Supplementary Figures:



Figure S1. TA-289 is growth inhibitory in the presence of a respiratory carbon source. Wild-type (wt) and $pdr\Delta$ cells treated with a serial dilution of TA-289 in the indicated concentrations in SC medium containing glycerol-ethanol as a carbon source, were incubated for 18 h at 30 °C and cell growth measured by determining OD at 590 nm.



Figure S2. Deletion of pro-apoptotic genes confers modest resistance to TA-289. Wild-type (wt) cells and strains deficient in the indicated prop-apoptotic genes were treated with a serial dilution of TA-289 in the indicated concentrations in SC medium, were incubated for 18 h at 30 °C and cell growth measured by determining OD at 590 nm.



gure S3. The cytotoxicity of TA-289 is dependent upon carbon source. A serial dilution of TA-289 (final concentration range $100 - 10 \mu$ M) was added to SC or SC-Gly-EtOH liquid media inoculated with cells (5 x 10^5 cells/mL). A 4 μ L aliquot of cells from each dilution was removed and spotted onto either SC or SC-Gly-EtOH solid media every 30 mins for 4 h. A) Cells treated with TA-289 in SC and spotted onto SC (fermentative to fermentative state), B) Cells treated with TA-289 in SC and spotted onto SC-Gly-EtOH (fermentative to respiratory state), C) Cells treated with TA-289 in SC-Gly-EtOH and spotted onto SC (respiratory to fermentative state), D) Cells treated with TA-289 in SC-Gly-EtOH and spotted onto SC-Gly-EtOH (respiratory to respiratory state).



Figure S4. TA-289 causes a late-phase cell cycle block. Cells ($pdr\Delta$, 2 x 10⁷ cells/mL) were treated with 100 μ M TA-289 for 4 h and stained with SYTOX green (x-axis) prior to analysis by flow cytometry. Histogram overlays were visualized using FlowJo 7.6.1 flow cytometry analysis software. Carrier solvent treated (1% DMSO) control cells (dark grey filled) and cells treated with TA-289 (light grey unfilled) distributions are as indicated.



Figure S5. TA-289 does not target microtubules. Heterozygous *TUB2* deletion strain ($tub2\Delta$::*KanR/TUB2* and wild-type diploid (BY4743) cells treated with a serial dilution of either TA-289 or benomyl in the indicated concentrations, incubated for 18 h at 30 °C and cell growth measured by determining OD at 590 nm.



Figure S6. The TA8 mutant is resistant to H_2O_2 induced cell death. TA-289 resistant mutant TA8 (A) and the parental $pdr\Delta$ (B) cells were treated with the indicated concentrations of H_2O_2 in the presence of glucose as a carbon source and incubated at 30 °C for 3 h. A 1 µL aliquot of cells were spotted onto SC plates every 30 mins and incubated for 2 days at 30 °C.



Figure S7. TA-289-induced growth inhibition persists in the presence of antioxidants. Cells (*pdr* Δ) were inoculated at 5 x 10⁵ cells/ml in SC medium buffered with 50 mM citrate (pH 4) containing ascorbate (Asc, 10 mM), L-cysteine (Cys, 5 mM), glutathione (GSH, 5 mM), N-acetyl-L-cysteine (NAC, 5 mM) or quercetin (Quer, 1 mM). After 2 h incubation at 30 °C, TA-289 was added at a final concentration of 50, 25 or 12.5 μ M, or 1% DMSO (solvent carrier control) and further incubated at 30 °C for 24 h. Growth was measured by absorbance at 590 nm on the Wallac EnVision 2102 Multilabel Plate Reader.



Figure S8. Sensitivity of yeast complex I homologues and complex II gene deletions to TA-289. Deletion strains of genes involved in complex I (*nde1* Δ , *ndi1* Δ) and complex II (*sdh1* Δ , *sdh2* Δ , and *emi5* Δ) were tested for sensitivity or resistance to 60 µM, 30 µM, and 15 µM TA-289, and residual growth relative to wild-type ($\Delta his3$) is presented. Resistance (asterisk) and sensitivity (diamond) of 1% significance are denoted.



Figure S9. Sensitivity of complex III genes to TA-289. Deletion strains of genes involved in complex III were tested for sensitivity or resistance to 60 μ M, 30 μ M, and 15 μ M TA-289, and residual growth relative to wild-type is presented. Resistance (asterisk) and sensitivity (diamond) of 1% significance are denoted. Notably, only the deletion of *QCR10* displayed significant sensitivity, with all other genes tested displaying either resistance or normal growth (\approx 1, wild-type growth).



Figure S10. Sensitivity of complex IV genes to TA-289. Deletion strains of genes involved in complex IV were tested for sensitivity or resistance to 60 μ M, 30 μ M, and 15 μ M TA-289, and residual growth relative to wild-type is presented. Resistance (asterisk) of 1% significance are denoted.



Figure S11. TA-289 causes mitochondrial fragmentation independent of its ability to generate ROS. Six ETC deletion strains and a wild-type control $\Delta his3$, expressing Aim17p-GFP, were treated with TA-289 (25 μ M and 50 μ M) and incubated for 30 min at 30 °C. Cells were visualised on the Opera[®] confocal microplate imaging reader. White arrows indicate cells with abnormal fragmented mitochondria in the form of multiple foci within the cell



Figure S12. TA-289 causes mitochondrial fragmentation in the presence of antioxidants. Aim17p-GFP expressing cells were exposed to 25 μ M TA-289 in the presence of ascorbate (Asc, 10 mM), L-cysteine (Cys, 5 mM), glutathione (GSH, 5 mM), N-acetyl-L-cysteine (NAC, 5 mM) or quercetin (Quer, 1 mM) in SC with 50 mM citrate buffer (pH 4). After 30 min incubation at 30 °C, cells were visualised on the Opera[®] high throughput confocal microscope. Cytoplasmic XX-RFP and NLS-mCherry are expressed to allow identification of the cytoplasm and nucleus respectively

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Figure S13. Atomic number assignments for TA-289.

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e S14. ¹H-NMR spectrum of TA-289



Figure S15. ¹³C-NMR spectrum of TA-289. Carbon assignments are as noted, refer to Figure S13 for structural assignments.

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| ΤΑ-289 (μΜ) | Relative size |
|-------------|----------------------------------|
| 30 | $1.15 (p = 1.15 \times 10^{-4})$ |
| 100 | 1.41 (p = 2.67 x 10^{-16}) |

Table S1. Cell sizes of $pdr\Delta$ cells treated with TA-289. Cells (5 x 10⁵ cells/mL) were treated with either 30 μ M or 100 μ M TA-289 for 18 h and visualized using light microscopy, and size of cells were measured using ImageJ and expressed relative to the solvent carrier control (1% DMSO). An un-paired student t-test was performed, and each value represents the fold decrease in cell size compared to untreated control cells. The corresponding p-values were calculated using the unpaired Student's t-test.