

Benzothiourea derivatives target the secretory pathway of the human fungal pathogen *Cryptococcus neoformans*

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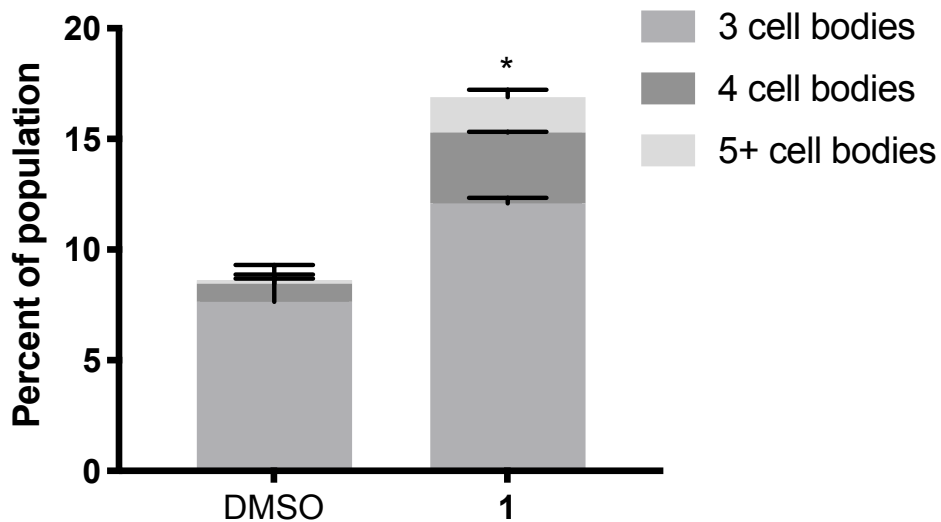
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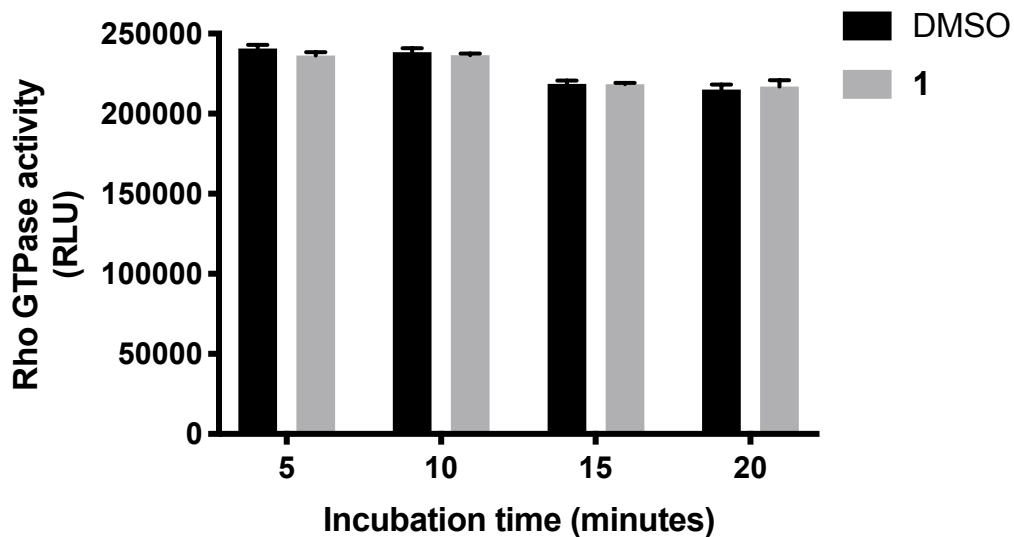
4 Pages: Supplementary Table 1 and Supplementary Figures 1-3

Clinical antifungal	1		Clinical antifungal		FICI
	MIC <sub>a</sub> (µg/mL)	MIC <sub>b</sub> (µg/mL)	MIC <sub>a</sub> (µg/mL)	MIC <sub>b</sub> (µg/mL)	
Amphotericin B	2	2	2	2	2
Fluconazole	4	2	2	1	1
5-Flucytosine	2	2	2	2	2

