Supporting information

Collateral sensitivity of multidrug resistant cells to the orphan drug tiopronin[a]

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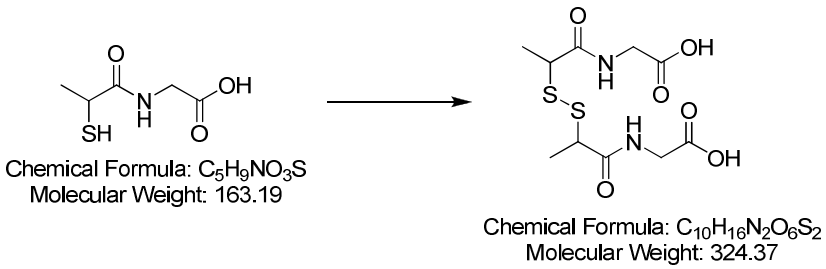
Supporting Materials and Methods

Tiopronin auto-oxidation. A 3 M solution of tiopronin was prepared by dissolving 500 mg of Tiopronin in 3.06 mL of distilled water in a 20 mL scintillation vial. The resulting solution was vortexed and allowed to stand at room temperature. The oxidation process of tiopronin was monitored by HPLC-MS using a Zorbax SB-C18 4.6x50 mm 3.5 μm reverse phase column and a 5→95% over 10min at 1.0 mL/min Acetonitrile/H_2O with 0.05% TFA, and APCI mass detection. Oxidation was measured at 5 intervals: T_0=0 hrs, T_1=17 hrs, T_2=25 hrs, T_3=42 hrs, T_4=65 hrs, and T_5=89 hrs.

Synthesis of oxidized tiopronin (10). Iodine (156 mg, 0.61 mmol) was added in portions to a solution of Tiopronin (100 mg, 0.61 mmol) in deionized water (5 mL). The resulting reaction mixture was heated to 110 ^oC under microwave irradiation for 35 minutes. Reaction mixture was quenched by addition of saturated sodium thiosulfate solution, and allowed to stir for 10 minutes. The reaction mixture was extracted with ethyl acetate twice and the combined organic layers washed with saturated sodium thiosulfate twice, dried over magnesium sulfate and concentrated. Product was crashed out of toluene to afford the cross-linked Tiopronin as an off-white solid (26 mg, 13%).

a.

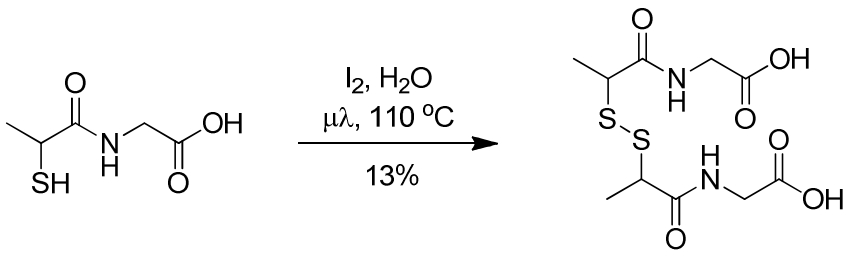


b.

