotection steps. In certain cases, quantities isolated were only sufficient to be characterized by ^{1}H and HRMS, as ^{13}C NMR data acquisition was not possible at such low concentrations.

<u>Characterization data (Scheme 3)</u>.

Compound 11:

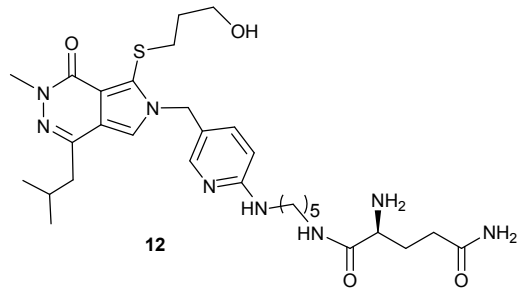


11

^{1}H NMR (CD_3OD, 400 MHz) 8.55 (d, J = 8.9 Hz, 1H), 8.03 (s, 2H), 7.84 (obscured t, 1H), 7.63 (s, 1H), 6.96 (s, 1H), 5.87 (s, 2H), 3.88 (t, J = 5.7 Hz, 1H), 3.80 (s, 3H), 3.57 (obscured q, 4H), 3.22-3.18 (m, 2H), 3.06 (t , J = 7.7 Hz, 2H), 2.53 (d, J = 7.3 Hz, 2H), 2.40 (br t, 2H), 2.29-2.15 (m, 2H), 1.89-

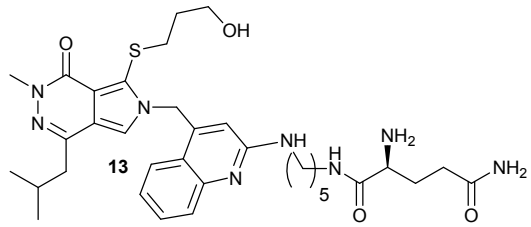
1.77 (m, 2H), 1.75-1.71 (m, 2H), 1.68-1.60 (m, 2H), 1.57-1.50 (m, 2H), 1.38 (obscured t, 2H), 0.98 ppm (d, J = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{34}H_{48}N_8O_4S$: 664.3519, Found: 665.3573.

Compound 12:



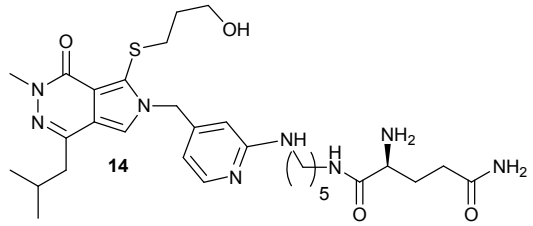
^{1}H NMR (d_6-DMSO, 400 MHz) 8.41 (br, 1H), 8.13 (br, 2H), 7.91 (s, 1H), 7.87 (s, 1H), 7.53 (br, 1H), 7.43 (s, 1H), 6.95 (s, 1H), 6.79 (br, 1H), 5.38 (s, 2h), 3.72 (br, 2H), 3.54 (s, 3H), 3.40 (t, J = 6.4 Hz, 2H), 3.23 (s, 2H), 2.95 (obscured t, 2 H), 2.15-2.10 (m, 3 H), 1.92-1.89 (m, 3H), 1.54-1.44 (m, 6H), 1.34 (br, 2H), 0.92 ppm (d, J = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{30}H_{46}N_8O_4S$: 614.3363, Found: 615.34018.

Compound 13:



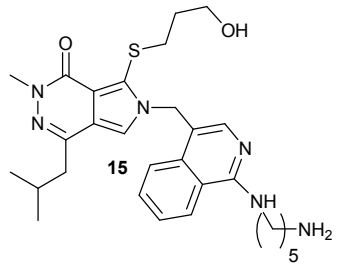
HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{34}H_{48}N_8O_4S$: 664.3519, Found: 665.3546; HPLC purity >95%.

