The Effect of Structural Modifications to Glyoxal-*bis*(thiosemicarbazonato)copper(II) Complexes on Cellular Copper Uptake, Copper-mediated ATP7A Trafficking and P-Glycoprotein Mediated Efflux

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Supporting Information

Contents

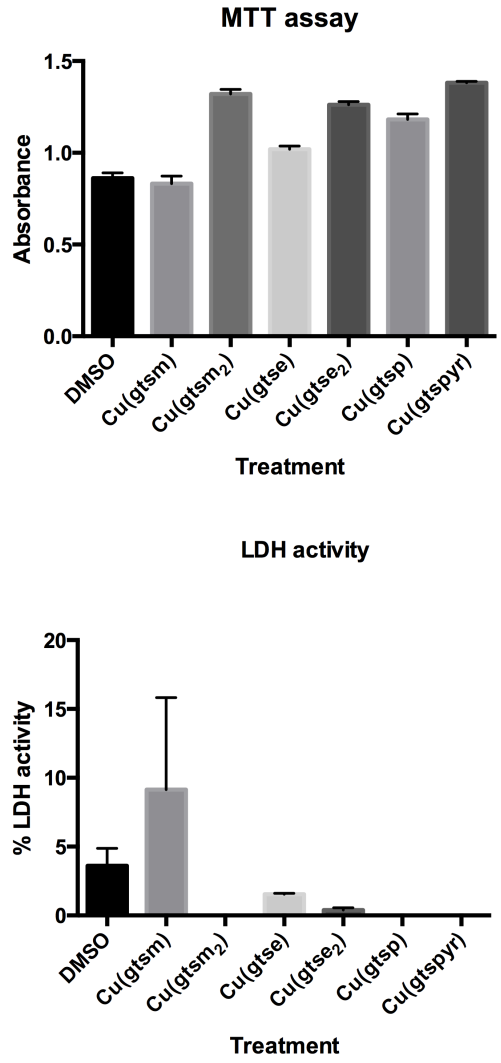


Figure S1. MTT assay and LDH release as a measure of cell viability

The health of SKOV3 cells treated with 1μM of Cu(gts*x*) or DMSO for two hours was assessed by measuring 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) reduction and the release of the enzyme lactate dehydrogenase (LDH). Results show that SKOV3 cells are not adversely affected by Cu(gts*x*) compounds (n=3).

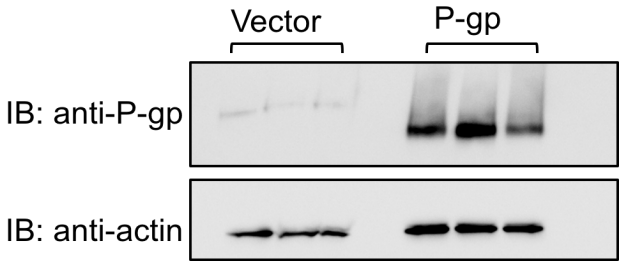
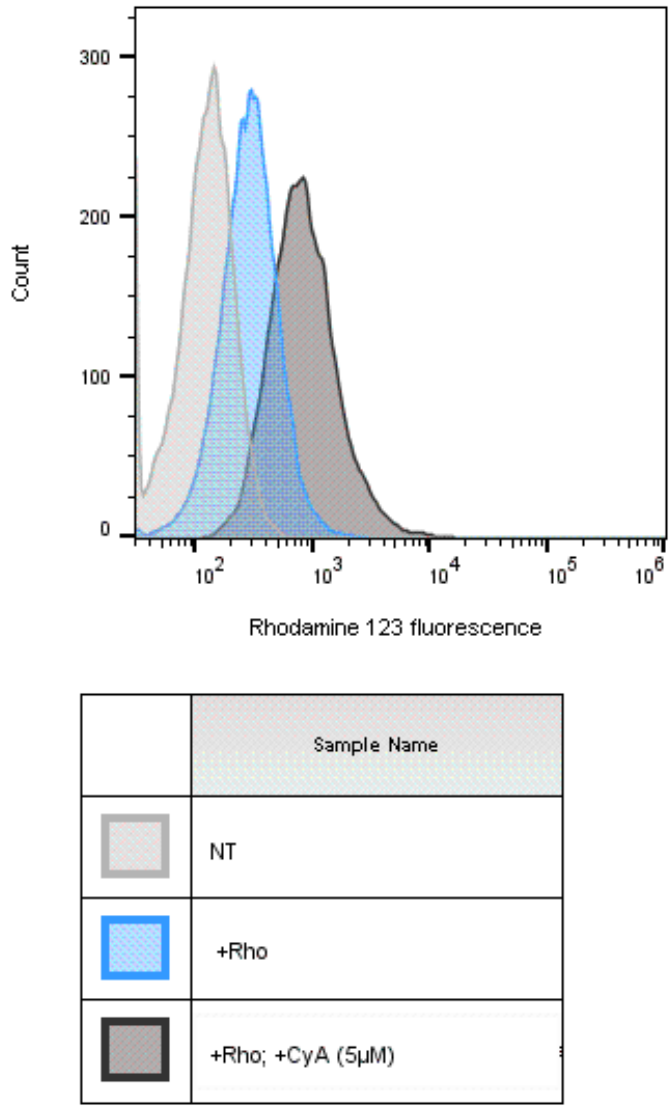


