

Supplemental Data

Title:

19-Substituted benzoquinone ansamycin heat shock protein-90 inhibitors: Biological activity and decreased off-target toxicity

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Journal Title:

Molecular Pharmacology

Legends for Supplemental Figures:

Supplemental Figure 1.

19-Substitutions protect TAMH cells from 17-DMAG-induced toxicity.

Phase-contrast microphotographs of TAMH cells were taken following treatment with DMSO, 50 μ M DMAG, 50 μ M 19Ph-DMAG, or 50 μ M 19Me-DMAG for 24 h.

Supplemental Figure 2

Inhibition of yeast Hsp90 ATPase activity by 19-methyl BQAs.

Yeast Hsp90 ATPase activity was measured in reactions with either vehicle (DMSO; Control), or 19-methyl substituted BQAs at indicated concentrations in the presence or absence of rhNQO1. The reactions were analyzed after 3 h and phosphate concentrations

were measured using the malachite green assay. Solid bars represent samples run in the absence of rhNQO1, while open bars represent samples run in the presence of rhNQO1. Data expressed as mean \pm SEM (n = 3). * p < 0.05 versus corresponding sample without rhNQO1 (two-tailed t-test).

Supplemental Figure 3

Molecular modeling of the interactions of 19Ph-DMAG with Hsp90.

Computational-based molecular docking of the (A) quinone (green sticks) and (B) hydroquinone (cyan sticks) forms of 19Ph-DMAG into the ATP binding domain of human Hsp90 (PDB ID: 2K5B; orange ribbons and gray sticks). Insets are magnified views from two vantage points forty-five degrees apart. Dashed lines represent predicted hydrogen bond interactions.

Supplemental Figure 4

NQO1 protein expression in human breast cancer cell lines.

Whole-cell sonicates of breast cancer cell lines MDA468 (NQO1 null), MDA468/NQ16 (NQO1 overexpressing), and BT474 were assayed for NQO1 protein expression by immunoblotting. β -actin was included as a loading control.

Supplemental Figure 5

Immunoblot analysis of biomarkers for Hsp90 inhibition on MDA468/NQ16 and MDA468 cells treated with BQAs and 19-phenyl BQAs. (A-B) Hsp70, Akt and Raf-1 protein levels were analyzed by immunoblot analysis after treatment of MDA468 and

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MDA468/NQ16 cells with BQA or 19BQA at indicated concentrations for 24 h. Results were confirmed in duplicate experiments.

2K5B&19phQuinone.pdb

Predicted protein-ligand complex consisting of Hsp90 (PDB ID: 2K5B; Chain A) and the quinone form of 19-phenyl-DMAG (Chain B).

