SUPPLEMENTARY INFORMATION

Niche-based screening identifies small-molecule inhibitors of leukemia stem cells

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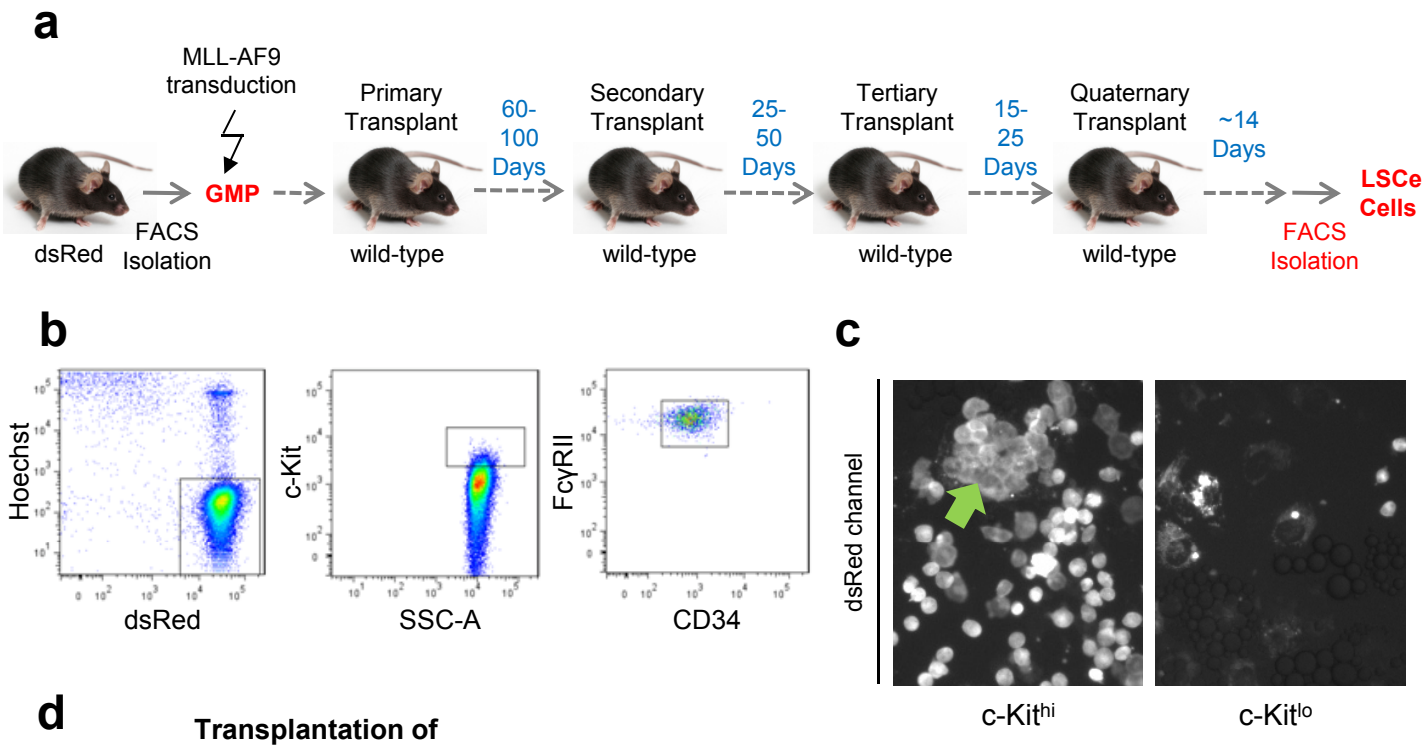
Supplementary Results

Supplementary Figures 1-5

Supplementary Tables 1-6

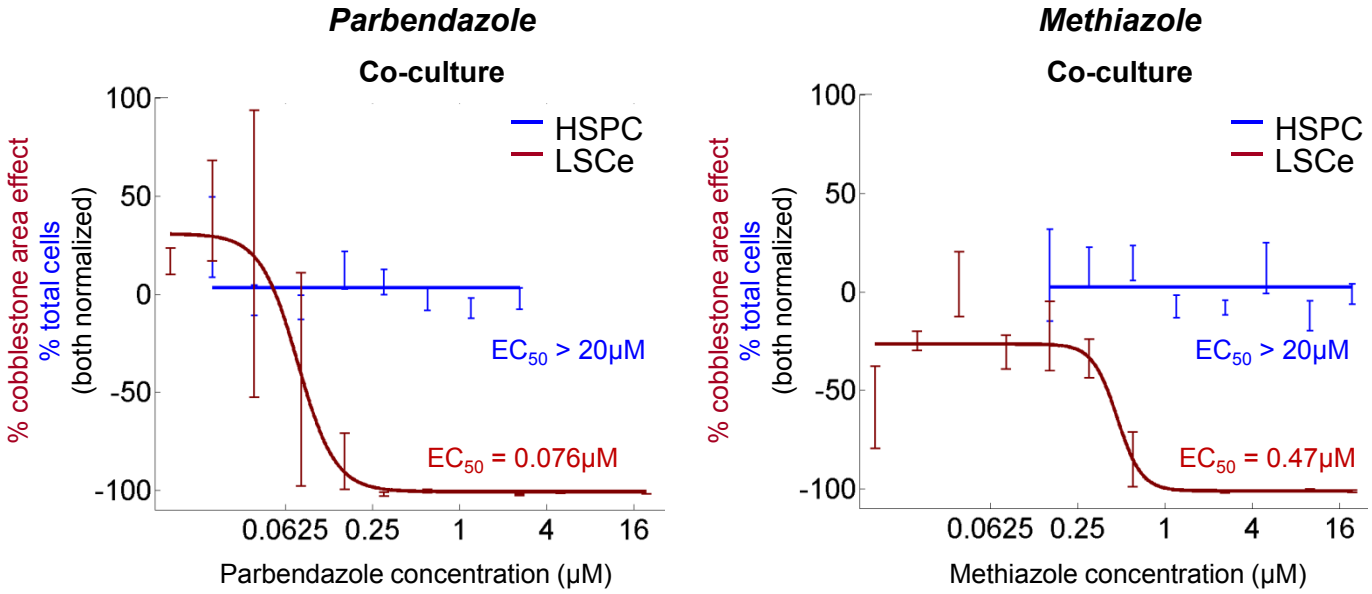
Supplementary Note 1


a
MLL-AF9
transduction
GMP
dsRed
FACS
Isolation
Primary
Transplant
wild-type
60-
100
Days
Secondary
Transplant
wild-type
25-
50
Days
Tertiary
Transplant
wild-type
15-
25
Days
Quaternary
Transplant
wild-type
~14
Days
FACS
Isolation
LSCe
Cells
b
Hoechst
dsRed
c-Kit
SSC-A
FcγRII
CD34
c
dsRed channel
c-Kithi
c-Kitlo
d
Transplantation of
LSCe Co-cultures
LSCe
500 cells
100 cells
25 cells
Percent survival
Days post transplant
e
original
dsRed image
dsRed image with
'objects' defined
f
Positive control (10μM XK469)
Neutral control (DMSO)
Count
Binned % cobblestone area effect
g
Stromal Toxicity
Lowest toxic concentration
(by compound)
none at ≤ 20 μM
20 μM
10 μM
5 μM
2.5 μM
1.25 μM

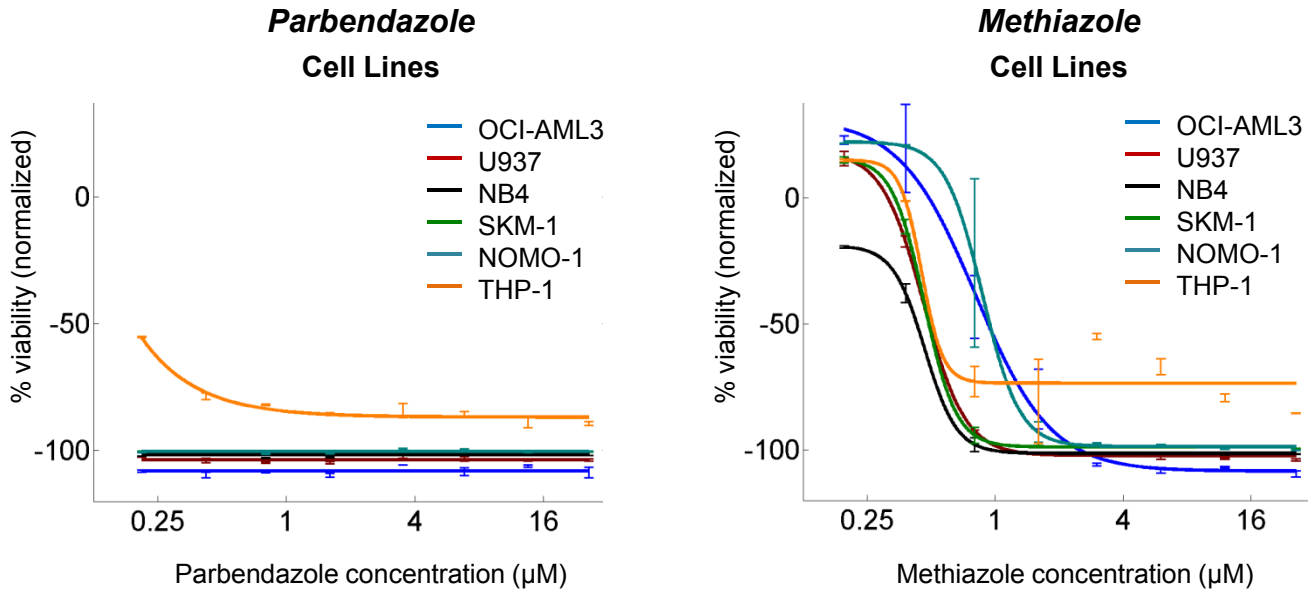
Supplementary Figure 1 | A High-Throughput System for Probing Primary, Stem-enriched Leukemia Cells Within a Stromal Niche

(**a**) Primary, stem cell-enriched, murine leukemia cells (LSCe cells) were generated as shown for high throughput screening. Granulocyte-Monocyte Progenitors (GMPs) were isolated by fluorescence-activated cell sorting (FACS) from β-actin dsRed mice, transduced with the MLL-AF9 oncogene, and transplanted into lethally-irradiated wild-type mice. At disease onset, splenocytes were transplanted through 3 additional rounds of recipient animals to generate quaternary leukemic mice. Whole bone marrow was harvested from these animals at disease onset, and (**b**) the LSCe population was isolated by flow cytometry using defined immunophenotypic markers Hoechst^{-} dsRed^{+} c-Kit^{hi} FcγRII^{hi} CD34^{hi} following Lin and Sca-1 depletion. Representative gating strategies are shown. Also see Methods. (**c**) When LSC-enriched (LSCe) cells (c-Kit^{hi}) and LSC-depleted cells (c-Kit^{lo}) are plated into co-culture with OP9 stroma at identical densities, the c-Kit^{hi} cells form CAFCs (arrow) with greater efficiency than the c-Kit^{lo} cells. Qualitative images representative of two independent experiments are shown. (**d**) Kaplan-Meier survival curves of mice transplanted with co-cultures generated from 500, 100, or 25 LSCe cells on bone marrow mesenchymal stromal cell (BMSC) stroma at the 6-day assay endpoint ($n \geq 4$ as shown). (**e**) The CellProfiler segmentation algorithm divides each individual cell into one or more subcellular areas (termed “objects;” See Methods), shown outlined in white. (**f**) Distribution of neutral control (DMSO only) and positive control (10 μM XK469, a topoisomerase IIβ inhibitor) wells. (**g**) The performance of 196 prioritized compounds in the stromal toxicity counterscreen, by lowest toxic concentration. A concentration range of 160 nM to 20 μM was examined with OP9 and primary BMSC stromal monolayers grown alone, at 8 concentrations, with a viability readout (CellTiter-Glo). Compounds showing toxicity towards either type of stroma at or below 10 μM were excluded from further study.

a

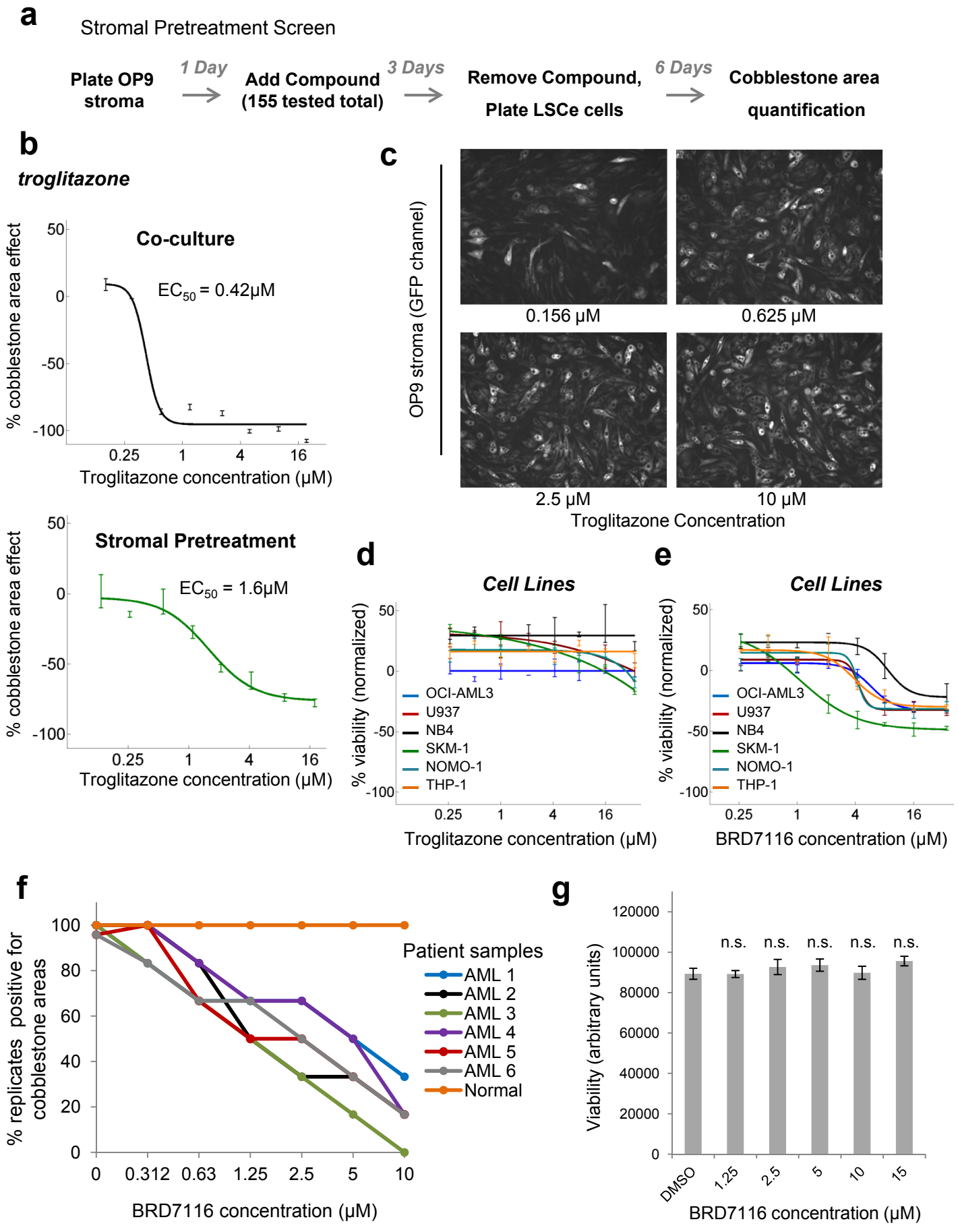

Parbendazole
Co-culture
HSPC
LSCe
EC50 > 20μM
EC50 = 0.076μM
% cobblestone area effect
% total cells
(both normalized)
100
50
0
-50
-100
0.0625
0.25
1
4
16
Parbendazole concentration (μM)
Methiazole
Co-culture
HSPC
LSCe
EC50 > 20μM
EC50 = 0.47μM
Methiazole concentration (μM)

b


Parbendazole
Cell Lines
OCI-AML3
U937
NB4
SKM-1
NOMO-1
THP-1
% viability (normalized)
0
-50
-100
0.25
1
4
16
Parbendazole concentration (μM)
Methiazole
Cell Lines
Methiazole concentration (μM)

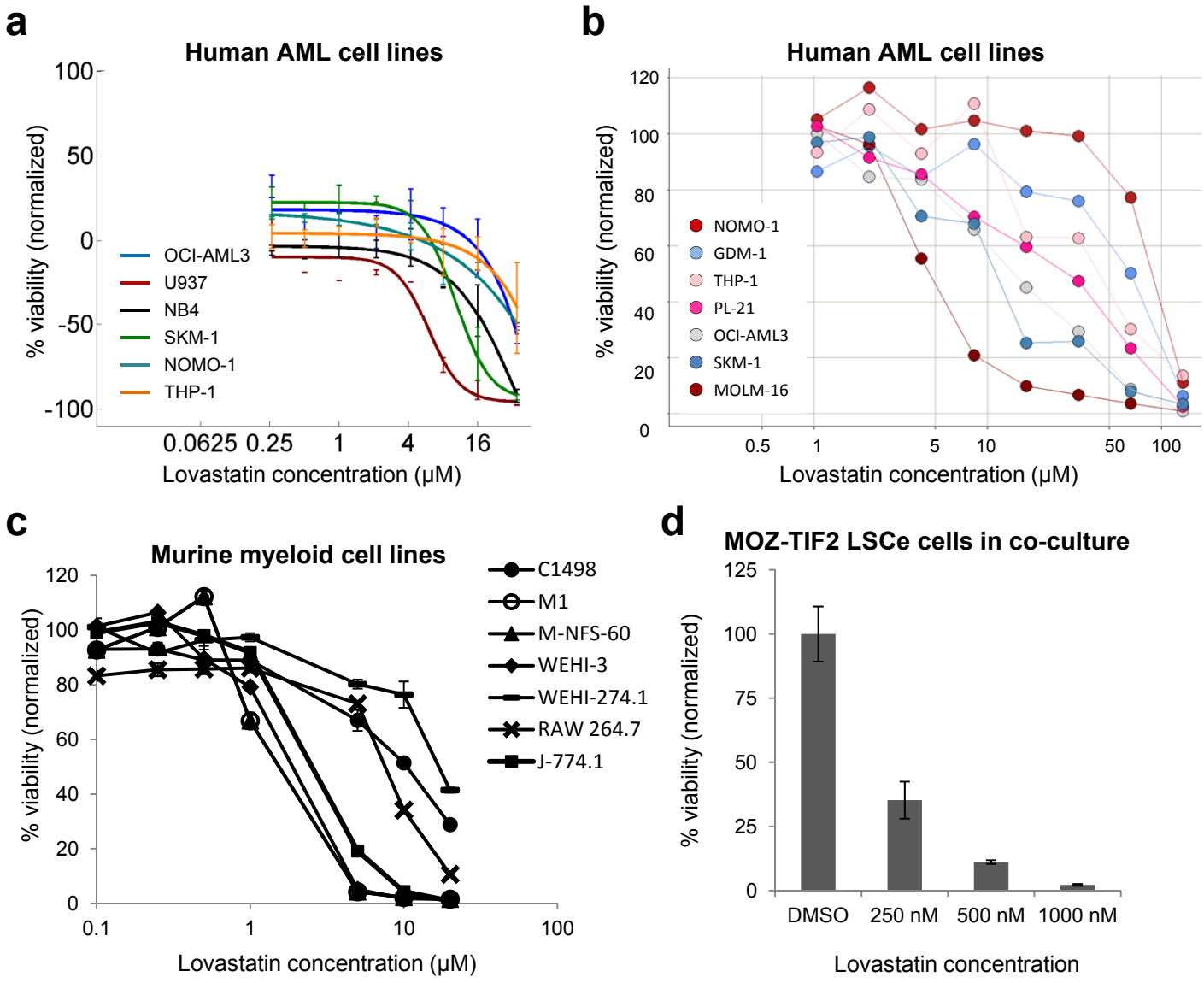
Supplementary Figure 2 | Prioritized Screening Hits Display LSCe Cell Selectivity Relative to HSPCs in Co-culture

