Supplementary Materials: A New Strategy for Glioblastoma Treatment: In Vitro and In Vivo Preclinical Characterization of Si306, a Pyrazolo[3,4-*d*]Pyrimidine Dual Src/P-Glycoprotein Inhibitor

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1. Cell Growth Inhibition by Dasatinib, Si306 and Pro-Si306

GB cell lines BT138, T98G and A172 were treated with Si306 and pro-Si306 at concentrations ranging from 1 to 100 μM, or with DMSO as a control. Cell viability was evaluated 72 h after treatment for T98G and A172 cell lines and after 24 h for BT-138. In table S1 the IC_{50} values are reported.

Table S1. Sensitivity of glioblastoma multiforme (GBM) cells to Si306 and pro-Si306 expressed as IC_{50} values ± S.D.

Compounds	BT-138 μM (24 h)	T98G μM (72 h)	A172 μM (72 h)
Si306	72.58 ± 6.21	0.46 ± 0.02	1.69 ± 0.60
Pro-Si306	30.71 ± 4.75	/	/

2. Rho 123 Accumulation (Proportional to the Level of P-gp Inhibition)

The inhibition of P-gp function illustrated by dose-dependent accumulation of Rho 123. Rho 123 accumulation was assessed in untreated non-MDR (U87) and MDR (U87-TxR) cells as well as in U87-TxR cells treated with increasing concentrations of Si306 (C), pro-Si306 (D), Dex-VER (B; 1, 2, 5, 10, 20 μM) and TQ (A; 1, 2, 5, 10, 20 nM). This Supplementary Figure S1 shows the methodology used for the calculation of IC50 values for P-gp inhibition presented in Figure S3 and Table S2.

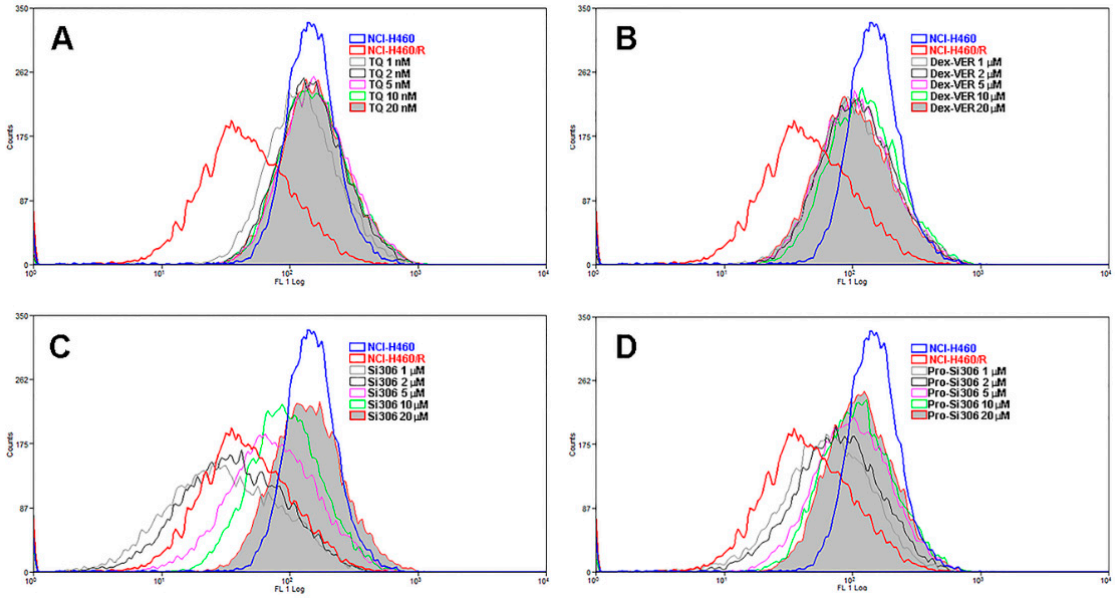

Figure S1. Rho 123 accumulation in U87-TxR cells treated with increasing concentrations of TQ (**A**), Dex-VER (**B**), Si306 (**C**) and pro-Si306 (**D**).

2. Si306 HPLC/MS Analysis

Si306 has been analyzed by HPLC-MS, using Si34 as internal standard. Representative LC-MS chromatograms (single ion monitoring) for Si306 and for Si34 are reported in Figure S3 and S4 respectively. In addition, mass spectra are shown in Figure S4.

