Supplemental Figure Methods and Tables

Methods

Calu-6 cells were plated at 300Kcells/well on sterile 6-well tissue culture plates in Eagle's Minimal Essential Medium (ATTC) supplemented with 10% heat inactivated FBS (Gibco) and allowed to attach overnight by incubating at 37 °C with 5% CO_2. Cells were treated with DMSO (0.1%), BSO (200 µM) or tasisulam (100 µM) for 0, 6, 24, 32, 52 and 75 hours. Cells were washed 2X prior to harvest with 2 mL of Ice-Cold PBS and lysed with 130 µL of lysis buffer (M-PER, Thermo Scientific) containing 10 mM HCl. Cells were lysed by shaking for two minutes on an orbital shaker and cell lysates were collected and stored at -80 °C until assayed. Protein concentration was determined using 20 µL of each sample (Coomassie Plus, Thermo Scientific). Total glutathione concentration was determined by mixing 28 µL of 15% sulfo-salicylic acid with 110 µLl of cell lysate and incubating on ice for 30 minutes. Samples were centrifuged for 10 minutes at 16,000 x g to pellet insoluble material. Glutathione concentration was determined for each sample using a glutathione recycling assay as previously described by Anderson (Anderson, M.E. (1985) Determination of Glutathione and glutathione disulfide in biological samples, Methods of Enzymology, vol. 113, 548-555). Data were plotted in GraphPad Prism and normalized to total protein. B) Reduced glutathione concentration was determined for Calu-6 cells treated as described above up to 24 hr using the GSH-Glo Glutathione assay (Promega) according to manufacturer's instructions. C) Calu-6 cells were plated at 38K cells/well on sterile 24-well tissue culture plates in Eagle's Minimal Essential Medium (ATTC) supplemented with 10% heat inactivated FBS (Gibco) and allowed to attach overnight by incubating at 37 °C with 5% CO_2. Cells were treated with FCCP, FCCP + N-acetyl-L-cysteine (NAC, 2.5 mM), tasisulam, or tasisulam + NAC (2.5mM) in medium. After 72 hours, 70 µLof 0.5% trypsin was added to each well and incubated until the cells detached from the plate. The trypsin was neutralized with the addition of 230 µL medium containing 10% FBS, and then 100 µL of 0.4% Trypan Blue added to wells and shaken for 1 minute. The cell number was determined by direct counting using a cytometer and the data were plotted in GraphPad Prism.

Calu-6 S-phase block and release. $1.25x10^6$ Calu-6 cells were plated in T150 flasks and incubated 7 hr in EMEM (ATCC 30-2003) supplemented with 10% FBS. 2 mM thymidine was added to each flask and incubated 16 hours. Cells were washed with PBS and released into growth medium for 8 hr. Cells were blocked a second time for 15 hours with 2 mM thymidine. Cells were washed with PBS and released into growth medium with or without 40 mM tasisulam as indicated in the figure legend. Cells were collected by trypsinization , fixed in 70% ethanol at -20°C, washed with PBS and stained with propidium iodide. Flow cytometry was used to determine cell cycle phase.

Histology	Sensitive	Resistant	Total
Bladder	2	1	66%
Breast	4	1	75%
CNS	6	2	75%
Colon	10	0	100%
Gastric	1	1	50%
Head and Neck	1	0	100%
Leukemia	4	0	100%
Liver	1	0	100%
Lung	16	5	76%
Melanoma	18	14	56%
Mesothelioma	1	1	50%
Ovarian	7	3	70%
Pancreatic	2	1	66%
Prostate	2	0	100%
Renal	10	2	83%
Sarcoma	1	1	50%
Uterine/Cervical	1	1	50%

Table S1 — Broad activity of tasisulam in tumor cell lines *in vitro*. Tasisulam was evaluated *in vitro* for anti-proliferative activity. "Sensitive" cell lines are those where the tasisulam anti-proliferative EC_{50} is <50 μM (corresponding to a free drug concentration of <5 μM).

	Cell Cycle State (%)		
	G1	S	G2/M
Control	63±2	7±4	30±5
Tasi 20 uM	62±3	12±3	25±5
Tasi 2 uM	59±1	18±4	24±4
Nocodazole	39±4	7±3	54±5

Table S2. HUVEC cell cycle percentages following tasisulam treatment. HUVEC cells cultured in 0.5% serum (n = 3) were treated with the indicated concentrations of tasisulam for 48 hoursprior to cell cycle analysis using Acumen Explorer based HCI per the manufacturer's instructions. Nocodazole concentration was 5 μM.