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Cytotoxic drugs that are mechanistically distinct from current chemotherapies are attractive components of personalized combination regimens for combating aggressive forms of cancer. To gain insight into the cellular mechanism of a highly potent platinum–acridine hybrid agent, we performed a correlation analysis of NCI-60 compound screening results and gene expression profiles. We discovered a plasma membrane transporter, human multidrug and toxin extrusion protein 1 (hMATE1, SLC47A1), as the dominant pan-cancer predictor for cancer cell chemosensitivity to the hybrid agent. We have validated the role of hMATE1 using transporter inhibition, gene knockdown, and chemical sensitization assays. The results suggest that hMATE1 may have applications as a molecular marker to identify and target tumors that are likely to respond to platinum–acridines. Furthermore, enhancement of hMATE1 expression by epigenetic drugs emerges as a potential co-treatment strategy to sensitize tumor tissue to platinum–acridines and other anticancer drugs transported by hMATE1.
A Membrane Transporter Determines the Spectrum of Activity of a Potent DNA-Targeted Hybrid Anticancer Agent

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ABSTRACT: Cytotoxic drugs that are mechanistically distinct from current chemotherapies are attractive components of personalized combination regimens for combatting aggressive forms of cancer. To gain insight into the cellular mechanism of a potent platinum–acridine anticancer agent (compound 1), a correlation analysis of NCI-60 compound screening results and gene expression profiles was performed. A plasma membrane transporter, the solute carrier (SLC) human multidrug and toxin extrusion protein 1 (hMATE1, SLC47A1), emerged as the dominant predictor for cancer cell chemosensitivity to low-nanomolar concentrations of the hybrid agent (Pearson correlation analysis, $p < 10^{-5}$) across a wide range of tissues of origin. The crucial role of hMATE1 was validated in an in vitro model of lung adenocarcinoma (A549), which expresses high levels of the membrane transporter. This was achieved with transporter inhibition assays and transient knockdown of the SLC47A1 gene, in conjunction with quantification of intracellular accumulation of compound 1 and cell viability screening. HCT-116 colon cancer cells, in which hMATE1 is epigenetically repressed, can be sensitized to compound 1 by priming the cells with the polycomb repressive complex 2 (PRC2)-targeted drugs EPZ-6438 (tazemetostat) and EED226. The results suggest that hMATE1 may have applications as a pan-cancer molecular marker to identify and target tumors that are likely to respond to platinum–acridines. Furthermore, enhancement of hMATE1 expression by epigenetic drugs may be a potential co-treatment strategy to efficiently deliver platinum–acridines and other clinical anticancer drugs transported by hMATE1 to tumor tissue.

KEYWORDS: drug discovery, gene expression, membrane transport, platinum–acridines, SLC47A1
INTRODUCTION

Since the FDA approval of cisplatin (Figure 1a), chemically unique approaches have been pursued to improve the efficacy and safety of platinum-based chemotherapy. The design of several of the newer-generation nonclassical metallodrugs is based on the premise that tumor resistance can be overcome at the DNA level as a consequence of the agents’ unique DNA binding modes and DNA damage response (DDR) patterns. This reasoning has redefined the landscape of platinum anticancer drug discovery and resulted in promising new clinical and preclinical candidates. One type of compound in preclinical development are platinum–acridine agents, represented by compound 1 (Figure 1b), the most potent derivative identified in this class of cytotoxics. Platinum–acridines bind to DNA by a mechanism that involves intercalation and platination of nucleobase nitrogen, causing a more severe form of DNA damage than the cross-links observed for cisplatin. On a per-adduct basis, the hybrid agents are more potent inhibitors of DNA synthesis than cisplatin, which induce replication fork arrest and a high level of DNA double-strand breaks requiring specialized DNA repair modules, and are more efficient transcription inhibitors. These mechanisms most likely contribute to the high cytotoxicity of platinum–acridines, particularly in non-small-cell lung cancer (NSCLC), where the hybrid agents show up to 1000-fold higher activity than cisplatin. Collectively, the results from mechanistic studies in cell-free systems, human cancer cells, and chemical genomic fitness profiling in S. Cerevisiae are consistent with nuclear DNA as the principal target of these agents.

Platinum–acridines show a dramatically higher activity than cisplatin in NSCLC, even though the hybrid adducts are repaired more rapidly than the classical cross-links in these notoriously DNA repair-proficient cells. These findings call into question whether the damage at the genome level and cellular response platinum–acridines cause alone overcome chemoresistance in NSCLC. In this article, we report the results of a study that combined activity screening and gene expression correlation analysis, as well as functional target validation performed on compound 1. We not only demonstrate a complete lack of similarity of the compound’s antitumor profile with that of the classical platinum drugs, but also
discovered a membrane transporter, human multidrug and toxin extrusion protein, hMATE1 (SLC47A1), as the single most predictive marker of chemosensitivity to platinum–acridines and demonstrate its potential utility as a target for personalized cancer treatment.

EXPERIMENTAL SECTION

**Compound Screening.** Compound 1 was tested by the NCI Developmental Therapeutics Program in a panel of 59 cancer cell lines in a one-dose screen at 10 μM test compound and in five-dose screens over a concentration range of 10⁻⁴ to 10⁻⁸ M. Five-dose screens were performed in duplicate. Reported GI₅₀ values and the chemosensitivity profiles (mean graph) are means of the two experiments. All correlation analyses were based on GI₅₀ assay endpoints.¹⁰

**Correlation and Gene Set Overlap Analysis.** Comparative analysis of NCI-60 activity profiles based on GI₅₀ end points was performed with the COMPARE analysis tools¹¹ (dtp.cancer.gov/private-compare) versions 20190306 and 20190828. Both the Standard Agents and Marketed Drugs databases were searched using GI₅₀ values as the endpoint and the following parameters: min. Pearson correlation coefficient, \( R = 0.00 \); min. number of common cell lines in seed and target vector, 55 or 56; min. standard deviation for seed and target vector, 0.05; number of results, 2000. Correlations between GI₅₀ values and gene expression patterns based on transcript levels (z-scores) from 5 different microarray platforms were analyzed in a similar manner for a total of 58 cell lines with a minimum correlation of \( R = ± 0.30 \) (for \( N = 58, R = ± 0.259 \) is statistically significant at \( p < 0.05 \)). The CellMiner tool¹²,¹³ was used to compare the gene expression, DNA copy number alteration, and DNA methylation status for SLC47A1 across NCI-60 cell lines (database version 2.2, https://discover.nci.nih.gov.cellminer; human genome version HG19, number of genes: 25683). Correlation analysis of SLC47A1 transcript levels (average log2 intensities) and DNA methylation (scores 0–1 for completely unmethylated to completely methylated gene promoters) was done with CellMinerCDB (version 1.1; discover.nci.nih.gov/cellminercdb), which implements the GDSC (Sanger Institute) cell line set and databases.¹² Correlations between ad-hoc
defined gene sets and the MSigDB gene sets encompassing a total of 38055 genes were calculated using hypergeometric distribution analysis with a false discovery rate q-value < 0.05 (gsea-msigdb.org).14

**Drugs, Reagents, siRNA, and Antibodies.** Compound 1 was synthesized according to a published procedure (analytical purity > 97% for NCI-60 and all cell-based assays). All biological assays were performed with appropriately (serially) diluted 10 mM stock solutions of compound 1 in dimethylformamide (DMF). DMF controls were included in all experiments to confirm that the solvent had no effect on cell viability and other assay parameters. The epigenetic drugs, EED226 (HY-101117), tazemetostat (EPZ-6438) (HY-13803), valproic acid (HY-10585) and decitabine (HY-A0004) were purchased from MedChemExpress (Monmouth Junction, NJ, USA). Pyrimethamine (46706), phenazine methosulfate (PMS, P9625), and bovine serum albumin solution (BSA, A8412) were purchased from Sigma Aldrich (St. Louis, MO, USA). MTS reagent was purchased from Promega (G1112) (Madison, WI, USA). RIPA buffer (89901), protease inhibitor mix (87785), and BCA Protein Assay Kit (23227) were purchased from Thermo Fisher (Waltham, MA, USA). Lipofectamine transfection reagent, RNAiMAX, was purchased from Invitrogen (13778100) (Carlsbad, CA, USA). Opti-Mem reduced serum media was purchased from Gibco (31985062) (Gaithersburg, MD, USA). The hMATE1 (SLC47A1)-specific pre-designed siRNA and scrambled RNA controls were purchased from Thermo Fisher (Life Sciences Solutions, Carlsbad, CA, USA): Silencer siRNA, ID: 140539; sense: 5’-CCGAGACAUCAUUAACUGtt-3’, antisense: 5’-CAGAUUAAUGAUGUCUCGgtc-3’; Silencer Select siRNA1, ID: s30533; sense: 5’-CAAACUUGAUUUCCAGUAtt-3’, antisense: 5’-UACUGGGAAUAAGUUGcc-3’; Silencer Select siRNA2, ID: s30534; sense: 5’-GAUCGUAACUGAUUGCCAtt-3’, antisense: 5’-UAGCAACUGAGAUCtgc-3’; Scrambled siRNA control: Silencer Negative Control #3 siRNA (AM4615); (V) Silencer Select Negative Control #1 siRNA (4390843). Antibodies were purchased from the following suppliers and used at the indicated dilutions: anti-MATE1 (SLC47A1) antibody (Abcam, ab104016, immunoblotting, 1:1,000); anti-GAPDH antibody (Bethyl, A300-639A-M, immunoblotting, 1:1,000); goat-anti-rabbit IgG-HRP secondary
antibody (Thermo Fisher, G-21234, immunoblotting, 1:10,000); anti-hMATE1 (SLC47A1) antibody (Thermo Fisher, PA5-25272, immunofluorescence, 1:300); goat-anti-rabbit IgG Alexa Fluor-635 secondary antibody (Invitrogen, A-31576, immunofluorescence, 1:400).

**LC-MS Analysis.** The chemical compatibility of pyrimethamine and compound 1 was tested in PBS-buffered solution at 37 °C for 72 hours. Prior to LC-MS analysis, buffer salts were removed using Pierce C18 spin columns (Thermo Fisher, Cat. No. 89870) and samples were redissolved in HPLC grade solvent. LC-MS profiles were analyzed on a Bruker Amazon-SL LC-MS system equipped with an electrospray source using an Agilent ZORBAX SB-C18 analytical column (5 mm, 4.6 × 150 mm, PN 883975-902). Pyrimethamine did not undergo undesired ligand substitution chemistry with compound 1 (data not shown).

**General Cell Culture Maintenance.** The human cell lines, A549 (lung adenocarcinoma, doubling time 21 h) and HCT-116 (colorectal carcinoma, doubling time 17 hours) were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). A549 cells were cultured in DMEM/F12K media (Thermo Fisher, 11330-032) supplemented with 10% FBS (Thermo Fisher, A3160601) and 10% penstrep (Thermo Fisher, 15070-063), unless stated otherwise. HCT-116 cells were cultured in RPMI 1640 (Gibco, A10491-01) with the same additives as above. Cells were incubated at a constant temperature at 37 °C in a humidified atmosphere containing 5% CO₂ and subcultured every 2–3 days to maintain cells in logarithmic growth. All experiments used cells with passage numbers of less than 20. Cells were tested periodically for mycoplasma infections using Hoechst 33258 DNA staining.

**Uptake of Compound 1 Studied by Confocal Fluorescence Microscopy.** Images were collected on an LSM 880 Confocal Microscope (Carl Zeiss Microscopy) using a 63×/1.4 NA Plan-Apochromatic objective. To allow comparative fluorescence intensity analysis, excitation power, pinhole settings, PMT gain, and offset values across and within imaging sessions for each respective channel were not changed. Zen software 2.5 (blue edition, Carl Zeiss Microscopy GmbH, 2018) was used for image
processing. Panels were assembled and annotated without any additional enhancements of images, unless explicitly stated, in Adobe Photoshop CC, version 2017.1.1.

For transporter inhibition assays, cells were seeded into 35-mm poly-D-lysine-coated glass bottom dishes (MatTek Corporation, Ashland, MA, USA) at a density of $10^5$ cells/mL in 2 mL of medium per dish. Cells were allowed to attached overnight prior to pre-treatment with 10 μM pyrimethamine or vehicle for 20 minutes and subsequent treatment with 10 μM compound 1 for 4 hours. Medium was removed and dishes were washed 3 times with 1 mL of pre-warmed PBS buffer. Cells were then fixed with 4% formaldehyde in PBS (Thermo Fisher) for 15 minutes at room temperature and washed an additional 3 times with PBS before imaging. The fluorescence of acridine was excited with a 405 nm (15 mW) laser at 4.4% and collected between 405-481 nm. The intensity of acridine-related fluorescence in treated cells was estimated by drawing a region of interest (ROI) around each cell, with the bright-field images assisting in identifying the cell perimeter. Signal intensities are averages from all pixels in the ROI minus the background fluorescence. A total of more than 100 individual cells across 4 views of 2 independent experiments were analyzed in this fashion. Automated quantification of fluorescence intensities was also performed using CellProfiler 3.0 with similar results (data not shown).

For RNAi knockdown of hMATE1 (SLC47A1) in imaging assays, A549 cells were harvested from T-75 cell culture flasks and seeded on a 6-well plate at a density of 150,000 cells per well. Silencer Select siRNA1, Silencer Select siRNA2, and scrambled siRNA (Silencer Select Negative Control #1) were thoroughly mixed with RNAiMAX in Opti-Mem media according to manufacturer’s protocol and added to each well. Cells transfected at a final siRNA concentration of 20 nM for 48 hours at 37 °C were detached with trypsin and subcultured at a 1:2 ratio into 35 mm MatTek plates and allowed to attach overnight. Cell culture medium was replaced and supplemented with 10 μM compound 1, and dishes were incubated for 4 hours at 37 °C. Cells were fixed with 4% formaldehyde, permeabilized with 0.5% Triton X-100, washed with PBS, incubated with 7.5% BSA) for 30 minutes at room temperature, and incubated with appropriately diluted primary antibody in 1% BSA for 1 hour at room temperature.
the cells were washed with PBS, they were incubated with the secondary antibody (Goat-anti-Rabbit IgG Alexa Fluor-635), diluted in 1% BSA (1:400 anti-rabbit) for 1 hour at 37 °C. After three PBS washes, samples were imaged immediately or stored in PBS at 4 °C for further testing.

