A small molecule that reverses glucocorticoid resistance in T-cell acute lymphoblastic leukemia (T-ALL)

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Experimental:

Maintenance of cell lines

CUTLL1 cells were cultured as previously described in RPMI1640 + 20% FBS and 1% Pen/Strep[1]. Glucocorticoid-receptor-overexpressing and knockdown cell lines were cultured in the same conditions with 1 µg/mL puromycin.

Screening/viability assays

CUTLL1 cells were seeded in 384-well clear bottom plates (Corning) at 6,000 cells/well. Before cell seeding, 1 µM dexamethasone was added to the "dex-treated" cells (an equivalent volume of DMSO was added to vehicle-treated cells). Cells were plated using a Biomek FX robot (Beckman Coulter) and were treated with 5.3 µg/mL of each library member for 72 h. Cell viability was measured after 16-hour incubation with the redox active fluorescent dye Alamar Blue. Fluorescence was measured on a Victor3 platereader (Perkin Elmer). LOC library compilation was previously described[2].

Dexamethasone (D4902) and RU486 (M8046) were purchased from Sigma Aldrich. Compound E (ALX- 270-415) was purchased from Enzo Life Sciences.

Western Blotting

Cells were harvested and washed in PBS before lysis in a buffer containing 150 mM NaCl, 50 mM Tris HCl, 0.1% NP40 and protease inhibitor. After 10 minutes of lysis on ice, the supernatant was collected and added to 5X SDS buffer and boiled for 5 minutes. Gels were run on precast bis-tris gels (Invitrogen) and blotting was done using an iBlot apparatus (Invitrogen). Antibodies used: glucocorticoid receptor (Cell Signaling cat no. 7437), intracellular NOTCH (Cell Signaling cat no. 4147) cleaved-PARP (Santa Cruz) and alpha-tubulin (Santa Cruz). For ICN and Cleaved-PARP, fluorescent secondary antibodies were used for visualization on an Odyssey (Li-Cor). Glucocorticoid receptor was visualized using the standard chemiluminescence techniques.

RT-qPCR

RT-qPCR was performed and analyzed as previously described[2]. All samples were normalized to actin. For analyzing glucocorticoid levels, the following primer pair was used**: 5'-3'F**: GAGCTACTGTGAAGGTTTCTGCG, **5'-3'R:** CGCTGCTTGGAGTCTGATTG

Microarray gene expression analysis.

CUTLL1 cells were treated with vehicle only (DMSO), dexamethasone (1 µM), J9 (30 µM) and dexamethasone (1 µM) plus J9 (30 µM) in combination for 24 hours in triplicate. RNA was isolated, labeled and hybridized to HumanHT-12 v4 Expression BeadChip (Illumina) oligonucleotide microarrays using standard procedures. Raw gene expression data from Illumina arrays were log2 transformed and quantile normalized using MATLAB.

Glucocorticoid-regulated transcripts in primary ALL were identified from expression data obtained from peripheral blood lymphoblasts from 13 pediatric ALL patients after 24 hr of treatment with glucocorticoids[3] (GEO ID GSE2677), and differentially expressed

transcripts were identified after log2 transformation and quantile normalization (t test, $p < 0.01$, and fold change >1.3). Enrichment of these glucocorticoid signature genes in dexamethasone plus J9 versus dexamethasone-only treated samples was analyzed by GSEA using the *t* test metric and 1,000 permutations of the genes[4] (Subramanian, et al. 2005).

Chemistry

All reactions were carried out under a nitrogen atmosphere under anhydrous conditions unless indicated otherwise. Anhydrous methylene chloride (DCM), tetrahydrofuran (THF), N,N-dimethylformamide (DMF) was purchased from Sigma-Aldrich. Reactions were magnetically stirred and monitored by thin layer chromatography carried out by Merck pre-coated 0.25 mm silica plates containing a 254 nm fluorescence indicator. Flash chromatography was preformed on a Teledyne combiflash companion automatic flash chromatography system. Preparative thin layer chromatography was performed on 1 mm. Spectroscopy: NMR spectra were obtained on a Bruker DPX 300 or 400 MHz spectrometer.

1-cyclopropyl-2-(pyridin-4-yl)ethanone

To a solution of 4-picoline (1.57 mL, 16.1 mmol) in THF at 0° C LDA (2 M, 8.86 mL, 17.7 mmol, 1.1 mol equiv) was added dropwise over 5 minutes. After 1 h a solution of ethyl cyclopropyl carboxylate (2.49 mL, 20.9 mmol, 1.3 mol equiv) was added and stirred at 0 °C for 30 min before being warmed to 25 °C and stirred for an additional 6 h. Upon completion, the reaction was quenched with water and the product was extracted with EtOAc (3 X). The combined organic layers were dried (sodium sulfate), concentrated, and the crude material was purified by combiflash 0-> 80% EtOAc in hexanes to provide X (0.505 g, 19.5 % yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.64-8.47 p.p.m. (m, 2H), 7.22-7.03 (m, 2H), 3.87 (s, 2H), 1.99 (tt, J = 7.8, 4.5 Hz, 1H), 1.16-1.01 (m, 2H), 0.94 (dt, J = 8.1, 3.5 Hz, 2H). HRMS (m/z): [M+] cald for C10H11NO, 161.20, found 161.89

(E)-1-cyclopropyl-3-(dimethylamino)-2-(pyridin-4-yl)prop-2-en-1-one

N,N-dimethylformamide dimethyl acetal (0.43 mL, 3.24 mmol, 2.0 mol equiv) was added 2 (261 mg, 1.62 mmol) and heated to 90 °C, neat for 6 h. Upon completion excess reagent was evaporated and the residue was dissolved in water and extracted with DCM (3 X). The combined organic layers were dried (sodium sulfate), concentrated and the crude material was pushed forward to the next step.