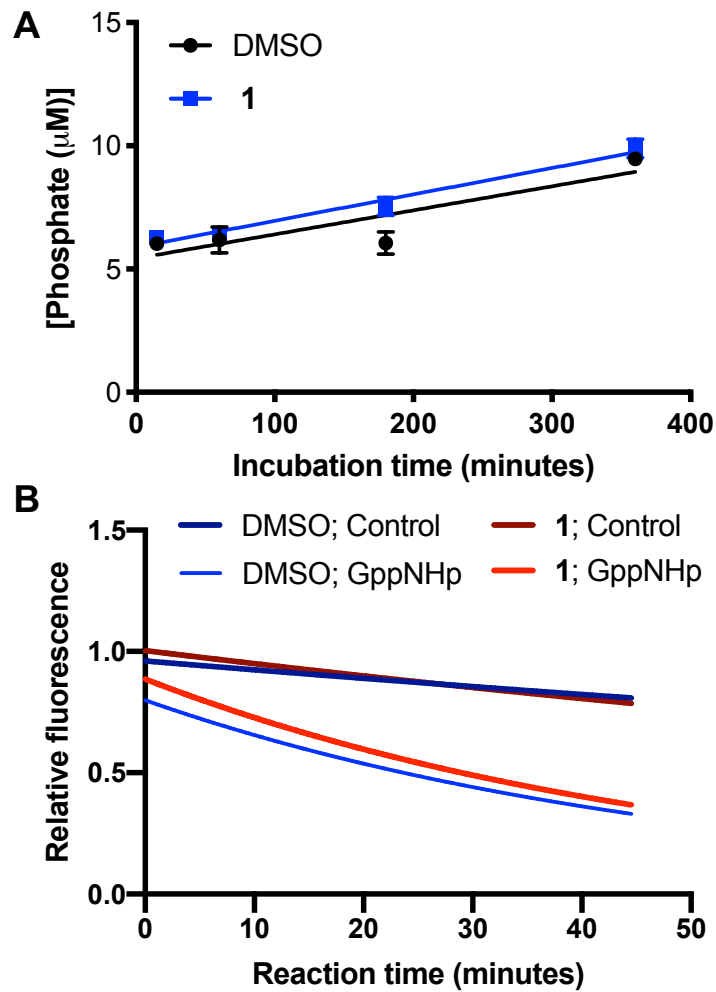
**Table S1. Fractional inhibitory concentration indices (FICI) of 1 with clinical antifungals.** FICI determined using standard checkerboard susceptibility assays under CLSI conditions. FICI = 1 – 4 is considered no interaction. MIC<sub>a</sub> = MIC of single drug, MIC<sub>b</sub> = MIC of drug in combination. Representative data from two independent experiments.



**Figure S1. BTU treatment induces a cell separation defect.** Cells grown in 10% Sab pH8 with **1** (1  $\mu\text{g}/\text{mL}$ ) have a two fold higher incidence of cells with 3 cell bodies or more. \* $p=0.0099$  by unpaired t-test of total 3+ cell bodies compared to control. Data represent mean and SD of triplicates with at least 150 cells counted per sample.



**Figure S2. BTU derivatives do not affect Rho1 GTP hydrolysis.** Incubation with **1** (25  $\mu\text{g}/\text{mL}$ ) did not affect the intrinsic GTP hydrolysis of *Cn*Rho1 as measured by GTPase-Glo assay. Data represent mean and SD of triplicates.



**Figure S3. Intrinsic GTPase activity or GDP/GTP exchange is not affected by BTU derivatives.** A) Purified Sav1 binds the fluorescently labeled mantGDP. Upon addition of GppNHp (5  $\mu\text{M}$ ), GDP is exchanged, as measured by decrease in fluorescence compared to control. Data shows non linear fit of a single reaction representative of duplicates from one of three independent experiments. B) *CnSav1* GTPase activity is not affected by **1** (50  $\mu\text{g}/\text{mL}$ ) as measured by Malachite green phosphate detection. Data represent mean and SD of duplicates. Lines are linear regression of each data set.