For sensitization assays, HCT-116 cells were harvested from T-75 flasks, seeded on a 24-well plate with glass-like polymer bottom (P24-1.5P, Cellvis, Sunnyvale, CA) with 25,000 cells per well, and allowed to attached overnight. Single drugs or drug combinations were tested in this assay at final concentrations of 2.5 μM EED226, 2.5 μM EPZ-6438, 500 μM valproic acid, and 10 μM decitabine (see assay layout 1, AL1, in the SI). Cells were incubated at 37 °C for 72 hours. Each well was replaced with fresh medium supplemented with 10 μM compound 1, and incubation was continued for 4 additional hours. Each well was washed with 3 times with warm PBS, before cells were fixed with 0.5 mL of 4% formaldehyde at room temperature for 15 minutes. After 3 PBS washes, plates were immediately imaged or stored at 4 °C until analyzed. Subsequent incubations of HCT-116 cells at escalating doses of EED226 and EPZ-6438 were performed analogously (see AL2 in the SI). Representative conditions that were screened for expression levels of hMATE1 were determined by immunofluorescence with anti-hMATE1 antibody as described in RNAi knockdown experiments.

**Uptake of Compound 1 Studied by ICP-MS.** Protocols for the quantification of intracellular platinum–acridines by ICP-MS have been described previously. Briefly, cells collected from the transporter inhibition and hMATE1 knockdown assays (see below) were pelleted and homogenized by microwave-assisted digestion (ETHOS UP Milestone, Sorisole, Italy) in a mixture of dilute, trace-metal grade HCl and HNO₃. Standard curves appropriate for quantification of platinum in specified uptake assays were generated using concentrations of 0 ppt, 20 ppt, 50 ppt, 100 ppt, 200 ppt, and 500 ppt of a diluted Pt standard (High-Purity Standards, Charleston, SC, USA). An 8800 Triple Quadrupole ICP-MS spectrometer (Agilent, Tokyo, Japan) equipped with a SPS 4 automatic sampler, a Scott-type double pass spray chamber operated at 2°C, and a Micromist concentric nebulizer was used for analysis. Helium gas
(≥99.999% purity, Airgas, Colfax, NC, USA) was used in the collision/reaction cell to minimize potential spectral interferences while monitoring the isotope $^{195}$Pt.

For transporter inhibition assays, 700,000 A549 cells in 2.5 mL of F12K media (ATCC 30-2004), supplemented with 10% FBS, and 10% penstrep, and 10% L-glutamine (Thermo Fisher, 25030-081), were seeded into T-25 flasks and allowed to attach overnight. Cells pre-treated with 100 nM pyrimethamine for 25 minutes and untreated cells were then dosed with 100 nM compound 1 for 3 hours. After treatment, medium was aspirated, and cells were washed 3 times with fresh media. Trypsin was added to detach cells, and 3 mL of fresh media were added to each flask to collect the cell suspensions, which were pelleted by centrifugation at 250 × g for 3 minutes. After the supernatant was aspirated, pellets were washed with 3 mL of PBS solution twice and centrifuged again at 250 × g for 3 minutes. Pellets were stored at -80 °C until analyzed by ICP-MS. The assay was performed in triplicate for each treatment group.

For uptake studies after hMATE1 (SLC47A1) knockdown, A549 cells were reverse-transfected with Silencer Select siRNA1 or Silencer Select Negative Control #1 scrambled RNA for 48 hours using the RNAiMAX system in Opti-Mem media. Media was replaced with fresh antibiotics-free DMEM/F12K medium and incubation was continued for an additional 24 hours. Cells were then incubated with 100 nM compound 1 at 37 °C for 4 hours, and cell pellets were prepared as described above. The assay was performed in triplicate for each treatment group. Microwave digestions and ICP-MS analysis for Pt were performed as described above.

**Cell Proliferation Assays.** The cytotoxicity studies were carried out on nonpyrogenic polystyrene 96-well cell culture plates (Corning Inc., Corning, NY, USA) according to a standard protocol using the colorimetric Celltiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI, USA). Relative cell viability was determined from the viability of treated and untreated (control) cells. IC$_{50}$ values were calculated from sigmoidal curve fits of log[compound 1] vs.
response in GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). For the number of replicates and level of significance in each assay, see figure captions in the Results section and information in specific sections below.

In pyrimethamine competition assays, A549 cells were seeded at a density of 5000 cells per well and allowed to attach for 24 hours. Cells were then pre-treated with 10 or 100 nM pyrimethamine for 20 minutes and subsequently incubated with 100 nM compound 1 or DMF-containing media (control) for 72 hours. No-treatment controls were also included. Assays were run in duplicate with 6 replicates per plate. Cell viability was assessed as described above.

Cell viability in RNAi knockdown assays was assessed by transfecting A549 cells on 96-well plates using a reverse transfection protocol. Briefly, Silencer siRNA, scrambled RNA (Silencer Negative Control #3 siRNA), and lipofectamine (RNAiMAX) were diluted with Opti-Mem prior to mixing in each well to generate a final siRNA concentration of 10 nM. Mixtures were incubated for 20 minutes at room temperature. Cells were then seeded into new wells in DMEM/F12 medium without antibiotics at a density of 5000 cells/well, incubated in the presence of transfection reagent for 24 hours at 37 °C in 5% CO₂, and finally treated with compound 1 at fixed concentrations of 100 nM or 1 μM (or DMF-containing media in control groups) for an additional 24 or 48 hours. Cell viability after 48 and 72 hours was assessed as described above.

In HCT-116 sensitization experiments, cells were seeded at a density of 1100 cells/well in 100 μL of media and allowed to attach overnight. Medium in each well was replaced with fresh medium containing a combination of EED226 and EPZ-6438 to generate final concentrations of 2.5 and 5 μM of each drug. Medium supplemented with epigenetic drugs was replaced every 24 hours and finally removed after 72 hours to begin treatment with compound 1 at concentrations of 1 μM and 10 μM for an additional 72 hours. Cell viability after 72 hours was assessed as described above.
**Immunoblotting.** Cells were lysed in RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) according to the manufacturer’s protocol. RIPA buffer was supplemented with protease inhibitors. Plated cells were washed twice with ice-cold PBS buffer and then lysed with cold RIPA buffer for 30 min on ice with occasional swirling. Lysed cells were collected with a cell scraper and transferred into a 15 mL microcentrifuge tube. Cell lysates were then sonicated using a Branson Digital Sonifier 450 (settings: 10% pulse, 1 second on/1 second off, for 20 seconds) and centrifuged at 14,000 × g for 15 minutes at 4 °C. Total protein concentrations were quantified using a BCA Protein Assay Kit.

Protein samples were denatured by incubation in a sample buffer (Thermo Fisher, 39001) supplemented with DTT (50 mM) at 46 °C for 30 min. Equal amounts of total protein were loaded per lane and separated by SDS-polyacrylamide electrophoresis in 4-15% Mini-PROTEAN TGX Precast Protein Gels (Bio-rad, 456-1083) in Tris-glycine SDS buffer (Fisher, BP13414) (30 min at 50 V and 30 min at 120 V). The proteins were wet-transferred to nitrocellulose membranes (Advansta, San Jose, CA, USA, L-08002-010) (2 hours at 100 V) (transfer buffer: 25 mM Tris-base, 190 mM glycine, 20% methanol. adjusted to pH 8.3). Membranes were then (i) blocked in TBST buffer (20 mM Tris, 150 mM NaCl and 0.05% Tween 20, adjusted to pH 7.6, 5% non-fat milk) at room temperature for 1 hour, (ii) incubated with primary anti-MATE1 antibody or GAPDH antibody in TBST buffer (2% non-fat milk) at 4 °C overnight, (iii) washed 4 times for 5 minutes with TBST buffer and incubated with goat-anti-rabbit IgG-HRP secondary antibody in TBST buffer (2% non-fat milk) at room temperature for 1 hour, (iv) washed with TBST 4 times for 5 minutes, and (v) finally incubated with SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo, 34580) at room temperature for 5 minutes. The protein bands were visualized alongside pre-stained protein ladder (PageRuler, Thermo Fisher) using an Amersham Imager 600 (GE Healthcare). Band intensities were integrated using Image J (version 1.52a, National Institutes of Health, Bethesda, MD).
To generate sufficient quantities of cell-free extract for Western blot analysis accompanying knockdown experiments, A549 cells were seeded at a density of 150,000 cells per well on 6-well plates, and transfections were performed with an optimized siRNA concentration of 2.5 nM. Likewise, to quantify hMATE1 (SLC47A1) in epigenetic sensitization assays, 100,000 HCT-116 cells were seeded into 60-mm dishes and treated with a 2.5 μM or 5 μM mixture of EPZ-6438 and EED226. Cell lysates were generated in both cases as described above.

RESULTS

Compound 1 Shows High Potency and a Unique Activity Profile Among DNA-Targeted Anticancer Agents. We took advantage of the 60-cell line screen maintained by the Developmental Therapeutics Program of the US National Cancer Institute (NCI-60) in combination with the COMPARE analysis tools\textsuperscript{11} to study the biological activity of compound 1 and to assess if mechanistic similarities exist with clinically relevant oncology drugs. Compound 1 was screened twice in a library of 59 cell lines from nine different tissues of origin. The 10 cell lines most sensitive to compound 1 (50% growth inhibition endpoint: logGI\textsubscript{50} < -7.75, which corresponds to GI\textsubscript{50} < 18 nM) were NCI-H460, NCI-H226, NCI-H522, and A549 (all NSCLC), SF-295 (glioblastoma), SN12C (renal cell carcinoma), SK-MEL-5 and UACC-62 (both melanoma), DU-145 (prostate), and T-47D (triple-negative breast cancer), representing cancer models from six different tissues of origin and of varying oncogene and tumor suppressor status (Table S1). In six of these cell lines (incl. 3 NSCLC), compound 1 resulted in 50% growth inhibition at single-digit nanomolar concentrations (logGI\textsubscript{50} < -8) (Table S1). Compound 1 showed approximately two orders of magnitude higher activity across the entire spectrum of cell lines than cisplatin, which results in an average growth inhibition similar to that achieved by doxorubicin and topotecan, two oncology drugs also acting through DNA damage-mediated mechanisms (Figure 1b). While the two topoisomerase poisons kill cancer cells at similar inhibitory concentrations as compound 1, they do not show the cell line-specific cytotoxic enhancement of our hybrid agent, which is most notable
in NSCLC. Of the four agents in comparison, compound 1 shows the widest range of activity from low-nanomolar to micromolar GI$_{50}$ values with a more than 2000-fold difference between the most sensitive and the most resistant cell lines (ΔlogGI$_{50}$ > 3.3, Figure S1).

We then used the COMPARE algorithm in conjunction with Pearson correlation analysis$^{17}$ to search the NCI database for test compounds that resulted in NCI-60 activity patterns similar to that of compound 1. The results demonstrate that the mechanism of compound 1 is unique among DNA-targeted cytotoxic drugs and other classes of cancer chemotherapeutics ($R < 0.5$) (Table 1). Of the approved oncology drugs tested in NCI-60, transcription inhibitors and topoisomerase poisons revealed the highest similarity with compound 1. Importantly, cisplatin and oxaliplatin were among the drugs that showed the lowest level of correlation. These results suggest that our hybrid molecule and the traditional platinum-based drugs may not share any relevant mechanistic features except their proven ability to form adducts with nuclear DNA. This raises the question as to whether the unique activity profile of compound 1 might be associated with specific molecular targets or gene expression patterns in cancer cells.
Figure 1. Platinum–acridine agent 1 shows a unique activity profile that correlates with hMATE1 (SLC47A1) expression levels in NCI-60 cell lines. a) Structures of cisplatin and hybrid agent 1. b) Comparative summary of NCI-60 screening results for cisplatin (CDDP), doxorubicin (DOX), topotecan (TOP), and compound 1 based on average growth inhibition (GI_{50} end point, average of 2 assays) for 59 cell lines of different tissues of origin. Asterisks indicate that compound 1 showed cell growth inhibition at log(GI_{50}) < −8 in one or multiple cell lines. A comparison of cell line-specific activity can be found in Figure S1. c) Growth inhibition by compound 1 and SLC47A1 expression are highly correlated in NCI-60 (mean centered profiles). Left panel: relative sensitivity and resistance to compound 1 at 50% growth inhibition (GI_{50}). Right panel: relative transcript intensities (z-scores) for SLC47A1 (z-scores for the cell line MDA-MB-468 were not available). Cell lines are color-coded by tissue of origin. d) Correlation of SLC47A1 expression with chemosensitivity in NCI-60 (Pearson correlation analysis) for positively correlated SLC transporters. A summary of all significantly correlated SLC genes and their (putative) mechanisms of action can be found in Table S3.
**Table 1.** COMPARE Analysis of Chemosensitivity Profiles for Compound 1 and Selected Anticancer Drugs

| test compound      | DNA damage | mechanism                                           | Pearson’s $R$ |
|--------------------|------------|-----------------------------------------------------|---------------|
| Compound 1         | Pt–ICa hybrid | Inhibitor of DNA synthesis and transcription        | 1             |
| Mitomycin C        | Alk, XL    | Inhibitor of rRNA synthesis                         | 0.499*        |
| Doxorubicin        | IC         | Topo II poison, oxidative stress                    | 0.449         |
| Topotecan          | IC         | Topo I poison                                       | 0.344         |
| Actinomycin D      | IC         | Transcription inhibitor                              | 0.286         |
| Bleomycin          | SC         | $O_2$-dependent DNA double-strand breaks             | 0.221*        |
| Erlotinib          | N/A        | Protein kinase inhibitor                             | 0.187         |
| Gemcitabine        | N/A        | Inhibitor of DNA synthesis                           | 0.18          |
| Rapamycin          | N/A        | Inhibitor of mTOR growth signaling                  | 0.123         |
| Paclitaxel (Taxol) | N/A        | Microtubule-targeted mitotic inhibitor               | 0.123*        |
| Cisplatin          | Pt, XL     | Transcription inhibitor                              | 0.116         |
| Vinblastine        | N/A        | Microtubule-targeted mitotic inhibitor               | 0.099*        |
| Oxaliplatin        | Pt, XL     | Inhibitor of replication and transcription, non-DNA damage mediated mechanisms | 0.015         |

*aAbbreviations: Pt, platinating agent; XL, cross-linker; IC, intercalator; Alk, alkylating agent. SC, strand cutter. Asterisks indicate drugs for which no five-dose NCI-60 data were available in the concentration range $−8 < \log[drug] < −4$ (used for screening compound 1). In these cases, correlations were based on analysis of alternative concentration ranges for test compounds.