Figure S2. The level of P-gp expressed in HEK293 vector only or HEK293 overexpressing P-gp cell lines as determined by western blot analysis. There is an approximate 12-fold increase (adjusted

for protein loading) in the level of P-gp erexpressed in P-gp overexpressing cells compared to vector-only cells (n=3; P<0.05).

A



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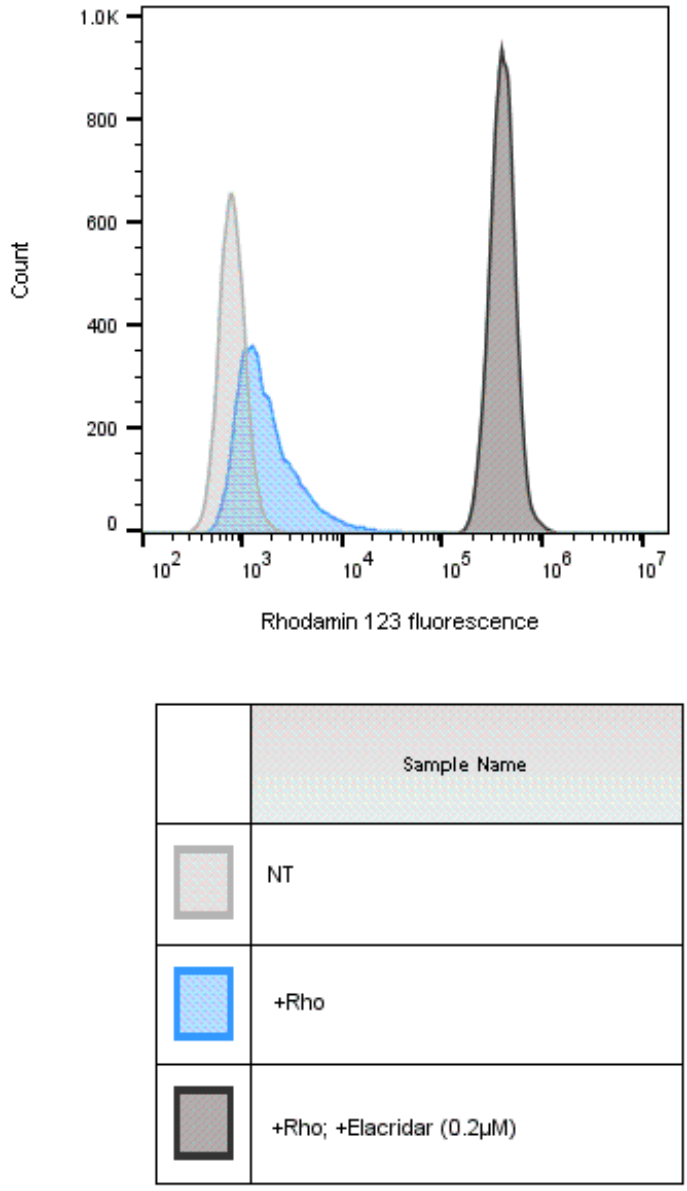
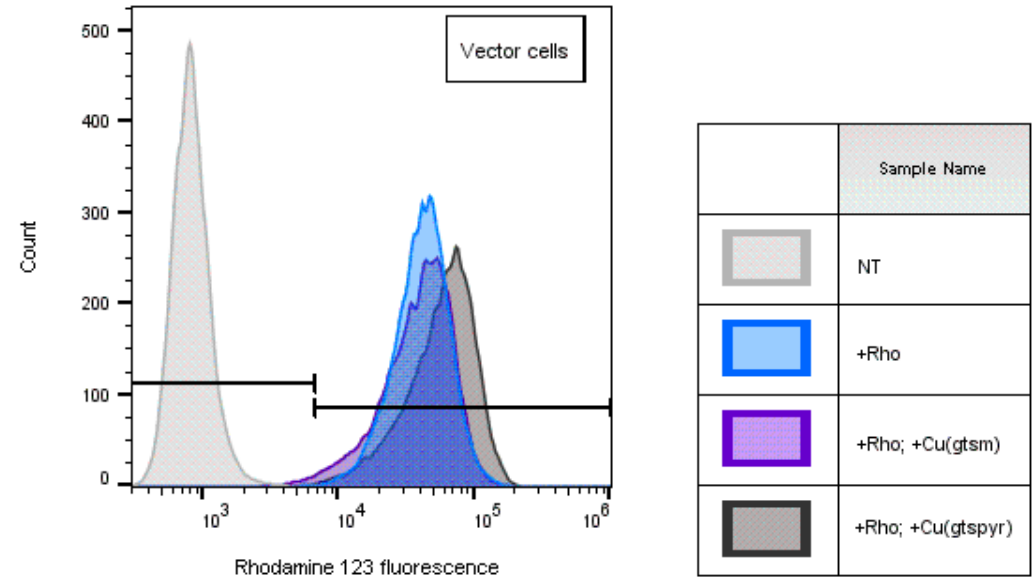


Figure S3. Functional P-glycoprotein assay. The retention of rhodamine 123 was measured in HEK293 cells expressing P-gp by flow cytometry. Rhodamine 123 (0.5µg/ml) was added for 30 min in the presence and absence of (A) cycloporin A (cyA; 5µM) or (B) elacridar (0.2µM). Overlays compare the rhodamine fluorescence of non-treated (grey), rhodamine (blue) and rhodamine + P-gp inhibitor (black) treated cells. The addition of inhibitor increases rhodamine fluorescence, suggesting P-gp is actively effluxing rhodamine dye at basal conditions. Elacridar is a potent inhitor of P-gp, as seen by high retention of rhodamine 123 dye.