(**a**) Dose-response curves for parbendazole and methiazole on LSCe cells (2 replicates per concentration) and normal HSPCs (6 replicates per concentration) in co-culture on primary BMSC stroma. (**b**) These benzimidazole carbamates also displayed strong activity against human AML cell lines (shown as mean +/- SEM of duplicate replicates).


a
Stromal Pretreatment Screen
Plate OP9 stroma
1 Day
Add Compound (155 tested total)
3 Days
Remove Compound, Plate LSCe cells
6 Days
Cobblestone area quantification
b
troglitazone
Co-culture
EC50 = 0.42μM
% cobblestone area effect
Troglitazone concentration (μM)
Stromal Pretreatment
EC50 = 1.6μM
c
OP9 stroma (GFP channel)
0.156 μM
0.625 μM
2.5 μM
10 μM
Troglitazone Concentration
d
Cell Lines
% viability (normalized)
OCI-AML3
U937
NB4
SKM-1
NOMO-1
THP-1
Troglitazone concentration (μM)
e
Cell Lines
BRD7116 concentration (μM)
f
% replicates positive for cobblestone areas
Patient samples
AML 1
AML 2
AML 3
AML 4
AML 5
AML 6
Normal
BRD7116 concentration (μM)
g
Viability (arbitrary units)
n.s.
DMSO
BRD7116 concentration (μM)

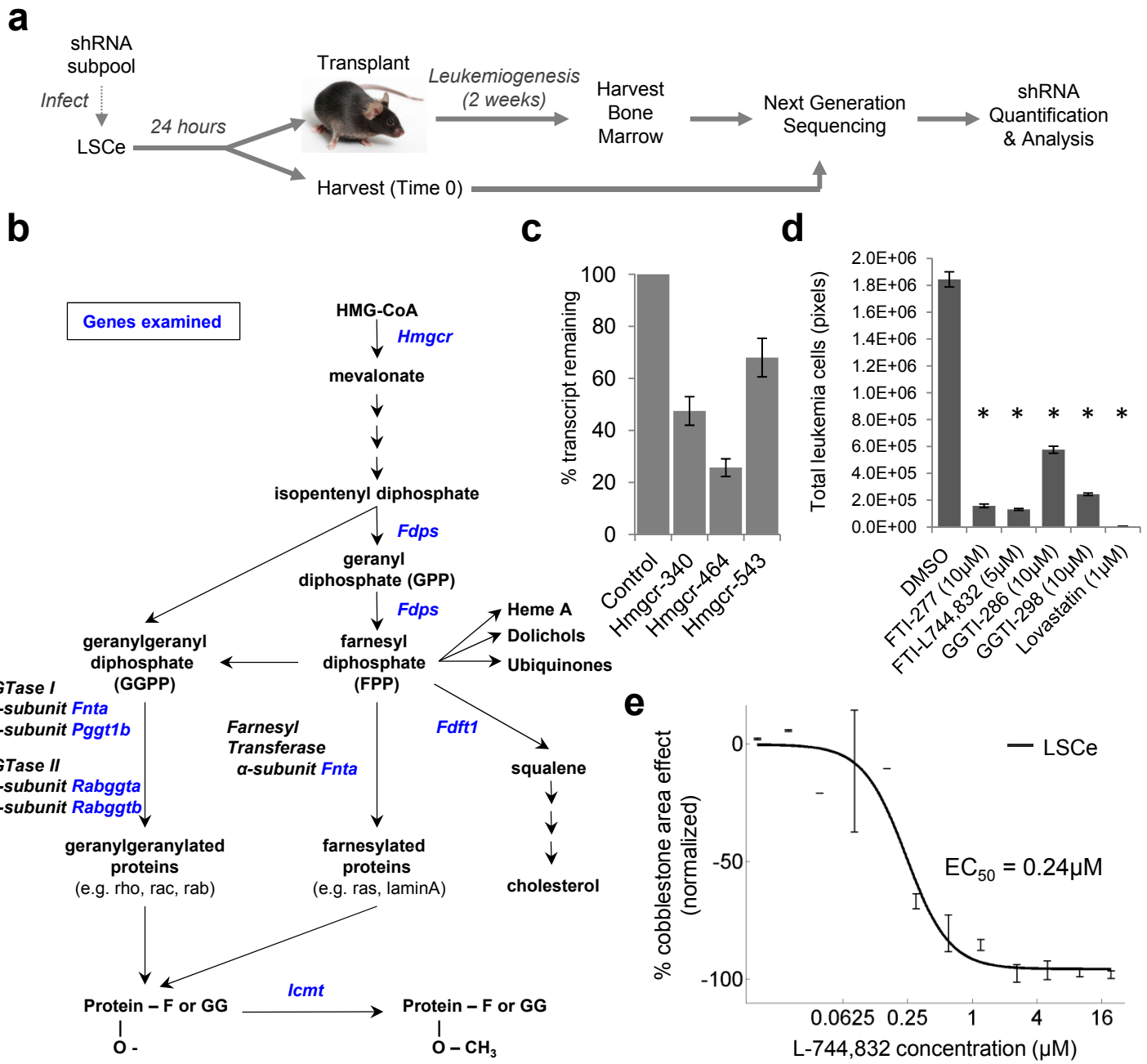
Supplementary Figure 3 | Novel Small Molecule BRD7116 Selectively Targets LSCe Cells by Both Cell-Autonomous and Cell-Non-Autonomous Mechanisms

(**a**) A stromal pretreatment secondary screen identified compounds that antagonize leukemia cobblestone area formation indirectly through the stroma. (**b**) Normalized dose-response curves for troglitazone, a PPAR-γ agonist in the LSCe cell retest and stromal pretreatment screens, both with OP9 stroma in duplicate. (**c**) Representative images of OP9 stroma treated with troglitazone, consistent with adipocytic differentiation. (**d**) Troglitazone dose-response curves for 6 human AML cell lines. Data is mean +/- SEM for duplicate replicates. (**e**) BRD7116 dose-response curves for 6 human AML cell lines, with incomplete inhibition relative to positive controls. (**f**) The effects of BRD7116 on CAFC activity of primary human $CD34^+$ cells isolated from either normal or leukemic patient samples. Co-cultures were treated for 6 days, and then rinsed. The fraction of replicate co-cultures containing cobblestone areas at the 5-week assay endpoint (2 weeks for FLT3-ITD sample) is shown ($n \geq 6$). The clinical characteristics of the AML samples are described in Figure 4b. (**g**) Viability of BMSC stromal cells pretreated with BRD7116 prior to the plating of admixed LSCe and HSPC cells (as mean +/- SEM of quadruplicate replicates), from stromal monolayers cultured in the absence of hematopoietic cells in parallel for the same length of time as the stromal pretreatment screen depicted in Figure 3c. n.s. = not significant relative to DMSO-treated controls.


a
Human AML cell lines
% viability (normalized)
100
50
0
-50
-100
OCI-AML3
U937
NB4
SKM-1
NOMO-1
THP-1
0.0625 0.25 1 4 16
Lovastatin concentration (μM)
b
Human AML cell lines
% viability (normalized)
120
100
80
60
40
20
0
NOMO-1
GDM-1
THP-1
PL-21
OCI-AML3
SKM-1
MOLM-16
0.5 1 5 10 50 100
Lovastatin concentration (μM)
c
Murine myeloid cell lines
% viability (normalized)
120
100
80
60
40
20
0
C1498
M1
M-NFS-60
WEHI-3
WEHI-274.1
RAW 264.7
J-774.1
0.1 1 10 100
Lovastatin concentration (μM)
d
MOZ-TIF2 LSCe cells in co-culture
% viability (normalized)
125
100
75
50
25
0
DMSO
250 nM
500 nM
1000 nM
Lovastatin concentration

Supplementary Figure 4 | Lovastatin Selectively Inhibits Murine and Human Leukemia Cells in Co-culture

(**a**) Lovastatin activity against human AML cell lines (in duplicate). (**b**) Dose-response curves for lovastatin from an additional, independent human AML cell line screen (see Methods). Effects were normalized between DMSO control, set at 100, and media only (no cells), set at zero. (**c**) Lovastatin dose-response curves for 7 murine myeloid cell lines. Data are represented as mean +/- SEM of duplicate replicates. (**d**) Quantification of lovastatin effects on MOZ-TIF2 LSCe cells in co-culture (normalized to DMSO control; as mean +/- SEM) by flow cytometry analysis ($n \geq 3$).


a
shRNA subpool
Infect
LSCe
24 hours
Transplant
Leukemiogenesis (2 weeks)
Harvest Bone Marrow
Next Generation Sequencing
shRNA Quantification & Analysis
Harvest (Time 0)
b
Genes examined
HMG-CoA
Hmgcr
mevalonate
isopentenyl diphosphate
Fdps
geranyl diphosphate (GPP)
Fdps
geranylgeranyl diphosphate (GGPP)
farnesyl diphosphate (FPP)
Heme A
Dolichols
Ubiquinones
GGTase I
α-subunit Fnta
β-subunit Pggt1b
GGTase II
α-subunit Rabggta
β-subunit Rabggtb
Farnesyl Transferase
α-subunit Fnta
Fdft1
squalene
cholesterol
geranylgeranylated proteins
(e.g. rho, rac, rab)
farnesylated proteins
(e.g. ras, laminA)
Protein – F or GG
O -
Icmt
Protein – F or GG
O – CH3
c
% transcript remaining
Control
Hmgcr-340
Hmgcr-464
Hmgcr-543
d
Total leukemia cells (pixels)
DMSO
FTI-277 (10μM)
FTI-L744,832 (5μM)
GGTI-286 (10μM)
GGTI-298 (10μM)
Lovastatin (1μM)
e
% cobblestone area effect (normalized)
LSCe
EC50 = 0.24μM
L-744,832 concentration (μM)
f
Total reconstitution
% donor cells in peripheral blood
n.s.
DMSO (HSPCs only)
Lovastatin
g
Multilineage repopulation
DMSO (HSPCs only)
Lovastatin
% of donor cells
n.s.
B lymphoid
Myeloid
T lymphoid

Supplementary Figure 5 | Sensitivity of LSCe Cells to HMGCR Inhibition

(**a**) Schematic representation of *in vivo* shRNA screening strategy. Primary LSCe cells were infected with a pool of shRNA lentiviruses targeting genes within the mevalonate pathway as well as control genes. After 24 hours, a portion of the cells were harvested and the remainder transplanted into recipient mice. After 2 weeks, at the onset of leukemiogenesis, the leukemia cells in the bone marrow were harvested and the change in shRNA frequency was determined relative to Time 0 (See Methods). (**b**) Schematic indicating the location of the genes examined *in vivo* by shRNA pooled screening within the mevalonate pathway. (**c**) Quantification of Hmgcr RNA knockdown in murine Ba/F3 cells for the Hmgcr shRNAs that scored in the screen relative to three control shRNAs (LUC-58, RFP-03, LacZ-29). (**d**) The effects of various chemical inhibitors of farnesyl and geranylgeranyl transferases on LSCe cells co-cultured with OP9 stromal cells at the 6 day assay endpoint. Data are mean +/- SEM of triplicate replicates. * $p < 0.001$ relative to DMSO-treated controls. (**e**) The farnesyltransferase inhibitor L-744,832 was also a hit in the co-culture screen and passed initial selectivity filtering steps. The dose-response curve for LSCe cells co-cultured with BMSC stroma is shown. (**f**) The long-term engraftment of admixed normal HSPCs quantified by flow cytometric analysis at 16 weeks post transplantation was not impaired by lovastatin treatment (compared to DMSO-treated co-cultures containing HSPCs alone). Mean +/- SEM is shown (n = 5). n.s. = not significant, relative to DMSO controls. (**g**) Multilineage repopulation (as frequency of cell types in peripheral blood) at 16 weeks post transplantation was also unaffected by lovastatin treatment. Mean +/- SEM is shown (n = 5). n.s. = not significant, relative to DMSO controls.

Supplementary Table 1 | Image-based Rules Defining the CAFC Phenotype

Rule #	Rule	Explanation
1	CellsdsRed_Neighbors_PercentTouching_2 > 69.2	Cell objects that have >69% of their perimeter touching other objects, after expanding 2 pixels
2	CellsdsRed_Texture_GaborY_CorrdsRed_3 < 13.9	Cell objects with texture feature (Gabor wavelet in Y direction) < 13.9 at a 3 pixel scale in the dsRed channel
3	CellsdsRed_Neighbors_NumberOfNeighbors_2 > 3.0	Cell objects with > 3 neighbor objects (within 2 pixels)
4	CellsdsRed_Texture_Contrast_CorrdsRed_3 < 9.55	Cell objects with low texture contrast at a 3 pixel scale in the dsRed channel
5	CellsdsRed_Intensity_MinIntensity_CorrdsRed > 0.104	Cell objects with the minimum intensity across all their pixels in the dsRed channel > 0.104
6	CellsdsRed_Intensity_StdIntensity_CorrdsRed < 0.0312	Cell objects with standard deviation of pixel intensities in dsRed channel < 0.03 (a measure of texture)
7	CellsdsRed_Intensity_MinIntensity_CorrStroma > 0.109	Cell objects with the minimum intensity across all their pixels in the stromal channel > 0.109
8	CellsdsRed_Neighbors_NumberOfNeighbors_2 > 2.0	Cell objects with >2 neighbor objects (within 2 pixels)
9	CellsdsRed_Zernike_9_9 > 0.0185	Cell objects with a 9^{th}/9th order Zernike shape feature > 0.018
10	CellsdsRed_Texture_SumEntropy_CorrdsRed_1 > 2.42	Cell objects with a low texture feature (Haralick's Sum of Entropy) at a 1 pixel scale in the dsRed channel
11	CellsdsRed_Texture_InverseDifferenceMoment_CorrdsRed_1 > 0.571	Cell objects with a texture feature (Haralick's Inverse Difference Moment) >0.571 at a 1 pixel scale in the dsRed channel
12	CellsdsRed_Neighbors_AngleBetweenNeighbors_2 < 77.3	Cell objects with nearby neighbor objects (within 2 pixels) that are within 77 degrees of each other
13	CellsdsRed_Zernike_5_3 > 0.0424	Cell objects with a 5^{th}/3rd order Zernike shape feature > 0.0424
14	CellsdsRed_Neighbors_SecondClosestXVector_2 > -7.82	Cell objects with their second closest neighbor having an X coordinate vector > -7.82
15	CellsdsRed_Location_Center_Y < 351.3	Cell objects with their centroid Y coordinate < 351 pixels from the origin
16	CellsdsRed_Texture_GaborX_CorrdsRed_3 < 29.6	Cell objects with texture feature (Gabor wavelet in X direction) < 29.6 at a 3 pixel scale in the dsRed channel
17	CellsdsRed_Intensity_MinIntensity_CorrdsRed > 0.104	Cell objects with the minimum intensity across all their pixels in the dsRed channel > 0.104
18	CellsdsRed_Texture_GaborY_CorrdsRed_3 < 18.2	Cell objects with texture feature (Gabor wavelet in Y direction) < 18.2 at a 3 pixel scale in the dsRed channel
19	CellsdsRed_Neighbors_SecondClosestYVector_2 < 8.75	Cell objects with their second closest neighbor having an Y coordinate vector < 8.75
20	CellsdsRed_Zernike_2_2 < 0.058	Cell objects with a 2nd/2nd order Zernike shape feature < 0.058
21	CellsdsRed_Neighbors_SecondClosestYVector_2 > -7.62	Cell objects with their second closest neighbor having an Y coordinate vector < -7.62
22	CellsdsRed_Zernike_0_0 > 0.615	Cell objects with a 0^{th}/0th order Zernike shape feature (even intensity) > 0.615
23	CellsdsRed_Neighbors_SecondClosestObjectNumber_2 > 636	Cell objects with their second closest neighbor having an object number > 636
24	CellsdsRed_Intensity_UpperQuartileIntensity_CorrdsRed > 0.0919	Cell objects with a pixel intensity of the upper quartile in the dsRed channel > 0.0919
25	CellsdsRed_Intensity_StdIntensity_CorrdsRed < 0.0154	Cell objects with a pixel intensity standard deviation in the dsRed channel < 0.0154
26	CellsdsRed_Texture_GaborX_CorrdsRed_1 > 2.44	Cell objects with texture feature (Gabor wavelet in X direction) > 2.44 at a 1 pixel scale in the dsRed