The Chemosensitivity of Cancer Cells, Regardless of Tissue of Origin, to Compound 1 is Highly Positively Correlated with hMATE1 (SLC47A1) Expression. To gain insight into the factors driving the unique activity profile of compound 1, a comparative analysis of cell growth inhibition data
and global gene expression in NCI-60 cell lines was performed, based on gene transcript (mRNA) levels determined on multiple microarray platforms, which are available as part of the COMPARE tools.\textsuperscript{18,19} COMPARE analysis yielded 806 unique genes correlated positively, and 849 genes correlated negatively ($p < 0.05$) with the growth inhibition of compound 1 (GI$_{50}$ endpoint) across the entire range of cell lines (Table S2). The by far strongest positive correlation ($R = 0.69, p < 10^{-5}$) was observed with the gene $SLC47A1$, which encodes a member of the solute carrier (SLC) family of proteins: human multidrug and toxin extrusion protein 1, hMATE1. hMATE1, a 13-helix transmembrane protein,\textsuperscript{20} shows high expression levels in normal liver and renal tissue (Figure S2), where it serves as a proton-coupled antiporter.\textsuperscript{21} Its primary function is the ATP-independent efflux of organic cations across apical membranes into the bile and urine, which renders hMATE1 an essential modulator of drug response, drug toxicity, and drug–drug interactions.\textsuperscript{22} Aberrantly high expression of hMATE1 is also observed in cancerous tissues (Figure S2).

The above analysis is consistent with a mechanism by which MATE promotes the uptake of compound 1 into cancer cells rather than acting as an efflux pump, which would cause a more resistant phenotype and would have resulted in a negative correlation. A comparison of the NCI-60 screening results for compound 1 with the $SLC47A1$ expression profile (Figure 1c) supports the findings of the COMPARE analysis and illustrates the extent to which the transport protein dominates chemosensitivity. With a few exceptions, cell lines showing high levels of $SLC47A1$ transcript are generally exquisitely sensitive to compound 1, while the opposite is true for cell lines expressing low levels (Figure 1c). Compound 1 performs poorly relative to other DNA-targeted drugs (e. g., doxorubicin and topotecan, Figure S1) across all leukemia cell lines, which invariably show low $SLC47A1$ expression. In cell lines representing solid tumors, considerable cell line-dependent variability exists. For instance, in the two prostate cancer cell lines tested, PC-3 (GI$_{50} \approx 5$ μM, low $SLC47A1$ expression) and DU-145 (GI$_{50} < 10$ nM, high $SLC47A1$ expression), compound 1 shows a more than 500-fold difference in growth inhibition, which is not observed for any other oncology drug in NCI-60. Likewise, the renal carcinoma cell line,
SN12C, which shows the highest level of SLC47A1 expression of all NCI-60 cell lines, most likely due to a gene copy number amplification\(^2\) (Figure S3), was also the most sensitive to compound 1.

SLC47A1 is not the only solute carrier gene whose expression showed a positive correlation with growth inhibition in NCI-60, but only SLC47A1 correlated at such a high level (\(p < 10^{-5}\) vs. \(p < 0.01\) for all other SLC genes; see Figure 1d and Table S3), suggesting a specific and dominant role of this transporter in the mechanism of compound 1. When calculating overlaps between the > 800 genes that were positively correlated with the activity of compound 1 and gene ontology (GO) gene sets deposited in the Molecular Signatures Database (MSigDB\(^1\)), GO terms such as plasma membrane function and components, and intracellular transport ranked highest (Table S4). This is in stark contrast to doxorubicin and topotecan, which showed the greatest overlap with GO sets annotated chromatin, DNA damage recognition and repair, and chromosome organization (data not shown), as would be expected for a genotoxic agent.\(^9\) These observations underpin the notion that, contrary to our expectation, the chemosensitivity of cancer cells to compound 1 is not controlled at the genome level, but by the transportome.

**Pyrimethamine, a Selective hMATE1 Inhibitor, Effectively Blocks Cellular Accumulation of Compound 1 and Quenches Its Cytotoxicity in A549 Cells.** To validate hMATE1 protein as a mediator of chemosensitivity, we first performed a transporter inhibition assay in A549 human lung adenocarcinoma cells. A549 expresses high levels of hMATE1 (SLC47A1) (The Human Genome Database; see Figure S2), which we confirmed by Western blot analysis (Figure S4). Unsurprisingly, the cell line proved to be highly sensitive to compound 1 in the NCI-60 screen (GI\(_{50}\) < 10 nM) and in previous colorimetric cell proliferation assays (IC\(_{50}\) = 3.9 nM).\(^4\) In this assay, prior to treatment with compound 1, cultured A549 cells were pre-treated with the antimalarial drug pyrimethamine (PM, Figure 2a), a potent and selective inhibitor of hMATE1 (reported \(K_i\) values: 77–93 nM\(^2\)). Since the assay required co-incubation of compound 1 and PM, we first confirmed that no undesired reactivity exists between the two agents (Supporting Information). When A549 cells were pre-treated with PM, followed
by a 4-hour exposure to compound 1, confocal microscopy images showed a reduction of intracellular acridine fluorescence by 60% relative to cells not treated with PM (Figure 2b,c). These results suggest that hMATE1-mediated transport across the plasma membrane is directly involved in the cellular uptake of compound 1. Because the microscopy experiments were performed at relatively high concentrations of platinum–acridine and PM (10 µM), contributions from non-specific transport by other membrane proteins cannot be ruled out under these conditions. To overcome this drawback, we took advantage of the parts-per-trillion-level limit of detection of inductively coupled plasma mass spectrometry (ICP-MS) and also quantified uptake of compound 1 from cellular platinum levels under therapeutically more relevant conditions. When cells were pre-incubated with 100 nM PM to avoid non-specific inhibition of other organic cation transporters and subsequently treated with 100 nM compound 1, corresponding to the compound’s IC₉₀ value in A549, a decrease of uptake by 85% was observed (Figure 2d). Together, these findings corroborate that compound 1 is selectively transported across the plasma membrane by hMATE1.

To determine if blocking hMATE1 by PM had an effect on the cytotoxicity of compound 1 in A549 cells, we performed a colorimetric cell proliferation assay (Figure 2e). Exposure to 100 nM compound 1 for 72 hours causes severe cell death with less than 10% of the cells surviving treatment. When A549 cells were pre-treated with PM at concentrations that did not compromise cell viability, a pronounced cytoprotective effect was observed. PM at a concentration of 10 nM was able to significantly (p < 0.01) increase the population of viable cells to 20%, while 100 nM inhibitor resulted in 90% survival (p < 0.0001) of cells treated with compound 1. The level of protection achieved at the latter concentration of PM correlates well with the reduced (85%) platinum levels determined by ICP-MS (Figure 2d), providing additional support for the notion that hMATE1-mediated transport is the key to compound 1’s high potency.
Figure 2. Pyrimethamine (PM) protects A549 lung adenocarcinoma cells from the cytotoxic effects of compound 1 by blocking its cellular uptake. a) PM, a high-affinity, selective inhibitor of hMATE1. b) Confocal fluorescence microscopy images of A549 cells treated for 4 hours with 10 μM compound 1 with or without PM. Scale bars: 20 μm. Acridine fluorescence in the blue channel is displayed in cyan. c) Mean fluorescence intensities in the acridine channel (arbitrary units) of > 100 selected A549 cells (treated according to the conditions in panel b) determined in 6 images from 2 independent experiments; P < 0.0001, mean ± S.D., two-tailed t-test with unequal variance. d) Accumulation of compound 1 (100 nM, 4 hours) in A549 cells in the absence and presence of PM (100 nM) determined by inductively coupled mass spectrometry, ICP-MS; P < 0.01, data are the mean of three independent experiments ± S.E.M, two-tailed t-test. e) Cytoprotective effect of PM-mediated inhibition of uptake of compound 1 into A549 cells monitored by a cell proliferation assay. The results were significant at p < 0.01 and p <
0.0001, respectively. Data are the mean of two experiments performed in sextuplicate ($n = 12$) ± S.E.M, two-tailed t-test.

Gene Knockdown by RNA Interference (RNAi) Further Validates the Role of hMATE1

Protein in the Mechanism of Compound 1. Ultimate evidence for a direct role of hMATE1 transporter in promoting the cellular accumulation and cytotoxicity of compound 1 came from gene knockdown experiments using RNA interference (RNAi). Such an assay is complicated by the non-trivial task of combining transient gene silencing with a long-term cell proliferation assay. Using transfection of appropriate siRNAs, we were able to generate an A549 model in which hMATE1 was transiently reduced by 40–50% relative to scrambled control, which is consistent with reported knockdown efficiencies achieved for the SLC47A1 gene in this cells line using RNAi.26 Knockdown was confirmed by Western blot analysis and immunofluorescence intensity evaluation of transfected cells (Figure 3a,b). The cellular uptake of compound 1 was studied under the same conditions as in the transporter inhibition assay using PM. In hMATE1 knockdown cells, accumulation of platinum was significantly ($p = 0.0091$) reduced by 50% relative to control cells transfected with a scrambled RNA sequence (Figure 3c). We then designed a 96-well plate assay that allowed us to assess the performance of compound 1 in A549 cells after hMATE1 knockdown. After 24 hours of continuous treatment, the dose- and time-dependent cytotoxicity of compound 1 was reduced in A549 cells at concentrations of 100 nM and 1 µM by 12% and 35%, respectively. At the higher concentration, the level of protection persists after 48 hours of treatment, which resulted in a 36% higher survival of hMATE1-silenced cells compared to mock-treated cells. These results unequivocally confirm that hMATE1 protein plays a direct role in the mechanism of compound 1 by mediating its cellular uptake, which ultimately controls the chemosensitivity of the lung cancer cell line.
Figure 3. Transient knockdown of the membrane transporter hMATE1 (SLC47A1) attenuates uptake and cytotoxicity of compound 1. a) Western blot analysis of hMATE1 and GAPDH (loading control) protein levels in A549 cells reverse-transfected with scrambled RNA sequence (“mock”) (left) or hMATE1 siRNA (right) (one 72-hour transfection at 2.5 nM siRNA). b) Immunofluorescence staining of fixed, permeabilized A549 cells 72 hours after siRNA knockdown or mock treatment. Scale bars: 20 μm. c) Uptake of compound 1 into A549 cells after siRNA or mock transfection determined by ICP-MS. Accumulated platinum (ng/10^6 cells) is shown as the mean ± S.E.M. of three independent experiments. The assay was performed several times under slightly varied conditions with similar results (see Figure S5); *p < 0.01, **. d) Effect of hMATE1 knockdown on the cytotoxicity of compound 1 in A549 cells assessed by a cell proliferation assay (MTS). Data are the mean ± S.E.M of two independent experiments performed in triplicate (n = 6; the results were significant at *p < 0.05 (*) and **p < 0.001 (***), respectively; two-tailed t-test). For additional data and replicates, see Figure S5.
Transcriptomics and Gene Set Overlap Analysis Suggest that hMATE1 Expression is Epigenetically Regulated in Many Types of Cancer. Significant correlations exist between SLC47A1 transcript levels and DNA methylation status (CpG islands, CGI) of the gene ($p < 0.001$), as well as correlations involving epigenetic repressors of gene expression, such as DNA methyltransferase I (DNMT1) and the histone methyltransferase, enhancer of zeste homolog 2 (EZH2) (Table S5). Thus, in addition to DNA copy number amplifications (Table S3), epigenetic alterations appear to dominate hMATE1 expression in cancer tissue. This was confirmed in an extended set of 963 cell lines in the Genomics of Drug Sensitivity in Cancer database (GDSC, Sanger Institute) for which SLC47A1 expression is strongly negatively correlated with CGI methylation (Pearson’s $R = -0.32$, $p = 4.9 \times 10^{-25}$) (Figure S6, Table S6). A recent study demonstrates that hMATE1 expression in normal liver tissue is attenuated epigenetically by promoter hypermethylation, which supports the above observations.

We also discovered a link between genes whose methylation status is negatively correlated with SLC47A1 transcript levels in NCI-60 (CellMiner), including SLC47A1 itself, and specific gene sets in the Molecular Signatures Database (MSigDB, gsea-msigdb.org) (Table S7). Hypergeometric distribution analysis of our list of genes (Table S8) showed the highest correlation with genes epigenetically silenced in embryonic stem cells. The process involves EZH2-mediated histone protein H3 trimethylation at lysine 27 (H3K27me3) by the polycomb repressive complex 2 (PRC2) and downstream promoter CGI hypermethylation. These data provide additional clues about hMATE1 regulation at the epigenome level and a potential link between hMATE1 expression, cancer stemness, and drug resistance. Taken together, these observations led us to hypothesize that epigenetic drugs reversing the repression of hMATE1 might increase the uptake of compound 1 and sensitize resistant cancer cells to this agent.
Treatment of HCT-116 Colon Cancer Cells with Epigenetic Drugs Activates hMATE1 Expression and Enhances the Cellular Uptake and Cytotoxicity of Compound 1. To test if cancer cells can be sensitized to compound 1 by priming with epigenetic drugs, we chose the colon cancer cell line HCT-116. HCT-116 cells show low hMATE1 expression caused by repressive modifications in its SLC47A1 promoter region (see Figure S6) and proved to be relatively resistant to compound 1 in NCI-60 (Figure 1c, Figure S1).

