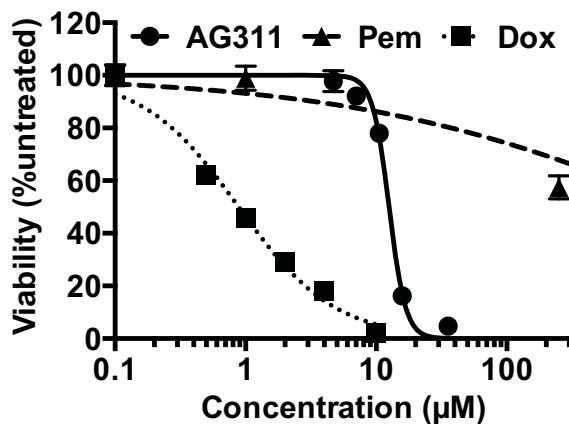


**A small molecule with anticancer and antimetastatic activities induces rapid mitochondrial-associated necrosis in breast cancer**

Anja Bastian, Jessica E. Thorpe, Bryan C. Disc, Lora C. Bailey-Down, Aleem Gangjee, Ravi K. Devambatla, Jim Henthorn, Kenneth M. Humphries, Shraddha S. Vadvalkar, Michael A. Ihnat

*The Journal of Pharmacology and Experimental Therapeutics*



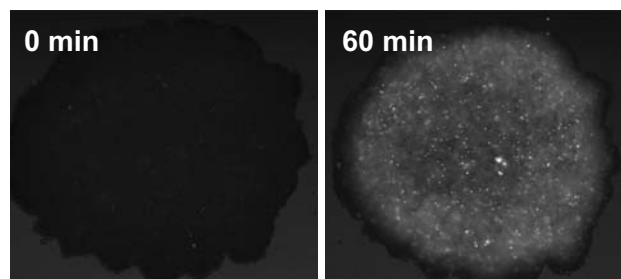
**Figure S1. AG311 shows a steep dose response curve.**

MDA-MB-435 cells were treated with multiple doses of AG311, pemetrexed (Pem), or doxorubicin (Dox) to establish a dose response. Viability was assessed after 48 hours with PrestoBlue.

**A small molecule with anticancer and antimetastatic activities induces rapid mitochondrial-associated necrosis in breast cancer**

Anja Bastian, Jessica E. Thorpe, Bryan C. Disc, Lora C. Bailey-Down, Aleem Gangjee, Ravi K. Devambatla, Jim Henthorn, Kenneth M. Humphries, Shraddha S. Vadvalkar, Michael A. Ihnat

*The Journal of Pharmacology and Experimental Therapeutics*



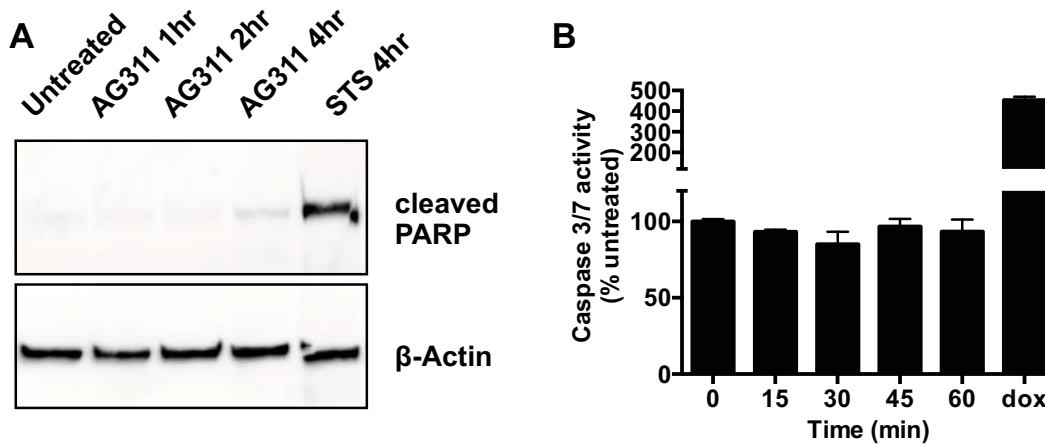
**Figure S2. AG311 induced membrane permeability in MDA-MB-435 spheroids.**

MDA-MB-435 spheroids were cultured for 12 days and treated with AG311 (25  $\mu$ M) in the presence of SYTOX Green. Images were acquired every 5 minutes.