A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Rhodamine 123 fluorescence
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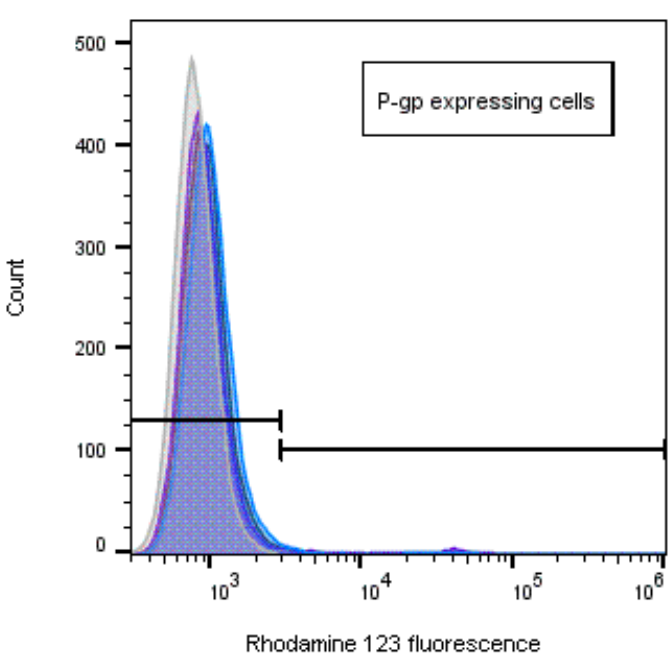
+Rho
+Rho; +Cu(gtsm)
+Rho; +Cu(gtspyr)

B


P-gp expressing cells
Count
Rhodamine 123 fluorescence

C

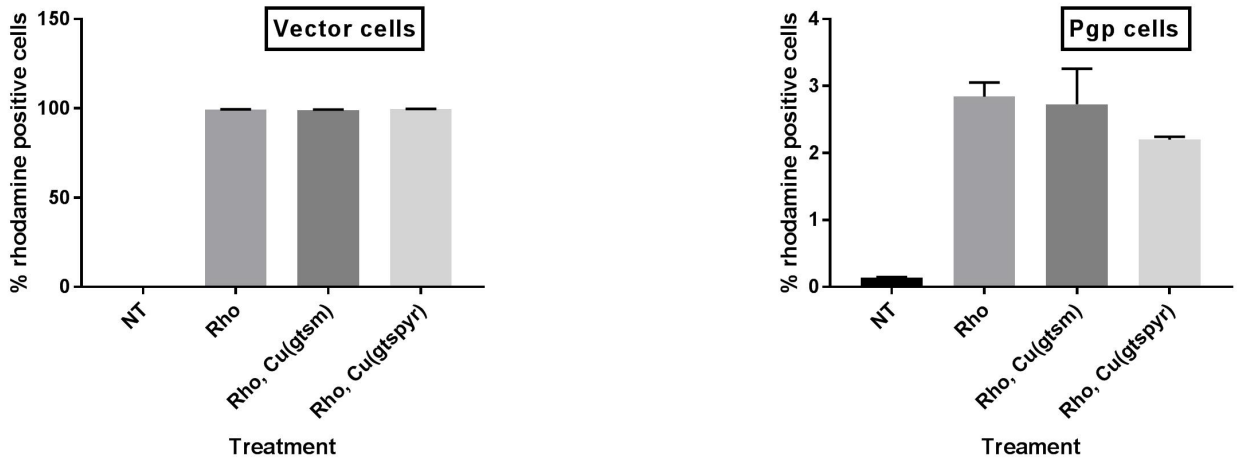


Figure S4. Treatment with Cu(gtsm) or Cu(gtspyr) does not affect the activity of P-gp in both vector and P-gp expressing cells as measured by P-gp functional assays. (A and B) Vector or P-gp expressing cells were incubated with Cu(gtsm) or Cu(gtspyr) at 1uM for 2 hour prior to addition of rhodamine 123 for a further 30min. Cells were washed and fresh media was added for 30min. Cells were then harvested and the level of rhodamine 123 remaining in cells analysed by flow cytometry using the CytoFlex analyser (Beckman coulter). (C) Graph shows percentage of rhodamine positive cells per treatment. No statistical significance in the percentage of rhodamine positive cells was found between rhodamine alone and rhodamine + Cu(gtsx) samples as determined by two-way anova and Dunnett's multiple comparison test. (n=3).