We first pre-screened several epigenetic drugs in cultured HCT-116 cells in a multi-well plate format for their ability to increase the uptake of compound 1 using fluorescence microscopy (Figure 4a, see caption for conditions). Cells were treated with four epigenetic drugs that are currently being studied in advanced phase clinical trials: EPZ-6438 (tazemetostat, a potent inhibitor of enhancer of zeste homolog 2, EZH2), EED226 (an allostERIC inhibitor of the polycomb repressive complex 2, PRC2), decitabine (a DNA methyltransferase I, DNMT1, inhibitor), and valproic acid (a histone deacetylase, HDAC, inhibitor), as well as combinations of these drugs (Figure 4b). Epigenetic drugs have previously been demonstrated to enhance the expression of epigenetically silenced genes in HCT-116, including the SLC47A1 gene. EPZ-6438 and EED226, alone or in combination, resulted in enhanced uptake of compound 1, based on the observation of increased acridine-associated, blue fluorescence in the confocal microscopy images, without causing changes in cell morphology and viability (Figure S7). These compounds were then tested again at escalating doses (2.5–20 mM) (Figure 4a). A combination of EPZ-6438 and EED226 (“E/E”) resulted in the most pronounced increase in uptake of compound 1 in a dose-dependent manner (Figure 4c). Additionally, images of representative cells stained with hMATE1 antibody showed a higher level of immunofluorescence compared to the no-treatment control, which was considered preliminary evidence of increased hMATE1 expression (Figure 4d).

We then used a cell proliferation assay to determine if pre-exposing HCT-116 cells to non-toxic concentrations of EPZ-6438 and EED226 sensitized them to compound 1. At higher concentrations, the epigenetic drugs alone also caused significant changes in the cells’ growth characteristics and significant
cell death. Because of this limitation, the experiments were performed with 2.5 µM and 5 µM E/E. When cells were treated with 10 µM compound 1, pre-exposure to E/E resulted in a pronounced decrease in cell viability that was dependent on the dose of epigenetic drug. At 5.0 µM E/E, the maximum enhancement in cell growth inhibition relative to unsensitized control was 45% (Figure 4e). Under these ad hoc conditions, Western blot analysis of lysates from HCT-116 cells show a 20% and 70% increase in hMATE1 levels relative to control at the lower and the higher concentration, respectively (Figure 4f). This observation in conjunction with the microscopy results (Figure 4c,d) strongly suggests that hMATE1 protein is the mediator of the chemosensitizing effect. The results of this proof-of-concept experiment demonstrate the feasibility of sensitizing cancer cells to compound 1 using nontoxic concentrations of epigenetic drugs.
Figure 4. Epigenetic, PRC2-targeted drugs sensitize HCT-116 colon cancer cells to compound 1. 

Schematic layout of drug screening assay. A darker cyan color indicates higher levels of drug accumulation based on fluorescence intensity. Wells labeled ‘C’ are no-treatment controls (DMSO) and crossed-out wells indicate tested concentrations of drug affect cell viability. 

b) Structures of epigenetic drugs used in this assay. 

c) Microscopy images of HCT-116 cells exposed to 10 µM compound 1 for 4 hours after pretreatment with varying concentrations of EPZ-6438 and EED226 (“E/E”) for 72 hours. Scale bars: 20 µm. 

d) Immunofluorescence staining of cells from the control and 10 µM treatment groups. Scale bars: 20 µm. 

e) Viability of HCT-116 cells pre-treated with epigenetic drugs determined using cell proliferation assays (MTS). Data are presented as the mean ± S.E.M. for an assay performed with triplicate wells (*, p < 0.05; **, p < 0.01; two-tailed t-test). For replicates of this assay, see Figure S8. 

f) Expression levels of hMATE1 and GAPDH (loading control) in HCT-116 cells determined by Western blot analysis under the same conditions as described in panel (e).
DISCUSSION

At physiological pH, compound 1 and its derivatives exist as 2+ charged, hydrophilic cations comprising a positively charged platinum(II) moiety and a protonated 9-aminoacridine chromophore ($pK_a = 9–10$). In earlier work, we have demonstrated that the most potent platinum-acridines accumulate in NSCLC cells at a 60–100-fold faster rate than cisplatin, which is consistent with the efficient, SLC transporter-mediated uptake mechanism established in this study. Compound 1 is the first chemotherapeutic agent for which bioinformatics and high-throughput screening tools have identified an overexpressed transport protein as a target that confers a high level of chemosensitivity to cancer cells.

Compound 1 has emerged from a pipeline of platinum–acridine agents that were designed based on the guiding principle that rapid formation of unique DNA adducts would overcome tumor resistance to DNA-targeted drugs, including platinum-based pharmaceuticals. While DNA damage indisputably is the ultimate cause of cancer cell death produced by the hybrid agent, its low-nanomolar activity critically depends on a transport protein, which is an unprecedented feature among anticancer drugs in the NCI-60 database. hMATE1 controls the pattern of activity with a high level of predictability. Cancer cells overexpressing the membrane transporter are highly sensitive to compound 1 regardless of genetic background and phenotypic abnormalities. Efficient transmembrane transport that leads to high intracellular drug concentrations has the potential to overcome common resistance mechanisms such as DNA repair or multidrug resistance-mediated drug efflux.

hMATE1 expression is high in most NSCLC cell lines (Fig. 1c), which explains why the advantage of platinum–acridines over cisplatin and other cytotoxic agents was first noted in this aggressive type of cancer. Membrane transporters that help drugs accumulate in diseased tissue may ultimately result in a more favorable therapeutic window for systemic treatment. Compound 1 has already demonstrated efficacy in xenograft models of A549 in mice when administered intravenously, both directly and as liposomal formulation. Using a non-optimized dosing schedule, the agent was able to reduce tumor growth by 65% with less than 20% weight loss in test animals, which was reversible,
without causing other signs of systemic toxicity. It is possible that hMATE1-enhanced uptake into tumors contributes to the efficacy of compound 1 in vivo.

A few cases have been reported of membrane transporters typically involved in drug elimination that may also enhance drug uptake into tumor tissue. Organic cation transporters (hOCT, SLC22A) are an example of such a dual pharmacokinetic role.\(^{45}\) hOCTs have been shown to enhance the cytotoxicity and efficacy of platinum-containing drugs.\(^ {43,46}\) For instance, in colorectal cancer tissue, high levels of hOCT assist in the cellular uptake of oxaliplatin, which has provided a rationale for the drug’s therapeutic use in this form of cancer.\(^ {47}\) hMATE1 protein, which mediates efflux of substrate from polarized epithelial cells in excretory organs, may play a similar role by transporting substrates across the plasma membrane into cells.\(^ {21}\) This has recently been demonstrated for the clinical kinase inhibitor imatinib (Gleevec) in chronic myeloid leukemia (CML) cells, which enhances the drug’s potency in this hematological cancer.\(^ {48}\) Importantly, in the same study hMATE1 expression levels have been validated as a predictor of interindividual differences in imatinib response and clinical outcome in CML patients.\(^ {48}\) These findings corroborate the critical role solute carrier (SLC) transporters may play in mediating delivery of pharmacologically relevant levels of drug to diseased tissue.\(^ {49}\)

Finally, we provide proof-of-concept data to demonstrate that colorectal cancer cells treated with epigenetic drugs can be sensitized to compound 1 and that the enhanced cytotoxicity is caused by hMATE1-mediated drug accumulation. A growing body of clinical evidence supports the utility of co-administering cytotoxic drugs with epigenetic drugs (see also clinicaltrials.gov). Liu et al.\(^ {50}\) recently demonstrated that renal cell carcinoma (RCC) cells can be sensitized to oxaliplatin by pre-treatment with the hypomethylating agent decitabine, which promotes hOCT2 expression and oxaliplatin accumulation. Another compelling case of epigenetic sensitization has been reported by Gardner et al.\(^ {51}\) for the Schlafen-11 protein (SLFN11), a putative RNA/DNA helicase that acts as a sensor of replicative stress and tumor suppressor.\(^ {52}\) In patient-derived small-cell lung cancer (SCLC) tissue, Schlafen-11, which sensitizes cancer cells to topoisomerase I poisons, was epigenetically silenced.\(^ {51}\) Treatment with
epigenetic drugs restores Schlafen-11 levels, which reverses resistance in SCLC and re-sensitizes cells to the drug topotecan.\textsuperscript{51} There also appears to be an epigenetic component to hMATE1 (\textit{SLC47A1}) expression in SCLC\textsuperscript{53} (sclcelllines.cancer.gov). Since topotecan is a substrate of hMATE1,\textsuperscript{54} the reported level of sensitization to the topoisomerase I poison in SCLC cell lines after treatment with EPZ-6438\textsuperscript{51} may also reflect higher drug accumulation due to increased levels of hMATE1. Using compound 1 as a cytotoxic component in similar combination regimens to treat SCLC and other cancers not responding optimally to our hybrid agent (e.g., leukemias, colorectal cancer, ovarian cancer, see Figure 1), would be an attractive opportunity.

**CONCLUSION**

In summary, the current study provides the mechanistic basis for the unique spectrum of anticancer activity of a platinum–acridine hybrid agent, compound 1. The data demonstrates that the fate of a cancer cell treated with compound 1 is decided at the plasma membrane. The results underscore the crucial role of hMATE1 in mediating intracellular delivery of oncology drugs and as a potential pan-cancer marker of drug responsiveness. In addition, epigenetic priming may present a new strategy for tackling intractable tumors with platinum–acridines and other oncology drugs targeting this membrane transporter. These features render compound 1 a unique cytotoxic agent, which may have applications as a component of personalized combination regimens to treat resistant tumors.

**ASSOCIATED CONTENT**

**Supporting Information**

Data from COMPARE correlation analysis; pattern comparisons for \textit{SLC47A1} expression, DNA methylation, and copy number variations in CellMiner (Tables S2 and S5); summary of gene set overlap
analysis; NCI-60 screening data; supplemental figures of SLC47A1 tissue expression, Western blot analysis (uncropped images), confocal microscopy images; results of assay replicates; schematic plate layouts with experimental details (PDF)

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The authors declare no competing financial interests.

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Supporting Information

for

A Membrane Transporter Determines the Spectrum of Activity of a Potent DNA-Targeted Hybrid Anticancer Agent

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| Tissue of Origin | Cell Line   | log$_{10}$GI$_{50}$ | CDKN2A | TP53 | PTEN | RB1 | PIK3CA | KRAS | BRAF |
|------------------|-------------|----------------------|--------|------|------|-----|-------|------|------|
| Lung             | NCI-H460    | < -8.00              | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
|                  | NCI-H226    | < -8.00              | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
|                  | NCI-H522    | -7.92                | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
|                  | A549        | < -8.00              | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
| CNS              | SF-295      | -7.88                | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
| Renal            | SN12C       | < -8.00              | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
| Melanoma         | SK-MEL-5    | -7.75                | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
|                  | UACC-62     | < -8.00              | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
| Prostate         | DU-145      | < -8.00              | ■      | ■    | ■    | ■   | ■     | ■    | ■    |
| Breast           | T-47D       | -7.83                | ■      | ■    | ■    | ■   | ■     | ■    | ■    |

■ homozygous mutation/deletion  ■ heterozygous mutation/deletion (based on refs. 1 and 2)
Figure S1. Comparison of NCI-60 chemosensitivity profiles (averages of at least 2 assays) for cisplatin (CDDP, NSC 119875), doxorubicin (DOX, NSC 123127), topotecan (TOP, NSC 609699), and compound 1 (NSC # not disclosed).
**Table S2.** Results of NCI COMPARE analysis: positive and negative correlations between chemosensitivity (NCI-60, logGI50) and microarray gene expression data (z-scores)