Compound 14:



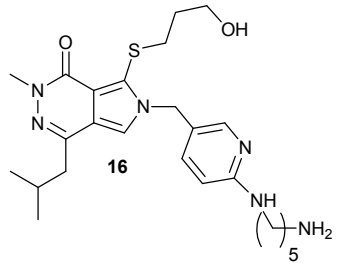
^{1}H NMR (CD_3OD, 400 MHz) δ 7.79 (d, *J* = 6.1 Hz, 1H), 7.73 (s, 1H), 6.70 (br, 1H), 6.23 (s, 1H), 5.65 (s, 2H), 3.87 (t, *J* = 5.8 Hz, 1H), 3.58 (s, 3H), 3.55 (obscured t, 2H), 3.26 (obscured m, 2H), 2.99 (t, *J* = 6.7 Hz, 2H), 2.61 (d, *J* = 7.4 Hz, 2H), 2.47-2.40 (m, 2H), 2.23-2.19 (m, 1H), 2.18-2.09 (m, 2H), 1.86-1.66 (m, 3H), 1.65-1.55 (m, 2H), 1.49-1.35 (m, 2H), 0.98 ppm (d, *J* = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{30}H_{46}N_8O_4S$: 614.3363, Found: 615.3408.

Compound 15:



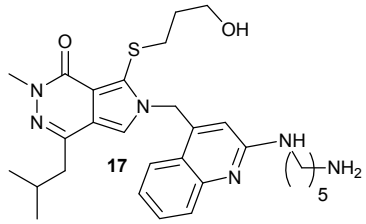
^{1}H NMR (CD_3OD, 400 MHz) 8.54 (d, *J* = 8.9 Hz, 1H), 8.03 (s, 2H), 7.84 (obscured t, 1H), 7.63 (s, 1H), 6.91 (s, 1H), 5.74 (s, 2H), 3.65 (s, 3H), 3.57 (obscured t, 4H), 3.09 (t , *J* = 7.7 Hz, 2H), 2.86 (obscured t, 2H), 2.54 (d, *J* = 7.3 Hz, 2H), 2.09-2.05 (m, 1H), 1.89-1.81 (m, 2H), 1.75-1.65 (m, 4H), 1.61-1.56 (m, 2H), 0.98 ppm (d, *J* = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{29}H_{40}N_6O_2S$: 536.2933, Found: 537.2983.

Compound 16:



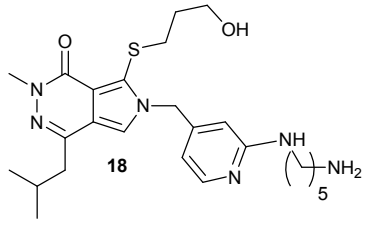
HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{25}H_{38}N_6O_2S$: 486.2777, Found: 487.2824; HPLC purity >95%.

Compound 17:



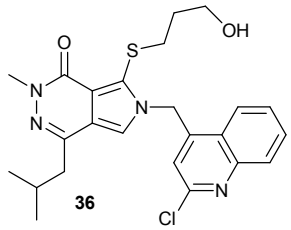
HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{29}H_{40}N_6O_2S$: 536.2933, Found: 537.2980; HPLC purity >95%.

Compound 18:



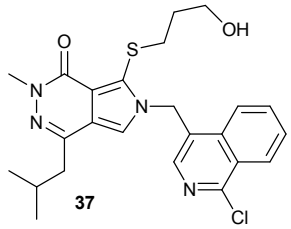
^{1}H NMR (C*D*$_3$O*D*, 400 MHz) δ 7.81 (d, *J* = 6.5 Hz, 1H), 7.74 (d, *J* = 6.2 Hz, 1H), 6.69 (br t, 1H), 6.26 (s, 1H), 5.64 (s, 2H), 4.43 (t, *J* = 5.6 Hz, 2H), 3.74 (s, 3H), 3.55 (t, *J* = 5.6 Hz, 2H), 3.05-2.99 (m, 2H), 2.93-2.89 (m, 2H), 2.60 (d, *J* = 7.4 Hz, 2H), 2.20-2.10 (m, 1H), 2.17-2.13 (m, 1H), 1.75 (br m, 5H), 1.53-1.40 (br m, 2H), 0.97 ppm (d, *J* = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{25}H_{38}N_6O_2S$: 486.2777, Found: 487.2827.