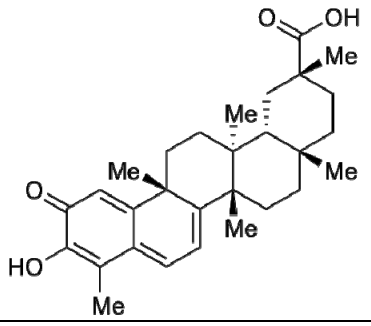
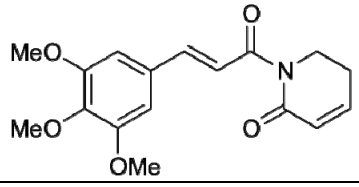
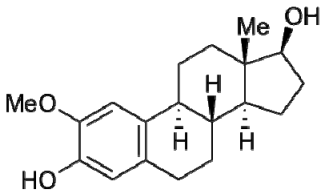
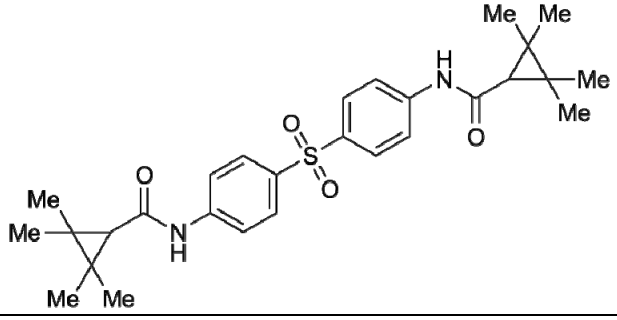
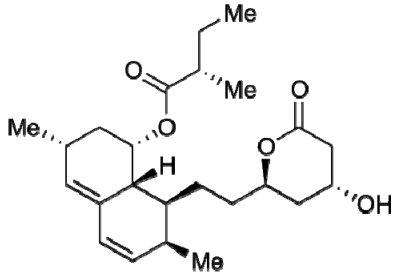
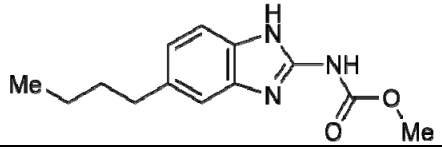
		channel
27	CellsdsRed_Neighbors_FirstClosestXVector_2 > -6.50	Cell objects with their closest neighbor having an X coordinate vector > -6.5
28	CellsdsRed_Neighbors_NumberOfNeighbors_2 > 2.0	Cell objects with >2 neighbor objects (within 2 pixels)
29	CellsdsRed_Texture_DifferenceEntropy_CorrdsRed_1 > 1.58	Cell objects with a texture feature (Haralick's Difference Entropy) > 1.58 at a 1 pixel scale in the dsRed channel
30	CellsdsRed_Texture_Contrast_CorrdsRed_1 < 2.88	Cell objects with a texture feature (Haralick's Contrast) < 2.88 at a 1 pixel scale in the dsRed channel
31	CellsdsRed_Neighbors_FirstClosestXVector_2 > 1.59	Cell objects with their closest neighbor having an X coordinate vector > 1.59
32	CellsdsRed_Intensity_MedianIntensity_CorrStroma < 0.220	Cell objects with a median pixel intensity in the stromal channel < 0.22
33	CellsdsRed_Intensity_MassDisplacement_CorrStroma > 3.88	Cell objects with a pixel intensity shift in the grayscale versus binary centroids in the stromal channel > 3.88
34	CellsdsRed_Texture_GaborX_CorrdsRed_3 < 19.7	Cell objects with texture feature (Gabor wavelet in X direction) < 19.7 at a 3 pixel scale in the dsRed channel
35	CellsdsRed_Texture_GaborY_CorrdsRed_1 > 1.91	Cell objects with texture feature (Gabor wavelet in Y direction) > 1.91 at a 1 pixel scale in the dsRed channel
36	CellsdsRed_Zernike_4_2 > 0.00394	Cell objects with a 4^{th}/2nd order Zernike shape feature > 0.00394
37	CellsdsRed_Texture_DifferenceEntropy_CorrdsRed_1 < 1.17	Cell objects with a texture feature (Haralick's Difference Entropy) < 1.17 at a 1 pixel scale in the dsRed channel
38	CellsdsRed_Texture_AngularSecondMoment_CorrdsRed_3 > 0.081	Cell objects with a texture feature (Haralick's Angular Second Moment) > 0.081 at a 3 pixel scale in the dsRed channel
39	CellsdsRed_Zernike_3_1 > 0.139	Cell objects with a 3rd/1st order Zernike shape feature > 0.139
40	CellsdsRed_Zernike_2_0 < 0.165	Cell objects with a 2nd/0th order Zernike shape feature < 0.165
41	CellsdsRed_Zernike_7_5 < 0.0515	Cell objects with a 7^{th}/5th order Zernike shape feature < 0.0515
42	CellsdsRed_Intensity_StdIntensityEdge_CorrdsRed < 0.0261	Cell objects with standard deviation of pixel intensities along their perimeter in dsRed channel < 0.0261
43	CellsdsRed_Intensity_MeanIntensityEdge_CorrdsRed > 0.146	Cell objects with mean pixel intensities along their perimeter in dsRed channel > 0.146
44	CellsdsRed_Zernike_5_1 > 0.0464	Cell objects with a 5^{th}/1st order Zernike shape feature > 0.0464
45	CellsdsRed_Neighbors_FirstClosestYVector_2 > -6.77	Cell objects with their closest neighbor having a Y coordinate vector > -6.77
46	CellsdsRed_Neighbors_SecondClosestYVector_2 > -7.32	Cell objects with their second closest neighbor having a Y coordinate vector > -7.32
47	CellsdsRed_AreaShape_Eccentricity < 0.872	Cell objects not very elliptical (on a scale of 0=circular to 1=linear/flattened) < 0.872
48	CellsdsRed_Zernike_1_1 < 0.363	Cell objects with a 1st/1st order Zernike shape feature < 0.363
49	CellsdsRed_Intensity_StdIntensityEdge_CorrdsRed < 0.0326	Cell objects with standard deviation of pixel intensities along their perimeter in dsRed channel < 0.0326
50	CellsdsRed_Intensity_MinIntensity_CorrdsRed > 0.0937	Cell objects with the minimum intensity across all their pixels in the dsRed channel > 0.0937

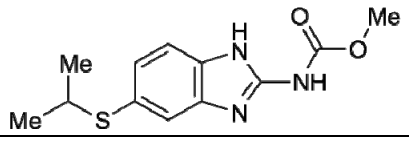
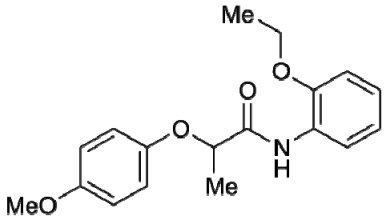
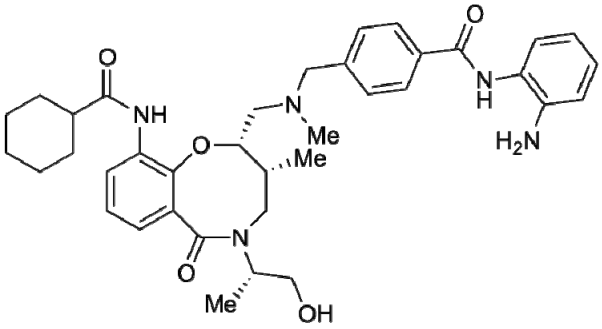
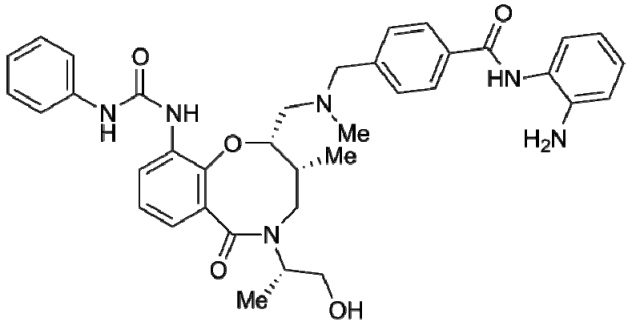
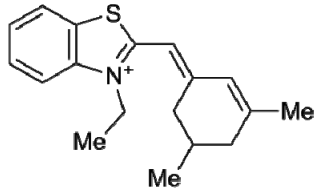
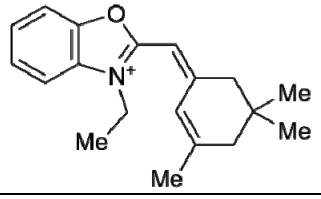
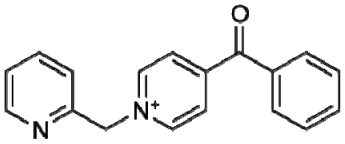
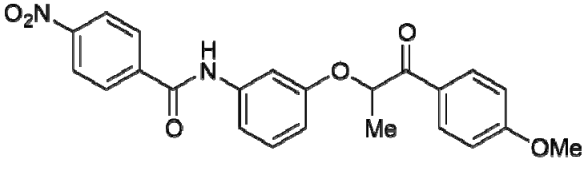
The 50 rules identified by the user-trained automated algorithm as the strongest image-based correlates of the cobblestone area phenotype are shown for a representative screening run. Rules are based on features of cell “objects” (see Supplementary Fig. 1d and Methods), which individual cells are segmented into for classification. While all 50 rules contribute to the classification algorithm, the rules are rank-ordered with those given the most weight listed first. Detailed explanations are shown for the purposes of illustration only, as the exact 50 rules will vary by image batch (see Methods).

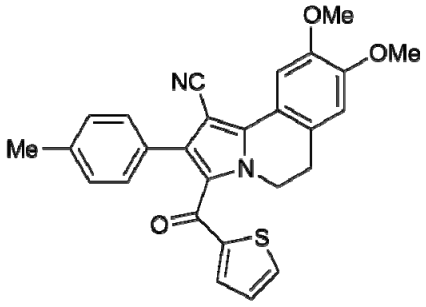
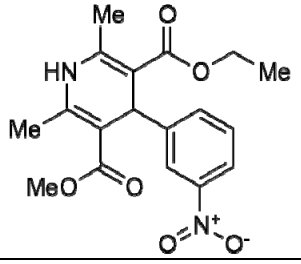
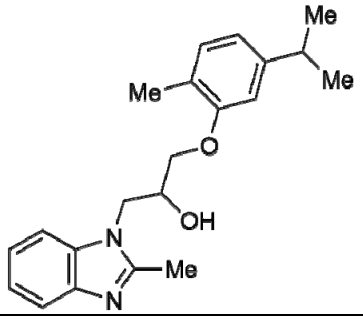
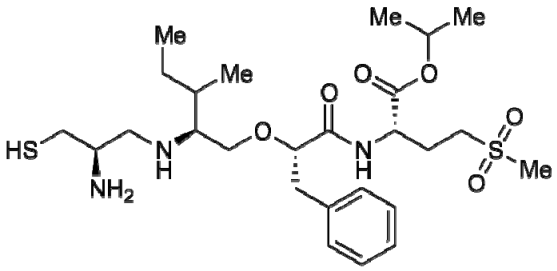
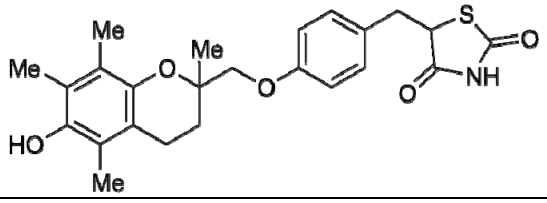
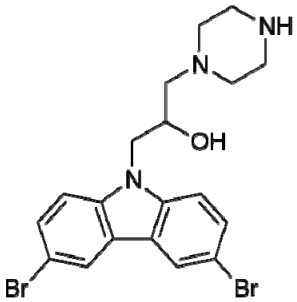
Supplementary Table 2 | Small Molecule Screening Data

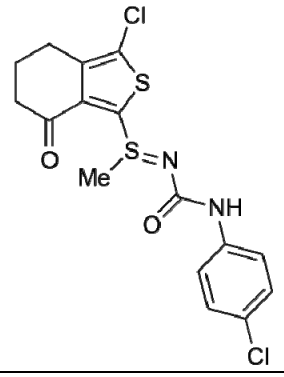
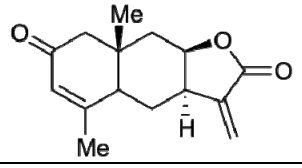
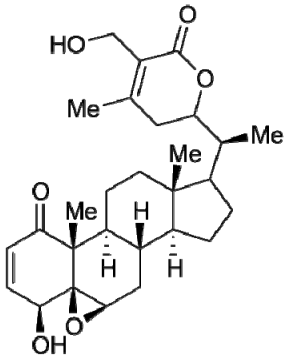
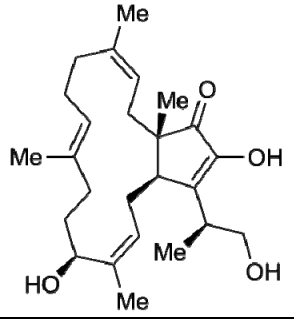
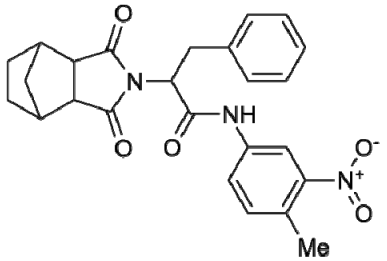
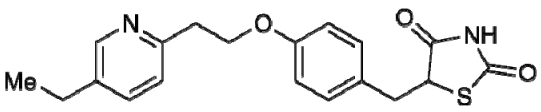
Category	Parameter	Description
Assay	Type of assay	Cell-based co-culture assay
	Target	Leukemic Cobblestone Area-Forming Cells (CAFCs)
	Primary measurement	Total CAFC area per well
	Key reagents	Primary stem-enriched leukemia cells, Bone marrow stromal cells (OP9 cell line or primary bone marrow mesenchymal stromal cells), Image analysis software algorithms (see http://www.cellprofiler.org/published_pipelines.shtml)
	Assay protocol	See Methods
	Additional comments	
Library	Library size	14,718 compounds screened
	Library composition	Compounds were selected from a series of chemically diverse commercially available and internally synthesized libraries, including ~1,920 known bioactive molecules, ~1,600 natural products, and 2,880 compounds generated via diversity oriented synthesis (DOS)
	Source	Broad Institute compound collection
	Additional comments	
Screen	Format	384-well (Corning 3712)
	Concentration(s) tested	5 μM in 0.2% DMSO
	Plate controls	Neutral control: DMSO carrier alone; Positive control: 10 μM XK469, a topoisomerase IIβ inhibitor
	Reagent/ compound dispensing system	Automated: Multidrop Combi (Thermo Scientific) for reagents, CyBi-Well Vario (CyBio) for compounds
	Detection instrument and software	Microscopy images captured using automated ImageXpress Micro (Molecular Devices; 10x total magnification; binning of 2); Images analyzed using CellProfiler software
	Assay validation/QC	Assay sensitivity and specificity for CAFCs were 86% and 87%, respectively. Retest rate was 71.5% in co-culture with OP9 stromal type (61.6% with primary bone marrow stromal cells); Additional retesting (Fig. 1e); Compounds previously known to have preferential activity against LSCs relative to HSPCs were recovered; Secondary assays
	Correction factors	N/A
	Normalization	A two-point normalization of total CAFC area per well was performed for compound treatments on each plate using the mean of neutral control wells (set at 0% effect) and the mean of positive control wells (set at -100% effect) on that plate. Each experimental compound was thus represented as a percent effect on CAFC area per well within this normalized range.
	Additional comments	All compounds were tested in duplicate
Post-HTS analysis	Hit criteria	The lowest (in magnitude) % inhibitory effect (see Normalization above) required to achieve statistical significance within a given screening run (z-score less than -3 relative to the neutral control in both replicates) was identified. This cutoff (-67% effect) was then used to permissively identify hits across the run that achieved at least this degree of inhibition.
	Hit rate	2.8%
	Additional assay(s)	Retesting (above); Secondary screening assays (Fig. 1e); Other secondary studies.
	Confirmation of hit purity and structure	Compounds were repurchased and retested in secondary experiments and verified analytically.
	Additional comments	

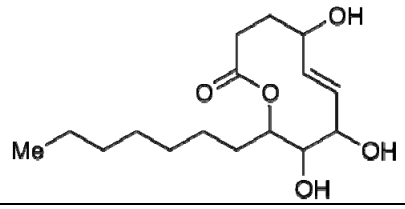
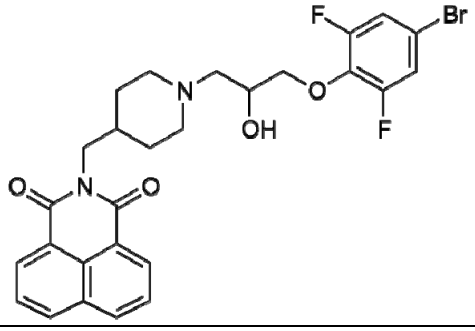
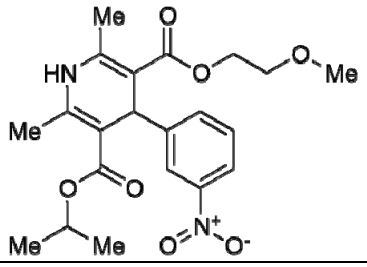
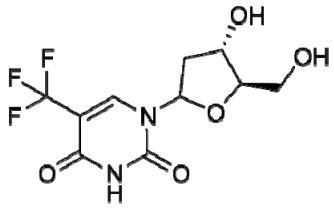
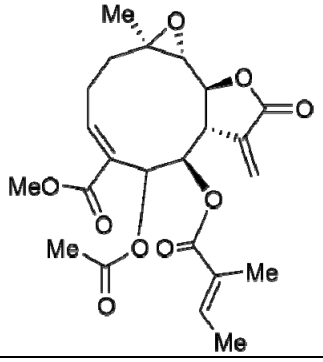
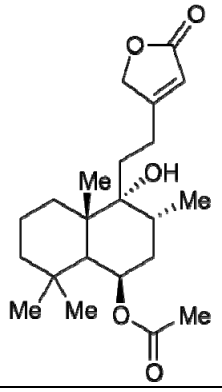
Supplementary Table 3 | The 155 Prioritized Screening Hits

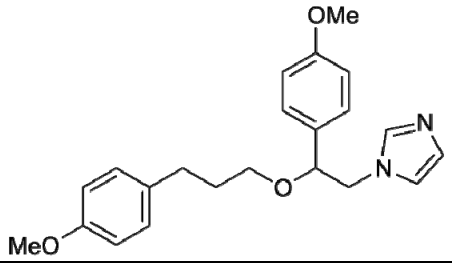
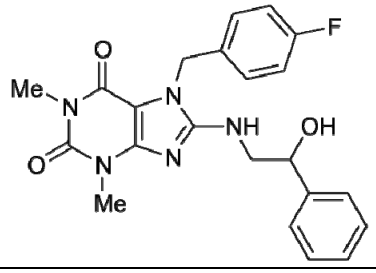
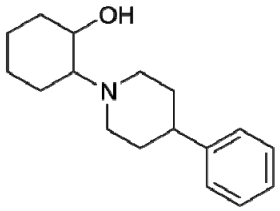
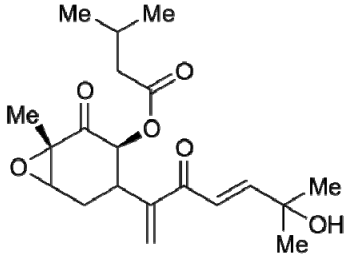
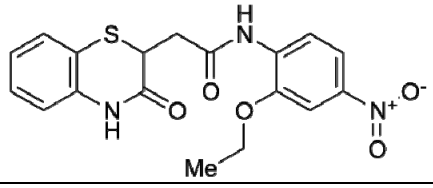
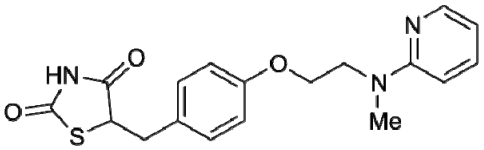
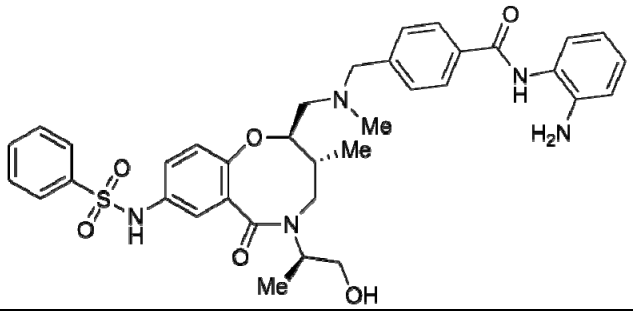
#	**Name** and **Source**	**Structure**	**SMILES**	**O**
1	celastrol*		OC(C(C=C1C2=CC=C3[C@]1(CC[C@]4([C@@H]5C[C@@](CC[C@@]5(CC[C@]34C)C)(C(O)=O)C)C)C)=O)=C2C	CC10
2	piperlongumine†		O=C(C=CCC1)N1C(/C=C/C2=CC(OC)=C(C(OC)=C2)OC)=O	
3	2-methoxy-estradiol‡		O[C@@H]1[C@@]2(C)CC[C@]3([H])C(C=C4OC)=C(C=C4O)CC[C@@]3([H])[C@]2([H])CC1	
4	BRD7116§		O=C(NC1=CC=C(S(=O)(C2=CC=C(C=C2)NC(C3C(C3(C)C)(C)C)=O)=O)C=C1)C(C4(C)C)C4(C)C	CC10; SPT
5	lovastatin‡		CCC(C)C(=O)OC1CC(C)C=C2C=CC(C)C(CCC(O)CC(O)CC(=O)O)C12	CC10
6	parbendazole†		O=C(OC)NC1=NC2=C(N1)C=CC(CCCC)=C2	