4-cyclopropyl-5-(pyridin-4-yl)pyrimidin-2-amine (1)

To a solution of sodium ethoxide (242 mg, 3.51 mmol, 1.1 mol equiv) in ethanol 8 mL, guanidine hydrochloride (335 mg, 3.51 mmol, 1.1 mol equiv) was added and stirred for 30 min before the addition of a solution of X (0.7 g, 3.24 mmol) in ethanol (2 mL) at 25 °C. The reaction was stirred at 40 °C for 4 h. Upon completion, the reaction was diluted

with water and extracted 3 times with ethyl acetate. The combined organic layers were dried (sodium sulfate), concentrated and purifed by combiflash 0->2% MeOH in DCM to provide Y (360 mg, X% yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.78 -8.59 p.p.m. (m, 2H), 8.08 (s, 1H), 7.43-7.33 (m, 2H), 5.11 (s, 2H), 1.97 (tt, J = 8.0, 4.7 Hz, 1H), 1.30-1.18 (m, 2H), 0.96 (dq, J = 8.2, 3.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.75, 162.56, 156.86, 149.96, 145.04, 124.33, 14.09, 11.32. HRMS (m/z): [M+] cald for C12H12N4, 212.25, found 212.4

1-cyclopropyl-2-(4-methoxyphenyl)ethanone

To a stirred solution of 4-methoxyphenacetyl chloride (0.5 g, 2.71 mmol) and copper bromide (0.389 g, 1.0 mol equiv) in THF at 0 °C, cyclopropylmagnesium bromide solution (0.5 M in THF, 6 mL, 2.98 mmol, 1.1 mol equiv) was added over 20 minutes. The resulting mixture was slowly warmed to room temp and stirred for an additional 4 h. Upon completion, the reaction was quenched with a saturated solution of ammonium chloride and the crude material was extracted three times with ethyl acetate. The combined organic layers were dried (sodium sulfate), concentrated, and purified by combiflash 0->50% EtOAc in hexanes to yield 1-cyclopropyl-2-(4-methoxyphenyl)ethanone (136 mg, 26% yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.20-7.08 p.p.m. (m, 2H), 6.97- 6.83 (m, 2H), 3.83 (s, 4H), 3.79 (s, 2H), 2.01- 1.94 (m, 1H), 1.08-0.99 (m, 2H), 0.87 (dt, J = 8.1, 3.4 Hz, 2H). HRMS (m/z): [M+] cald for C12H14O2, 190.24, found 212.4.

4-cyclopropyl-5-(pyridin-3-yl)pyrimidin-2-amine (2)

Prepared according to scheme 1 using 3-picoline in place of 4-picoline in step (a).^{1}H NMR (400 MHz, Chloroform-d) δ 8.69 p.p.m. (dd, J = 2.3, 0.9 Hz, 1H), 8.63 (dd, J = 4.8, 1.7 Hz, 1H), 8.07 (s, 1H), 7.74 (dt, J = 7.8, 2.0 Hz, 1H), 7.39 (ddd, J = 7.8, 4.8, 0.9 Hz, 1H), 5.10 (s, 2H), 1.90 (tt, J = 8.0, 4.7 Hz, 1H), 1.25-1.18 (m, 2H), 0.99-0.90 (m, 2H). HRMS (m/z): [M+] cald for C12H12N4, 212.25, found 212.4.

4-cyclopropyl-5-(pyridin-2-yl)pyrimidin-2-amine (3)

Prepared according to scheme 1 using 2-picoline in place of 4-picoline in step (a). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.72 p.p.m. (ddd, J = 4.9, 1.9, 0.9 Hz, 1H), 8.29 (s, 1H), 7.75 (td, J = 7.7, 1.9 Hz, 1H), 7.49 (dt, J = 7.9, 1.1 Hz, 1H), 7.29- 7.16 (m, 1H), 5.20 (s, 2H), 2.25 (tt, J = 8.0, 4.7 Hz, 1H), 1.21 (dq, J = 4.7, 3.6 Hz, 2H), 0.94 (dq, J = 8.1, 3.6 Hz, 2H). HRMS (m/z): [M+] cald for C12H12N4, 212.25, found 213.11.