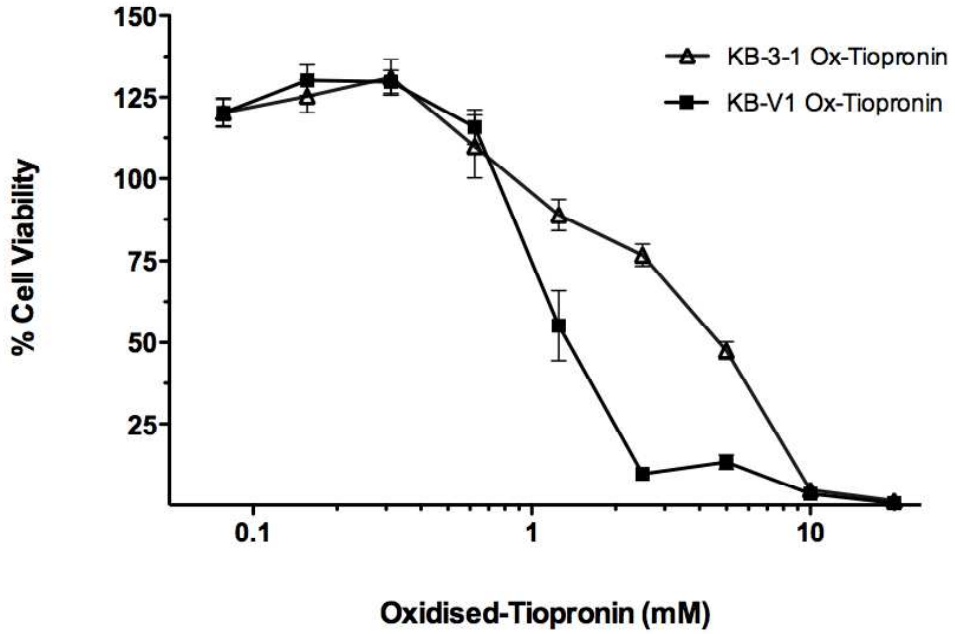
Time (h)	% 1	% 10
0	96.6	3.4
17	96.3	3.7
25	94.2	5.7
42	95.6	4.4
65	93.0	7.0
89	92.2	7.7

Supporting Figure S1a. Scheme showing the structure and molecular weight of **1** (RSH) and its oxidised (RSSR) form **10**. **S1b.** Tabulation showing percentage of **1** and % oxidised **1** in solution over time. The HPLC peak corresponding to **1** was assigned based on an APCI+ mass spectrum peak of 164.1 (**1**+H). The HPLC peak corresponding to **10** was assigned based on an APCI+ mass spectrum peak at 325.0 ((**1**-H)$_2$+H). No other significant metabolites were observed in the HPLC trace.