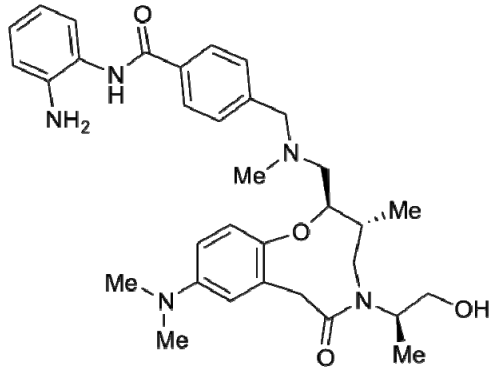
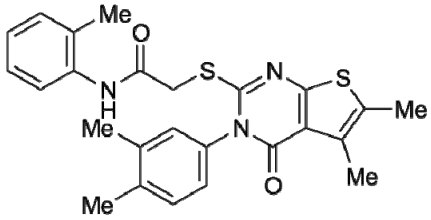
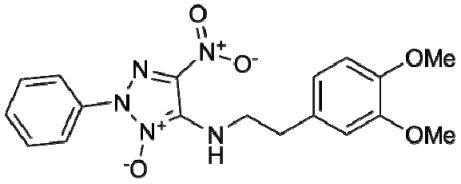
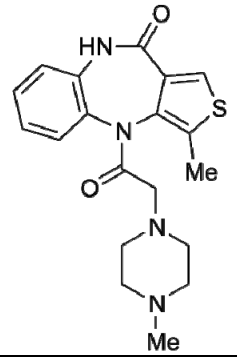
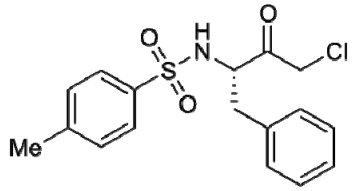
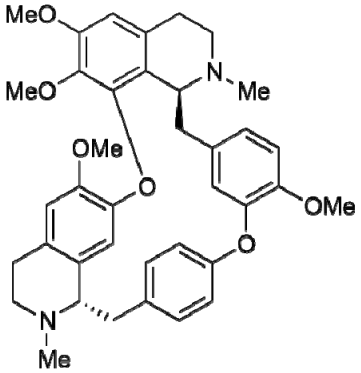
7	methiazole[†]		O=C(NC1=NC2=C(N1)C=CC(SC(C)C)=C2)OC	
8	BRD1686**		O=C(C(OC1=CC=C(C=C1)OC)C)NC2=C(C=CC=C2)OCC	
9	BRD9608[a]		O=C(C1=CC=CC(NC(C2CCCCC2)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(C=CC=C5)N)=O)N3[C@H](CO)C	
10	BRD6708[a]		O=C(C(C=CC=C1NC(NC2=CC=CC=C2)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=CC=CC=C5N)=O)C=C4)N3[C@H](CO)C	
11	BRD1319[††]		CC[N+]1=C(/C=C(C2)/C=C(CC2C)C)SC3=C1C=CC=C3	CC10
12	BRD0638[††]		CC[N+]1=C(/C=C(C2)\C=C(CC2(C)C)C)OC3=C1C=CC=C3	CC10
13	BRD1856[§]		O=C(C1=CC=CC=C1)C2=CC=[N+](CC3=NC=CC=C3)C=C2	CC10
14	BRD6491**		O=C(C1=CC=C([N+]([O-])=O)C=C1)NC2=CC=CC(OC(C)C(C3=CC=C(C=C3)OC)=O)=C2	CC10

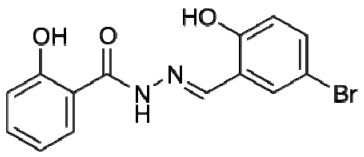
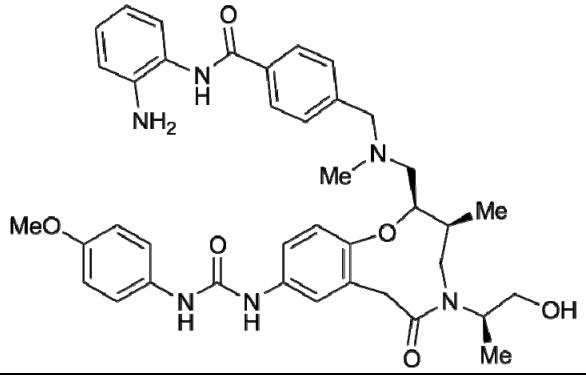
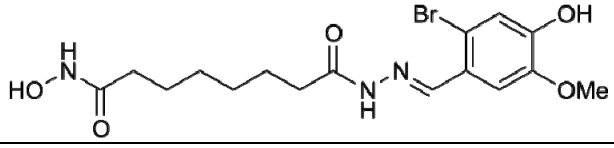
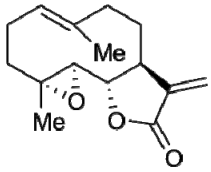
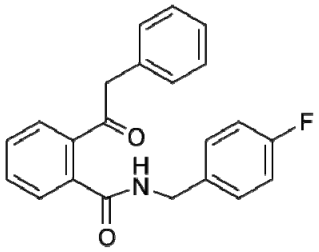
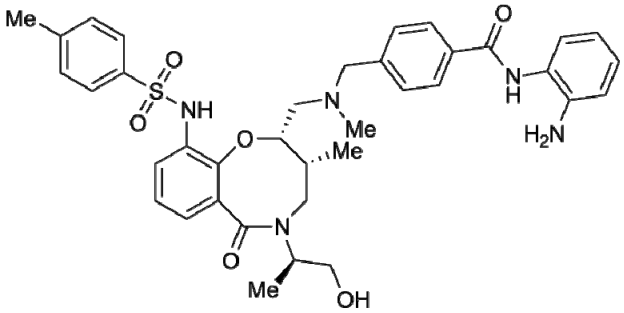
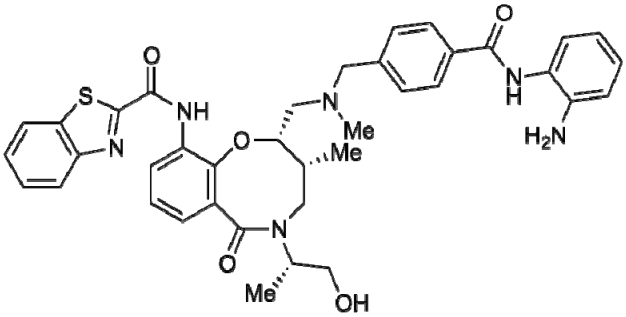
15	BRD8404[‡‡]		O=C(C1=CC=CS1)C2=C(C3=CC=C(C=C3)C)C(C#N)=C(N2CC4)C5=C4C=C(C(OC)=C5)OC	CC10
16	nitrendipine[§§]		O=[N+]([O-])C1=CC=CC(C2C(C(OCC)=O)=C(C)NC(C)=C2C(OC)=O)=C1	
17	BRD53501**		OC(CN1C(C=CC=C2)=C2N=C1C)COC(C=C3C(C)C)=C(C=C3)C	CC5
18	L-744,832[§§]		O=C(N[C@@H](C(OC(C)C)=O)CCS(=O)(C)=O)[C@H](CC1=CC=CC=C1)OC[C@@H](NC[C@H](CS)N)C(C)CC	
19	troglitazone***		O=C(NC1=O)SC1CC(C=C2)=CC=C2OCC3(C)OC(C(C)=C4C)=C(C(C)=C4O)CC3	CC10; SPT
20	Bax channel blocker[†††]		OC(CN1CCNCC1)CN2C(C=CC(Br)=C3)=C3C4=C2C=CC(Br)=C4	

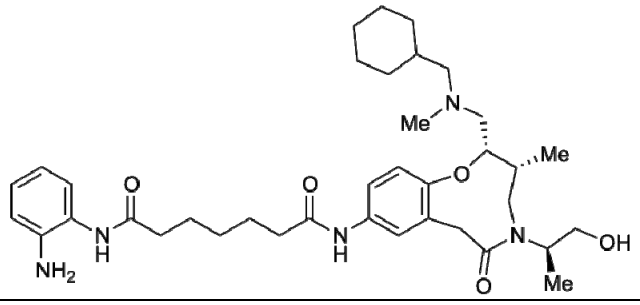
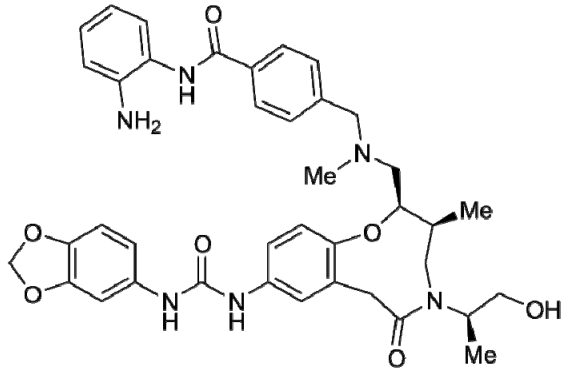
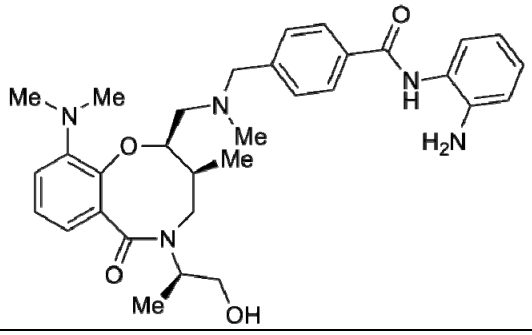
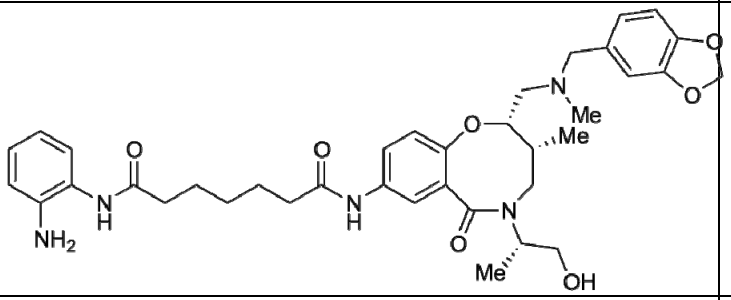
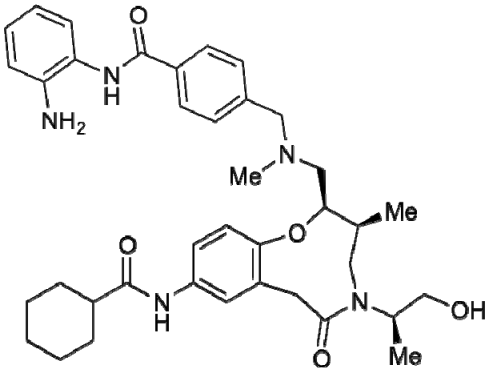
21	BRD1059‡‡		ClC(C=C1)=CC=C1NC(/N=S(C2=C(C(CCC3)=O)C3=C(S2)Cl)\C)=O	
22	BRD7506‡		O=C1C[C@@]2(C)C(C[C@@](C(C(O3)=O)=C)([H])[C@H]3C2)C(C)=C1	
23	BRD3807‡		O=C(C(CO)=C(C1)C)OC1[C@H]([C@H]2[C@@]3([C@](CC2)([H])[C@@]4([H])[C@]([C@]([C@]5(O6)[C@H]6C4)(C(C=C[C@H]5O)=O)C)([H])CC3)C)C	
24	BRD0602‡		OC[C@H](C([C@H]1C/C=C([C@H](CC/C(C)=C/CC/C(C)=C\C[C@@]12C)O)/C)=C(O)C2=O)C	
25	BRD7359**		O=[N+]([O-])C(C=C(C=C1)NC(C(CC2=CC=CC=C2)N3C(C(C4CC5CC4)C5C3=O)=O)=O)=C1C	CC10
26	pioglitazone hydrochloride†††		O=C(NC(S1)=O)C1CC2=CC=C(C=C2)OCCC(C=C3)=NC=C3CC	SPT

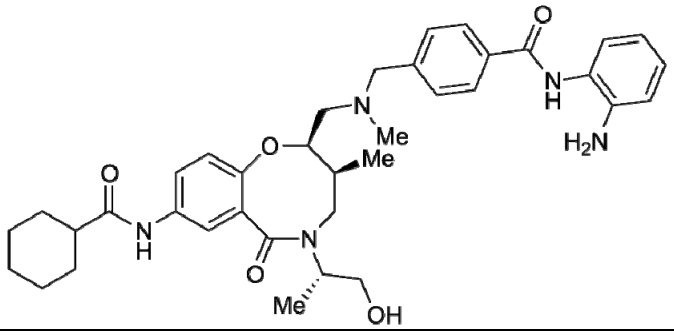
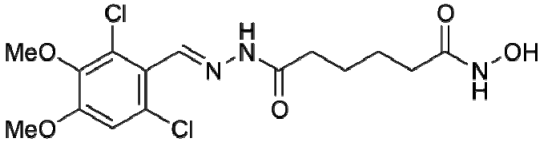
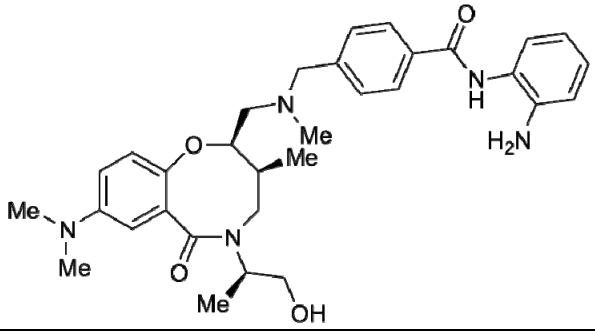
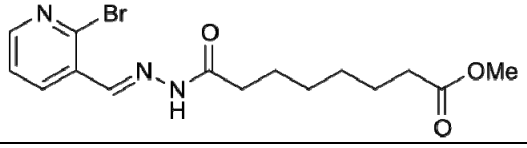
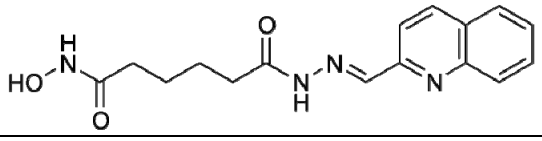
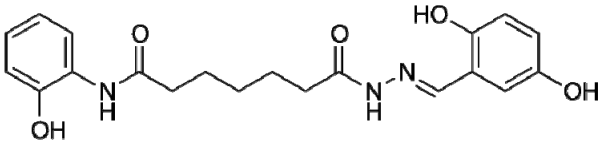
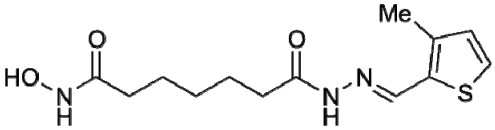
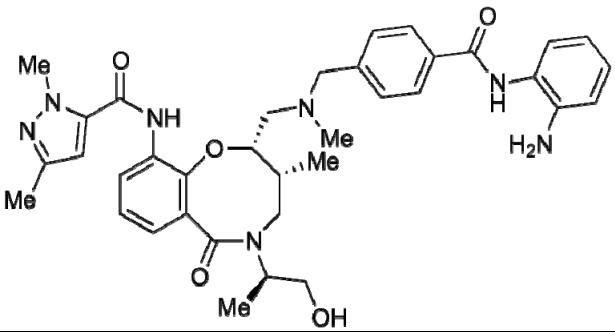
27	BRD6436*		O=C(CCC(/C=C/C(C1O)O)O)OC1CCCCCCC	CC5
28	BRD6574**		OC(COC1=C(F)C=C(Br)C=C1F)CN2CCC(CC2)CN3C(C4=CC=CC5=C4C(C3=O)=CC=C5)=O	
29	nimopidine[§§]		O=[N+]([O-])C1=CC=CC(C2C(C(OCCOC)=O)=C(C)NC(C)=C2C(OC(C)C)=O)=C1	
30	trifluridine[†]		OC[C@@H]1[C@H](CC(O1)N2C(NC(C(C(F)(F)F)=C2)=O)=O)O	
31	BRD3636[‡]		O=C(/C1=C/CC[C@]2(O[C@@H]2[C@H]3OC(C([C@@H]3[C@H](C1OC(C)=O)OC(/C(C)=C/C)=C)=C)=O)C)OC	
32	BRD4560[‡]		O=C1OCC(CC[C@@](O)([C@]([C@H]2[C@@H](C3)OC(C)=O)(CCCC2(C)C)C)[C@H]3C)=C1	