4-cyclopropyl-5-(quinolin-4-yl)pyrimidin-2-amine (4)

Prepared according to scheme 1 using lepidine in place of 4-picoline in step (a). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.01 p.p.m. (d, J = 4.3 Hz, 1H), 8.21 (dt, J = 8.5, 1.0 Hz, 1H), 8.11 (s, 1H), 7.84-7.71 (m, 2H), 7.57 (ddd, J = 8.3, 6.8, 1.2 Hz, 1H), 7.38 (d, J = 4.3 Hz,

1H), 5.02 (s, 2H), 1.50 (tt, J = 8.0, 4.6 Hz, 1H), 1.27-1.09 (m, 2H), 0.83 (dddd, J = 6.8, 5.7, 4.3, 1.9 Hz, 2H). HRMS (m/z): [M+] cald for C16H14N4, 262.31, found 262.12.

4-cyclopropyl-5-phenylpyrimidin-2-amine (5)

Prepared according to scheme 1, using 1-cyclopropyl-2-phenylethanone (prepared using the procedure for 1-cyclopropyl-2-(4-methoxyphenyl)ethanone) for step (b) in place of 1-cyclopropyl-2-(pyridin-4-yl)ethanone.^{1}H NMR (300 MHz, Chloroform-d) δ 8.08 p.p.m. (s, 1H), 7.55-7.30 (m, 6H), 4.90 (s, 2H), 2.01 (ddd, J = 8.1, 4.8, 3.3 Hz, 1H), 1.25-1.13 (m, 2H), 0.99-0.82 (m, 2H). HRMS (m/z): [M+] cald for C13H13N3, 211.26, found 212.14.

4-cyclopropyl-5-(4-methoxyphenyl)pyrimidin-2-amine (6)

Prepared according to scheme 1, using 1-cyclopropyl-2-(4-methoxyphenyl)ethanone for step (b) in place of 1-cyclopropyl-2-(pyridin-4-yl)ethanone. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.03 p.p.m. (s, 1H), 7.33-7.26 (m, 2H), 6.97 (d, J = 8.7 Hz, 2H), 4.93 (s, 2H), 3.84 (s, 3H), 1.98 (tt, J = 8.0, 4.7 Hz, 2H), 1.24-1.06 (m, 2H), 0.88 (dq, J = 8.1, 3.5 Hz, 2H). HRMS (m/z): [M+] cald for C14H15N3O, 241.29, found 242.13.

4-cyclopropyl-5-(4-fluorophenyl)pyrimidin-2-amine (7)

Prepared according to scheme 1, using 1-cyclopropyl-2-(4-fluorophenyl)ethanone (prepared using the procedure for 1-cyclopropyl-2-(4-methoxyphenyl)ethanone) for step (b) in place of 1-cyclopropyl-2-(pyridin-4-yl)ethanone. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.04 p.p.m. (s, 1H), 7.42-7.32 (m, 2H), 7.14 (t, J = 8.7 Hz, 2H), 5.06 (s, 2H), 1.94 (tt, J = 8.0, 4.7 Hz, 1H), 1.23-1.14 (m, 2H), 0.97-0.80 (m, 2H). HRMS (m/z): [M+] cald for C13H12FN3, 229.25, found 229.10.

5-(3-bromopyridin-4-yl)-4-cyclopropylpyrimidin-2-amine (8)

Prepared according to scheme 1, starting from 3-bromo-4-methylpyridine for step (a) in place of 4-picoline.^{1}H NMR (300 MHz, Chloroform-d) δ 8.86 p.p.m. (d, J = 0.6 Hz, 1H), 8.60 (d, J = 4.9 Hz, 1H), 7.99 (s, 1H), 7.28 (d, J = 1.5 Hz, 2H), 5.01 (s, 2H), 1.59-1.47 (m, 1H), 1.36-1.06 (m, 3H), 0.94 (d, J = 21.4 Hz, 2H). HRMS (m/z): [M+] cald for C12H11BrN4, 291.15, found 290.50.

4-cyclopropyl-5-(3-vinylpyridin-4-yl)pyrimidin-2-amine (9)

To a solution of 5-(3-bromopyridin-4-yl)-4-cyclopropylpyrimidin-2-amine (60 mg, 0.206 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.0103 mmol, 5%), tributylvinyl tin (0.309 mmol, 1.5 mol equiv) was added and heated to 80 °C for 24 h. Upon completion, a 1 M solution of KF (0.309 mL, 0.309 mmol, 1.5 mol equiv) was added and stirred for 12 h at 25 °C. The mixture was then filtered over celite, diluted with water and extracted 3X with ethyl acetate. The combined organic layers were dried (sodium sulfate),

concentrated, and purified by preparative TLC 2% MeOH in DCM to provide 4-cyclopropyl-5-(3-vinylpyridin-4-yl)pyrimidin-2-amine (15 mg, 31% yield). ^{1}H NMR (300 MHz, Chloroform-d) δ 8.87 p.p.m. (s, 1H), 8.54 (dd, J = 5.0, 1.3 Hz, 1H), 7.94 (d, J = 1.3 Hz, 1H), 7.17 (dd, J = 5.0, 0.8 Hz, 1H), 6.57 (dd, J = 17.7, 11.2 Hz, 1H), 5.78 (dd, J = 17.7, 1.0 Hz, 1H), 5.33 (dd, J = 11.2, 1.0 Hz, 1H), 5.21-4.88 (m, 2H), 1.56 (ddd, J = 9.2, 4.6, 3.2 Hz, 1H), 1.22-1.03 (m, 2H), 0.99- 0.76 (m, 2H). HRMS (m/z): [M+] cald for C14H14N4, 238.29, found 238.12.