| Entry | Positive Correlation | Negative Correlation | p-value |
|-------|----------------------|----------------------|---------|
| 1     | 0.692 SLC47A1        | -0.525 ZNF330        | p < 0.00001 ****** |
| 2     | 0.541 CCDC104        | -0.52 CCT8           | p < 0.01 **    |
| 3     | 0.535 FAM120AOS      | -0.511 MYSM1         | p < 0.001 ***  |
| 4     | 0.533 CCDC113        | -0.504 TEMM222       | p < 0.05 *     |
| 5     | 0.531 LOC100268168   | -0.501 CIDEB         |         |
| 6     | 0.527 DOLK           | -0.489 MRPL41        |         |
| 7     | 0.522 NQO1           | -0.485 RQCD1         |         |
| 8     | 0.519 POLDIP2        | -0.477 ADCY10P1      |         |
| 9     | 0.513 ARL8A          | -0.477 DUSP7         |         |
| 10    | 0.509 PHLP2P2        | -0.476 WRAP73        |         |
| 11    | 0.508 MOCS2          | -0.475 GK2           |         |
| 12    | 0.499 LOC100287590   | -0.474 NDST1         |         |
| 13    | 0.497 DOCK1          | -0.474 SMU1          |         |
| 14    | 0.495 AKAP10         | -0.473 PEX5          |         |
| 15    | 0.495 TNPO1          | -0.47 DR1            |         |
| 16    | 0.489 LOC728431      | -0.47 FOX3           |         |
| 17    | 0.487 ULK1           | -0.47 RFX3           |         |
| 18    | 0.486 ZNF652         | -0.469 LARS          |         |
| 19    | 0.484 TMTC3          | -0.468 WASF2         |         |
| 20    | 0.478 CLIP1          | -0.466 SYNPO2        |         |
| 21    | 0.476 TENC1          | -0.462 SYNCRIP        |         |
| 22    | 0.476 KIAA1841       | -0.461 SMIM8         |         |
| 23    | 0.476 C5AR1          | -0.453 ZNF451        |         |
| 24    | 0.475 SQSTM1         | -0.451 MAP3K19       |         |
| 25    | 0.474 TMEM218        | -0.449 RBM15         |         |
| 26    | 0.474 LOC100287525   | -0.447 KHSRP         |         |
| 27    | 0.473 ANKRD40        | -0.446 LRRC3B        |         |
| 28    | 0.47 SNRNP27         | -0.446 PVT1          |         |
| 29    | 0.468 TACO1          | -0.444 ADH1B         |         |
| 30    | 0.467 FBXL20         | -0.441 CSRNP3        |         |
| 31    | 0.465 IFT27          | -0.44 IPOS           |         |
| 32    | 0.464 ZNF219         | -0.44 DNAH2          |         |
| 33    | 0.464 UNC119         | -0.44 CSF2           |         |
| 34    | 0.463 HFE            | -0.44 DPH2           |         |
| 35    | 0.463 PSMD11         | -0.438 MKI67IP       |         |
|   |   |   |   |   |
|---|---|---|---|---|
| 36 | 0.462 | TAOK1 | -0.438 | TARP |
| 37 | 0.462 | SEMA3C | -0.435 | BABAM1 |
| 38 | 0.459 | SKP1 | -0.434 | NCS1 |
| 39 | 0.457 | TXNRD1 | -0.434 | EXOSC3 |
| 40 | 0.456 | TMLHE | -0.433 | FAM186B |
| 41 | 0.455 | LRRC23 | -0.433 | DIAPH2 |
| 42 | 0.454 | HSF4 | -0.431 | EPAG |
| 43 | 0.453 | ZBTB40 | -0.431 | VCPIP1 |
| 44 | 0.453 | MAN1A2 | -0.43 | FAM91A1 |
| 45 | 0.452 | ADSSL1 | -0.428 | TTYH1 |
| 46 | 0.452 | ACACB | -0.428 | RBM33 |
| 47 | 0.452 | PCGF2 | -0.427 | SLC24A2 |
| 48 | 0.451 | BCAS4 | -0.427 | SLC24A2 |
| 49 | 0.451 | TOB1 | -0.427 | BTF3L4 |
| 50 | 0.45 | RBPMS | -0.425 | TNFRSF10C |
| 51 | 0.449 | SNAPIN | -0.425 | LOC100129250 |
| 52 | 0.449 | RNPEP | -0.424 | ST6GAL1 |
| 53 | 0.445 | PTGR1 | -0.424 | JPH3 |
| 54 | 0.443 | NIN1 | -0.424 | TFAP4 |
| 55 | 0.443 | SRXN1 | -0.422 | ZNF473 |
| 56 | 0.443 | NFYB | -0.421 | FHI |
| 57 | 0.441 | RUBDL3 | -0.421 | XAB2 |
| 58 | 0.439 | WBP1 | -0.421 | ZMYM2 |
| 59 | 0.437 | PSMD3 | -0.42 | VAPB |
| 60 | 0.437 | NTSC3B | -0.42 | KCNQ5 |
| 61 | 0.437 | STAT3 | -0.42 | ZNF800 |
| 62 | 0.437 | FBXW4 | -0.418 | WDR52 |
| 63 | 0.435 | COPRS | -0.416 | KRT77 |
| 64 | 0.434 | FAM188B | -0.416 | ZRANB2 |
| 65 | 0.434 | F2RL2 | -0.416 | CCL16 |
| 66 | 0.434 | GOLGA1 | -0.416 | LOC284998 |
| 67 | 0.433 | VEZT | -0.415 | SLC43A3 |
| 68 | 0.433 | ZBTB41 | -0.415 | POLR1E |
| 69 | 0.433 | NFU1 | -0.415 | CDKN3 |
| 70 | 0.432 | MFSD5 | -0.415 | KIAA1009 |
| 71 | 0.431 | RUFY2 | -0.414 | RIN3 |
| 72 | 0.431 | GABARAPL2 | -0.414 | TPP2 |
| 73 | 0.43 | TMEM5 | -0.414 | ORMDL1 |
| 74 | 0.43 | OLFM1 | -0.414 | CHRAC1 |
| 75 | 0.43 | TRNP1 | -0.414 | CCDC18 |
|   |   |   |   |
|---|---|---|---|
| 76 | 0.43 | PQLC3 | -0.414 |
| 77 | 0.43 | HOXC13 | -0.414 |
| 78 | 0.43 | ABHD15 | -0.413 |
| 79 | 0.429 | TRIM23 | -0.413 |
| 80 | 0.429 | ZNF268 | -0.413 |
| 81 | 0.429 | FBXO21 | -0.413 |
| 82 | 0.429 | HOXA-A52 | -0.412 |
| 83 | 0.428 | KIAA0319 | -0.412 |
| 84 | 0.428 | KYNU | -0.412 |
| 85 | 0.428 | TBK1 | -0.412 |
| 86 | 0.428 | TMEM132A | -0.412 |
| 87 | 0.427 | MPP3 | -0.411 |
| 88 | 0.427 | BLVRA | -0.41 |
| 89 | 0.427 | EGF | -0.41 |
| 90 | 0.427 | IRX2 | -0.41 |
| 91 | 0.426 | WIPF2 | -0.41 |
| 92 | 0.425 | RHBD2 | -0.409 |
| 93 | 0.425 | SETD1A | -0.409 |
| 94 | 0.425 | UNKL | -0.409 |
| 95 | 0.425 | FDXR | -0.409 |
| 96 | 0.424 | G6PD | -0.409 |
| 97 | 0.424 | LETM1 | -0.408 |
| 98 | 0.424 | NLGN4Y | -0.407 |
| 99 | 0.424 | WISP2 | -0.406 |
| 100 | 0.423 | MMP28 | -0.406 |
| 101 | 0.422 | TUBG1 | -0.406 |
| 102 | 0.422 | MTA3 | -0.406 |
| 103 | 0.421 | ERAL1 | -0.406 |
| 104 | 0.421 | MED13 | -0.406 |
| 105 | 0.421 | LRIG2 | -0.405 |
| 106 | 0.421 | VMO1 | -0.405 |
| 107 | 0.42 | DAZAP2 | -0.405 |
| 108 | 0.42 | ATXN1L | -0.405 |
| 109 | 0.42 | JOSD2 | -0.404 |
| 110 | 0.42 | AP1G1 | -0.404 |
| 111 | 0.419 | IDNK | -0.403 |
| 112 | 0.419 | SPATS2 | -0.402 |
| 113 | 0.419 | SLC35E3 | -0.402 |
| 114 | 0.419 | CD163 | -0.402 |
| 115 | 0.419 | DYNC2LI1 | -0.402 |
|   |     |          |       |            |
|---|-----|----------|-------|------------|
| 116| 0.419| TLE2     | -0.4  | C19orf70   |
| 117| 0.419| MAP2K4   | -0.399| CCNB1IP1   |
| 118| 0.419| VMP1     | -0.398| DBT        |
| 119| 0.418| GATA5    | -0.397| LYNX1      |
| 120| 0.418| TP53I11  | -0.397| DHR54-A51  |
| 121| 0.418| SDF2     | -0.397| DNAJA1     |
| 122| 0.417| FBXO15   | -0.397| SLC22A7    |
| 123| 0.417| PCDHA    | -0.397| STX12      |
| 124| 0.417| CDK3     | -0.396| CSPG4      |
| 125| 0.417| NR1H2    | -0.396| PLVAP      |
| 126| 0.416| KIAA0100 | -0.396| PTER       |
| 127| 0.416| TSSK6    | -0.396| CEP152     |
| 128| 0.416| TRADD    | -0.395| ZER1       |
| 129| 0.415| MARK3    | -0.393| DDX18      |
| 130| 0.415| MSL1     | -0.393| LINC00312  |
| 131| 0.415| GSR      | -0.393| SMIM12     |
| 132| 0.415| FAM134B  | -0.393| NDUFB6     |
| 133| 0.414| ATG10    | -0.393| PFKFB1     |
| 134| 0.414| MLIP     | -0.393| PLAG1      |
| 135| 0.413| GJC2     | -0.392| SRIF3      |
| 136| 0.412| CCDC121  | -0.391| GRK1       |
| 137| 0.411| XYL12    | -0.391| LOC100505964 |
| 138| 0.411| MEGF9    | -0.391| HEMK1      |
| 139| 0.411| FLJ12120 | -0.391| NES        |
| 140| 0.411| SLC30A5  | -0.391| OBP2B      |
| 141| 0.41  | TRIM16   | -0.391| ZMYND11    |
| 142| 0.41  | AMFR     | -0.39  | DDX49      |
| 143| 0.41  | SPAG5    | -0.39  | PHIP       |
| 144| 0.409| FAM155B  | -0.389| MPPE1      |
| 145| 0.409| SRGAP3   | -0.389| PREP       |
| 146| 0.409| MOB3B    | -0.389| GATA5      |
| 147| 0.409| SNF8     | -0.389| ZNF544     |
| 148| 0.408| RABGAP1  | -0.389| E4F1       |
| 149| 0.408| BLVRB    | -0.389| RIOK3      |
| 150| 0.408| CLTC     | -0.388| SCN2B      |
| 151| 0.407| NKIRAS2  | -0.388| LRRC14     |
| 152| 0.407| SIRT3    | -0.388| MON1A      |
| 153| 0.406| BMPR1A   | -0.388| CCDC58     |
| 154| 0.406| LOC399884| -0.387| METTL8     |
|   |   |   |   |   |
|---|---|---|---|---|
| 156 | 0.406 | PSMG4 | -0.387 | TMEM51-AS1 |
| 157 | 0.405 | BLOC1S2 | -0.387 | ANPEP |
| 158 | 0.405 | IDE | -0.387 | APPL1 |
| 159 | 0.405 | DHX29 | -0.386 | PPP1R3E |
| 160 | 0.404 | FAM45A | -0.386 | SIRT6 |
| 161 | 0.404 | C7orf25 | -0.386 | WDR27 |
| 162 | 0.404 | ANKRD32 | -0.386 | RASSF1 |
| 163 | 0.404 | S1PR3 | -0.386 | KCNJ4 |
| 164 | 0.403 | ARHGEF26 | -0.385 | UFM1 |
| 165 | 0.403 | ADORA3 | -0.385 | FAM184B |
| 166 | 0.402 | C8orf42 | -0.385 | ACOT11 |
| 167 | 0.402 | RNASEH1 | -0.385 | RPF1 |
| 168 | 0.402 | AMER2 | -0.384 | SAMD8 |
| 169 | 0.402 | SREBF1 | -0.384 | WDR48 |
| 170 | 0.402 | PIGS | -0.383 | GTF2B |
| 171 | 0.401 | THSD4 | -0.383 | GTPBP5 |
| 172 | 0.401 | SH3BGR | -0.382 | PDE12 |
| 173 | 0.401 | LRRC46 | -0.382 | ATP11C |
| 174 | 0.401 | FAM218A | -0.382 | MTUS2 |
| 175 | 0.4 | MIEN1 | -0.382 | TSPO |
| 176 | 0.4 | DLGAP1 | -0.382 | CLCN6 |
| 177 | 0.4 | KCNA1 | -0.382 | ARHGAP27 |
| 178 | 0.4 | ACP1 | -0.381 | PMAIP1 |
| 179 | 0.399 | TVP23B | -0.381 | VPS13A |
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| 194 | 0.396 | TMEM178B | -0.379 | PLA2G2E |
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| Gene      | Correlation | Gene      | Correlation |
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| EPHX1     | 0.387       | TGS1      | -0.373      |
| EXOC6B    | 0.386       | EIF3G     | -0.373      |
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| FM05      | 0.384       | TNN       | -0.371      |
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| SDK2      | 0.383       | GRIN1     | -0.37       |
| KDM5B     | 0.383       | C1orf228  | -0.37       |
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| 277 | 0.38 | CHRM4 | -0.367 | MYOT |
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| 279 | 0.38 | ABHD4 | -0.367 | PADI4 |
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| 303 | 0.377 | ALPK1 | -0.363 | ADO |
| 304 | 0.377 | TMEM254-AS1 | -0.363 | MAP2K7 |
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| 306 | 0.376 | RAB5C | -0.363 | CMKLR1 |
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| 311 | 0.375 | GPC1 | -0.363 | NHP2L1 |
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| 325 | 0.373 | PRDM15 | -0.361 | PTPN22 |
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| 328 | 0.372 | CCNB1 | -0.361 | DPH3 |
| 329 | 0.372 | C12orf10 | -0.361 | MED30 |
| 330 | 0.372 | AMZ2 | -0.361 | KLC4 |
| 331 | 0.372 | CISD3 | -0.361 | DUSP15 |
| 332 | 0.371 | KCTD20 | -0.36 | SRSF11 |
| 333 | 0.371 | HCP5 | -0.36 | SLC26A8 |
| 334 | 0.371 | SLC6A2 | -0.36 | USP37 |
| 335 | 0.371 | SETDB1 | -0.36 | WNT10A |
| 336 | 0.371 | TRIM3 | -0.36 | BRD4 |
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| 341 | 0.37 | BAZ2A | -0.359 | C9orf123 |
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| 350 | 0.369 | FOXN3-AS1 | -0.358 | SASS6 |
| 351 | 0.369 | CSPG5 | -0.358 | C17orf105 |
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| 399 | 0.363 | SIX3-AS1 | -0.352 | DCLRE1C |
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| 404 | 0.362 | AMACR | -0.352 | DIS3 |
| 405 | 0.362 | STAMBPA | -0.352 | HIVEP3 |
| 406 | 0.362 | SLC38A7 | -0.352 | DCN |
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| 410 | 0.362 | GLI4 | -0.351 | NUP214 |
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| 413 | 0.361 | SNHG10 | -0.351 | SYNPO2L |
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| 423 | 0.36 | PRPSAP2 | -0.35 | ZNF292 |
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| 445 | 0.358 | SERF1B | -0.349 | BZRAP1 |
| 446 | 0.357 | CTSL1 | -0.349 | GOLGA8A |
| 447 | 0.357 | CCDC19 | -0.349 | MMP9 |
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| 450 | 0.357 | THNSL2 | -0.348 | RREB1 |
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| 453 | 0.357 | METAP1 | -0.348 | HCG18 |
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| 458 | 0.356 | C5orf51 | -0.347 | MLLT1 |
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| 460 | 0.356 | ALOXE3 | -0.347 | TOE1 |
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| 463 | 0.355 | ZFPNM1 | -0.347 | TRIM25 |
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| CCKBR | 0.348   | -0.341      | OXSR1       |
| PLAC4 | 0.348   | -0.341      | UBE2L6      |
| RAB1A | 0.348   | -0.341      | EIF2S3      |
| ZFP1  | 0.348   | -0.341      | DST         |
| PBXIP1 | 0.348   | -0.341      | ZNHIT6      |
| DPF1  | 0.348   | -0.341      | SLC30A7     |
| IGHMBP2 | 0.347   | -0.341      | RPL5        |
| TMEM107 | 0.347   | -0.341      | PYDC1       |
| IAH1  | 0.347   | -0.341      | SLC25A32    |
| TVP23C | 0.347   | -0.341      | NPFF        |
| RHBDL1 | 0.347   | -0.341      | PARP8       |
| GIP   | 0.347   | -0.341      | ABCA9       |
| PDNP  | 0.347   | -0.339      | ZEB2        |
| MBTPS1 | 0.347   | -0.339      | PPIE        |
| PTGES | 0.347   | -0.339      | UBAP2L      |
| REEP5 | 0.347   | -0.339      | SSU72       |
| GPR25 | 0.347   | -0.339      | MGC12488    |
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| 562 | 0.346 | C11orf92 | -0.339 |
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| 565 | 0.345 | NDRG4 | -0.338 |
| 566 | 0.345 | AKT1 | -0.338 |
| 567 | 0.345 | BAI2 | -0.338 |
| 568 | 0.345 | XPR1 | -0.338 |
| 569 | 0.345 | C5orf15 | -0.338 |
| 570 | 0.345 | PTH1R | -0.338 |
| 571 | 0.345 | RFX3 | -0.338 |
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| 583 | 0.344 | COL4A6 | -0.336 |
| 584 | 0.344 | UBE2K | -0.336 |
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| 589 | 0.343 | FBXO4 | -0.335 |
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| 592 | 0.343 | CEP112 | -0.335 |
| 593 | 0.343 | B3GALT5 | -0.335 |
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| 599 | 0.342 | KRT10 | -0.335 | ADAM7 |
| 600 | 0.342 | KCNF1 | -0.335 | TXNL4A |
| 601 | 0.342 | UNC79 | -0.335 | PURB |
| 602 | 0.342 | DNAH1 | -0.335 | TMEM68 |
| 603 | 0.342 | TP53I13 | -0.335 | ZNRD1-AS1 |
| 604 | 0.342 | MPHOSPH9 | -0.335 | SCLT1 |
| 605 | 0.342 | POLR2J4 | -0.335 | IGF1 |
| 606 | 0.342 | RNF187 | -0.335 | SAFB |
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| 618 | 0.34 | CNTN1 | -0.334 | HAUS6 |
| 619 | 0.34 | CABYR | -0.334 | NOTCH4 |
| 620 | 0.34 | SOBP | -0.334 | CPPED1 |
| 621 | 0.34 | TMEM231 | -0.334 | SERPINB8 |
| 622 | 0.34 | LONP2 | -0.334 | PVRL3 |
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| 624 | 0.34 | TMEM245 | -0.333 | GORASP2 |
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| 626 | 0.34 | CRLS1 | -0.333 | TBL1XR1 |
| 627 | 0.34 | DNAJC28 | -0.333 | SLC26A6 |
| 628 | 0.34 | DHX8 | -0.333 | ALOX12 |
| 629 | 0.339 | ARFGEF2 | -0.333 | DNAJC8 |
| 630 | 0.339 | ABCA8 | -0.333 | NUPL2 |
| 631 | 0.339 | PDCL2 | -0.333 | UGT2B15 |
| 632 | 0.339 | GFOD2 | -0.333 | OSCAR |
| 633 | 0.339 | RASGEF1C | -0.333 | CNPPD1 |
| 634 | 0.339 | PXN | -0.333 | NOP16 |
| 635 | 0.339 | C1orf27 | -0.333 | LOC401176 |
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| 637 | 0.339 | TCEA2 | -0.333 | MIR143HG |
| 638 | 0.339 | ODF3L1 | -0.332 | KCNK10 |
| 639 | 0.339 | MON2 | -0.332 | ZNF266 |
| 640 | 0.339 | ZNF226 | -0.332 | FUS |
| 641 | 0.338 | CDK12 | -0.332 | ATP10A |
| 642 | 0.338 | GTF2H2 | -0.332 | TPSG1 |
| 643 | 0.338 | ATP6V0A1 | -0.332 | NCAHP2 |
| 644 | 0.338 | FGG | -0.332 | CSPP1 |
| 645 | 0.338 | AK7 | -0.332 | PPIL3 |
| 646 | 0.338 | DPY19L1P1 | -0.332 | KRTDAP |
| 647 | 0.338 | TFE3 | -0.332 | AMD1 |
| 648 | 0.338 | TEMEM8B | -0.332 | STK17B |
| 649 | 0.338 | GAA | -0.332 | PARL |
| 650 | 0.338 | CHD3 | -0.332 | HLF |
| 651 | 0.338 | C17orf97 | -0.331 | RORA |
| 652 | 0.338 | ZDHHHC12 | -0.331 | ADRA1A |
| 653 | 0.338 | CYB5D2 | -0.331 | TLE4 |
| 654 | 0.337 | NSF | -0.331 | DIO2 |
| 655 | 0.337 | JAK3 | -0.331 | MTFR1 |
| 656 | 0.337 | TUBD1 | -0.331 | CHD4 |
| 657 | 0.337 | PI4KB | -0.331 | DCAF13 |
| 658 | 0.337 | TAF10 | -0.331 | GNG5 |
| 659 | 0.337 | RNF112 | -0.331 | ABCB1 |
| 660 | 0.337 | FCER1A | -0.331 | WNK2 |
| 661 | 0.337 | IGFBP4 | -0.331 | RAB8A |
| 662 | 0.337 | KCNK15 | -0.331 | YME1L1 |
| 663 | 0.337 | CD160 | -0.331 | ZBTB7A |
| 664 | 0.337 | NES | -0.331 | TSC2 |
| 665 | 0.337 | PPAP2A | -0.331 | ZFPL1 |
| 666 | 0.337 | SULT4A1 | -0.331 | OSBPL2 |
| 667 | 0.337 | MTFMT | -0.331 | ZRANB1 |
| 668 | 0.337 | AP4E1 | -0.33 | NIPAL3 |
| 669 | 0.337 | C2orf72 | -0.33 | CHRM2 |
| 670 | 0.337 | TRPV5 | -0.33 | DOCK8 |
| 671 | 0.337 | ITLN1 | -0.33 | FAR2 |
| 672 | 0.336 | LLGL1 | -0.33 | ZNF454 |
| 673 | 0.336 | ASTN2 | -0.33 | CDX4 |
| 674 | 0.336 | COX7A2L | -0.33 | EMILIN2 |
| 675 | 0.336 | CACNA1D | -0.33 | RD3 |
|    |   |     |     |                  |
|----|---|-----|-----|------------------|
| 676| 0.336 | AKT1S1 | -0.33 | DNASE1L1 |
| 677| 0.336 | PTPLAD1 | -0.33 | SNRPE |
| 678| 0.336 | ZNF609 | -0.33 | STX4 |
| 679| 0.336 | FAM177A1 | -0.33 | LOC100996694 |
| 680| 0.336 | MF12-AS1 | -0.329 | KCNQ2 |
| 681| 0.336 | CCDC74A | -0.329 | ISY1 |
| 682| 0.336 | MAR6 | -0.329 | LOC146880 |
| 683| 0.336 | WWC2-AS2 | -0.329 | REG3A |
| 684| 0.336 | ACBD6 | -0.329 | UTP23 |
| 685| 0.336 | GOSR1 | -0.329 | C15orf39 |
| 686| 0.336 | VPS41 | -0.329 | SSCA1 |
| 687| 0.336 | SULT1A3 | -0.329 | SDR16C5 |
| 688| 0.336 | AP3B1 | -0.329 | CHD7 |
| 689| 0.336 | CUEDC1 | -0.329 | ARID1B |
| 690| 0.335 | AP2B1 | -0.329 | PRCD |
| 691| 0.335 | TBX3 | -0.329 | WDR81 |
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| 698| 0.335 | LINC00674 | -0.328 | WNT6 |
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| 700| 0.334 | FLOT2 | -0.328 | KPNA1 |
| 701| 0.334 | KIF3A | -0.328 | GATAD2A |
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| 703| 0.334 | YIPF4 | -0.327 | BMP8B |
| 704| 0.334 | TEF | -0.327 | PPP2R3C |
| 705| 0.334 | ADCY1 | -0.327 | TFB1M |
| 706| 0.334 | ZNF711 | -0.327 | TRIM27 |
| 707| 0.334 | LRRC37A | -0.327 | IFNK |
| 708| 0.334 | SYNGR4 | -0.327 | CMSS1 |
| 709| 0.334 | TRIM32 | -0.327 | NCAN |
| 710| 0.334 | RNF103 | -0.327 | CDRT1SL2 |
| 711| 0.334 | LOC100506746 | -0.327 | YPEL2 |
| 712| 0.334 | CLPTM1L | -0.327 | DHX30 |
| 713| 0.334 | LOC101060027 | -0.327 | HOXB-AS5 |
| 714| 0.334 | FAM102A | -0.327 | WBSCR27 |
| 715| 0.334 | ZFR2 | -0.326 | OB2P2A |
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| 716| 0.333 | TTY2 | -0.326 | MRPL3 |
| 717| 0.333 | THSD7A | -0.326 | IL9 |
| 718| 0.333 | ACRY | -0.326 | QRS11 |
| 719| 0.333 | LOC100505938 | -0.326 | ZNF430 |
| 720| 0.333 | EGFL7 | -0.326 | LARP1B |
| 721| 0.333 | MTX3 | -0.326 | IGHG1 |
| 722| 0.333 | ABCB6 | -0.326 | LOC100106009 |
| 723| 0.333 | RAB3C | -0.326 | CCNT2 |
| 724| 0.333 | SKI | -0.326 | SEP15 |
| 725| 0.333 | METTL9 | -0.326 | HMGA1 |
| 726| 0.333 | ZNF638 | -0.326 | RAB27A |
| 727| 0.332 | NUP98 | -0.326 | PDX1 |
| 728| 0.332 | AKR1C2 | -0.326 | DENND2A |
| 729| 0.332 | C1QTNF3 | -0.326 | GCSHP3 |
| 730| 0.332 | IFI6 | -0.326 | TEX12 |
| 731| 0.332 | PAIP1 | -0.326 | AP2B1 |
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| 733| 0.332 | H2AFY2 | -0.325 | PTGER1 |
| 734| 0.332 | ALDH7A1 | -0.325 | FBXL6 |
| 735| 0.332 | PMS2P3 | -0.325 | PDLIM5 |
| 736| 0.332 | KPNB1 | -0.325 | STX3 |
| 737| 0.332 | CLDN8 | -0.325 | EBNA1BP2 |
| 738| 0.332 | GLI1 | -0.325 | HSPA8 |
| 739| 0.332 | B9D1 | -0.325 | GMPR2 |
| 740| 0.331 | RSBN1L | -0.325 | UGT8 |
| 741| 0.331 | SCN8A | -0.325 | RNPS1 |
| 742| 0.331 | GKPAP1 | -0.325 | RPL23AP7 |
| 743| 0.331 | ZNF701 | -0.325 | RSF1 |
| 744| 0.331 | MST1 | -0.325 | SNX31 |
| 745| 0.331 | HTRA1 | -0.325 | OPRM1 |
| 746| 0.331 | TREM1 | -0.324 | HIPK2 |
| 747| 0.331 | LOC100129935 | -0.324 | ESRRA |
| 748| 0.331 | LOC100507367 | -0.324 | FBXO41 |
| 749| 0.33 | AKIP1 | -0.324 | TEKT2 |
| 750| 0.33 | LINC00473 | -0.324 | ZMYM6 |
| 751| 0.33 | USP42 | -0.324 | EIF1AD |
| 752| 0.33 | IQCH | -0.324 | SSBP1 |
| 753| 0.33 | PDZD4 | -0.324 | HDAC2 |
| 754| 0.33 | ATMIN | -0.324 | PEX14 |
| 755| 0.33 | CCDC53 | -0.324 | ARPC2 |
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| 757 | 0.33 | GTF2I | -0.324 | THBS1 |
| 758 | 0.33 | SNX21 | -0.324 | HECA |
| 759 | 0.33 | GLP1R | -0.324 | CYP2A7 |
| 760 | 0.33 | DIABLO | -0.323 | ATAD3B |
| 761 | 0.33 | PARP6 | -0.323 | TMEM209 |
| 762 | 0.33 | OR2W1 | -0.323 | RPL27A |
| 763 | 0.33 | SDHA | -0.323 | PDZD4 |
| 764 | 0.329 | SF3B4 | -0.323 | TNRC6B |
| 765 | 0.329 | CRTAP | -0.323 | FAM24A |
| 766 | 0.329 | HCO2 | -0.323 | NME8 |
| 767 | 0.329 | SFSWAP | -0.323 | DLEU2 |
| 768 | 0.329 | MAR7 | -0.323 | SMG1 |
| 769 | 0.329 | ZNF501 | -0.323 | PRKCSH |
| 770 | 0.329 | UBE2B | -0.323 | UBE2G2 |
| 771 | 0.329 | EPHX2 | -0.323 | DEFA1 |
| 772 | 0.329 | FAM110C | -0.323 | GRIN2D |
| 773 | 0.329 | LRRRC73 | -0.323 | FCR2 |
| 774 | 0.328 | DYNLRB2 | -0.323 | HAUS2 |
| 775 | 0.328 | SLC11A2 | -0.323 | KCN7 |
| 776 | 0.328 | KLK2 | -0.323 | PABPC1 |
| 777 | 0.328 | TNKS | -0.322 | PXDNL |
| 778 | 0.328 | TRMT1L | -0.322 | PTCH2 |
| 779 | 0.328 | RNFT2 | -0.322 | FAM43A |
| 780 | 0.328 | NAP1L2 | -0.322 | ZNF75A |
| 781 | 0.328 | CDC14A | -0.322 | A1BG-AS1 |
| 782 | 0.328 | TOR3A | -0.322 | MAG1 |
| 783 | 0.328 | ABCC3 | -0.322 | RN182 |
| 784 | 0.328 | SP2 | -0.322 | CHORDC1 |
| 785 | 0.328 | ACER3 | -0.322 | LOC100506088 |
| 786 | 0.328 | PTPRZ | -0.322 | CHCHD7 |
| 787 | 0.328 | SLC7A11 | -0.321 | FKBP6 |
| 788 | 0.327 | PLC8D4 | -0.321 | KCNJ5 |
| 789 | 0.327 | STRADA | -0.321 | PIGA |
| 790 | 0.327 | CIAPIN1 | -0.321 | KRT75 |
| 791 | 0.327 | RBM24 | -0.321 | CPSF1 |
| 792 | 0.327 | ARL3 | -0.321 | POLRMT |
| 793 | 0.327 | PTRHD1 | -0.321 | C17orf104 |
| 794 | 0.327 | PSMD7 | -0.321 | PKNOX1 |
|    | 0.327 | CHD1L  | -0.321 | MIS188P1 |
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| 797| 0.327 | AKTIP  | -0.321 | GLYCTK   |
| 798| 0.327 | ERMAP  | -0.321 | ELOF1    |
| 799| 0.327 | ATP6V1E2 | -0.32  | EXTL3    |
| 800| 0.327 | PRKAA1 | -0.32  | RBM44    |
| 801| 0.327 | PDIA6  | -0.32  | PRDM2    |
| 802| 0.327 | PIP4K2C | -0.32  | TRIM4    |
| 803| 0.326 | ANKD54 | -0.32  | ACRBP    |
| 804| 0.326 | AHCYL1 | -0.32  | MICAL3   |
| 805| 0.326 | HAUS3  | -0.32  | PLEK     |
| 806| 0.326 | ACACA  | -0.32  | PPP6R1   |
| 807|       |        | -0.32  | PPP4R1   |
| 808|       |        | -0.32  | FUOM     |
| 809|       |        | -0.32  | ACTR3    |
| 810|       |        | -0.319 | ENAM     |
| 811|       |        | -0.319 | NUDT4    |
| 812|       |        | -0.319 | C2CD4A   |
| 813|       |        | -0.319 | EXTL2    |
| 814|       |        | -0.319 | SMIM1    |
| 815|       |        | -0.319 | ETV6     |
| 816|       |        | -0.319 | HTR4     |
| 817|       |        | -0.319 | PPP1R12C |
| 818|       |        | -0.319 | GLMN     |
| 819|       |        | -0.319 | LSM4     |
| 820|       |        | -0.319 | KLF4     |
| 821|       |        | -0.319 | FCRL4    |
| 822|       |        | -0.318 | TCP11    |
| 823|       |        | -0.318 | UBA52    |
| 824|       |        | -0.318 | EEF2     |
| 825|       |        | -0.318 | SP8      |
| 826|       |        | -0.318 | SLC41A3  |
| 827|       |        | -0.318 | L3MBTL2  |
| 828|       |        | -0.318 | FAM209A  |
| 829|       |        | -0.318 | PPIH     |
| 830|       |        | -0.318 | MRPL37   |
| 831|       |        | -0.318 | MRQH1    |
| 832|       |        | -0.318 | MLEC     |
| 833|       |        | -0.318 | PTPN4    |
| 834|       |        | -0.318 | C1OA     |
| 835|       |        | -0.318 | LOC90499 |
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| 836 |   | -0.318 | ATG5 |
| 837 |   | -0.318 | CCDC61 |
| 838 |   | -0.318 | AQPEP |
| 839 |   | -0.318 | REXO4 |
| 840 |   | -0.318 | LILRB2 |
| 841 |   | -0.317 | GPR171 |
| 842 |   | -0.317 | C16orf95 |
| 843 |   | -0.317 | HSPA6 |
| 844 |   | -0.317 | HMGCLL1 |
| 845 |   | -0.317 | FAM194A |
| 846 |   | -0.317 | FLJ43663 |
| 847 |   | -0.317 | VPS13A-AS1 |
| 848 |   | -0.317 | STX16 |
| 849 |   | -0.317 | DGAT1 |
Table S3. NCI-60/COMPARE analysis: summary of SLC genes whose mRNA expression is positively or negatively correlated with chemosensitivity to compound 1 (logGI<sub>50</sub>)

