Supporting Methods

Analysis of MGd Uptake and Retention within A549 Cells. To assess drug retention, plateau phase cultures of A549 cells were exposed to 50 μM MGd for 24 h, and medium exchanged for drug-free spent medium (i.e., medium removed from parallel cultures). Cultures were returned to the incubator for the duration of the chase period. For uptake, cells were treated with MGd for the indicated time interval without a chase period. Following MGd treatment, cells were washed twice with PBS, and a single-cell suspension was prepared by exposure to 3 mL trypsin/EDTA for 7 min. Trypsin was inhibited using complete medium, and cells isolated by centrifugation. The resulting cell pellets were resuspended in Hank's buffered sterile saline and counted using a Coulter counter. No significant differences in cell number were detected. Cell suspensions were analyzed using a flow cytometer (Becton-Dickinson FACSCalibur) to measure average fluorescence in the red channel (>650 nm) as a surrogate for MGd concentration, which has an emission maximum of 760 nm following excitation with 488 nm light (14). A portion of each cell suspension was centrifuged and stored at –80 ^{o}C for subsequent extraction and analysis using reversed-phase HPLC. In brief, cell pellets (ca. 3 x 10^6 cells) were suspended in 0.15 mL PBS. MGd was extracted by the addition of a 50/50 v/v solution of methanol/acetonitrile containing 0.16 M glacial acetic acid and zinc sulfate. Extraction efficiency was corrected using an internal standard. HPLC was performed using an Agilent HP1100 chromatography system with detection based on intrinsic MGd fluorescence.

Analysis of MGd Stability. MGd (50 μM final) was added to complete medium obtained from 10-day plateau phase A549 cultures, distributed into loosely capped 1.5 mL microcentrifuge tubes, and placed into a humidified CO_2 incubator set at 37 °C. At chosen time intervals, samples were removed from the incubator and frozen on dry ice for subsequent analysis. After thawing, MGd was extracted from the medium samples while on ice and analyzed by HPLC as described above, except that MGd absorbance at 470 nm was used for detection.