4-cyclopropyl-5-(3-phenylpyridin-4-yl)pyrimidin-2-amine (10)

To a stirred solution of 5-(3-bromopyridin-4-yl)-4-cyclopropylpyrimidin-2-amine (60 mg, 0.206 mmol), phenyboronic acid (38 mg, 0.309 mmol, 1.5 mol equiv), Tetrakis(triphenylphosphine)palladium(0) (0.0103 mmol, 5%), a 2M solution of sodium carbonate (0.525 mL, 1.03 mmol, 5.0 mol equiv) was added. The resulting mixture was heated to 80 °C for 24 h. Upon completion, the reaction was diluted with water and extracted with ethyl acetate 3X. The combined organic layers were dried (sodium sulfate), concentrated and purified by preparative TLC, 1:1 ethylacetate in hexanes to yield 4-cyclopropyl-5-(3-phenylpyridin-4-yl)pyrimidin-2-amine (35 mg, 58% yield).^{1}H NMR (300 MHz, Chloroform-d) δ 8.74-8.62 p.p.m. (m, 2H), 7.85 (d, J = 1.2 Hz, 1H), 7.35-7.23 (m, 4H), 7.23 -7.11 (m, 2H), 4.99 (s, 2H), 3.52-3.43 (m, 1H), 2.05 (d, J = 9.1 Hz, 1H), 1.43-0.45 (m, 5H). HRMS (m/z): [M+] cald for C18H16N4, 288.35, found 289.10.

4-cyclopropyl-5-(3-(furan-3-yl)pyridin-4-yl)pyrimidin-2-amine (11)

Prepared from compound 8, according to the conditions described for compound 10.

^{1}H NMR (300 MHz, Chloroform-d) δ 8.74 p.p.m. (d, J = 0.8 Hz, 1H), 8.58 (d, J = 5.0 Hz, 1H), 7.93 (s, 1H), 7.38 (t, J = 1.7 Hz, 1H), 7.33-7.15 (m, 3H), 6.26 (dd, J = 1.9, 0.9 Hz, 1H), 5.01 (s, 2H), 1.50 (td, J = 8.0, 3.9 Hz, 1H), 0.87 (dd, J = 84.0, 44.1 Hz, 5H). HRMS (m/z): [M+] cald for C16H14N4O, 278.31, found 279.10.

5-(2-bromopyridin-4-yl)-4-cyclopropylpyrimidin-2-amine (12)

Prepared according to scheme 1, starting from 2-bromo-4-methylpyridine for step (a) in place of 4-picoline.^{1}H NMR (300 MHz, Chloroform-d) δ 8.42 p.p.m. (dd, J = 5.1, 0.7 Hz, 1H), 8.29 (d, J = 5.0 Hz, 1H), 8.05 (s, 1H), 7.56 (dd, J = 1.6, 0.7 Hz, 1H), 7.45-7.35 (m, 1H), 7.37-7.27 (m, 1H), 7.17 (dd, J = 5.1, 1.5 Hz, 1H), 5.11 (d, J = 9.2 Hz, 2H), 1.91 (tt, J = 8.0, 4.7 Hz, 1H), 1.24 (ddt, J = 6.5, 4.6, 2.5 Hz, 3H), 1.10- 0.86 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) 169.80, 162.76, 156.87, 150.05, 148.01, 142.58, 128.25, 123.44, 121.20, 14.15, 11.46. HRMS (m/z): [M+] cald for C13H12FN3, 229.25, found 229.91.

4-cyclopropyl-5-(2-(furan-3-yl)pyridin-4-yl)pyrimidin-2-amine (13)

Prepared from compound 12, using the conditions described for compound 10.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.65 p.p.m. (dd, J = 5.1, 0.9 Hz, 1H), 8.14-8.06 (m, 2H), 7.57-7.50 (m, 2H), 7.23 (dd, J = 5.1, 1.7 Hz, 1H), 6.94 (dd, J = 1.8, 0.9 Hz, 1H), 5.00 (s, 2H), 2.00 (tt, J = 8.0, 4.7 Hz, 1H), 1.30-1.15 (m, 3H), 1.07-0.94 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) 169.34, 162.17, 156.89,150.63, 134.93, 131.03, 130.52, 130.36, 128.46, 123.45, 121.80, 14.01, 11.11. HRMS (m/z): [M+] cald for C16H14N4O, 278.31, found 278.01.

4-isopropyl-5-(pyridin-4-yl)pyrimidin-2-amine (14)

Prepared according to scheme 1, using ethyl isobutyrate for step (a) in place of ethyl cyclopropyl carboxylate. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.70-8.58 (m, 2H), 8.09 (s, 1H), 7.23-7.18 (m, 2H), 5.06 (s, 2H), 3.03 (hept, J = 6.7 Hz, 1H), 1.18 (d, J = 6.7 Hz, 6H). HRMS (m/z): [M+] cald for C12H14N4, 214.27, found 214.12.