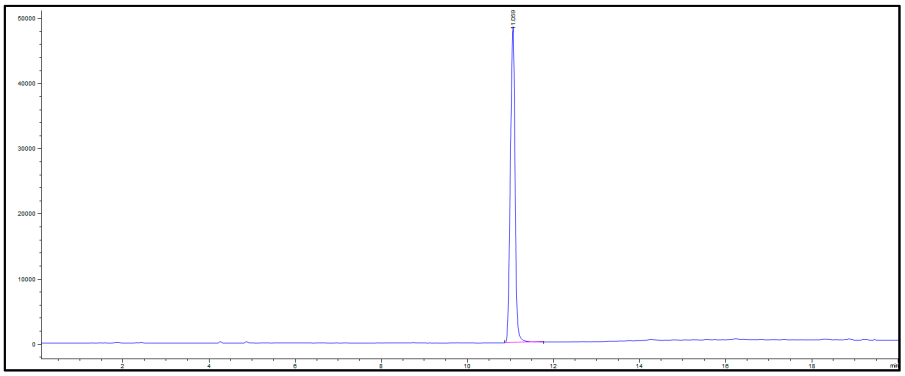

Figure S2. LC-MS chromatogram for Si306.

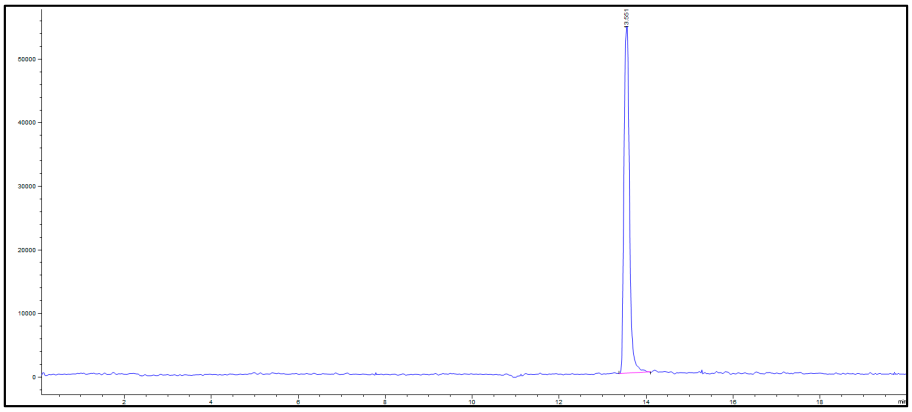

Figure S3. LC-MS chromatogram for Si34, used as internal standard for Si306.

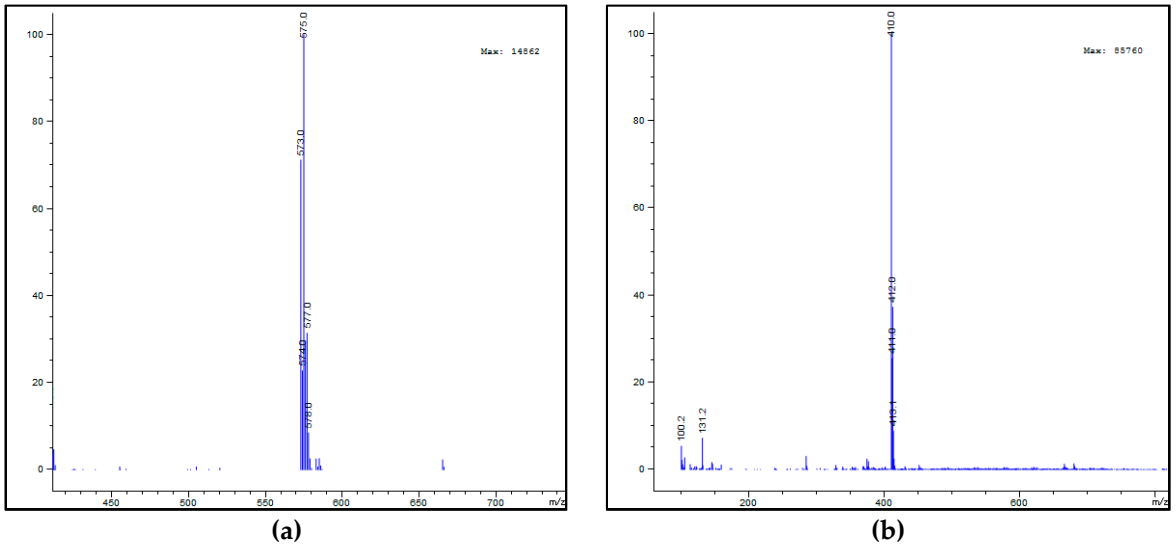

Figure S4. Mass spectra of (**a**) Si306 and (**b**) Si34.