a



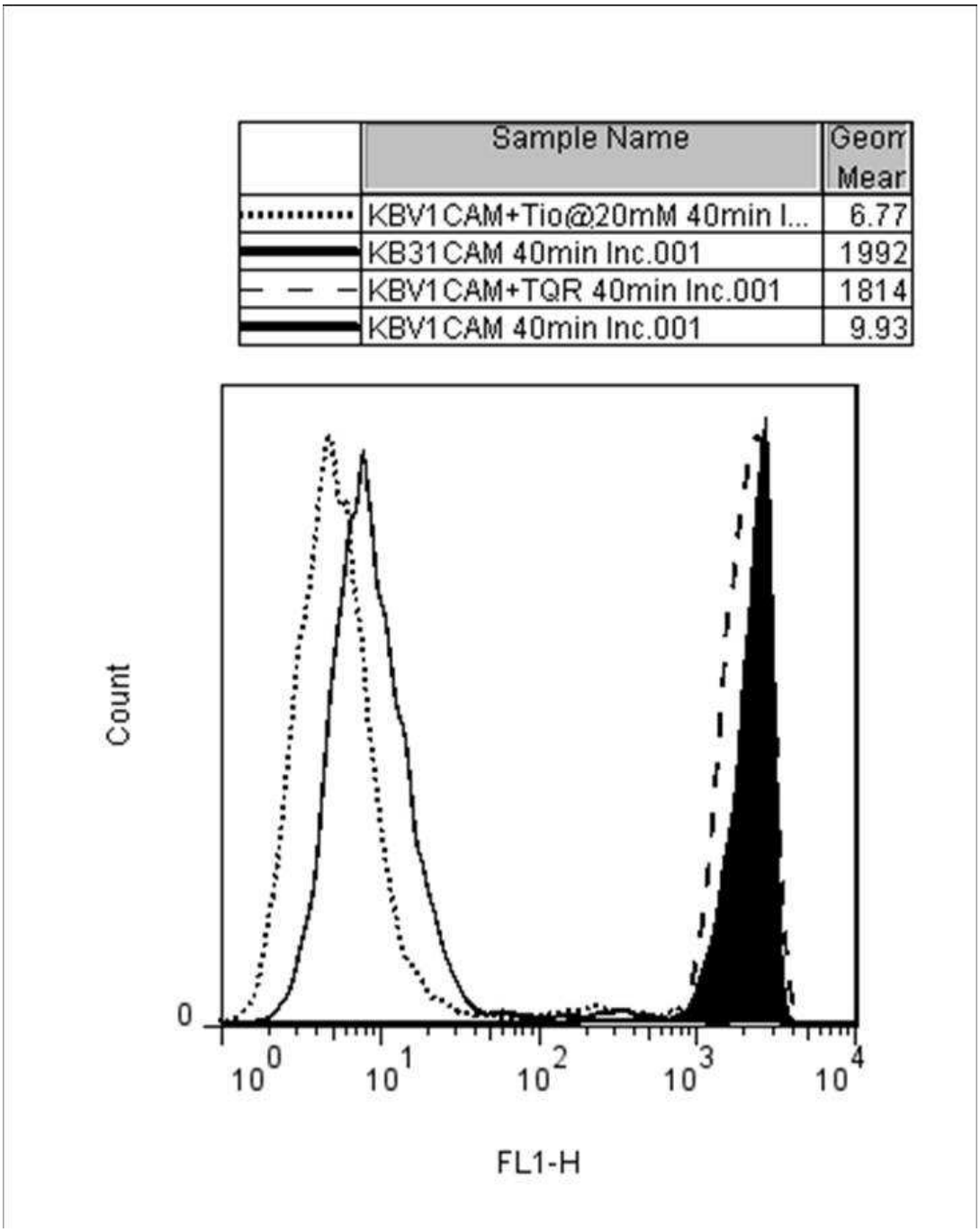
b



c

	KB-3-1	KB-V1	RR
1	7.5 ± 0.2	0.15 ± 0.02	51
10	4.8 ± 0.4	1.4 ± 0.3	3.4

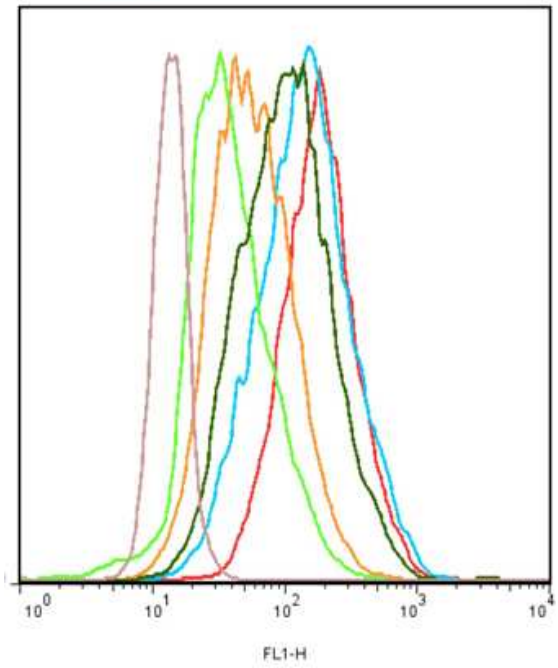
Supporting Figure S2a. Schematic for synthesis of oxidized **1** (**10**, RSSR), and **S2b.** Cytotoxicity of **10** against P-gp-expressing KB-V1and parental KB-3-1 human adenocarcinoma cells. **S3c.** Comparison of cytotoxicity (IC_{50}) of reduced (**1**) and oxidised (**10**) forms of tiopronin.



Supporting Figure S3. 1 does not interfere with P-gp function. KB-V1 cells were incubated with the P-gp substrate calcein-AM (1 μM) alone (solid line) or in the presence of **1** (20 mM, dotted line) or with the positive control P-gp inhibitor tariquidar (200 nM, dashed line) and compared with the fluorescence of the parental cell line KB-3-1 (black filled histogram).