4-methyl-5-(pyridin-4-yl)pyrimidin-2-amine (15)

Prepared according to scheme 1, using ethyl acetate for step (a) in place of ethyl cyclopropyl carboxylate.^{1}H NMR (300 MHz, Chloroform-d) δ 8.71- 8.58 p.p.m. (m, 2H), 8.14 (s, 1H), 7.24-7.18 (m, 2H), 5.07 (s, 2H), 2.37 (s, 3H). HRMS (m/z): [M+] cald for C10H10N4, 186.21, found 187.10.

5-(pyridin-4-yl)pyrimidin-2-amine (16)

Prepared from 2-amino 5-bromopyrimidine and 4-pyridinyl boronic acid, using the conditions described for compound 10. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.75-8.66 p.p.m. (m, 2H), 8.63 (s, 2H), 7.47-7.38 (m, 2H), 5.37 (s, 2H). HRMS (m/z): [M+] cald for C9H8N4, 172.19, found 173.08.

4-cyclobutyl-5-(pyridin-4-yl)pyrimidin-2-amine (17)

Prepared according to scheme 1, using ethyl cyclobutanecarboxylate for step (a) in place of ethyl cyclopropyl carboxylate.^{1}H NMR (400 MHz, Chloroform-d) δ 8.70-8.60 p.p.m. (m, 2H), 8.08 (s, 1H), 7.19- 7.11 (m, 2H), 5.11 (s, 2H), 3.70 -3.50 (m, 1H), 2.57-2.35 (m, 2H), 2.16-1.99 (m, 2H), 1.99-1.83 (m, 2H). HRMS (m/z): [M+] cald for C13H14N4, 226.28, found 226.12.

4-phenyl-5-(pyridin-4-yl)pyrimidin-2-amine (18)

Prepared according to scheme 1, using ethyl benzoate for step (c) in place of ethyl cyclopropyl carboxylate. ^{1}H NMR (400 MHz, Chloroform-d) δ 8.57- 8.48 p.p.m. (m, 2H), 8.37 (s, 1H), 7.43- 7.30 (m, 5H), 7.10- 7.04 (m, 2H), 5.38 (s, 2H). HRMS (m/z): [M+] cald for C15H12N4, 248.28, found 247.10.

4-(furan-2-yl)-5-(pyridin-4-yl)pyrimidin-2-amine (19)

Prepared according to scheme 1, using ethyl 3-furoate for step (a) in place of ethyl cyclopropyl carboxylate.^{1}H NMR (400 MHz, chloroform-d) δ 8.74-8.64 p.p.m. (m, 2H), 8.21 (s, 1H), 7.45 (dd, J = 1.8, 0.8 Hz, 1H), 7.27- 7.20 (m, 2H), 6.57 (dd, J = 3.6, 0.8 Hz, 1H), 6.41 (dd, J = 3.5, 1.7 Hz, 1H). HRMS (m/z): [M+] cald for C13H10N4O, 238.24, found 239.1.

4-cyclopropyl-2-methyl-5-(pyridin-4-yl)pyrimidine (20)

Prepared according to scheme 1, using acetamidine hydrochloride for step (c) in place of guanidine hydrochloride.^{1}H NMR (300 MHz, Chloroform-d) δ 8.77- 8.67 p.p.m. (m, 2H), 8.34 (s, 1H), 7.40- 7.35 (m, 2H), 2.66 (s, 3H), 1.97 (tt, J = 8.0, 4.7 Hz, 1H), 1.35-1.23 (m, 2H), 1.07- 0.94 (m, 2H). HRMS (m/z): [M+] cald for C13H13N3, 211.22, found 212.12.

4-cyclopropyl-5-(2-fluoropyridin-4-yl)pyrimidin-2-amine (21)

Prepared according to scheme 1 using 2-fluoro-4-methylpyridine for step (a). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.29 (d, J = 5.2 Hz, 1H), 8.09 (s, 1H), 7.38-7.19 (m, 1H), 7.01 (t, J = 1.6 Hz, 1H), 5.38-4.92 (m, 2H), 1.94 (td, J = 8.0, 4.0 Hz, 1H), 1.31-1.13 (m, 2H), 1.07 ? 0.94 (m, 2H). HRMS (m/z): [M+] cald for $C_{12}H_{11}FN_4$, 230.25, found 231.1.

5-(2-chloropyridin-4-yl)-4-cyclopropylpyrimidin-2-amine (22)

Prepared according to scheme 1 using 2-chloro-4-methylpyridine for step (a).^{1}H NMR (400 MHz, Chloroform-d) δ 8.45 (dd, J = 5.2, 0.7 Hz, 1H), 8.07 (s, 1H), 7.41 (dd, J = 1.5, 0.7 Hz, 1H), 7.33-7.25 (m, 1H), 5.12 (s, 2H), 1.92 (tt, J = 8.0, 4.7 Hz, 1H), 1.34 -1.16 (m, 2H), 1.00 (dt, J = 8.0, 3.3 Hz, 2H). HRMS (m/z): [M+] cald for $C_{12}H_{11}ClN_4$, 246.70, found 247.07.

