Ryan et al., *Intravital multiphoton microscopy with fluorescent bile salts in rats as an in vivo biomarker for hepatobiliary transport inhibition*, Drug Metabolism and Disposition

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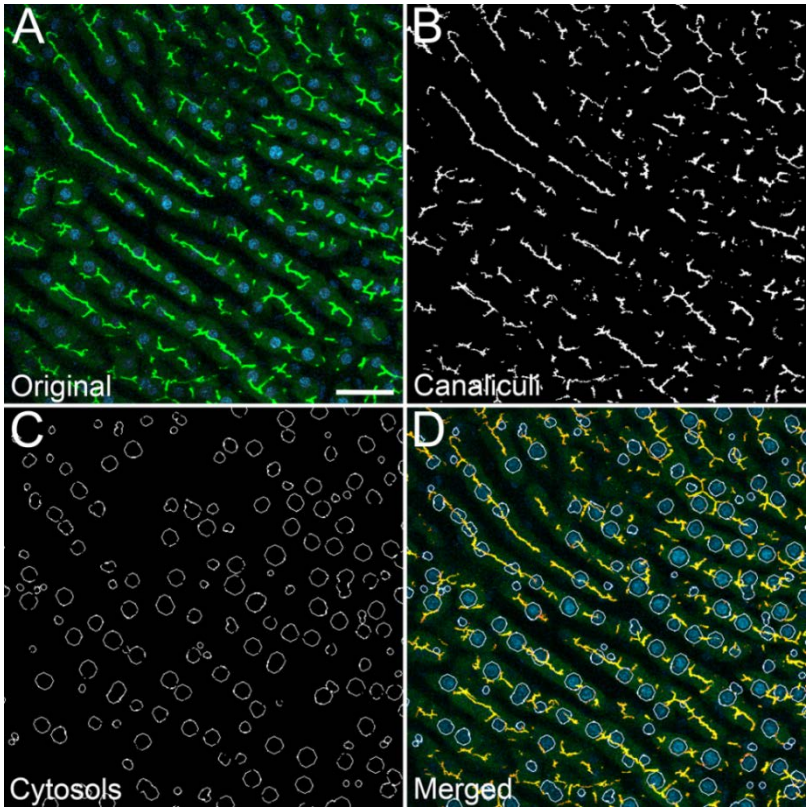
Supplementary Figure 1 – Image analysis procedures



Automated methods of digital image analysis (described in “Materials and methods”) were applied to original projected image volumes (panel A, CGamF displayed in green, Hoechst-labeled nuclei displayed in blue) to automatically segment regions of canaliculi (panel B) or hepatocyte cytosols (panel C) for automated quantification of the kinetics of secretion and uptake, respectively. Panel D shows canalicular regions of interest (red) and cytosolic regions of interest (white) overlaid over the original image. The nearly-complete overlap between the green canalicular fluorescence of the original image with the red signal of the segmented image (appearing as yellow) demonstrates the effective segmentation of the canalicular regions. Scale bar is 50 microns in length.

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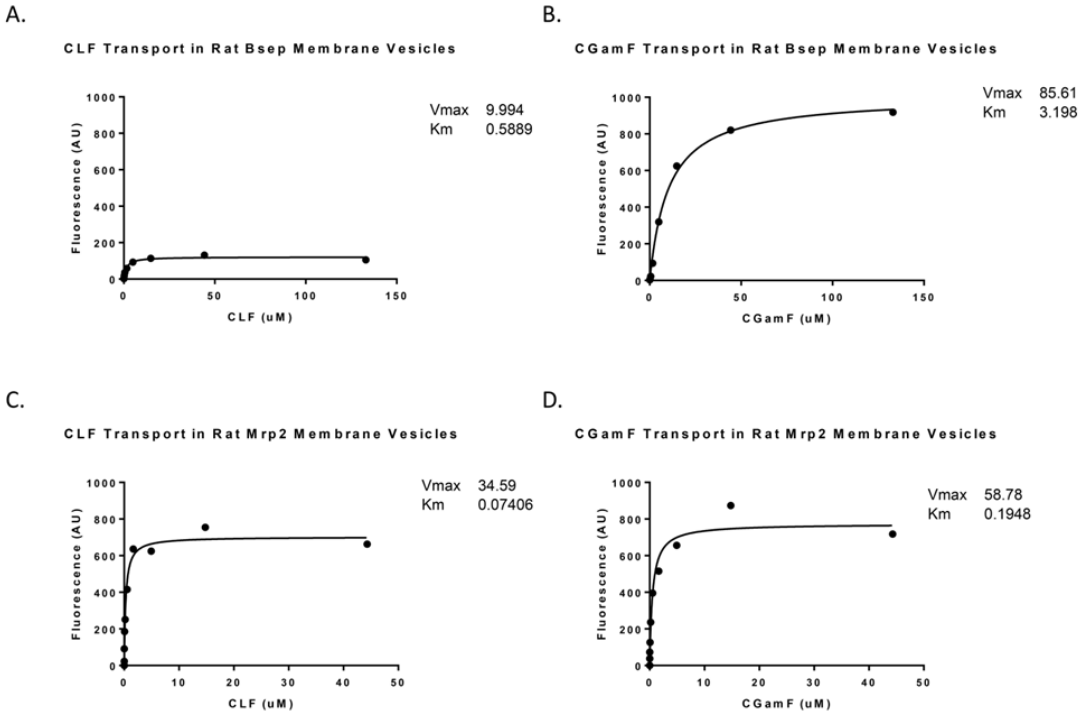
Supplementary Figure 2 - Transport of CGamF or CLF assessed in rat Bsep or Mrp2 membrane vesicles.