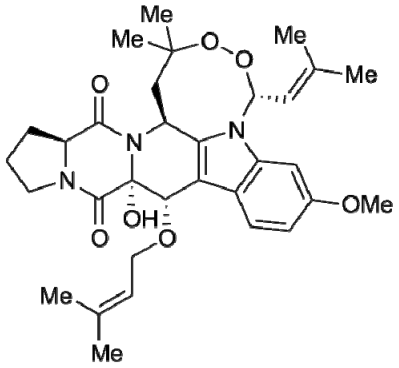
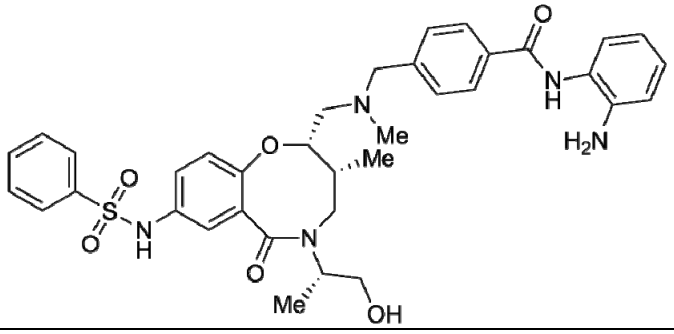
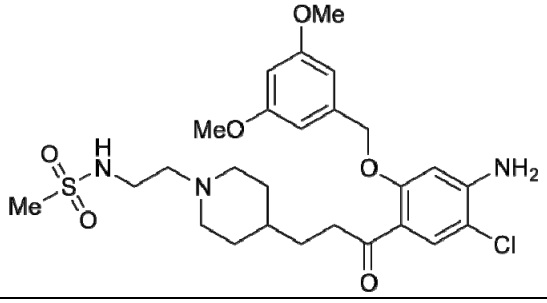
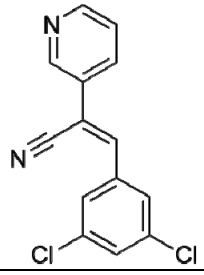
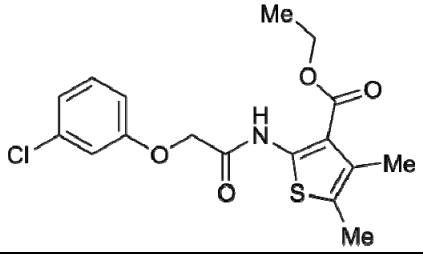
33	SKF-96365[§§]		COC1=CC=C(CCCOC(CN2C=NC=C2)C3=CC=C(C=C3)OC)C=C1	
34	BRD3808**		O=C(N(C1=O)C)C2=C(N=C(N2CC3=CC=C(C=C3)F)NCC(O)C4=CC=CC=C4)N1C	CC5
35	vesamicol hydrochloride*		OC1C(CCCC1)N2CCC(CC2)C3=CC=CC=C3	CC5
36	BRD0471[‡]		O=C([C@@]([C@H]1C[C@@H]2C(C(/C=C\C(C)(C)O)=O)=C)(O1)C)[C@@H]2OC(CC(C)C)=O	
37	BRD9886**		O=C(NC(C=CC=C1)=C1S2)C2CC(NC(C(OCC)=C3)=CC=C3[N+]([O-])=O)=O	
38	rosiglitazone*		O=C(NC(S1)=O)C1CC2=CC=C(C=C2)OCCN(C3=NC=CC=C3)C	
39	BRD2476[b]		O=C(C(C=C(C=C1)NS(C2=CC=CC=C2)(=O)=O)=C1O[C@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@@H](CO)C	

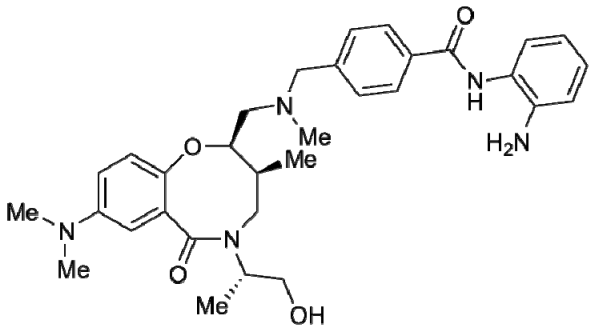
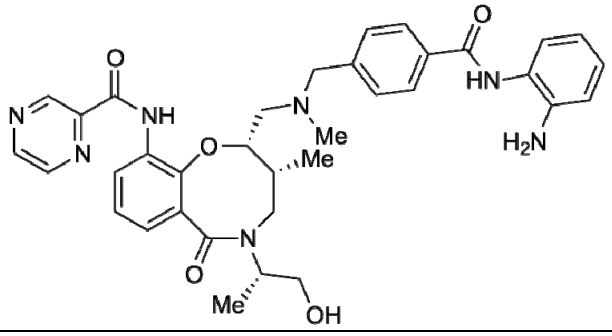
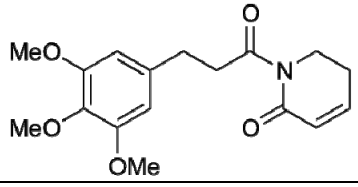
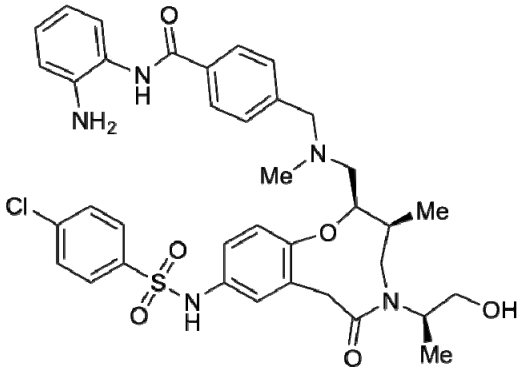
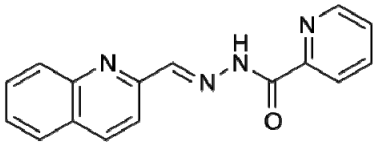
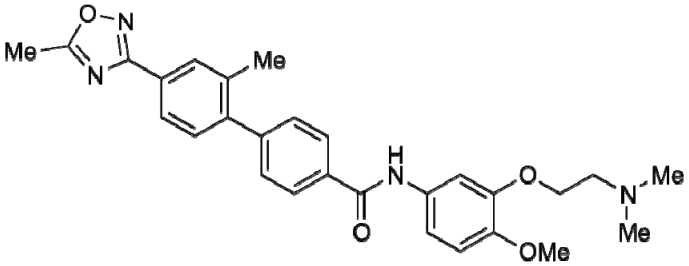
40	BRD2327[b]		O=C(CC(C=C(C=C1)N(C)C)=C1O[C@H]([C@@H](C2)C)CN(C)CC3=CC=C(C=C3)C(NC4=CC=CC=C4N)=O)N2[C@@H](CO)C	
41	BRD0666**		O=C1N(C2=CC=C(C)C(C)=C2)C(SCC(NC(C=CC=C3)=C3C)=O)=NC4=C1C(C)=C(S4)C	
42	BRD8436**		[O-][N+](N(N=C1[N+]([O-])=O)C2=CC=CC=C2)=C1NCCC3=CC=C(C(OC)=C3)OC	CC10
43	telenzepine dihydrochloride[†]		O=C(C1=CSC(C)=C12)NC3=C(C=CC=C3)N2C(CN(CC4)CCN4C)=O	CC10
44	tosyl-phe-CMK[§§]		ClCC([C@H](CC1=CC=CC=C1)NS(C2=CC=C(C=C2)C)(=O)=O)=O	
45	tetrandrine[§§]		COC1=C(OC(C=C2)=CC=C2C[C@H]3C(C(CCN3C)=C4)=CC(OC5=C([C@@H]6C7)C(CCN6C)=CC(OC)=C5OC)=C4OC)C=C7C=C1	

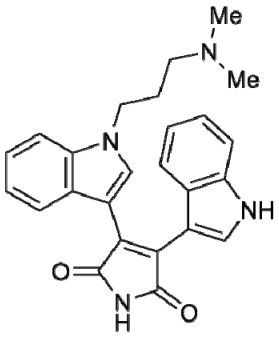
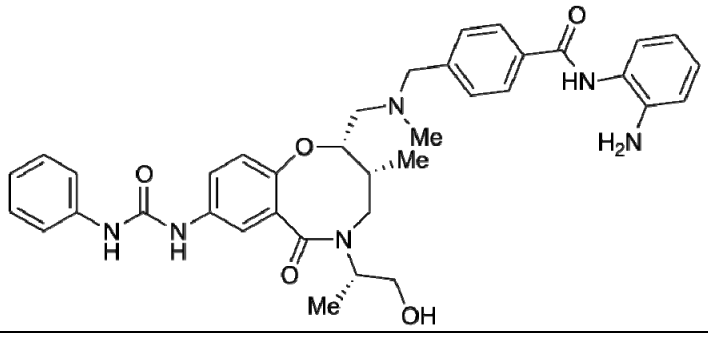
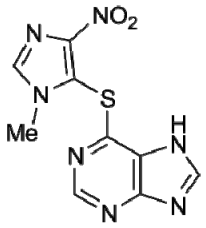
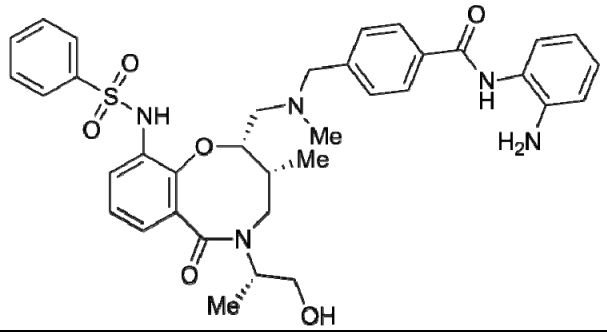
46	BRD9912[c,e,g]		OC1=CC=C(Br)C=C1/C=N/NC(C2=CC=CC=C2O)=O	CL5
47	BRD8012[b]		O=C(N(C[C@@H]([C@@H](O1)CN(C)CC2=CC=C(C=C2)C(NC3=C(N)C=CC=C3)=O)C)[C@H](C)CO)CC4=C1C=CC(NC(NC5=CC=C(C=C5)OC)=O)=C4	
48	BRD3408[c,e,g]		OC(C(OC)=C1)=CC(Br)=C1/C=N/NC(CCCCCCC(NO)=O)=O	CL5
49	parthenolide[‡‡‡]		CC1=CCC[C@]2(C)O[C@@H]2[C@H]3OC(=O)C(=C)[C@@H]3CC1	SPT
50	BRD4115[§]		FC1=CC=C(C=C1)CNC(C2=C(C=CC=C2)C(CC3=CC=CC=C3)=O)=O	
51	BRD3999[a]		O=C(C1=CC=CC(NS(C2=CC=C(C=C2)C)(=O)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@@H](CO)C	
52	BRD1478[a]		O=C(C(C=CC=C1NC(C2=NC3=C(C=CC=C3)S2)=O)=C1O[C@@H]([C@@H](C4)C)CN(C)CC5=CC=C(C(NC6=CC=CC=C6N)=O)C=C5)N4[C@H](CO)C	SPT

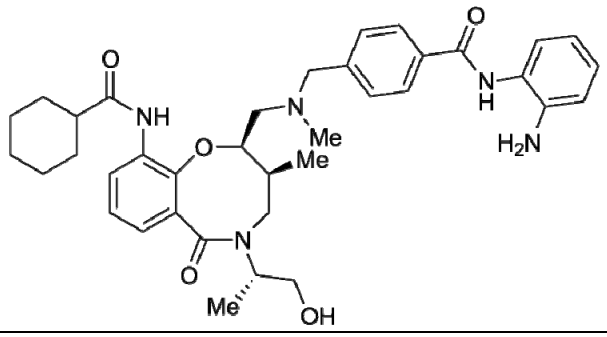
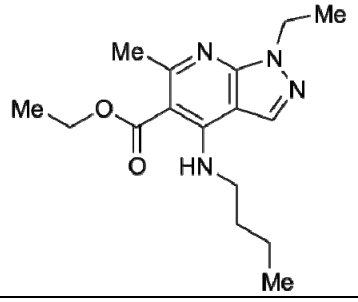
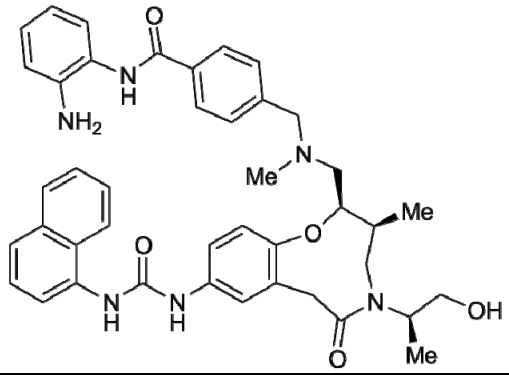
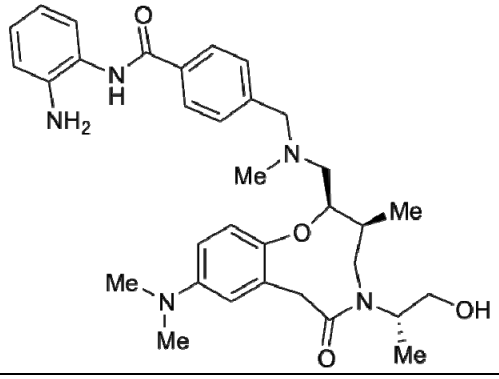
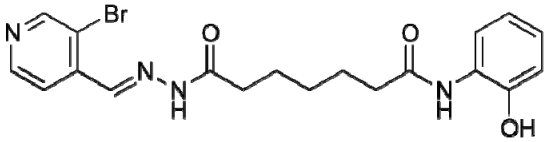
53	BRD0193[b]		O=C(CC(C=C(C=C1)NC(CCCCCC(NC2=CC=CC=C2N)=O)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4CCCCC4)N3[C@@H](CO)C	CC5
54	BRD9122[b]		O=C(CC(C=C(C=C1)NC(NC2=CC(OCO3)=C3C=C2)=O)=C1O[C@H]([C@H](C4)C)CN(C)CC5=CC=C(C(NC6=CC=CC=C6N)=O)C=C5)N4[C@@H](CO)C	CC5
55	BRD6332[a]		O=C(C1=CC=CC(N(C)C)=C1O[C@H]([C@H](C2)C)CN(C)CC3=CC=C(C(NC4=CC=CC=C4N)=O)C=C3)N2[C@@H](CO)C	
56	BRD3719[b]		O=C(C1=CC(NC(CCCCCC(NC2=C(N)C=CC=C2)=O)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC(OCO5)=C5C=C4)N3C(CO)C	
57	BRD2023[b]		O=C(CC(C=C(C=C1)NC(C2CCCCC2)=O)=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(N)C=CC=C5)=O)N3[C@@H](CO)C	SPT

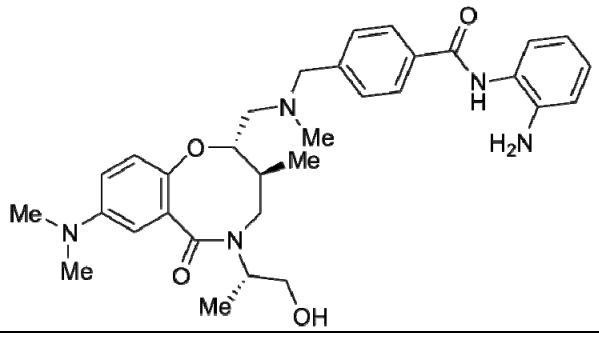
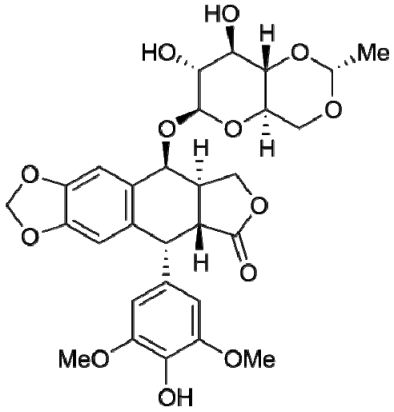
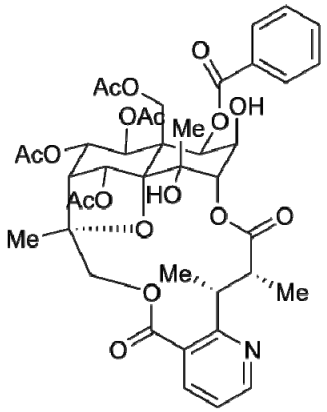
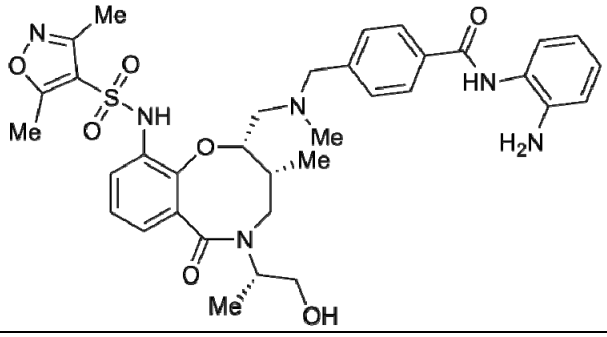
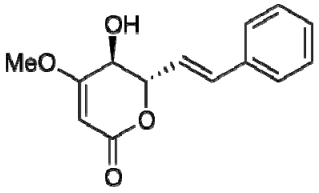
58	BRD8170[b]		O=C(C1=CC(NC(C2CCCCC2)=O)=CC=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(C=CC=C5)N)=O)N3[C@H](CO)C	
59	BRD9834[c,e,g]		ClC1=C(OC)C(OC)=CC(Cl)=C1/C=N/NC(CCCCC(NO)=O)=O	
60	BRD6819[b]		O=C(C1=CC(N(C)C)=CC=C1O[C@H]([C@H](C2)C)CN(C)CC3=CC=C(C(NC4=C(C=CC=C4)N)=O)C=C3)N2[C@@H](CO)C	
61	BRD0350[c,e,g]		BrC1=NC=CC=C1/C=N/NC(CCCCCCC(OC)=O)=O	
62	BRD6376[c,e,g]		ONC(CCCCC(N/N=C/C1=NC(C=CC=C2)=C2C=C1)=O)=O	CC5
63	BRD1484[c,e,g]		OC1=CC(/C=N/NC(CCCCCC(NC2=CC=CC=C2O)=O)=O)=C(C=C1)O	
64	BRD4247[c,e,g]		ONC(CCCCCC(N/N=C/C(SC=C1)=C1C)=O)=O	
65	BRD6258[a]		O=C(C(C=CC=C1NC(C2=CC(C)=NN2C)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@@H](CO)C	