| Gene       | Pearson's R<sup>a</sup> | Function                                      | P value<sup>b</sup> |
|------------|------------------------|-----------------------------------------------|---------------------|
| SLC11A2    | 0.328                  | H<sup>+</sup>-coupled Cu and M<sup>2+</sup> symporter | *                   |
| SLC12A4    | 0.352                  | K<sup>+</sup>/Cl<sup>-</sup> coupled transporter | **                  |
| SLC16A1    | 0.327                  | lactate/pyruvate transporter                  | *                   |
| SLC16A4    | 0.358                  | monocarboxylic acid transporter              | **                  |
| SLC17A9    | -0.366                 | ATP/mononucleotide vesicular uptake           | **                  |
| SLC22A17   | 0.374                  | multi-specific cation transporter (brain)     | **                  |
| SLC22A5    | 0.38                   | carnitine transporter                         | **                  |
| SLC22A7    | -0.397                 | multi-specific anion transporter              | **                  |
| SLC24A2    | -0.427                 | Ca<sup>2+</sup>/cation antiporter             | ***                 |
| SLC25A32   | -0.318                 | mitochondrial folate transporter              | *                   |
| SLC26A8    | -0.36                  | anion transporter                             | **                  |
| SLC27A7    | -0.352                 | fatty acid transporter                        | **                  |
| SLC29A9    | -0.374                 | uric acid transporter GLUT9                   | **                  |
| SLC30A1    | 0.371                  | cation transporter                            | **                  |
| SLC30A5    | 0.404                  | Zn<sup>2+</sup> transporter                   | **                  |
| SLC35E3    | 0.419                  | putative transporter                          | **                  |
| SLC35F6    | 0.353                  | carbohydrate/H<sup>+</sup> symporter         | **                  |
| SLC38A7    | 0.362                  | Na<sup>+</sup> coupled amino acid transporter | **                  |
| SLC39A8    | 0.381                  | Zn<sup>2+</sup> and Cd<sup>2+</sup> transporter | **                  |
| SLC3A2     | 0.392                  | amino acid transporter                        | **                  |
| SLC41A3    | -0.318                 | cation transporter                            | *                   |
| SLC43A3    | -0.415                 | putative transporter                          | **                  |
| **SLC47A1**| **0.692**              | **H<sup>+</sup>-coupled organic cation antiporter** | *****               |
| SLC48A1    | 0.38                   | heme transporter                              | **                  |
| SLC51B     | 0.393                  | bile acid transporter                         | **                  |
| SLC6A19    | -0.38                  | Na<sup>+</sup> dependent neutral amino acid transporter | ** |
| SLC6A2     | 0.371                  | Na<sup>+</sup>/neurotransmitter symporter     | **                  |
| SLC7A11    | 0.328                  | anionic amino acid transporter                | *                   |
| SLC8A1     | 0.364                  | Na<sup>+</sup>/Ca<sup>2+</sup> exchanger       | **                  |
| SLC04A1    | -0.328                 | organic anion transporter                      | *                   |