Fluorescence resulting from the without ATP control condition (background) was subtracted from the with ATP control condition to generate supplementary figure 2A – D. CGamF and CLF appear to be transported by both Bsep and Mrp2. In supplementary figures 2C and D, the data for the 133 µM concentration of CGamF were not included because background fluorescence was approximately equal to the with ATP condition (data not shown). These data suggest that CLF and CGamF have similar activity in rat Mrp2 transport, however CGamF appears to have higher activity in rat Bsep as compared to CLF. Two technical replicates for each concentration were evaluated. Curve fitting, Vmax and Km calculations performed in GraphPad Prism 7.

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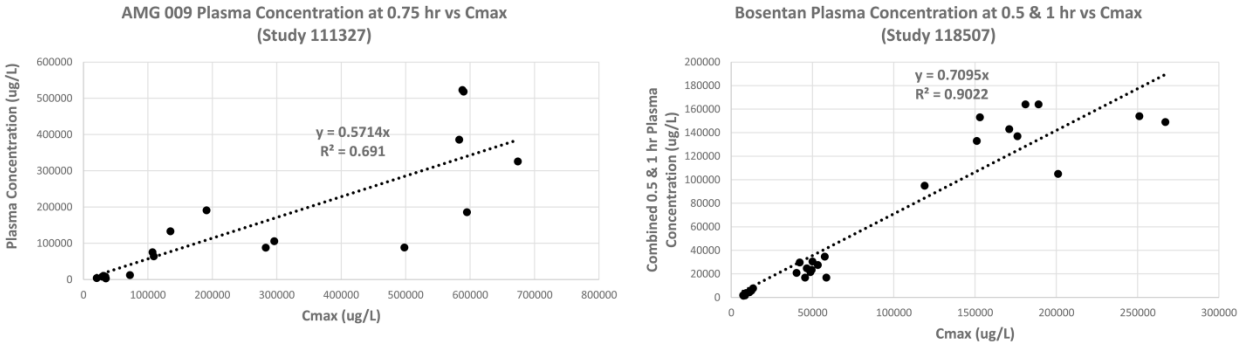
Supplementary Figure 3 – Rat plasma drug concentrations



From the previously conducted serum total bile acid studies in rats, plasma concentrations of AMG 009 at 45 minutes post dose (A), or bosentan at 30 and 60 minutes post dose (B) were correlated with C_{max} values for individual animals. Linear regression was performed to allow for the extrapolation of estimated C_{max} values for the single 50 minute time point taken during the intravital imaging studies.

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Video legends

Video 1 – Effects of AMG 009 on CGamF transport in rat liver – Time-series of MIP images of the livers of living rats collected over 5 minutes after IV injection of CGamF. Rats were injected with vehicle (left) or 30 mg/kg AMG 009 (right), twenty minutes prior to imaging. Video plays at ~100x speed.

Video 2 – Effects of AMG 009 on CLF transport in rat liver – Time-series of MIP images of the livers of living rats collected over 5 minutes after IV injection of CLF. Rats were injected with vehicle (left) or 30 mg/kg AMG 009 (right), twenty minutes prior to imaging. Video plays at ~100x speed.

Video 3 –Effects of bosentan on CGamF transport in rat liver – Time-series of MIP images of the livers of living rats collected over 5 minutes after IV injection of CGamF. Rats were injected with vehicle (left) or 30 mg/kg bosentan (right), twenty minutes prior to imaging. Video plays at ~100x speed.

Video 4 –Effects of bosentan on CLF transport in rat liver – Time-series of MIP images of the livers of living rats collected over 5 minutes after IV injection of CLF. Rats were injected with vehicle (left) or 30 mg/kg bosentan (right), twenty minutes prior to imaging. Video plays at ~100x speed.