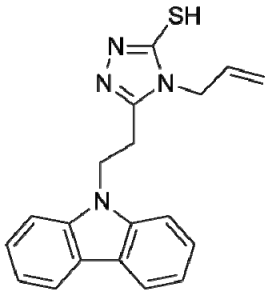
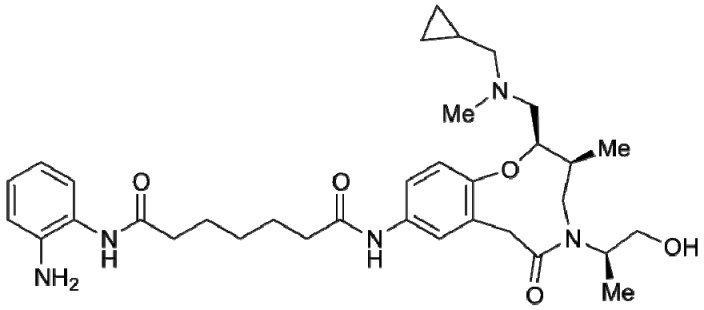
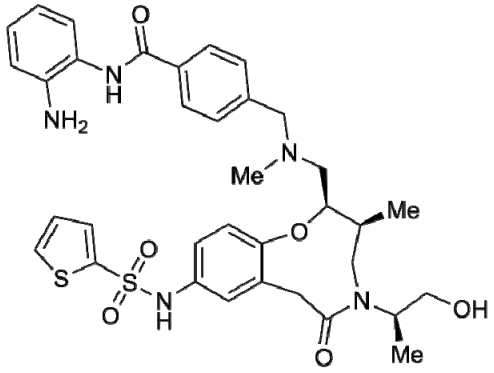
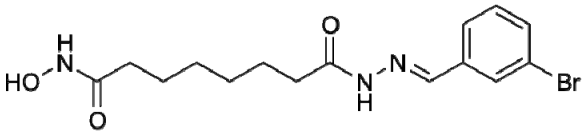
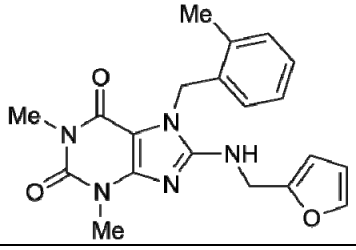
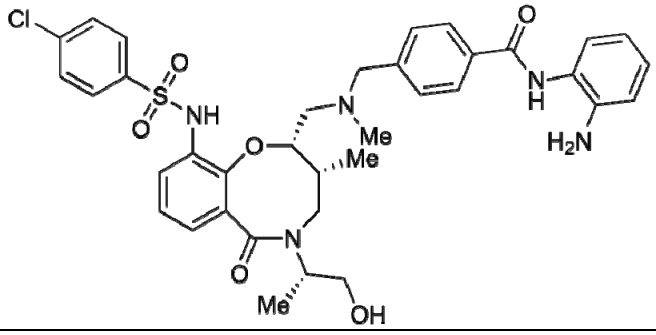
66	BRD8008[‡]		O=C(N(CCC1)[C@@H]1C2=O)[C@](O)([C@H]3OC/C=C(C)/C)N2[C@@H]4C5=C3C(C=C6)=C(N5[C@H](OOC(C4)(C)C)/C=C(C)/C)C=C6OC	
67	BRD0837[a]		O=C(C1=CC(NS(C2=CC=CC=C2)(=O)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	
68	BRD2498[§§]		O=S(NCCN(CC1)CCC1CCC(C(C=C(C(N)=C2)Cl)=C2OCC3=CC(OC)=CC(OC)=C3)=O)(C)=O	
69	RG-14620[§§]		ClC1=CC(Cl)=CC(/C=C(C#N)/C2=CC=CN=C2)=C1	
70	BRD8275**		ClC1=CC(OCC(NC2=C(C(C)=C(S2)C)C(OCC)=O)=O)=CC=C1	

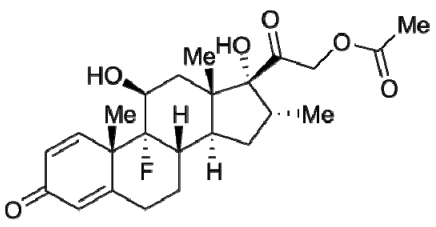
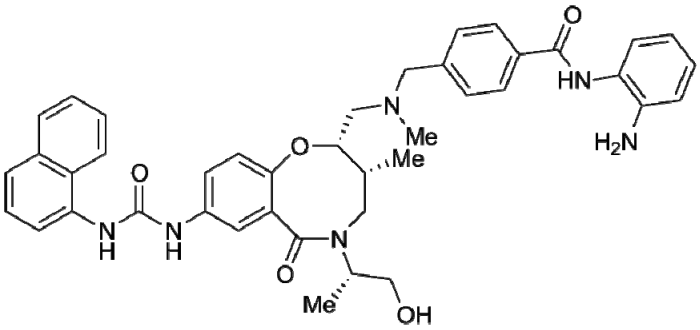
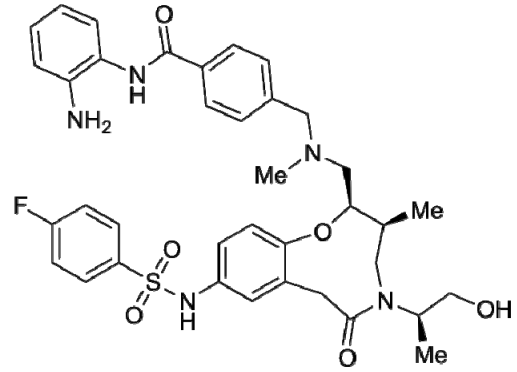
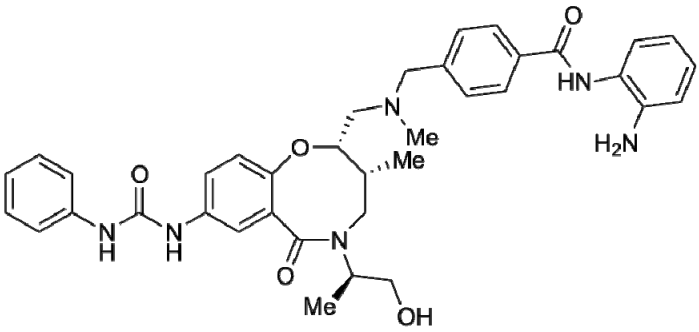
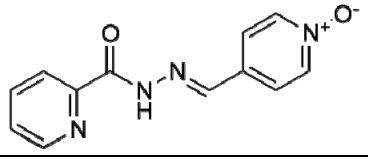
71	BRD2736[b]		O=C(C1=CC(N(C)C)=CC=C1O[C@H]([C@H](C2)C)CN(C)CC3=CC=C(C=C3)C(NC4=C(C=CC=C4)N)=O)N2[C@H](CO)C	
72	BRD9456[a]		O=C(C1=CC=CC(NC(C2=NC=CN=C2)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	
73	BRD1177[‡]		O=C(C=CCC1)N1C(CCC2=CC(OC)=C(C(OC)=C2)OC)=O	
74	BRD7475[b]		O=C(CC(C=C(C=C1)NS(C2=CC=C(C=C2)Cl)(=O)=O)=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=CC=CC=C5N)=O)N3[C@@H](CO)C	
75	BRD4053[c,e,g]		O=C(N/N=C/C1=NC(C=CC=C2)=C2C=C1)C3=NC=CC=C3	
76	SB-216641[§§]		O=C(C1=CC=C(C2=C(C)C=C(C3=NOC(C)=N3)C=C2)C=C1)NC(C=C4OCCN(C)C)=CC=C4OC	CC5; SPT

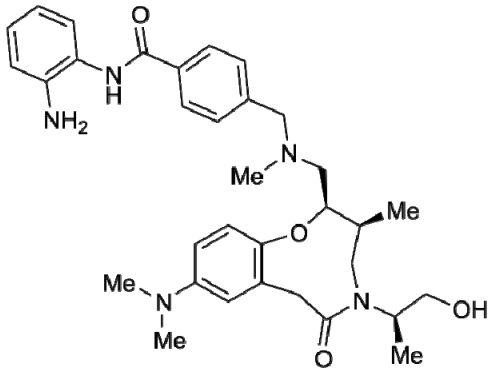
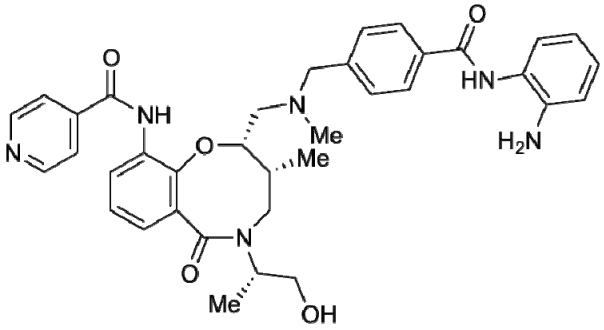
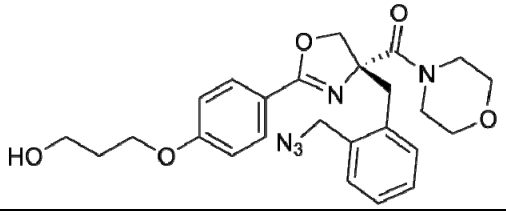
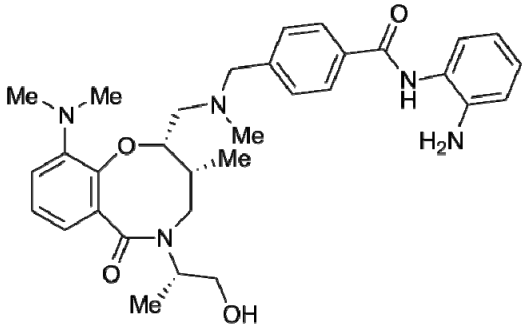
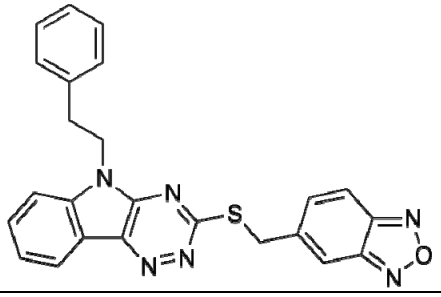
77	GF-109203X[§§]		O=C(N1)C(C2=CN(C3=C2C=CC=C3)CCCN(C)C)=C(C4=CNC5=C4C=CC=C5)C1=O	CC10
78	BRD5418[b]		O=C(C1=CC(NC(NC2=CC=CC=C2)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	
79	Azathioprine[†]		CN1C(SC2=NC=NC3=C2NC=N3)=C(N=C1)[N+]([O-])=O	
80	BRD6790[a]		O=C(C(C=CC=C1NS(C2=CC=CC=C2)(=O)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	

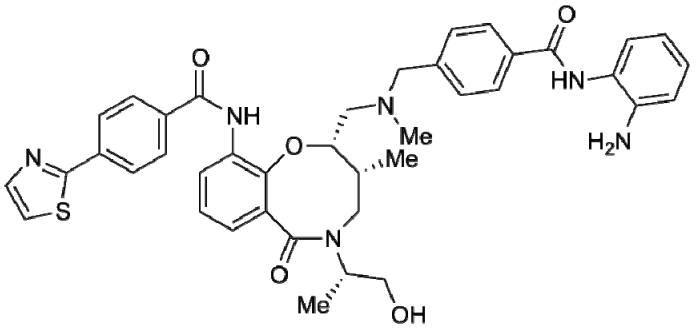
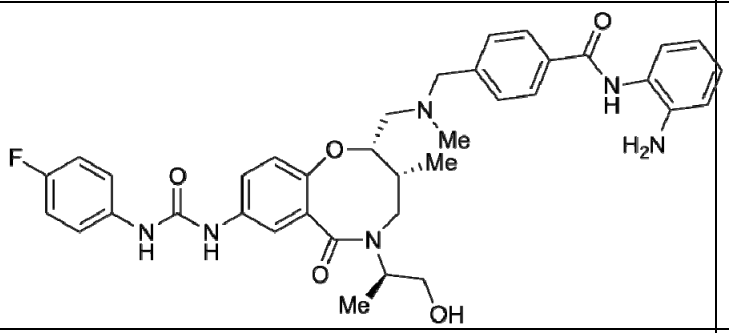
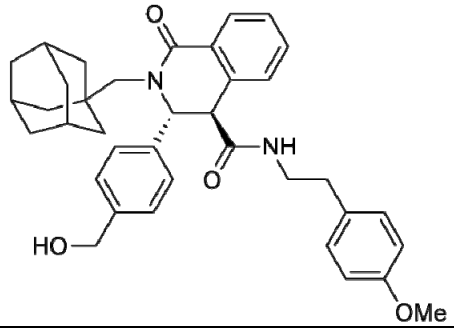
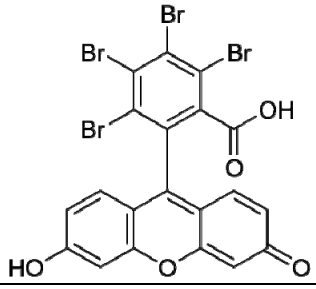
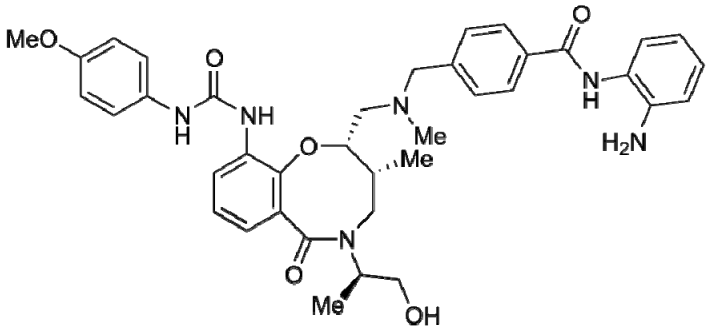
81	BRD2011[a]		O=C(C1=CC=CC(NC(C2CCCCC2)=O)=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C(NC5=CC=CC=C5N)=O)C=C4)N3[C@H](CO)C	
82	Tracazoloate[†]		O=C(OCC)C1=C(C(C=NN2CC)=C2N=C1C)NCCCC	
83	BRD27137[b]		O=C(CC(C=C(C=C1)NC(NC2=CC=CC3=C2C=CC=C3)=O)=C1O[C@H]([C@H](C4)C)CN(C)CC5=CC=C(C=C5)C(NC6=C(N)C=CC=C6)=O)N4[C@@H](CO)C	
84	BRD4586[b]		O=C(CC1=CC(N(C)C)=CC=C1O[C@H]([C@H](C2)C)CN(C)CC3=CC=C(C(NC4=C(N)C=CC=C4)=O)C=C3)N2[C@H](CO)C	
85	BRD1684[c,e,g]		BrC(C=NC=C1)=C1/C=N/NC(CCCCCC(NC2=CC=CC=C2O)=O)=O	

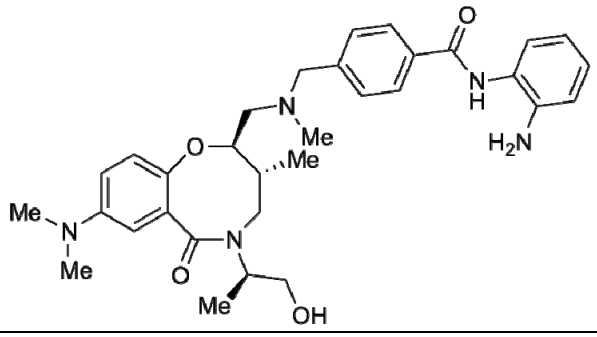
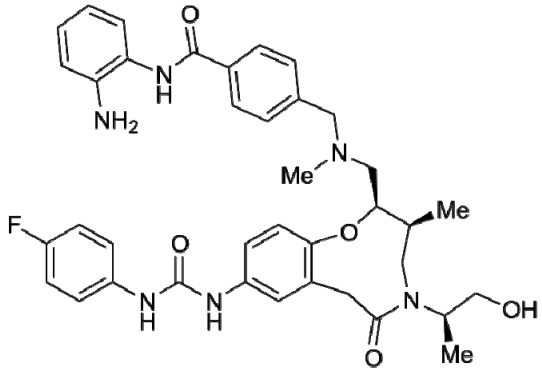
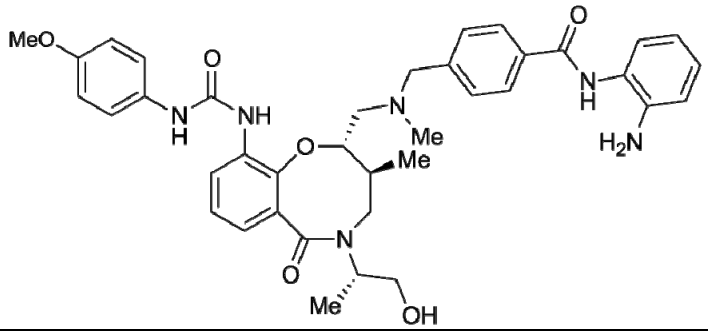
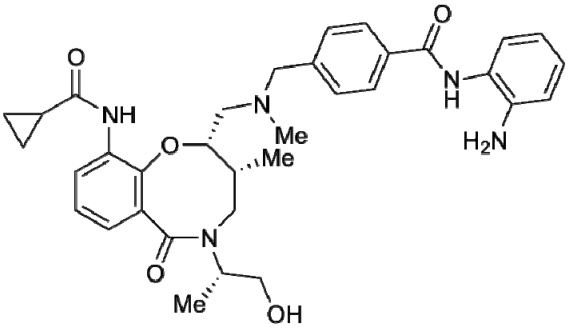
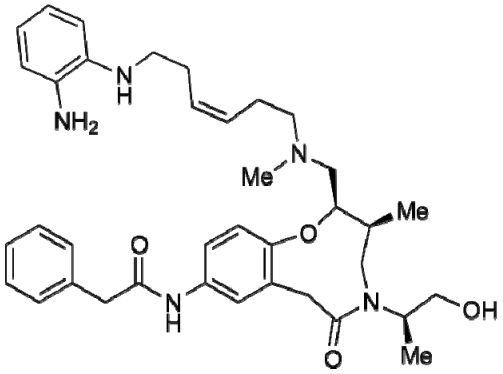
86	BRD3259[b]		O=C(C(C=C(C=C1)N(C)C)=C1O[C@@H]([C@H](C2)C)CN(C)CC3=CC=C(C(NC4=C(C=CC=C4)N)=O)C=C3)N2[C@H](CO)C	
87	Etoposide[†]		O=C(OC1)[C@]([C@@]1([H])[C@@H]2O[C@@H]3O[C@@](CO[C@@H](C)O4)([H])[C@]4([H])[C@H](O)[C@H]3O)([H])[C@H](C5=CC(OC)=C(O)C(OC)=C5)C6=C2C=C(OCO7)C7=C6	
88	BRD1486[‡]		O[C@H]1[C@H](OC([C@H](C)[C@@H](C)C2=C3C=CC=N2)=O)[C@@](O)(C)[C@@]4(O[C@@]5(C)COC3=O)[C@H](OC(C)=O)[C@H]5[C@H](OC(C)=O)[C@@H](OC(C)=O)[C@]4(COC(C)=O)[C@H]1OC(C6=CC=CC=C6)=O	
89	BRD3119[a]		O=C(C1=CC=CC(NS(C2=C(ON=C2C)C)(=O)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(C=CC=C5)N)=O)N3[C@H](CO)C	
90	BRD1831[‡]		O[C@@H]([C@@H](O1)/C=C/C2=CC=CC=C2)C(OC)=CC1=O	