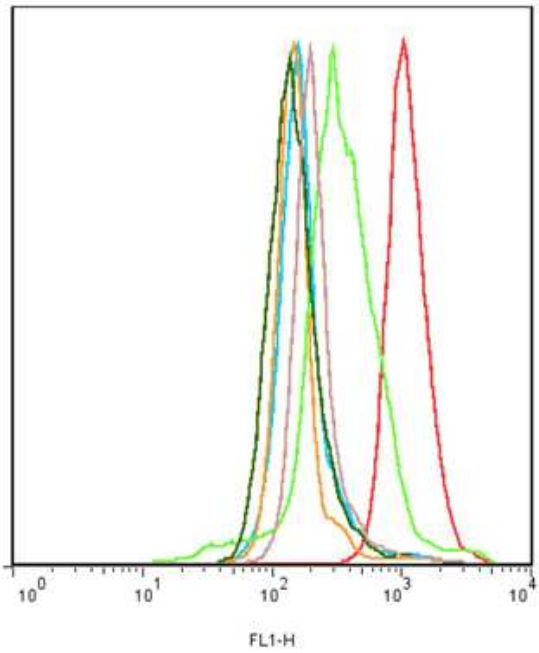
a.

Sample Name	Geom. Mean ,
KB31+MRK16.001	13.7
KBV1-VBL+MRK16.001	99.9
KBV1+5mMTio+MRK16.001	37.5
KBV1+1mMTio+MRK16.001	56.6
KBV1+0.1mMTio+MRK16.001	138
KBV1+MRK16.001	180



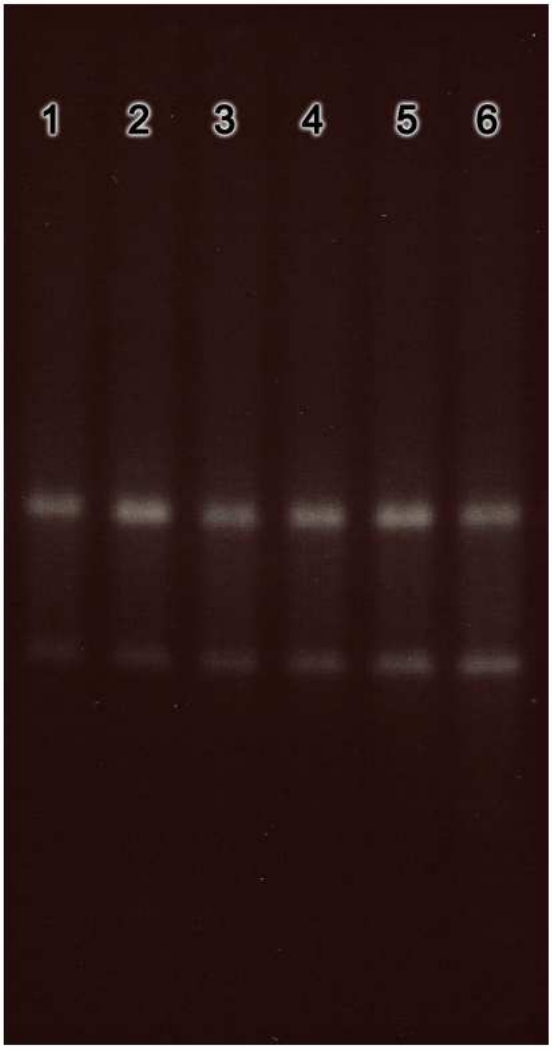
b.

Sample Name	Geom. Mean ,
KBV1+0.1mMTio+50ugmlRh123.001	216
KBV1+1mMTio+50ugmlRh123.001	151
KBV1+5mMTio+50ugmlRh123.001	335
KBV1+50ugmlRh123.001	155
KBV1-VBL+50ugmlRh123.001	173
KB31+50ugmlRh123.001	1098