**A small molecule with anticancer and antimetastatic activities induces rapid mitochondrial-associated necrosis in breast cancer**

Anja Bastian, Jessica E. Thorpe, Bryan C. Disc, Lora C. Bailey-Down, Aleem Gangjee, Ravi K. Devambatla, Jim Henthorn, Kenneth M. Humphries, Shraddha S. Vadvalkar, Michael A. Ihnat

*The Journal of Pharmacology and Experimental Therapeutics*



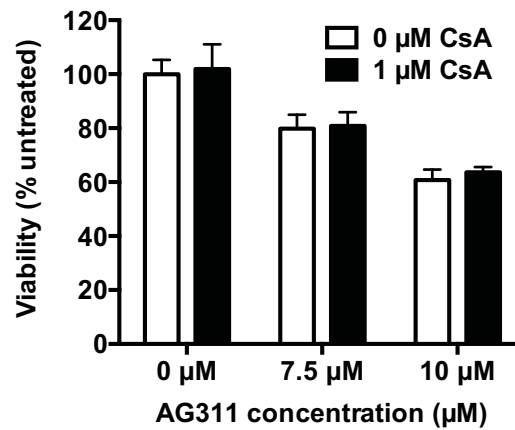
**Figure S3. AG311 does not induce apoptosis.**

**A)** Whole cell lysates were collected from AG311 (20  $\mu$ M) or staurosporine (STS, 1  $\mu$ M) treated MDA-MB-435 cells and western blot analysis performed for the presence of cleaved PARP. **B)** Cells were treated with AG311 (25  $\mu$ M) in 15-minute intervals or doxorubicin (dox) (1  $\mu$ M, 12 hours), followed by measuring caspase 3 and 7 activity.

**A small molecule with anticancer and antimetastatic activities induces rapid mitochondrial-associated necrosis in breast cancer**

Anja Bastian, Jessica E. Thorpe, Bryan C. Disc, Lora C. Bailey-Down, Aleem Gangjee, Ravi K. Devambatla, Jim Henthorn, Kenneth M. Humphries, Shraddha S. Vadvalkar, Michael A. Ihnat

*The Journal of Pharmacology and Experimental Therapeutics*



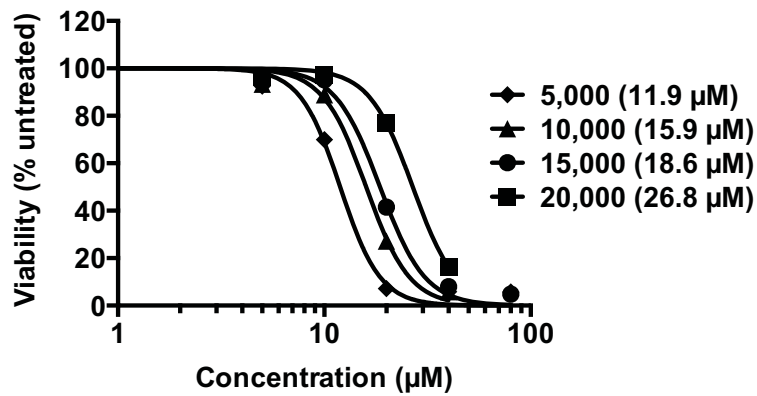
**Figure S4. AG311-induced cell death is not inhibited by cyclosporine A**

MDA-MB-435 cells were treated with AG311 or cyclosporine A (CsA), or cotreated with both agents. Viability was assessed with PrestoBlue 24 hours after treatment.

**A small molecule with anticancer and antimetastatic activities induces rapid mitochondrial-associated necrosis in breast cancer**

Anja Bastian, Jessica E. Thorpe, Bryan C. Disc, Lora C. Bailey-Down, Aleem Gangjee, Ravi K. Devambatla, Jim Henthorn, Kenneth M. Humphries, Shraddha S. Vadvalkar, Michael A. Ihnat

*The Journal of Pharmacology and Experimental Therapeutics*



**Figure S5. IC<sub>50</sub> values of AG311 on different cell densities**

Cells (4T1-luc2-GFP) were plated at different densities and treated with AG311 for 30 hours. Viability was assessed with PrestoBlue 30 hours after treatment. IC<sub>50</sub> are shown in parentheses.