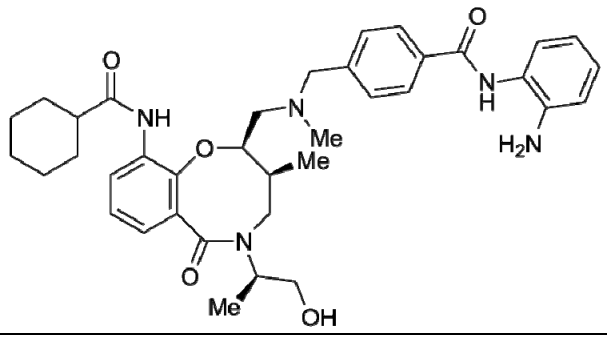
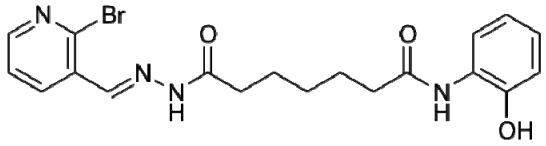
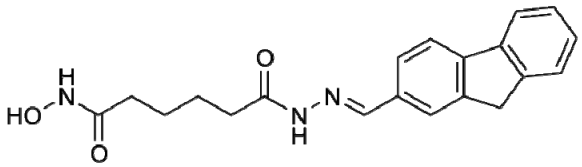
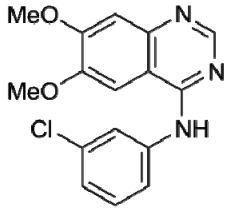
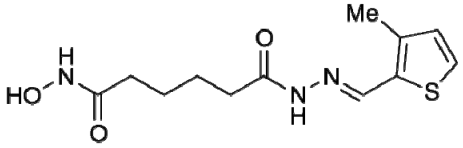
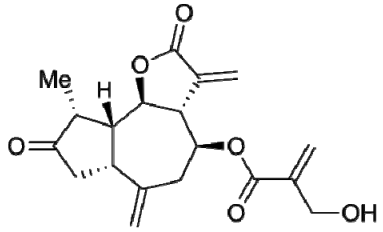
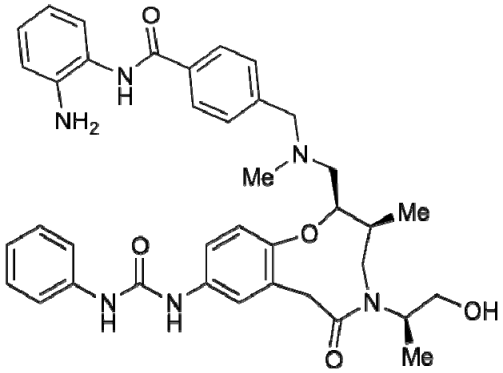
91	BRD7355[††]		SC1=NN=C(CCN2C(C=CC=C3)=C3C4=C2C=CC=C4)N1CC=C	
92	BRD9545[b]		O=C(CC(C=C(C=C1)NC(CCCCCC(NC2=C(N)C=CC=C2)=O)=O)=C1O[C@H]([C@H](C3)C)CN(C)CC4CC4)N3[C@@H](CO)C	
93	BRD33679[b]		O=C(CC1=CC(NS(C2=CC=CS2)(=O)=O)=CC=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(N)C=CC=C5)=O)N3[C@@H](CO)C	
94	BRD7348[c,e,g]		ONC(CCCCCCC(N/N=C/C1=CC=CC(Br)=C1)=O)=O	
95	BRD8827**		O=C(N(C(N1C)=O)C)C2=C1N=C(NCC3=CC=CO3)N2CC4=CC=CC=C4C	CC10
96	BRD1859[a]		O=C(C1=CC=CC(NS(C2=CC=C(Cl)C=C2)(=O)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(C=CC=C5)N)=O)N3[C@H](CO)C	

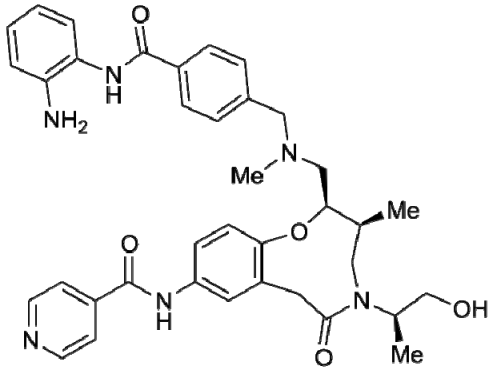
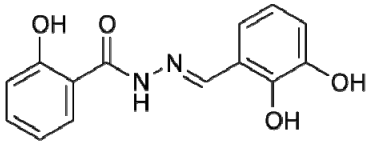
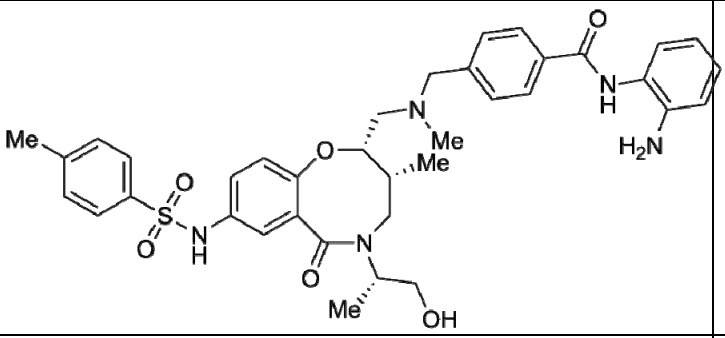
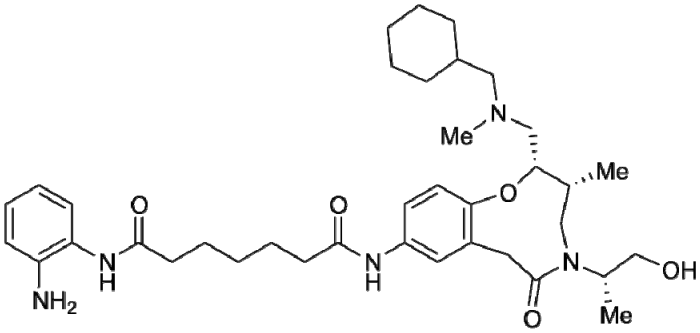
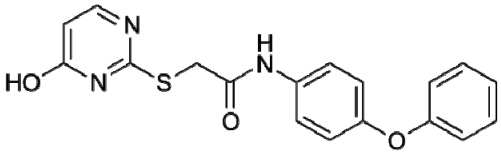
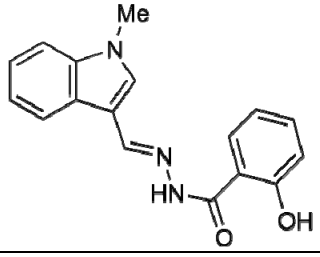
97	dexamethasone acetate[†]		O=C(C)OCC([C@](O)([C@@]1([C@@]2([H])[C@]3([H])[C@@](F)([C@](C(CC3)=CC4=O)(C=C4)C)[C@@H](O)C1)C)[C@@H](C2)C)=O	
98	BRD4339[b]		O=C(C1=CC(NC(NC2=CC=CC3=C2C=CC=C3)=O)=CC=C1O[C@@H]([C@@H](C4)C)CN(C)CC5=CC=C(C(NC6=C(C=CC=C6)N)=O)C=C5)N4[C@H](CO)C	
99	BRD5229[b]		O=C(CC1=CC(NS(C2=CC=C(C=C2)F)(=O)=O)=CC=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(N)C=CC=C5)=O)C=C4)N3[C@@H](CO)C	
100	BRD0010[b]		O=C(C1=CC(NC(NC2=CC=CC=C2)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(C=CC=C5)N)=O)N3[C@@H](CO)C	
101	BRD6218[c,e,g]		[O-][N+]1=CC=C(C=C1)/C=N/NC(C2=NC=CC=C2)=O	

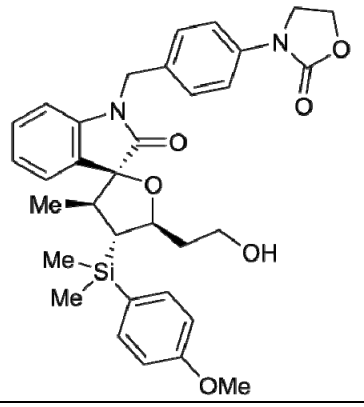
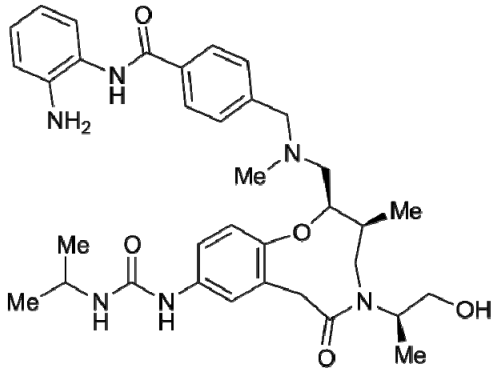
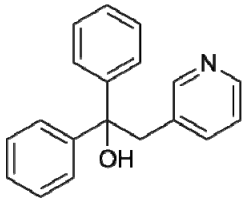
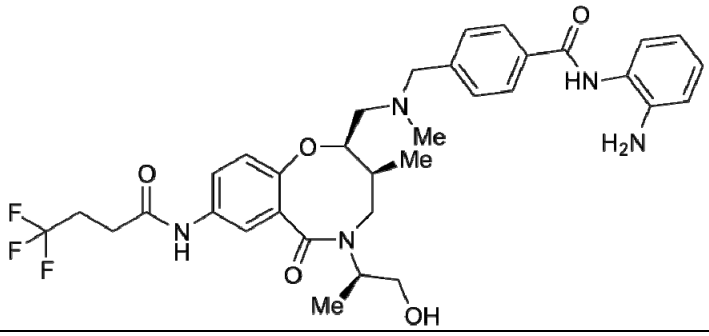
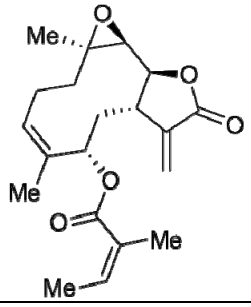
102	BRD8430[b]		O=C(CC(C=C(C=C1)N(C)C)=C1O[C@H]([C@H](C2)C)CN(C)CC3=CC=C(C(NC4=C(N)C=CC=C4)=O)C=C3)N2[C@@H](CO)C	
103	BRD3521[a]		O=C(C(C=CC=C1NC(C2=CC=NC=C2)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	
104	BRD1413[d]		OCCCOC1=CC=C(C2=N[C@](C(N3CCOCC3)=O)(CC4=CC=CC=C4CN=[N+]=[N-])CO2)C=C1	
105	BRD5757[a]		O=C(C(C=CC=C1N(C)C)=C1O[C@@H]([C@@H](C2)C)CN(C)CC3=CC=C(C(NC4=CC=CC=C4N)=O)C=C3)N2[C@H](CO)C	
106	BRD0257**		C1(CCN2C(N=C(N=N3)SCC4=CC5=NON=C5C=C4)=C3C6=C2C=CC=C6)=CC=CC=C1	

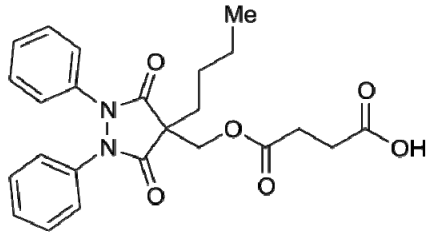
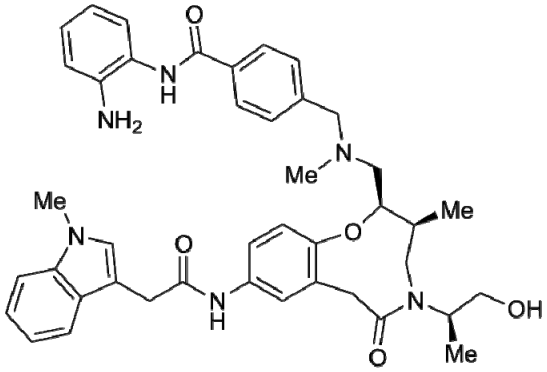
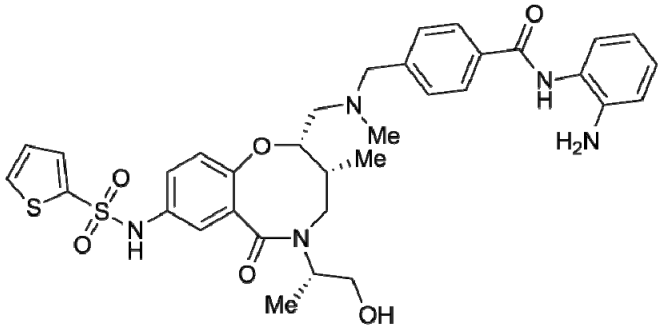
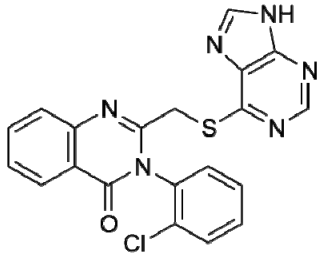
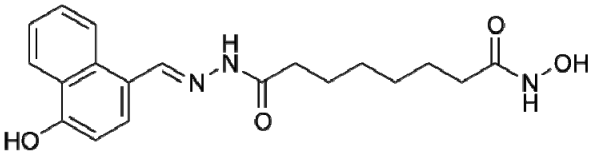
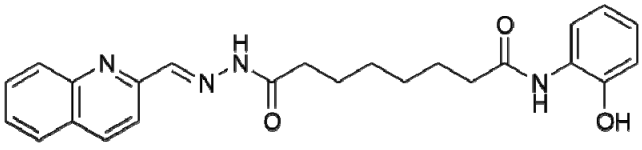
107	BRD6992[a]		O=C(C1=CC=CC(NC(C2=CC=C(C=C2)C3=NC=CS3)=O)=C1O[C@@H]([C@@H](C4)C)CN(C)CC5=CC=C(C(NC6=CC=CC=C6N)=O)C=C5)N4[C@H](CO)C	SPT
108	BRD6975[b]		O=C(C1=CC(NC(NC2=CC=C(C=C2)F)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@@H](CO)C	
109	BRD58870[e]		O=C(N([C@H]1C2=CC=C(CO)C=C2)CC3(C4)C[C@@H](C[C@@H]4C5)C[C@@H]5C3)C6=C(C=CC=C6)[C@H]1C(NCCC(C=C7)=CC=C7OC)=O	
110	NSC 119889[§§§]		OC(C1=C(C(Br)=C(C(Br)=C1Br)Br)C2=C(C=C3)C(OC4=C2C=CC(O)=C4)=CC3=O)=O	CC10
111	BRD9907[a]		O=C(N(C[C@H]([C@H](O1)CN(C)CC2=CC=C(C(NC3=C(C=CC=C3)N)=O)C=C2)C)[C@H](C)CO)C4=C1C(NC(NC(C=C5)=CC=C5OC)=O)=CC=C4	

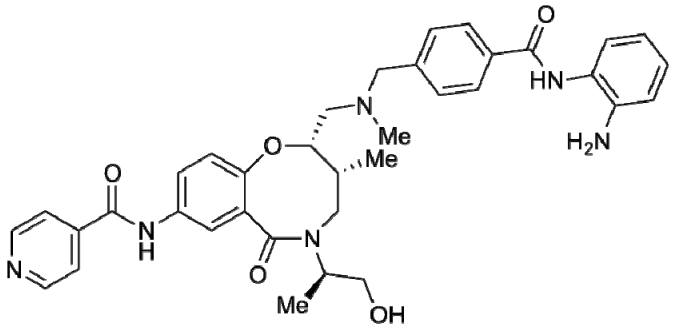
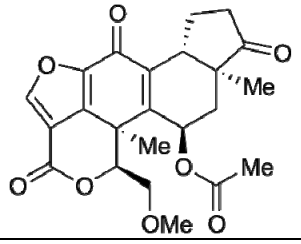
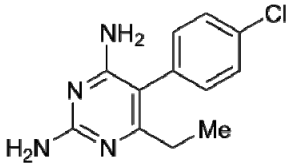
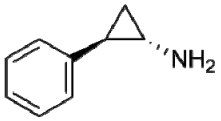
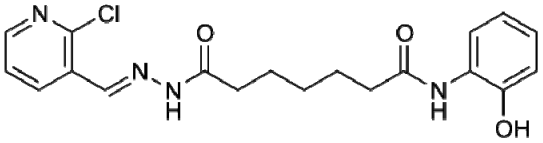
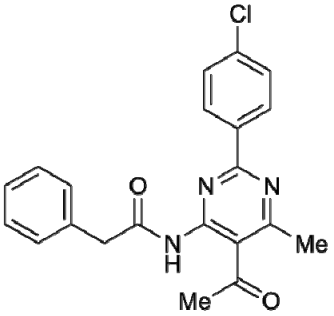
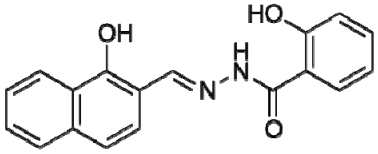
112	BRD5081[b]		O=C(C1=CC(N(C)C)=CC=C1O[C@H]([C@@H](C2)C)CN(C)CC3=CC=C(C(NC4=C(C=CC=C4)N)=O)C=C3)N2[C@@H](CO)C	CC5
113	BRD31108[b]		O=C(CC(C=C(C=C1)NC(NC2=CC=C(C=C2)F)=O)=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(N)C=CC=C5)=O)N3[C@@H](CO)C	CC10
114	BRD0508[a]		O=C1C2=CC=CC(NC(NC3=CC=C(C=C3)OC)=O)=C2O[C@H](CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)[C@@H](C)CN1[C@@H](C)CO	
115	BRD9652[a]		O=C(C(C=CC=C1NC(C2CC2)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	
116	BRD6350[b]		O=C(CC(C=C(C=C1)NC(CC2=CC=CC=C2)=O)=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(N)C=CC=C5)=O)C=C4)N3[C@@H](CO)C	