4-cyclopropyl-5-(2,6-dichloropyridin-4-yl)pyrimidin-2-amine (23)

Prepared according to scheme 1 using 2,6-dichloro-4-methylpyridine for step (a).^{1}H NMR (400 MHz, Chloroform-d) δ 8.06 (s, 1H), 7.34 (s, 2H), 5.16 (s, 2H), 1.89 (td, J = 8.0, 4.0 Hz, 1H), 1.33 ? 1.19 (m, 2H), 1.03 (dt, J = 8.0, 3.4 Hz, 2H). HRMS (m/z): [M+] cald for $C_{12}H_{10}Cl_2N_4$, 281.14, found 281.04.

Supplemental Figures:

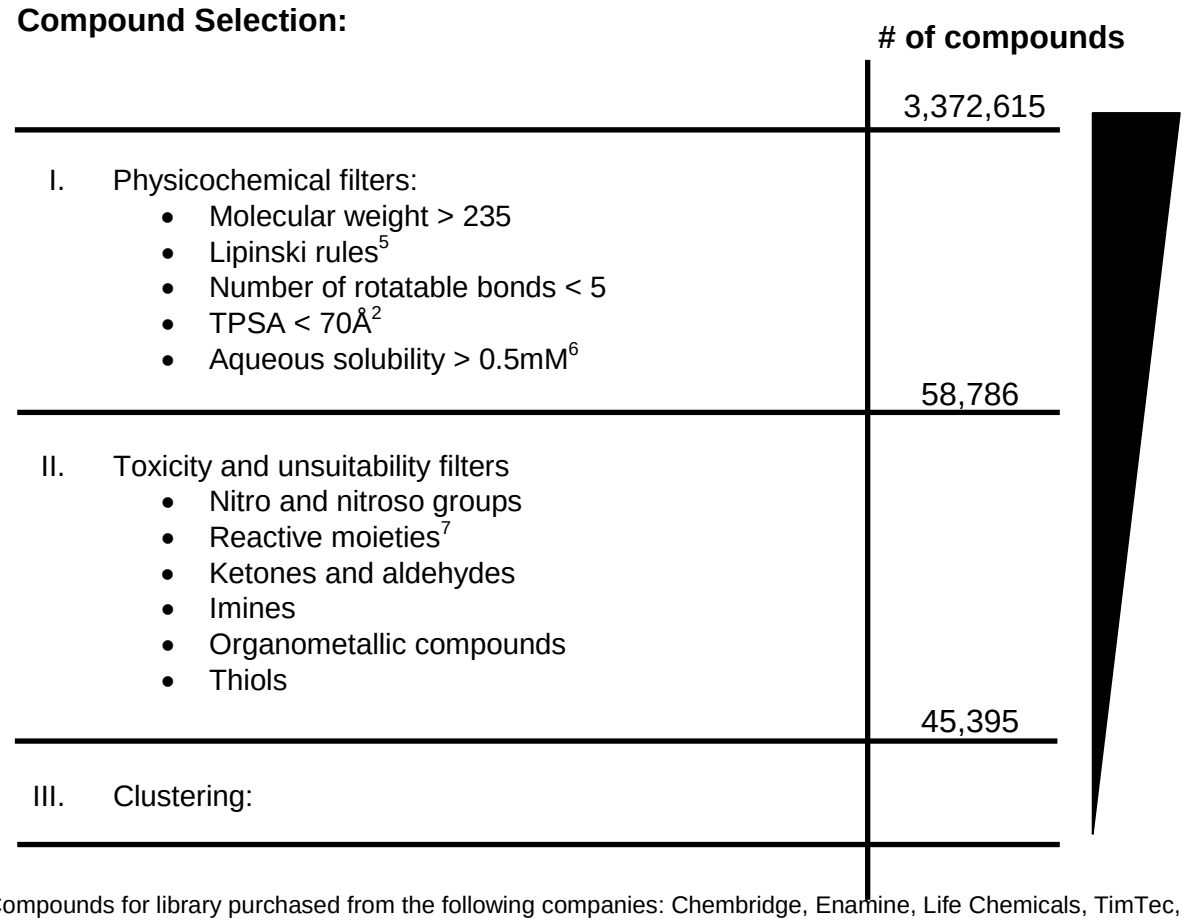


Figure S1. Compound selection process.