<sup>a</sup>N = 58. <sup>b</sup>* P < 0.05; ** P < 0.01; *** P < 0.001; ***** P < 0.00001.
Figure S2. Expression of hMATE1 (*SLC47A1*) in normal human tissue (A) and in cancer cells (B); The Human Protein Atlas, version 19.1, 12/2019, www.proteinatlas.org.
Figure S3. SLC47A1 gene copy numbers (left) and transcript levels (right) in NCI-60 (NCI CellMiner analysis tool, database version 2.2)
### Table S4. Summary of top 10 overlaps\(^a\) between gene ontology (GO) gene sets of the Molecular Signatures Database (MSigDB)\(^b\) and the input gene set\(^c\) derived from NCI-60 COMPARE correlation between activity of compound 1 (GI\(_{50}\)) and gene transcript levels for SLC47A1

| MSigDB Gene Set | \(K^d\) | Description | \(k^e\) | \(k/K\) | \(p\text{-value}^f\) | FDR q-value |
|-----------------|--------|-------------|--------|-------|----------------|-------------|
| GO_INTRACELLULAR_TRANSPORT | 1825 | The directed movement of substances within a cell. [GOC:ai] | 101 | 0.056 | 2.07 \(\times\) 10\(^{-19}\) | 2.07 \(\times\) 10\(^{-15}\) |
| GO_CELLULAR_MACROMOLECULE_LOCALIZATION | 1897 | Any process in which a macromolecule is transported to, and/or maintained in, a specific location at the level of a cell. Localization at the cellular level encompasses movement within the cell, from within the cell to the cell surface, or from one location to another at the surface of a cell. [GOC:mah] | 102 | 0.054 | 1.01 \(\times\) 10\(^{-18}\) | 5.02 \(\times\) 10\(^{-15}\) |
| GO_CELL_PROJECTION_PART | 1440 | Any constituent part of a cell projection, a prolongation or process extending from a cell, e.g. a flagellum or axon. [GOC:jl] | 85 | 0.059 | 4.35 \(\times\) 10\(^{-18}\) | 1.45 \(\times\) 10\(^{-14}\) |
| GO_WHOLE_MEMBRANE | 1653 | Any lipid bilayer that completely encloses some structure, and all the proteins embedded in it or attached to it. Examples include the plasma membrane and most organelle membranes. [GOC:gos] | 91 | 0.055 | 2.14 \(\times\) 10\(^{-17}\) | 5.34 \(\times\) 10\(^{-14}\) |
| GO_INTRACELLULAR_PROTEIN_TRANSPORT | 1164 | The directed movement of proteins in a cell, including the movement of proteins between specific compartments or structures within a cell, such as organelles of a eukaryotic cell. [GOC:mah] | 73 | 0.063 | 4.97 \(\times\) 10\(^{-17}\) | 9.93 \(\times\) 10\(^{-14}\) |
| GO_PROTEOLYSIS | 1762 | The hydrolysis of proteins into smaller polypeptides and/or amino acids by cleavage of their peptide bonds. [GOC:bf, GOC:mah] | 93 | 0.053 | 1.3 \(\times\) 10\(^{-16}\) | 2.16 \(\times\) 10\(^{-13}\) |
| 7 | GO_NEURON_PART | 1715 | Any constituent part of a neuron, the basic cellular unit of nervous tissue. A typical neuron consists of a cell body (often called the soma), an axon, and dendrites. Their purpose is to receive, conduct, and transmit impulses in the nervous system. [GOC:pr, http://en.wikipedia.org/wiki/Neuron] | 91 | 0.053 | 2.07 e^{-16} | 2.95 e^{-13} |
| 8 | GO_CELL_PROJECTION_ORGANIZATION | 1512 | A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of a prolongation or process extending from a cell, e.g. a flagellum or axon. [GOC:jl, GOC:mah, http://www.cogsci.princeton.edu/~wn/] | 84 | 0.056 | 2.43 e^{-16} | 3.04 e^{-13} |
| 9 | GO_RIBONUCLEOTIDE BINDING | 1891 | Interacting selectively and non-covalently with a ribonucleotide, any compound consisting of a ribonucleoside that is esterified with (ortho)phosphate or an oligophosphate at any hydroxyl group on the ribose moiety. [GOC:mah] | 95 | 0.050 | 1.29 e^{-15} | 1.43 e^{-12} |
| 10 | GO_MICROTUBULE_BASED_PROCESS | 734 | Any cellular process that depends upon or alters the microtubule cytoskeleton, that part of the cytoskeleton comprising microtubules and their associated proteins. [GOC:mah] | 53 | 0.072 | 4.34 e^{-15} | 4.34 e^{-12} |

\(^a\) Number of genes in comparison (n): 785, number of genes in universe (N): 38055. \(^b\) MSigDB database v6.2, updated July 2018; GSEA/MSigDB website v6.3, released 01/2018 by the Broad Institute Inc. \(^c\) See Table S2. Number of positively correlated genes: 806, \(P < 0.05\). \(^d\) Number of genes in gene set. \(^e\) Number of genes in overlap. \(^f\) For hypergeometric distribution.
Figure S4. Western blot analysis of A549 cell lysate for hMATE1 expression. The blot shows a band consistent with the 63-kDa full-length, 586-amino acid protein (The Human Protein Atlas, version 19.1, 12/2019, www.proteinatlas.org).
**Figure S5.** (A) Western blot for optimized RNAi conditions. The full-length protein (63 kDa) and the 34-kDa splice variant are observed. Transfection with 2.5 nM of both siRNA1 and siRNA2 provided the best knockdown efficiency relative to scrambled control and empty vector (lipofectamine). The GAPDH loading control is also shown. The gel image (30 sec. exposure) was contrast enhanced but otherwise not altered. (B) Rabbit–anti-human hMATE1 antibodies used in this study and aligned amino-acid sequences of the 63-kDa full-length protein and the 34-kDa splice variant (NCBI). Specific antibodies/epitopes: underlined: Thermo Fisher PA5-25272 and AVIVA OAAB02770 (residues 492-519, based on full length protein); bold: Abcam, ab104016 (residues 500-530, based on full length protein). (C,D) Results for two additional drug uptake experiments after hMATE1 (SLC47A1) knockdown, quantified by ICP-MS, showing reduction in uptake of compound 1 by 39% ($p < 0.01$) and 32% ($p < 0.01$), respectively.
Table S5. Pattern comparisons for $SLC47A1$ expression in NCI CellMiner

