Supplemental Data:

Evidence for dual mode of action of a thiosemicarbazone, NSC73306: A potent substrate of the multidrug resistance-linked ABCG2 transporter

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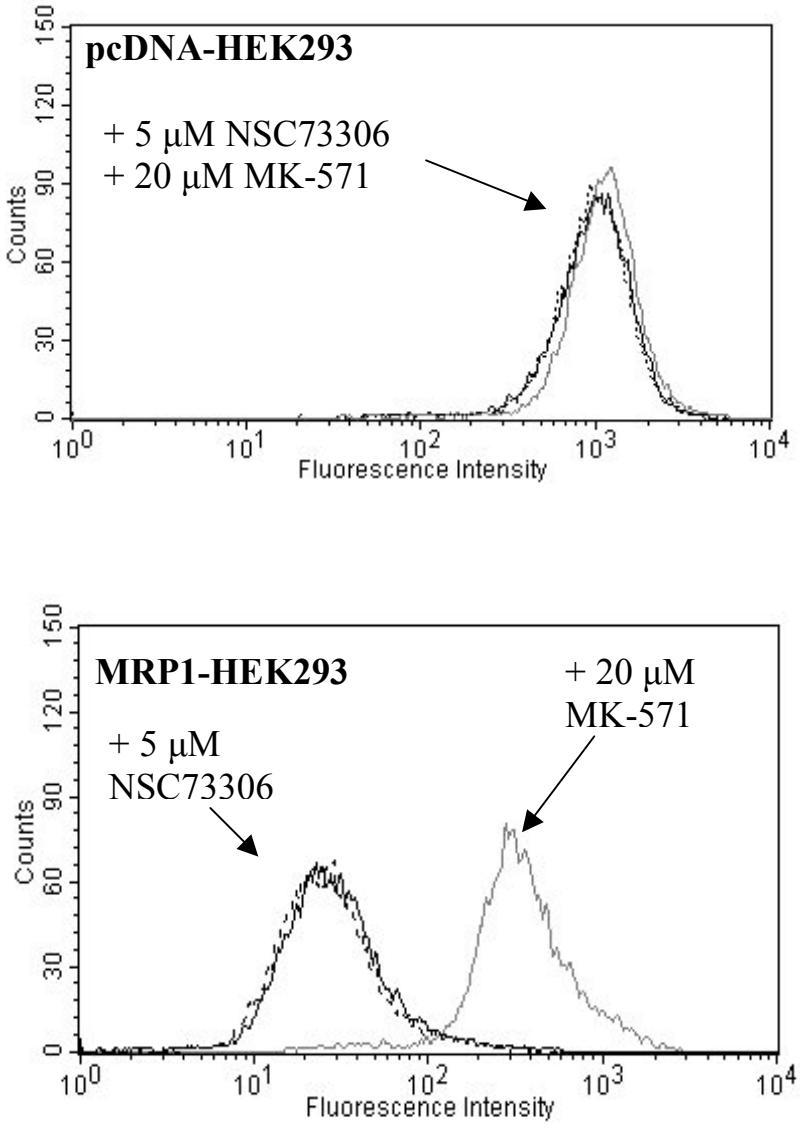
Wu et al, Supplementary, Figure 1