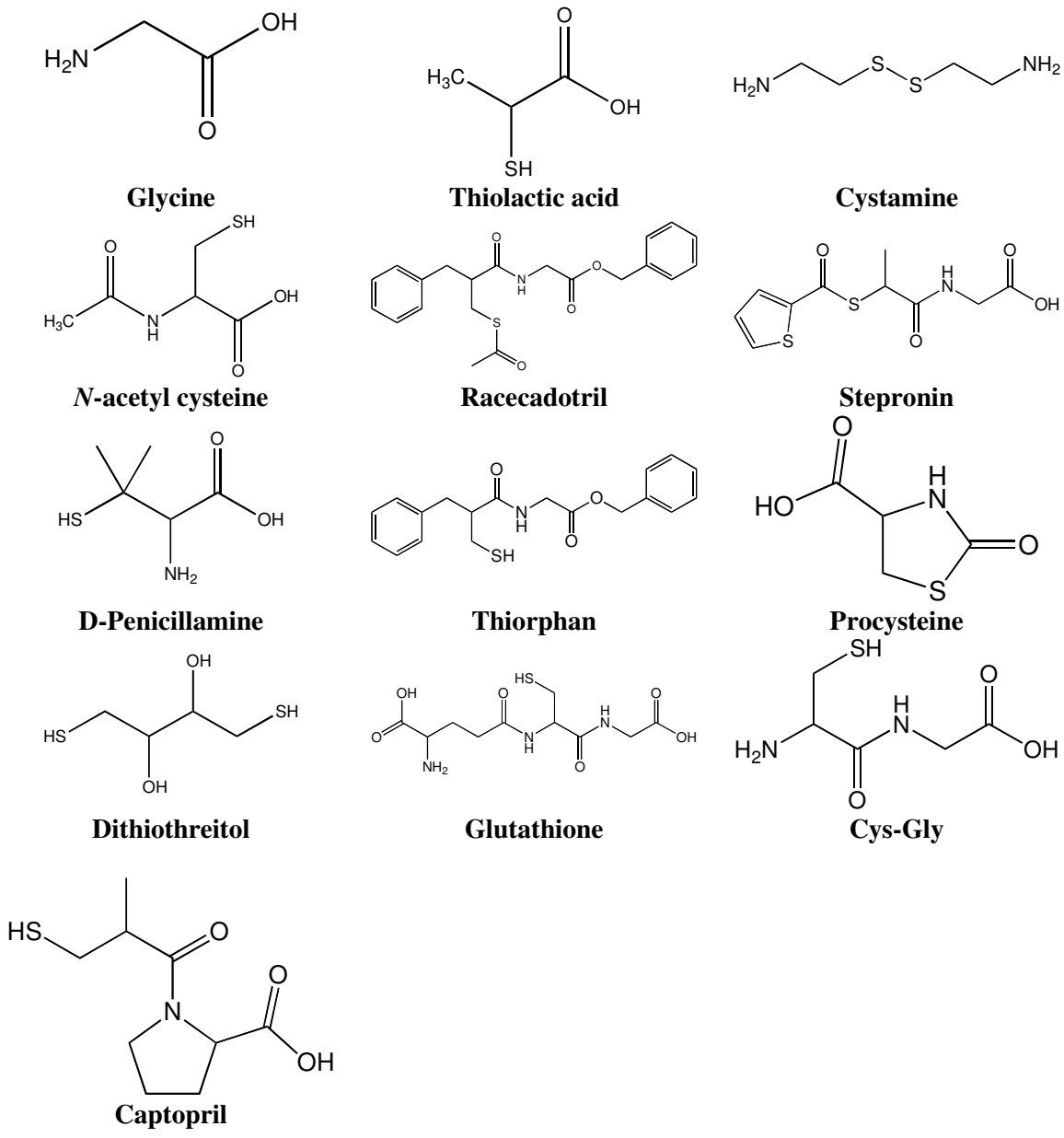


Supporting Figure S4a. FACS assay using the anti-P-gp antibody MRK16, that recognizes an extracellular epitope of P-gp, measuring total cell surface P-gp in live cells. Cells incubated in 1 (green curve) and 5 (orange curve) mM **1** show decreased P-gp expression after long-term treatment with **1**.

S4b. Rh123 efflux assay on long-term culture. At the standard concentration of Rh123 (0.5 μg/mL), all conditions showed complete efflux of Rh123 - in other words the assay cannot discriminate between the levels of P-gp expression due to functional redundancy present in the system (this assay is normally not used to 'measure' P-gp expression). We hypothesized that a higher concentration of substrate (50 μg/mL Rh123) might 'overwhelm' the efflux transporter and reveal differences in cellular acccumulation. In this case, only cells treated with 5 mM **1** (green curve) showed a diminished ability to preclude Rh123 accumulation.



Supporting Figure S5. Ethidium bromide staining of the RNA gel for the Northern blot showing in Figure 5a, demonstrating approximately equal RNA per lane, exemplified by the 18S/28S rRNA band intensities.



Supporting Figure S6. Structures of compounds described in Table 2.