Supplemental Material to:

Venkata Yenugonda, Yali Kong, Tushar Deb, Yonghang Yong, Rebecca Riggins and Milton Brown

Trans-resveratrol boronic acid exhibits enhanced anti-proliferative activity in estrogen-dependent MCF-7 breast cancer cells

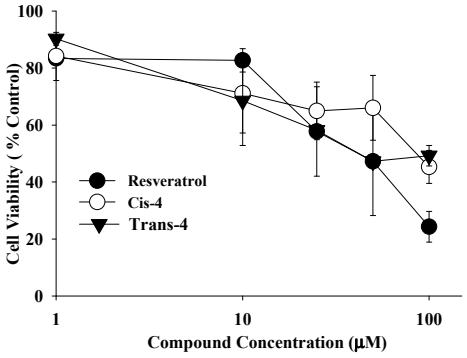
Cancer Biology & Therapy 2012; 13(10)
http://dx.doi.org/10.4161/cbt.20845

http://www.landesbioscience.com/journals/cbt/article/20845/

Supplementary Material

Table of Contents:

Figure S1. Effects of *cis*-4, *trans*-4 or resveratrol on the cell growth inhibition in ER-, estrogen-independent human breast cancer MDA-MB-231cell line.



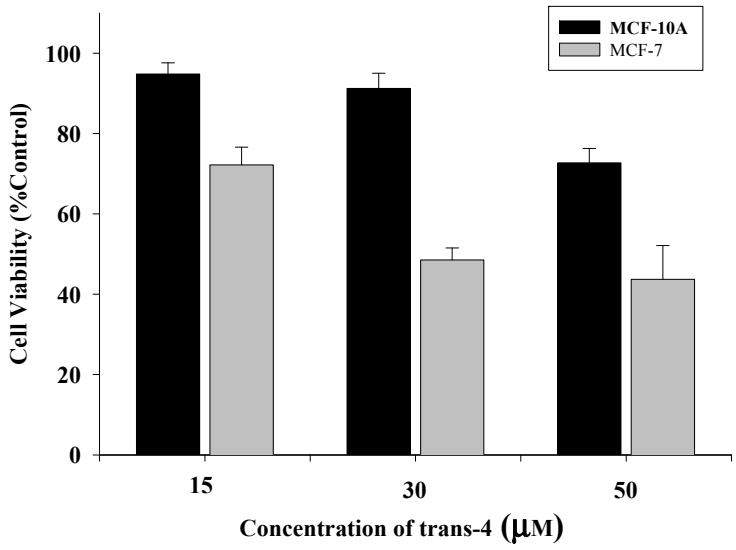
MDA-MB-231 Cell Growth Inhibiton (WST-1 assay, GI_{50}, μM)	
Compound	**72 hours**
Trans-4	46.0 ± 1.23
Cis-4	66.2 ± 1.25
Resveratrol[a]	37.9 ± 1.2

Note: Cell growth inhibition was estimated 72 hours after the addition of each compound using the WST-1 reduction assay. The GI_{50} value (the concentration yielding 50% growth inhibition) was interpolated from the graph of the log of compound concentration versus the fraction of surviving cells. The GI_{50} was calculated using Graph Pad Prism. Data are expressed as mean (±SEM) of two independent experiments.

a= Reported resveratrol data is also consistent with studies published by Murias, M et al., in which the GI_{50} for resveratrol is 38 μM in MDA-MB-231cells.

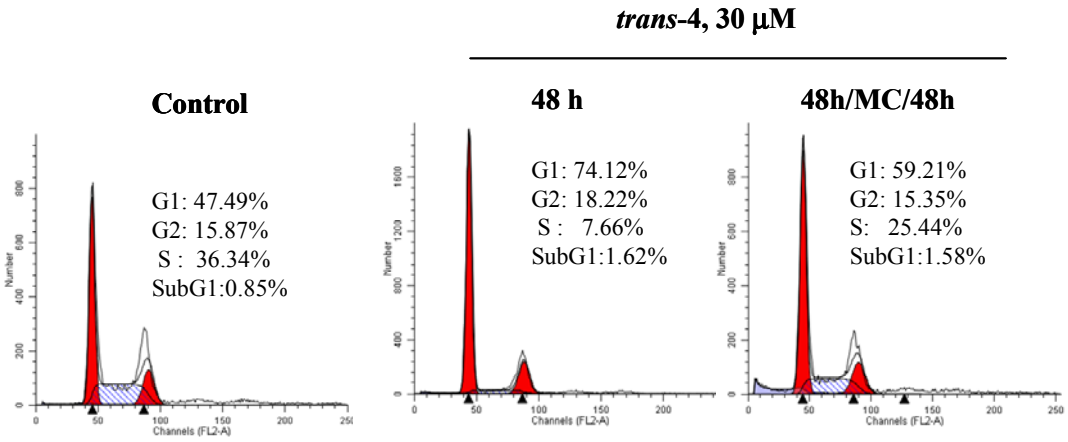
Murias M, Miksits M, Aust S, Spatzenegger M, Thalhammer T, Szekeres T, Jaeger M. Metabolism of resveratrol in breast cancer cell lines: impact of sulfo transferase 1A1 expression on cell growth inhibition. Cancer Lett. 2008;261: 172-182.