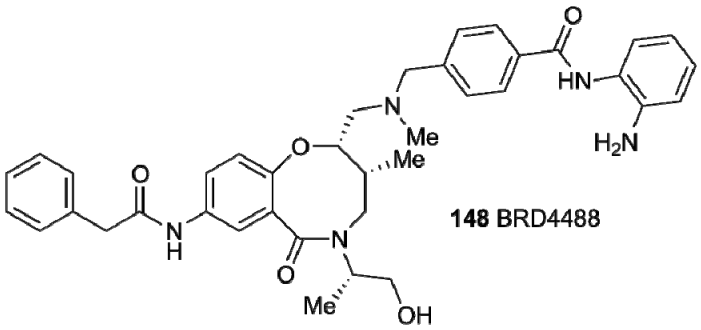
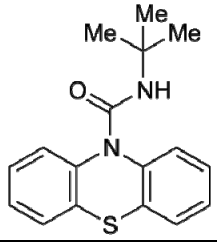
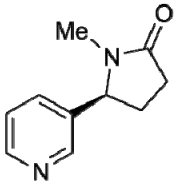
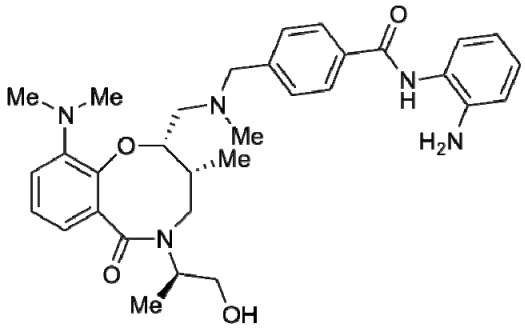
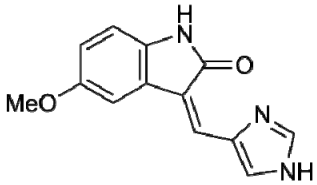
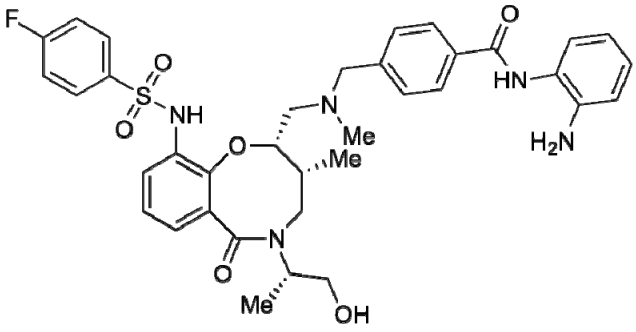
117	BRD0983[a]		O=C(C(C=CC=C1NC(C2CCCCC2)=O)=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(C=CC=C5)N)=O)N3[C@@H](CO)C	
118	BRD8258[c,e,g]		BrC(N=CC=C1)=C1/C=N/NC(CCCCCC(NC2=C(O)C=CC=C2)=O)=O	
119	BRD2553[c,e,g]		ONC(CCCCC(N/N=C/C1=CC2=C(C=C1)C(C=CC=C3)=C3C2)=O)=O	
120	tryphostin AG-1478[§§]		ClC1=CC(NC2=NC=NC3=C2C=C(OC)C(OC)=C3)=CC=C1	
121	BRD1698[c,e,g]		ONC(CCCCC(N/N=C/C(SC=C1)=C1C)=O)=O	
122	BRD6717[‡]		O=C(C[C@@H]1[C@H]2[C@@H]3[C@H](C(C(O3)=O)=C)[C@@H](OC(C(CO)=C)=O)CC1=C)[C@@H]2C	
123	BRD3438[b]		O=C(CC1=CC(NS(C2=CC=CS2)(=O)=O)=CC=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(N)C=CC=C5)=O)N3[C@@H](CO)C	

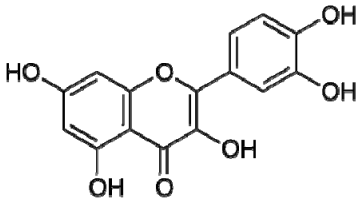
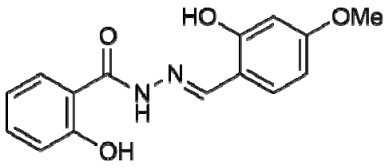
124	BRD4729[b]		O=C(CC(C=C(C=C1)NC(C2=CC=NC=C2)=O)=C1O[C@H]([C@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(N)C=CC=C5)=O)N3[C@@H](CO)C	
125	BRD1581[c,e,g]		OC1=CC=CC(/C=N/NC(C2=CC=CC=C2O)=O)=C1O	CL10
126	BRD5099[b]		O=C(C1=CC(NS(C2=CC=C(C)C=C2)(=O)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	
127	BRD8711[b]		O=C(CC(C=C(C=C1)NC(CCCCCC(NC2=C(N)C=CC=C2)=O)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4CCCCC4)N3[C@H](CO)C	
128	BRD4192**		OC1=CC=NC(SCC(NC2=CC=C(OC3=CC=CC=C3)C=C2)=O)=N1	
129	BRD6276[c,e,g]		O=C(C1=CC=CC=C1O)N/N=C/C2=CN(C3=C2C=CC=C3)C	

130	BRD7016[f]		O=C(OCC1)N1C(C=C2)=CC=C2CN(C3=O)C4=C(C=CC=C4)[C@@]3(O[C@H]5CCO)[C@H](C)[C@H]5[Si](C)(C)C6=CC=C(C=C6)OC	
131	BRD7283[b]		O=C(N(C[C@@H]([C@@H](O1)CN(C)CC2=CC=C(C=C2)C(NC3=C(N)C=CC=C3)=O)C)[C@H](C)CO)CC4=C1C=CC(NC(NC(C)C)=O)=C4	
132	BRD1933[‡‡]		OC(C1=CC=CC=C1)(CC2=CC=CN=C2)C3=CC=CC=C3	
133	BRD7942[b]		O=C(C1=CC(NC(CCC(F)(F)F)=O)=CC=C1O[C@@H]([C@H](C2)C)CN(C)CC3=CC=C(C(NC4=C(C=CC=C4)N)=O)C=C3)N2[C@@H](CO)C	
134	BRD3137[‡]		C/C=C(/C)\C(=O)O[C@H]1C[C@@H]2[C@H](OC(=O)C2=C)[C@@H]3O[C@]3(C)CCC=C1C	

135	Suxibuzone[†]		O=C(N(N(C1=O)C2=CC=CC=C2)C3=CC=CC=C3)C1(COC(CCC(O)=O)=O)CCCC	
136	BRD3661[b]		O=C(CC(C=C(C=C1)NC(CC2=CN(C)C3=C2C=CC=C3)=O)=C1O[C@H]([C@H](C4)C)CN(C)CC5=CC=C(C(NC6=C(N)C=CC=C6)=O)C=C5)N4[C@@H](CO)C	
137	BRD2745[b]		O=C(C1=CC(NS(C2=CC=CS2)(=O)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	CL5
138	BRD3842**		ClC1=C(C=CC=C1)N2C(C(C=CC=C3)=C3N=C2CSC4=NC=NC5=C4N=CN5)=O	
139	BRD0122[c,e,g]		ONC(CCCCCCC(N/N=C/C1=CC=C(O)C2=C1C=CC=C2)=O)=O	CL5
140	BRD8422[c,e,g]		O=C(N/N=C/C1=NC(C=CC=C2)=C2C=C1)CCCCCCC(NC3=CC=CC=C3O)=O	CC10

141	BRD9825[b]		O=C(C1=CC(NC(C2=CC=NC=C2)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C=C4)C(NC5=C(C=CC=C5)N)=O)N3[C@@H](CO)C	
142	wortmannin[§§]		O=C(O1)C2=COC(C(C3=C4[C@@H](C[C@]5([C@H]3CCC5=O)C)OC(C)=O)=O)=C2[C@]4(C)[C@H]1COC	CC5
143	pyrimethamine[†]		ClC(C=C1)=CC=C1C(C(N)=NC(N)=N2)=C2CC	
144	tranylcypromine[†]		N[C@@H]1[C@H](C1)C2=CC=CC=C2	
145	BRD0686[c,e,g]		ClC(N=CC=C1)=C1/C=N/NC(CCCCCC(NC2=CC=CC=C2O)=O)=O	
146	BRD8085**		O=C(CC1=CC=CC=C1)NC(N=C(N=C2C)C3=CC=C(Cl)C=C3)=C2C(C)=O	
147	BRD4355[c,e,g]		OC1=C(C=CC=C1)C(N/N=C/C2=C(C(C=CC=C3)=C3C=C2)O)=O	

148	BRD4488[b]	 **148** BRD4488	O=C(C1=CC(NC(CC2=CC=CC=C2)=O)=CC=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	
149	BRD4081[††]		O=C(N1C(C=CC=C2)=C2SC3=C1C=CC=C3)NC(C)(C)C	
150	cotinine[†]		O=C(CC[C@H]1C2=CC=CN=C2)N1C	
151	BRD5036[a]		O=C(C1=CC=CC(N(C)C)=C1O[C@@H]([C@@H](C2)C)CN(C)CC3=CC=C(C(NC4=C(C=CC=C4)N)=O)C=C3)N2[C@@H](CO)C	
152	SU 9516[§§§]		O=C(N1)/C(C2=C1C=CC(OC)=C2)=C\C3=CNC=N3	
153	BRD7637[a]		O=C(C1=CC=CC(NS(C2=CC=C(F)C=C2)(=O)=O)=C1O[C@@H]([C@@H](C3)C)CN(C)CC4=CC=C(C(NC5=C(C=CC=C5)N)=O)C=C4)N3[C@H](CO)C	

154	quercetin[†]		OC1=CC2=C(C(O)=C1)C(C(O)=C(O2)C3=CC=C(O)C(O)=C3)=O	
155	BRD6379[c,e,g]		OC1=CC(OC)=CC=C1/C=N/NC(C2=C(C=CC=C2)O)=O	CL10

* MicroSource Discovery Systems, Inc.
[†] Prestwick Chemical
[‡] Analyticon
[§] TimTec
** ChemBridge
[††] ChemDiv
[‡‡] Maybridge
[§§] Biomol
*** Sigma
[‡‡‡] MPBio
[§§§] CalBioChem

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[c] http://chembank.broadinstitute.org/chemistry/search/execute.htm?id=5685018
[d] Mitchell, J.M. & Shaw, J.T. A Structurally diverse library of polycyclic lactarns resulting from systematic placement of proximal functional groups. *Angew Chem Int Ed Engl* **45**, 1722-6 (2006).
[e] Vegas, A.J. et al. Fluorous-based small-molecule microarrays for the discovery of histone deacetylase inhibitors. *Angew. Chem., Int. Ed.,* **46**, 7960-4 (2007).
[f] Franz, A.K., Dreyfuss, P.D. Schreiber, S.L. Synthesis and cellular profiling of diverse organosilicon small molecule. *J Am Chem Soc* **129**, 1020-1 (2007).
[g] Tang, W., Luo T., Greenberg, E.F., Bradner, J.E. & Schreiber, S.L. Discovery of histon deacetylace & selective inhibitors. *Bioorg Med Chem Lett* **21**, 2601-5 (2011).

O Hits showing stromal pretreatment effects for LSCe cells cultured with OP9 stroma are noted (SPT), as are compounds that displayed ten-fold or five-fold greater potency against LSCe cells in co-culture relative to hAML cell lines (CC10, CC5, respectively; see Methods). Compounds that displayed ten-fold or five-fold greater potency against the hAML cell lines relative to LSCe cells in co-culture are also noted (CL10, CL5, respectively).

Supplementary Table 4 | Representative raw data for human CAFC assays

Lovastatin	AML 3	AML 6	Normal
0 (DMSO)	**16/16** (100%)	**23/24** (95.8%)	**24/24** (100%)
0.125 μM	**6/6** (100%)	**5/6** (83.3%)	**6/6** (100%)
0.25 μM	**6/12** (50%)	**6/12** (50%)	**12/12** (100%)
0.5 μM	**5/12** (41.7%)	**4/12** (33.3%)	**12/12** (100%)
0.75 μM	**3/12** (25%)	**3/12** (25%)	**12/12** (100%)
1 μM	**1/6** (16.7%)	**0/6** (0%)	**6/6** (100%)
2 μM	**0/6** (0%)	**0/6** (0%)	**6/6** (100%)

The data is shown as the ratio of the number of replicate wells positive for cobblestone areas relative to the total number of replicates.

Supplementary Table 5 | EC50 values for additional statins in co-culture with BMSC stromal cells

Statin	LSCe EC_{50} (nM)	HSPC EC_{50} (nM)
Cerivastatin	< 10	> 20,000
Simvastatin	15	> 20,000
Fluvastatin	28	> 20,000
Rosuvastatin	1,200	> 20,000
Atorvastatin	1,900	> 20,000

Supplementary Table 6 | Number of different shRNAs depleted by 20-fold relative to control shRNAs during *in vivo* leukemiogenesis

Gene	# shRNAs scoring
Hmgcr	3
Fnta	2
Icmt	2
Fdft1	1
Rabggtb	1
Fdps	1
Pggt1b	0
Rabggta	0

Supplementary Note 1 | High-Throughput Co-Culture Assay Methods

LSCe Co-culture Screen. Liquid dispensing was performed using an automated liquid dispenser (Multidrop Combi) or a multichannel pipettor, and liquid removal was performed with a Microplate washer (ELx405, BioTek) or 24-channel wand aspirator (VP186L, V and P Scientific). In 384-well plates (3712, Corning), wells were coated with 10 µl of 0.1% gelatin (ES006B, Chemicon International), incubated for 15 minutes, then washed with PBS. 6,750 OP9 cells in 50 µl of OP9 media were then added to each well with time spent in suspension at plating kept to a minimum for these cells. All incubation steps included the addition of a breathable plate cover (B90112, VWR) to avoid evaporation. After 24 hours of incubation at 37˚C/ 5% CO_2, the media was aspirated and 300 freshly isolated LSCe cells were added to each well in 50 µl of 50% OP9 media (pre-conditioned on OP9 cells for 3 days), and 50% co-culture media (500 ml DMEM (11965-092, Gibco), 10% Horse Serum (26050-088, Gibco), 1:100 Hydrocortisone (07904, StemCell Technologies), 2.5 ml Beta-mercaptoethanol (ES-007-E, Chemicon International), 10% FBS (10082-147, Gibco) and 1% Pen-Strep). After a 24 hour incubation, the plates were briefly centrifuged for 30 seconds (60 x gravity, slow braking) and 100 nl of test compounds in dimethyl sulfoxide (DMSO), DMSO alone, or XK469 (X3628, Sigma) were pin transferred

to a final concentration of 5 μM in 0.2% DMSO and the plates were re-incubated for 3 days. The media was aspirated, 50 μl of fresh media (the same 50/50 media mix added at LSCe cell plating) was added and 100 nl of compound was again added to the appropriate wells. Plates were returned to incubator for 2 days then imaged at 10x total magnification in the dsRed and GFP channels.

HSPC Co-Culture Screen. 2,000 Primary murine BMSCs were plated in 30μL per well in 384-well plates (3712, Corning) pretreated with fibronectin (20 μg/mL fibronectin (Millipore) for 30 minutes at 37°C). Breathable plate covers were added and plates were spun at approximately 60 x gravity, incubated at room temperature for 60-90 minutes then kept at 33°C/ 5% CO_2 for 3 days. Murine HSPCs were plated in phenol-red free alpha-MEM with 20% FBS (20 μL containing 200 cells per well) and incubated at 33°C/ 5% CO_2 overnight, after which 100 nl of test compounds in DMSO, or control compounds were pin transferred to a final concentration of 20 μM in 0.2% DMSO. The plates were then incubated at 33˚C/ 5% CO_2 for 6 days. The co-cultures were imaged using dsRed and GFP filters at 4x total magnification. The total number of HSPCs per well was quantified using CellProfiler software.

Quantification of Cobblestone Areas. An image analysis pipeline comprised of multiple algorithms for CAFC quantification was developed using CellProfiler[25] software (http://www.cellprofiler.org/published_pipelines.shtml). First, each of the nine dsRed images capture per well at 10x magnification was processed by masking the well boundary and correcting for illumination variation (a consequence of optical hardware irregularities, illumination patterns, or shading). Next, each individual dsRed-positive region (LSCe cells) was segmented within the well into one or more subcellular areas termed cell “objects” (see Supplementary Fig. 1d). Each object was then assessed for hundreds of characteristics including intensity, area, shape, object neighbors, and texture. These per-object measurements served as input to guide a biologist-supervised machine learning routine in the classification of CAFC versus non-CAFC objects. A gentle boosting classifier[26] was iteratively trained to learn rules to distinguish between the two phenotypes. Every object in every image of a given image set was then scored as either CAFC or non-CAFC using the set of 50 measurement rules returned from

the classifier. Each of nine sites imaged per well was analyzed independently, and the image processing was parallelized. The measurements were automatically merged and stored in a MySQL database (Oracle, Inc.). As cell objects did not correspond 1:1 to the number of cells, 'total cobblestone area per well' was used as a suitable proxy for total CAFC count per well. To determine the sensitivity and specificity of the pipeline, sets of more than 100 representative objects were presented to expert biologists for direct, manual classification. The biologists' classification was compared to the classification determined by the machine learning routine. The full confusion matrix for a representative example was as follows: True positives (i.e. True CAFC) = 36, True negatives (i.e. False CAFC) = 88, False positives (i.e. True nonCAFC) = 14, False negatives (i.e. False non-CAFC) = 5.

Filtering and Retest. 415 compounds scored in the LSCe primary screen. Of these, compounds that decreased total HSPC number by greater than 80% in both replicates relative to DMSO controls (Stewart, A.L., Scadden, D.T., et al., in preparation) or that overtly killed stromal cells in the primary HSPC screen were excluded, yielding 270 compounds. 240 of these were retested on LSCe cells co-cultured with OP9 stroma (8 concentrations per compound) and with BMSCs (4 concentrations per compound). For the BMSC co-culture, 300 LSCe cells in 20μL of fresh BMSC medium were added per 384-well to the existing wells containing stroma in 30μL of media plated 3 days prior). 196 compounds demonstrated an $EC_{50} \leq 5$ μM against LSCe cells in the presence of one stromal type (with 139 showing activity on both types) and were selected for further study.

Curve Fitting and Determination of EC_{50} Values. Refined curve fitting and EC_{50} values (Figs. 3a, 3b, 4a, and Supplementary Figs. 2a, 2b, 3b, 3d, 3e, 4a, 5e) were computed and visualized using MATLAB. For each experiment combining an experimental design, cell line or primary cell population, and small molecule, we extracted n value pairs (x,y) corresponding to n independent measurements of effect (y, normalized % of positive control) *versus* concentration ($x = \log_2[\mu M]$) across all experiments, $12 \leq n \leq 48$ (median $n = 24$) value pairs. For each experiment, we fit two types of models to each concentration response: 4-parameter sigmoid functions and 1-parameter constant functions. In some cases,

individual data points appeared significantly aberrant from the others, so we also computed a version of each model, sigmoid or constant, after censoring individual points that failed to meet an outlier condition defined by Cook's Distance[1,2], with $D_{cook} > 4/n$ [2]. For each model, we evaluated the fit parameters and their confidence intervals (CIs) to determine which of the four models was most appropriate to use for a given experiment. Models for which any of the parameter CIs were infinite (i.e., those models that did not converge) were rejected, as were sigmoid models whose height parameter was less than 25% of the positive control effect; conversely, we rejected constant models whose parameter CI range was more than 25% of the positive control effect. These filters were sufficient to indicate whether a sigmoid or constant model should be used, and we always selected the uncensored version of constant models if both were available. Some uncensored sigmoid models retained unrealistic slope parameters (too steep), so we chose the corresponding censored sigmoid model in cases where censoring improved the CI range on the slope parameter by at least 10-fold. Dose-response curves were plotted using the best available model according to these criteria. To report EC_{50} values for sigmoid models, we used the *x* value of the sigmoid inflection point when that point fell within the tested concentration range. When the sigmoid inflection point fell outside the tested concentration range, we reported EC_{50} values as inequalities (e.g. "< 10 nM"), rather than extrapolating. For all other experiments, data were normalized and EC_{50} values were calculated using Pipeline Pilot (Accelerys, Inc.) and GeneData Screener (GeneData).

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