Compound 36:



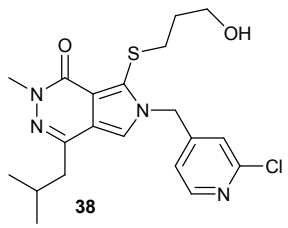
White solid (0.021 g, 52 % yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13 (d, *J* = 8.1 Hz, 1H), 7.98 (d, *J* = 9.8 Hz, 1H), 7.85 (t, *J* = 7.2 Hz, 1H), 7.70 (t, *J* = 9 Hz, 1H), 7.18 (s, 1H), 6.41 (s, 1H), 6.01 (s, 2H), 3.90 (t, *J* = 6.4 Hz, 2H), 3.79 (s, 3H), 3.15 (t, *J* = 5.1 Hz, 2H), 2.58 (d, *J* = 3.8 Hz, 2H), 2.18-2.11 (m, 1H), 1.81-1.74 (m, 2H), 0.97 ppm (d, *J* = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{24}H_{28}N_4O_2ClS$: 471.1621, Found: 471.1604.

Compound 37:



Buff solid (0.015 g, 80% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.46 (d, *J* = 8.4 Hz, 1H), 7.90 (s, 1H), 7.84-7.81 (m, 2H), 7.77-7.74 (obscured m, 1H), 6.97 (s, 1H), 5.89 (s, 2H), 3.93 (t, *J* = 5.6 Hz, 2H), 3.72 (s, 3H), 3.18 (t, *J* = 6.4 Hz, 2H), 2.43 (d, *J* = 7.4 Hz, 2H), 2.04-1.97 (m, 1H), 1.86-1.80 (m, 2H), 0.97 ppm (d, *J* = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{24}H_{28}N_4O_2ClS$: 471.1621, Found: 471.1303.

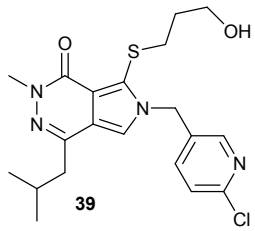
Compound 38:



38

Light brown oil (0.024 g, 70% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (s, 1H), 7.33 (obscured d, 1H), 7.15 (s, 1H), 5.52 (s, 2H), 3.67 (s, 3H), 3.54 (t, *J* = 6.1 Hz, 2H), 2.93 (d, *J* = 7.2 Hz, 2H), 2.60 (d, *J* = 7.3 Hz, 2H), 2.20-2.09 (m, 1H), 1.65-1.58 (m, 2H), 0.96 ppm (d, *J* = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{20}H_{26}N_4O_2ClS$: 421.1465, Found: 421.1454.

Compound 39:



39

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.29 (d, *J* = 4.7 Hz, 1H), 7.73 (s, 1H), 7.12 (s, 1H), 6.99 (d, *J* = 6.3 Hz, 1H), 5.65 (s, 2H), 3.94 (t, *J* = 6.1 Hz, 2H), 3.74 (s, 3H), 3.08 (obscured t, 2H), 2.55 (d, *J* = 6.6 Hz, 2H), 2.15-2.08 (m, 1H), 1.83-1.77 (m, 2H), 0.96 ppm (d, *J* = 6.6 Hz, 6H); HRMS (ES-TOF) m/z: [M + H]+ Calc'd for $C_{20}H_{26}N_4O_2ClS$: 421.1465, Found: 421.1452.