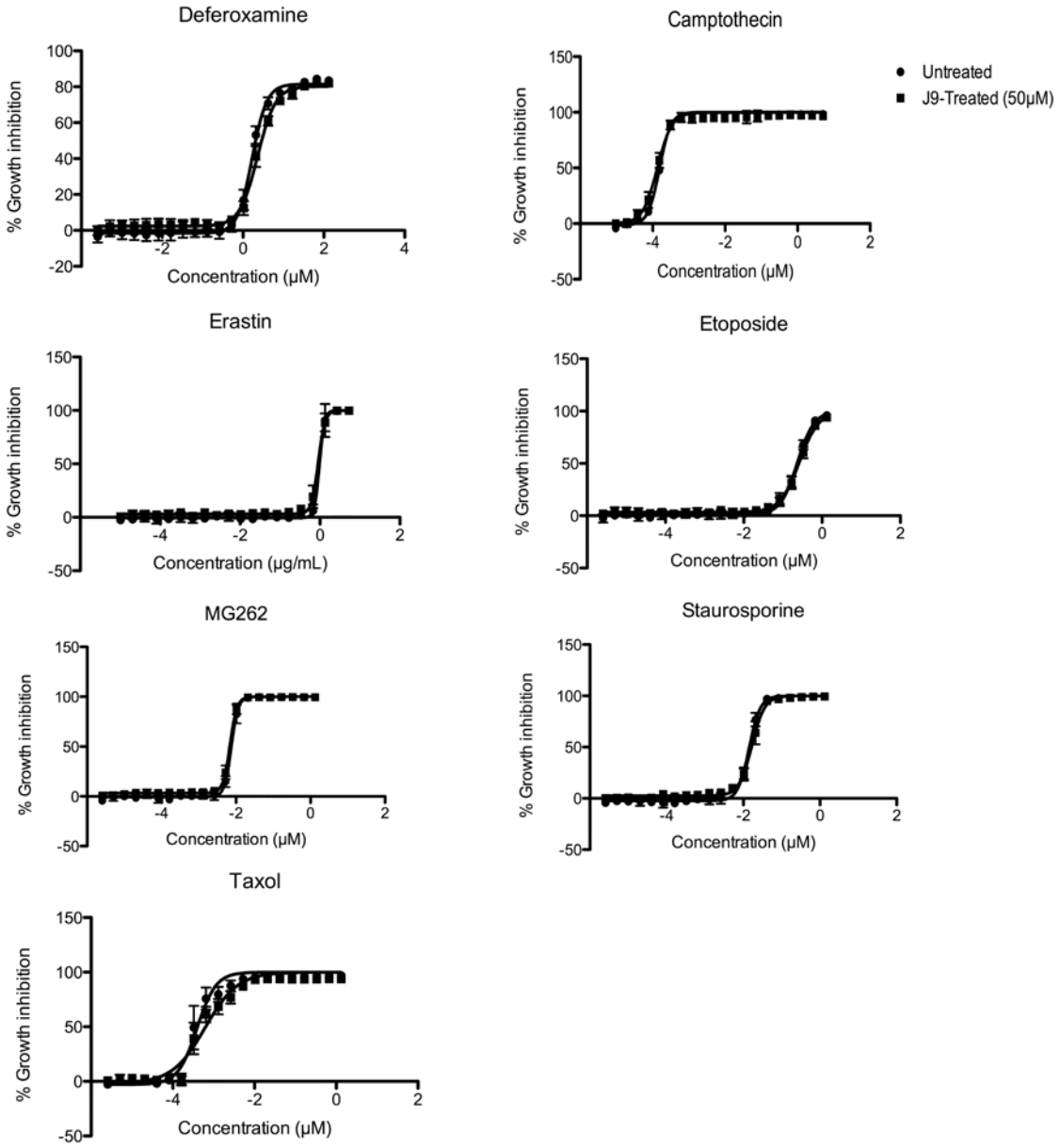
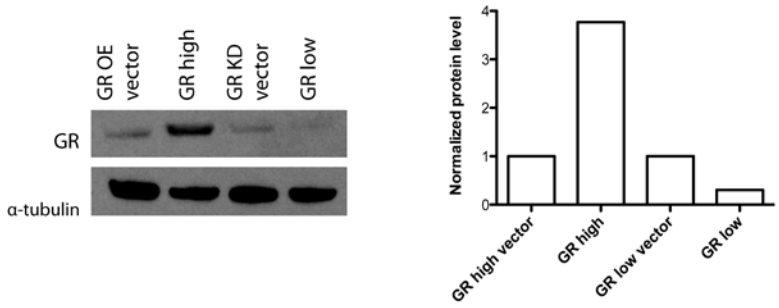
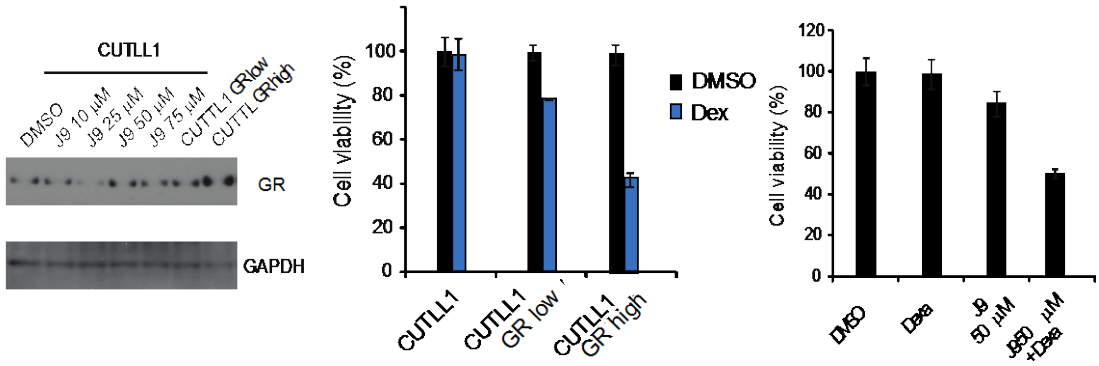


Figure S2: Co-treatment of CUTLL1 cells with J9 (50 μM) and a variety of cytotoxic agents. Cells were incubated for 72 hours.