Wu et al, Supplementary, Figure 2

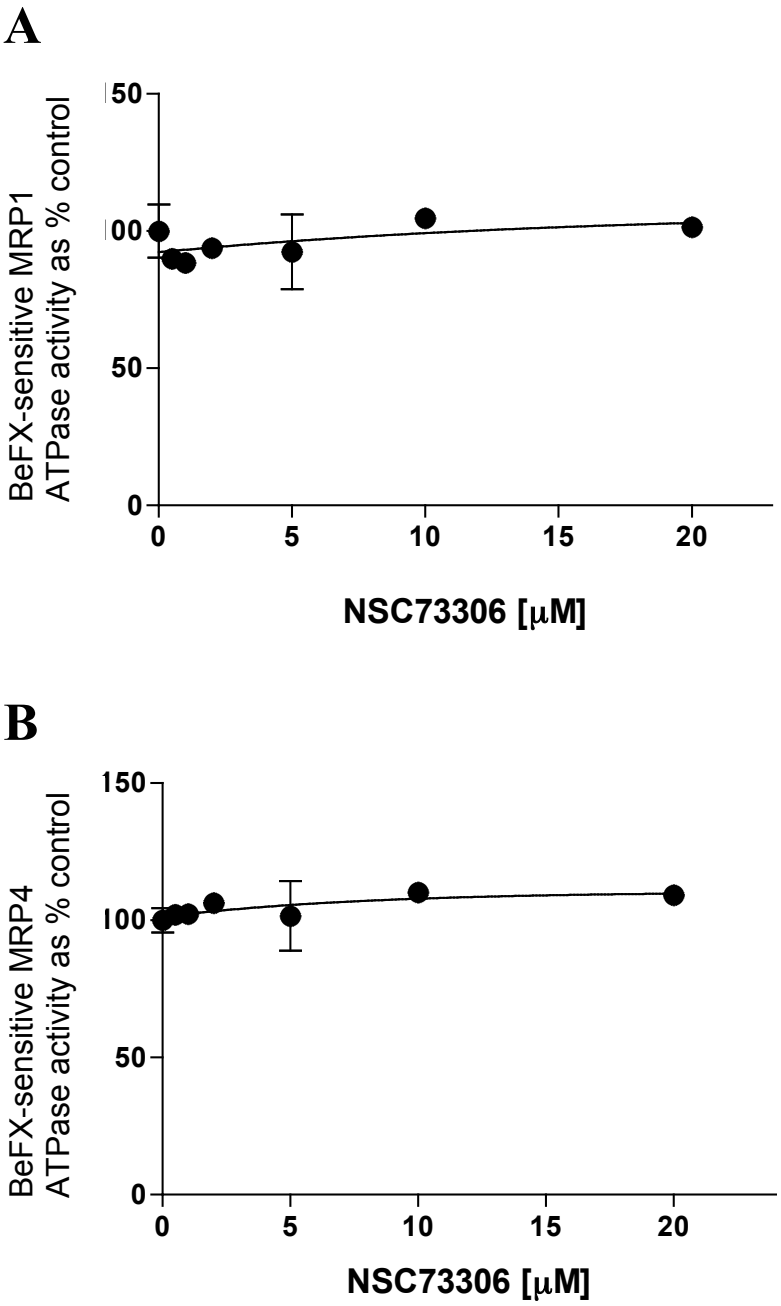


Figure Legends: Supplementary data.

Figure 1. Effect of NSC73306 on calcein accumulation in MRP1-HEK293 cells. Cells (control pcDNA-HEK293 and MRP-transfected MRP1-HEK293) were resuspended in IMDM supplemented with 5% fetal bovine serum. 0.25 μM calcein-AM was added to the cells in the presence or absence of NSC73306 and MK-571. Cells were incubated at 37ºC in the dark for 10 min. The cells were pelleted by centrifugation at 500× *g* and resuspended in 300 μL of PBS containing 0.1% bovine serum albumin. Thin solid lines represent cells in the absence of drug, dotted lines represent cells in the presence of 5 μM NSC73306 and grey solid lines represent cells in the presence of 20 μM MK-571. Samples were analyzed immediately by using FACS. Representative histograms from three independent experiments are shown.

Figure 2. Effect of NSC73306 on MRP1- and MRP4-mediated hydrolysis. Crude membranes of MRP1 (*A*) or MRP4 (*B*) baculovirus-infected High Five insect cells (100 μg/mL protein) were incubated at 37ºC for 5 min with NSC73306 in the presence or absence of BeFx. The reaction was initiated by the addition of 5 mM ATP and terminated with SDS (2.5% final concentration) after 20 min incubation at 37ºC. The amount of P_i released was quantitated using a colorimetric method (1, 2). Values represent mean ± S.E.M. from at least three independent experiments.

1. Kerr KM, Sauna ZE, Ambudkar SV. Correlation between steady-state ATP hydrolysis and vanadate-induced ADP trapping in Human P-glycoprotein. Evidence for ADP release as the rate-limiting step in the catalytic cycle and its modulation by substrates. J Biol Chem 2001;276:8657-64.
2. Sauna ZE, Nandigama K, Ambudkar SV. Multidrug resistance protein 4 (ABCC4)-mediated ATP hydrolysis: effect of transport substrates and characterization of the post-hydrolysis transition state. J Biol Chem 2004;279:48855-64.