**Deposited as separate file**
Figure S6. A highly significant correlation ($R = -0.32$, $p = 4.9 \times 10^{-25}$) between CPI methylation status and expression levels of the SLC47A1 gene is observed in 963 cancer cell lines of the Genomics of Drug Sensitivity in Cancer (GDSC) database (CellMinerCDB, version 1.1, discover.nci.nih.gov/cellminercdb). The data point for the colorectal cancer cell line HCT-116 used in this study is highlighted.
**Table S6.** Summary of significant ($p < 0.05$) correlations identified between CPI methylation status and expression levels of the *SLC47A1* gene in 963 cancer cell lines of different tissues of origin and cell types

| Cell Line Origin                  | Pearson’s $R$ | $P$ value   |
|----------------------------------|---------------|-------------|
| Multiple Myeloma                 | −0.55         | 0.028       |
| Colon                            | −0.33         | 0.025       |
| Esophagus/Stomach                | −0.41         | $7.6 \times 10^{-4}$ |
| Liver                            | −0.56         | 0.02        |
| **Lung**                         | **−0.32**     | **8.2 \times 10^{-6}** |
| **NSCLC**                        | **−0.43**     | **1.1 \times 10^{-5}** |
| **Lung Adenocarcinoma**          | **−0.47**     | **4.7 \times 10^{-4}** |
| Ovaries                          | −0.32         | 0.032       |
| Pancreas                         | −0.47         | $8.1 \times 10^{3}$ |
| Epithelial                       | −0.16         | $4.4 \times 10^{3}$ |
| Epithelial–Mesenchymal           | −0.43         | $2.7 \times 10^{7}$ |
| Mesenchymal                      | −0.23         | $1.3 \times 10^{5}$ |

*a* Genomics of Drug Sensitivity in Cancer (GDSC) database (CellMinerCDB, version 1.1, discover.nci.nih.gov/cellminercdb).
Table S7. Summary of correlations observed for chemosensitivity and omics data for compound 1.

| Data in Comparison                      | Pearson’s R | P value               |
|----------------------------------------|-------------|-----------------------|
| NCI-60, logGI<sub>50</sub>             | SLC47A1 transcript level | 0.692<sup>a</sup> | < .00001 (*****)<sup>a</sup> |
| NCI-60, logGI<sub>50</sub>             | DNMT1 transcript level   | -0.378<sup>a</sup>  | 0.0034 (**)<sup>a</sup>    |
| SLC47A1 transcript level               | SLC47A1 CGI methylation | -0.416<sup>b</sup>  | < 0.001 (***)<sup>b</sup>  |
| SLC47A1 transcript level               | SLC47A1 gene copy number | 0.398<sup>b</sup>  | < 0.001 (***)<sup>b</sup>  |
| SLC47A1 transcript level               | EZH2 transcript level    | -0.289<sup>b</sup>  | 0.025 (*)<sup>b</sup>      |
| SLC47A1 CGI methylation                | DNMT1 transcript level   | 0.311<sup>b</sup>  | 0.015 (*)<sup>b</sup>      |
| EZH2 transcript level                  | DNMT1 transcript level   | 0.479<sup>b</sup>  | < 0.001 (***)<sup>b</sup>  |

<sup>a</sup> NCICOMPARE analysis, n = 58.  <sup>b</sup> NCI CellMiner analysis tool, database version 2.2, n = 60.
Table S8. Summary of top 10 overlaps\(^a\) between all gene sets of the molecular signatures database (MSigDB)\(^b\) and the input gene set\(^c\) of hypermethylated genes negatively correlated with SLC47A1 expression.

| MSigDB Gene Set                     | \(K\) | Description                                                                                                                                                                                                 | \(k\)  | \(k/K\) | \(p\)-value\(^d\) | FDR \(q\)-value |
|-------------------------------------|-------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|---------|----------------------|-----------------|
| 1 BENPORATH_ES_WITH_H3K27ME3        | 1118  | Set 'H3K27 bound': genes possessing the trimethylated H3K27 (H3K27me3) mark in their promoters in human embryonic stem cells, as identified by ChIP on chip.                                                 | 115   | 0.1029  | 1.21 \(\times\) 10^{-92} | 2.15 \(\times\) 10^{-88} |
| 2 BENPORATH_EED_TARGETS             | 1062  | Set 'Eed targets': genes identified by ChIP on chip as targets of the Polycomb protein EED [GeneID=8726] in human embryonic stem cells.                                                                   | 111   | 0.1045  | 5.70 \(\times\) 10^{-90}  | 5.08 \(\times\) 10^{-86} |
| 3 MIKKELSEN_MEF_HCP_WITH_H3K27ME3   | 590   | Genes with high-CpG-density promoters (HCP) bearing histone H3 trimethylation mark at K27 (H3K27me3) in MEF cells (embryonic fibroblast).                                                                   | 91    | 0.1542  | 1.23 \(\times\) 10^{-88}  | 7.28 \(\times\) 10^{-85} |
| 4 BENPORATH_SUZ12_TARGETS           | 1038  | Set 'Suz12 targets': genes identified by ChIP on chip as targets of the Polycomb protein SUZ12 [GeneID=23512] in human embryonic stem cells.                                                               | 108   | 0.104   | 3.62 \(\times\) 10^{-87}  | 1.61 \(\times\) 10^{-83} |
| 5 BENPORATH_PRC2_TARGETS            | 652   | Set 'PRC2 targets': Polycomb Repression Complex 2 (PRC) targets; identified by ChIP on chip on human embryonic stem cells as genes that: posess the trimethylated H3K27 mark in their promoters and are bound by SUZ12 [GeneID=23512] and EED [GeneID=8726] Polycomb proteins. | 75    | 0.115   | 7.73 \(\times\) 10^{-63}  | 2.75 \(\times\) 10^{-59} |
| 6 MEISSNER_BRAIN_HCP_WITH_H3K4ME3_AND_H3K27ME3 | 1069 | Genes with high-CpG-density promoters (HCP) bearing histone H3 dimethylation at K4 (H3K4me2) and trimethylation at K27 (H3K27me3) in brain.                                                              | 89    | 0.0833  | 1.21 \(\times\) 10^{-62}  | 3.60 \(\times\) 10^{-59} |
|   | Description                                                                 | Count | P-val  | q-val  |
|---|------------------------------------------------------------------------------|-------|--------|--------|
| 7 | GO_INTRINSIC_COMPONENT_OF_PLASMA_MEMBRANE                                      | 1649  | 0.0631 | 7.53 e-62 | 1.92 e-58 |
|   | The component of the plasma membrane consisting of the gene products and protein complexes having either part of their peptide sequence embedded in the hydrophobic region of the membrane or some other covalently attached group such as a GPI anchor that is similarly embedded in the membrane. |       |        |        |
| 8 | MEISSNER_NPC_HCP_WITH_H3K4_ME2_AND_H3K27ME3                                  | 349   | 0.1719 | 7.26 e-61 | 1.62 e-57 |
|   | Genes with high-CpG-density promoters (HCP) bearing histone H3 dimethylation mark at K4 (H3K4me2) and trimethylation mark at K27 (H3K27me3) in neural precursor cells (NPC). |       |        |        |
| 9 | MIKKELSEN_NPC_HCP_WITH_H3K27ME3                                             | 341   | 0.1701 | 1.54 e-58 | 3.04 e-55 |
|   | Genes with high-CpG-density promoters (HCP) bearing histone H3 trimethylation mark at K27 (H3K27me3) in neural progenitor cells (NPC). |       |        |        |
| 10| MIKKELSEN_MCV6_HCP_WITH_H3K27ME3                                            | 435   | 0.1379 | 6.82 e-55 | 1.21 e-51 |
|   | Genes with high-CpG-density promoters (HCP) bearing the tri-methylation mark at H3K27 (H3K27me3) in MCV6 cells (embryonic fibroblasts trapped in a differentiated state). |       |        |        |

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Number of genes in comparison (n): 402, number of genes in universe (N): 38055. b MSigDB database v6.2, updated July 2018; GSEA/MSigDB website v6.3, released 01/2018 by the Broad Institute Inc. c See Table S5. Number of negatively correlated genes: 452, P < 0.05. d Number of genes in gene set. e Number of genes in overlap. f For hypergeometric distribution.
**Figure S7.** Pre-screening of HCT-116 colon cancer cells for the effects of epigenetic drugs on cell viability and accumulation of compound 1.

Cells were treated with single drugs or combinations of up to four epigenetic drugs at 2.5 μM for 72 hours. Cells were then fixed and imaged in the bright-field and blue fluorescence channels. (A) Bright-field images show intact monolayers of viable cells after incubation with EPZ-6438 and EED226 similar to DMSO-treated cells (control) but show changes in morphology and cell death after treatment with valproic acid and decitabine (only selected images of single treatments are shown). (B) Treatment at 2.5 μM EPZ-6438 and EED226, or a combination of the two drugs, for 72 hours leads to enhanced cellular accumulation of compound 1, based on increased acridine-associated blue fluorescence localized to regions previously identified as vesicular structures (see the main text). Scale bars in (A) and (B) are 20 μm.
**Figure S8.** (A) Western blot analysis of hMATE1 protein in HCT-116 cell lysates pre-treated with a combination of EPZ-6438 and EED226 (E/E). Bands for GAPDH (36 kDa), full-length hMATE1 (63 kDa), and a splice variant of hMATE1 containing the antibody epitope (34 kDa) are labeled. (B) Cell proliferation assay for HCT-116 cells pre-exposed to 2.5 µM or 5 µM E/E for 72 hours and subsequently treated with 1 µM or 10 µM compound 1 for another 72 hours. A pronounced decrease in cell viability was observed at higher doses of epigenetic drugs. The data is presented as the mean ± S.E.M. for an assay performed in sextuplicate (***, p < 0.001; ***, p < 0.001; two-tailed t-test).
**Assay Layouts**

**AL1.** Uptake of compound 1 into HCT-116 cells after sensitization with epigenetic drugs\(^a\) monitored by confocal fluorescence microscopy (pre-screening).

| Control | VA | VA EPZ | VA | VA EED EPZ | VAL DEC EPZ |
|---------|----|--------|----|------------|-------------|
| Control | VA | EPZ    | EPZ VA | EPZ EED DEC | EPZ DEC VAL EED |
| Control | EED | EED EPZ | EED VA | EED | EED DEC EPZ |
| Control | DEC | DEC EPZ | DEC VA | DEC EED | DEC |

\(^a\) VA, valproic acid; DEC, decitabine; EPZ, EPZ-6438; EED, EED226.

**AL2.** Uptake of compound 1 into HCT-116 cells after sensitization with EPZ-6438, EED226, and a combination of both drugs at escalating doses monitored by confocal fluorescence microscopy.

| Control | 2.5 \(\mu\)M | 5 \(\mu\)M | 10 \(\mu\)M | 20 \(\mu\)M | 50 \(\mu\)M |
|---------|---------------|------------|------------|------------|------------|
| Control | 2.5 \(\mu\)M | 5 \(\mu\)M | 10 \(\mu\)M | 20 \(\mu\)M | 50 \(\mu\)M |
| Control | 2.5 \(\mu\)M | 5 \(\mu\)M | 10 \(\mu\)M | 20 \(\mu\)M | 50 \(\mu\)M |

Green: EPZ-6438, Blue: EED226, Red: EPZ-6438 + EED226 (“E/E”), 1:1

**AL3.** Cell proliferation assay for A549 cells after hMATE1 (SLC47A1) knockdown.

|   | 1   | 2   | 3   | 4   | 5   | 6   | 7   |
|---|-----|-----|-----|-----|-----|-----|-----|
| A | PBS | PBS | PBS | PBS | PBS | PBS | PBS |
| B | PBS | Comp. 1 | Comp. 1 | blank | PBS |
| C | PBS | PBS | PBS | PBS | PBS | PBS | PBS |
| D | PBS | PBS | PBS | PBS | PBS | PBS | PBS |
| E | PBS | PBS | PBS | PBS | PBS | PBS | PBS |

**Assembly:** Add 20 \(\mu\)L Opti-Mem into wells B2-5, C2-6, and D2-5. Add 23.2 \(\mu\)L of Opti-Mem into wells B6, C6, D6. Then add siRNA to B2-3, C2-3, and D2-3. Add scrambled RNA to B4-5, C4-5, and D4-5. Add diluted lipofectamine (siRNA:lipo 6:1) to B2-5, C2-5, and D2-5. Transfer cells to B2-5, C2-5, and D2-5 at a density of 5000 cells/well. Add same amount of medium into well B6-D6 as blank control. Add PBS into the outermost wells to prevent evaporation of transfection reactions. B2, C2, and D2 are siRNA
+ compound 1; B3, C3, and D3 are siRNA only; B4, C4, and D4 are scrambled RNA + compound 1; B5, C5, and D5 are scrambled RNA only; B6, C6, and D6 are blanks.

**AL4.** Cell proliferation assay for HCT-116 cells after exposure to epigenetic drugs.

|   | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A | PBS | PBS | PBS | PBS | PBS | PBS | PBS | PBS | PBS | PBS | PBS |
| B | PBS | No drug | 1 µM | 10 µM | No drug | 1 µM | 1 µM | 10 µM | 10 µM | PBS |
| C | PBS | PBS | PBS | PBS | PBS | PBS | PBS | PBS | PBS | PBS |

**Assembly:** Add EED-226 and EPZ-6438 (“E/E”) to the cells in wells B5-G5, B7-G7, and B9-G9 to produce a final concentration of 2.5 µM, and to wells B6-G6, B8-G8, and B10-G10 to a final concentration of 5 µM. Incubate for a total of 72 hours and replace medium with fresh epi-drug(s) every 24 hours. Replace epi-drug after 72 hours with compound 1 at a final concentration of 1 µM in wells B3-G3, B7-G7, and B8-G8, and 10 µM in wells B4-G4, B9-G9, and B10-G10. Yellow columns: 2.5 µM E/E; Blue columns: 5 µM E/E. Column 2 is a no-treatment control, columns 3 and 4 are no-sensitization controls.
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