S3. Glucocorticoid receptor protein levels in CUTLL1 cells with high and low levels of glucocorticoid receptor. Cell lines with empty vectors are included as controls.



S4. Far left: GR protein levels in CUTLL1 cells treated with different concentrations of J9 compared to CUTLL1 cells expressing low and high levels of GR. Sensitivity of CUTLL1 GR high and low cells to dexamethasone (middle) compared to CUTLL1 cells treated with 50 μM J9 and dexamethasone (right).

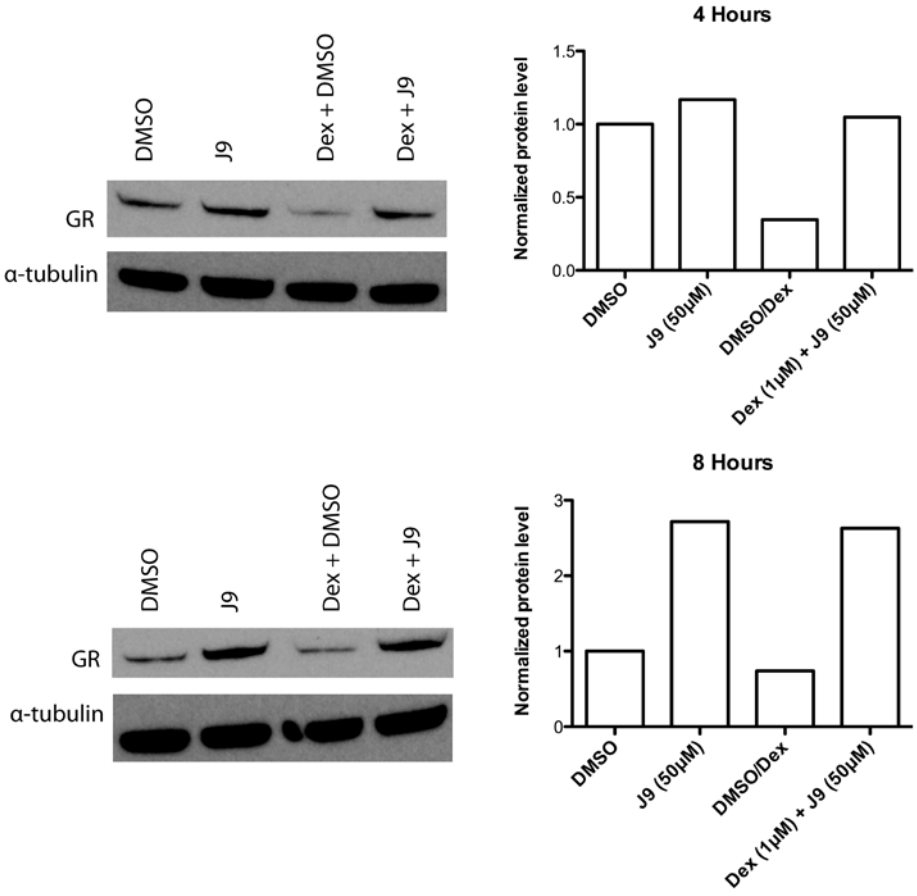


Figure S5. Protein levels of CUTLL1 cells treated as described for 4 hours (top) or 8 hours (bottom). Quantification shown on the right.

References:

(1) Palomero, T., Barnes, K. C., Real, P. J., Glade Bender, J. L., Sulis, M. L., Murty, V. V., Colovai, A. I., Balbin, M., and Ferrando, A. A. (2006) CUTLL1, a novel human T-cell lymphoma cell line with t(7;9) rearrangement, aberrant NOTCH1 activation and high sensitivity to γ-secretase inhibitors. *Leukemia 20*, 1279–1287.
(2) Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., Patel, D. N., Bauer, A. J., Cantley, A. M., Yang, W. S., Morrison, B., and Stockwell, B. R. (2012) Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell 149*, 1060–1072.
(3) Schmidt, S., Rainer, J., Riml, S., Ploner, C., Jesacher, S., Achmüller, C., Presul, E., Skvortsov, S., Crazzolara, R., Fiegl, M., Raivio, T., Jänne, O. A., Geley, S., Meister, B., and Kofler, R. (2006) Identification of glucocorticoid-response genes in children with acute lymphoblastic leukemia. *Blood 107*, 2061–2069.
(4) Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., and Mesirov, J. P. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U.S.A. 102*, 15545–15550.
(5) Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev. 46*, 3–26.
(6) Cheng, A., and Merz, K. M. (2003) Prediction of aqueous solubility of a diverse set of compounds using quantitative structure-property relationships. *J. Med. Chem. 46*, 3572–3580.
(7) Hann, M., Hudson, B., Lewell, X., Lifely, R., Miller, L., and Ramsden, N. (1999) Strategic Pooling of Compounds for High-Throughput Screening. *J. Chem. Inf. Model. 39*, 897–902.