Figure S2. Effects of *trans*-4 compound on cell growth inhibition in MCF-10A, immortalized human breast epithelial cells and MCF-7, human breast cancer cells



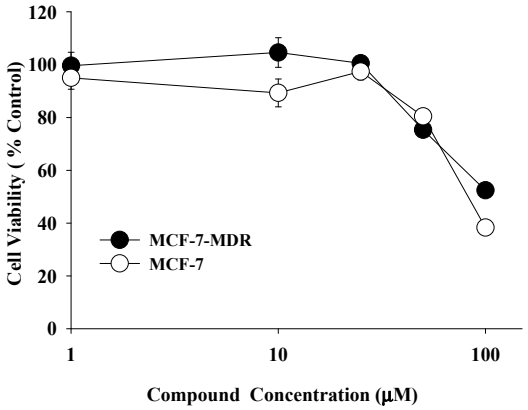
Legend: Trans-4 was tested at various concentrations (15, 30, 50 μM) for effects on cell growth inhibition of MCF-7 breast cancer cells. Cell growth inhibition was estimated 48 hours after the addition of each compound using the WST-1 reduction assay. Bar graph was plotted against the cell viability on Y-axis Vs concentration of *trans*-4. Data are expressed as mean (±SEM) of triplicate experiments.

Figure S3. Anti-proliferative effect of *trans*-4 is irreversible/reversible.



Legend: Exponentially growing MCF-7 cells were treated with *trans*-4 at 30 µM. Cells were treated with *trans*-4 for 48 hours and incubated further in fresh media without *trans*-4 for an additional 48 hours (a total of 96 hours) (48h/MC/48h). Cell cycle distributions were determined by flow cytometry using propidium iodide staining of 70% ethanol fixed cells.

Figure S4: Effect of resveratrol on the cell growth inhibition of multidrug resistant breast cancer cell line.



Cell Growth Inhibition **(WST-1 assay, GI_{50}, μM, 72 hours)**		
Cell line	Origin	Resveratrol
MCF-7	Breast	105.2 ± 1.23
CL 10.3	Breast (MDR)	158.5 ± 1.34
MDR GI_{50}/ non-MDR GI_{50}		1.5

Note: Resveratrol was tested at various concentrations for effects on cell growth inhibition of breast cancer cells. Cell growth inhibition was estimated 72 hours after the addition of each compound using the WST-1 reduction assay. The GI_{50} value (the concentration yielding 50% growth inhibition) was interpolated from the graph of the log of compound concentration versus the fraction of surviving cells. The GI_{50} was calculated using Graph Pad. Data are expressed as mean (±SEM) of two independent experiments.

The above results of resveratrol convincingly support the hypothesis (resveratrol enhances the cytotoxic profile of docetaxel and doxorubicin in solid tumor cell lines in vitro) of the published data by AI-Abd et al. Cell Prolif. 2011, 44, 591-601.

Figure S5: Proton NMR of *Cis*-4

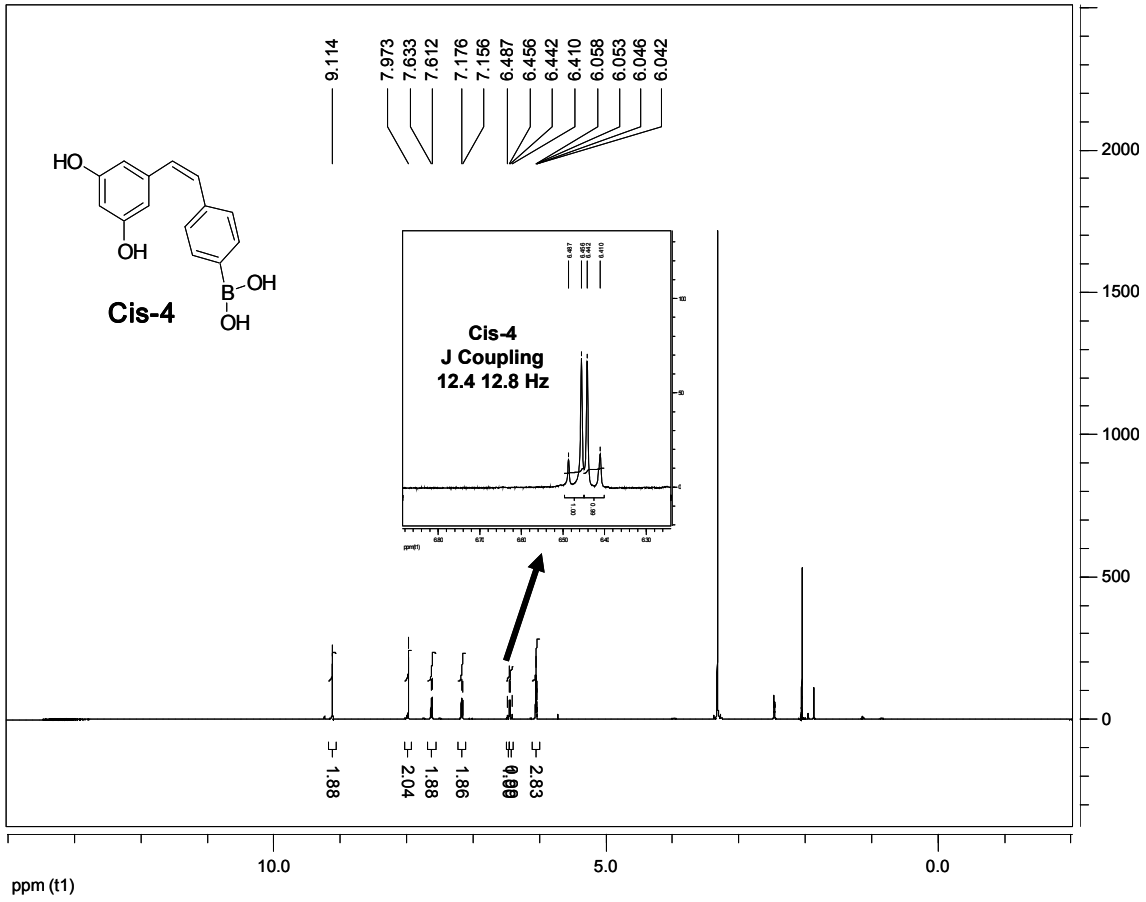
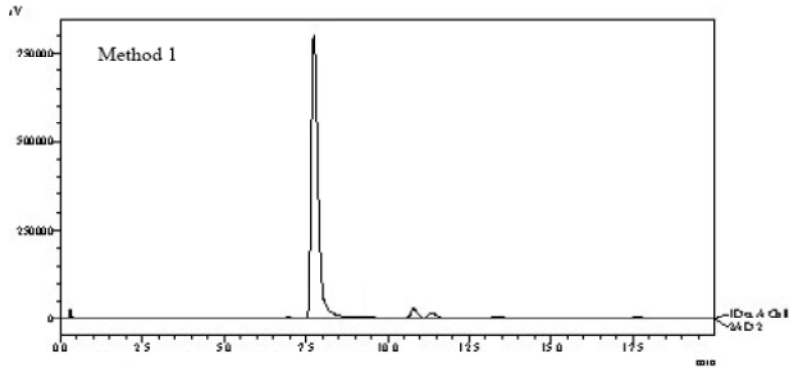
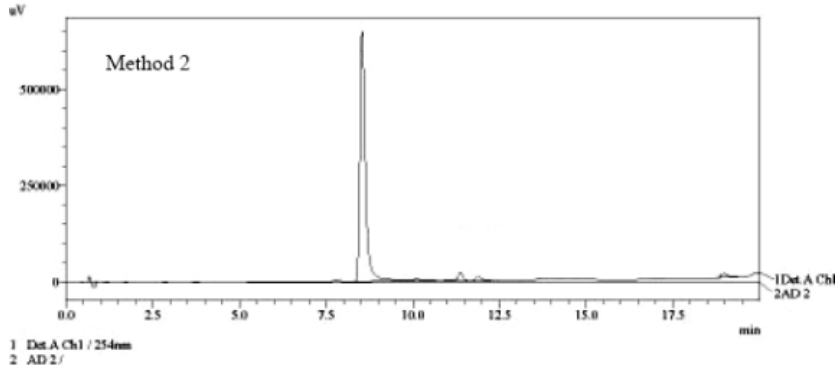


Figure S6. HPLC Chromatograms for *trans*-4





Conditions:

Reverse phase HPLC was performed on Restek's Ultra IBD C18 (5 μm, 4.6 × 50 mm) using two Shimadzu LC-20AD pumps and a SPD-20A-vis detector set at 330 nm: method **1**, 10%-40% acetonitrile in H_2O (v/v), flow rate at 1 mL/min over 20 mins; method **2**, 8%-40% methanol in H2O (v/v), flow rate at 1 mL/min over 20 mins.

Figure S7: Proton NMR of *Trans*-4