2K5B&19phHydroquinone.pdb

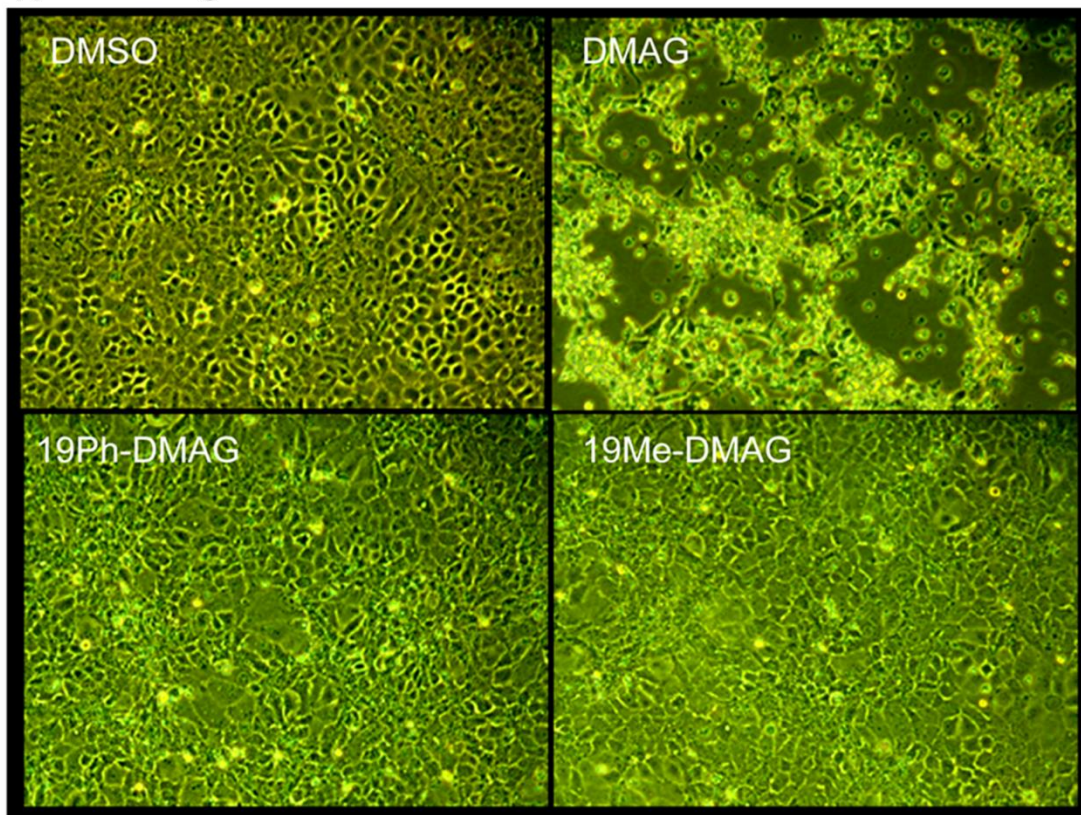
Predicted protein-ligand complex consisting of Hsp90 (PDB ID: 2K5B; Chain A) and the hydroquinone form of 19-phenyl-DMAG (Chain B).

Supplemental Table 1.

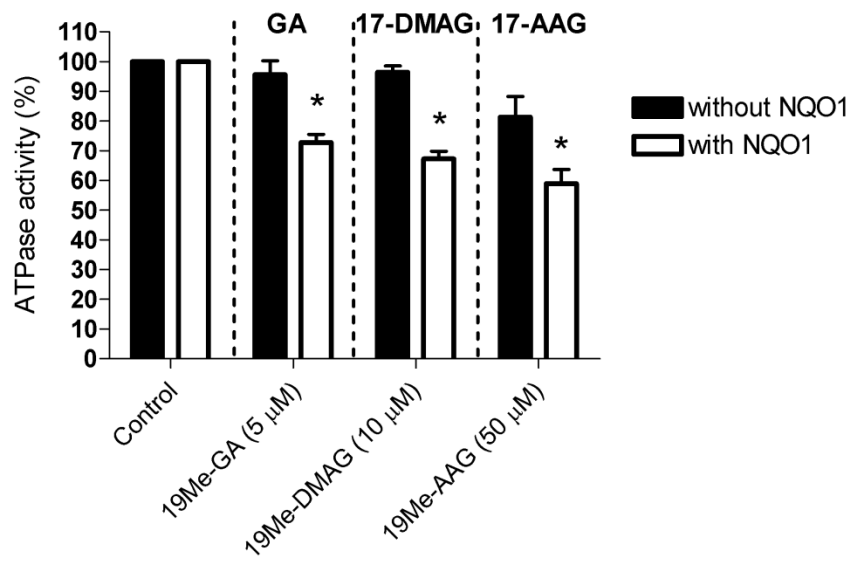
Predicted interactions of 19Ph-DMAG quinone and hydroquinone forms with Hsp90.

Compound	19Ph-DMAG Quinone	19Ph-DMAG Hydroquinone
Predicted Binding Energies (kcal/mol)	-18.9	-30.2
Predicted Hydrogen Bonds	Asn51 (x2; 1 sidechain, 1 backbone carbonyl) Lys58 (sidechain) Lys112 (sidechain) Gly135 (backbone carbonyl)	Asn51 (x2; 1 sidechain, 1 backbone carbonyl) Asp54 (x2; 2 sidechain) Lys58 (sidechain) Asp93 (sidechain) Lys112 (sidechain) Gly135 (backbone carbonyl)

Supplemental Figure 1

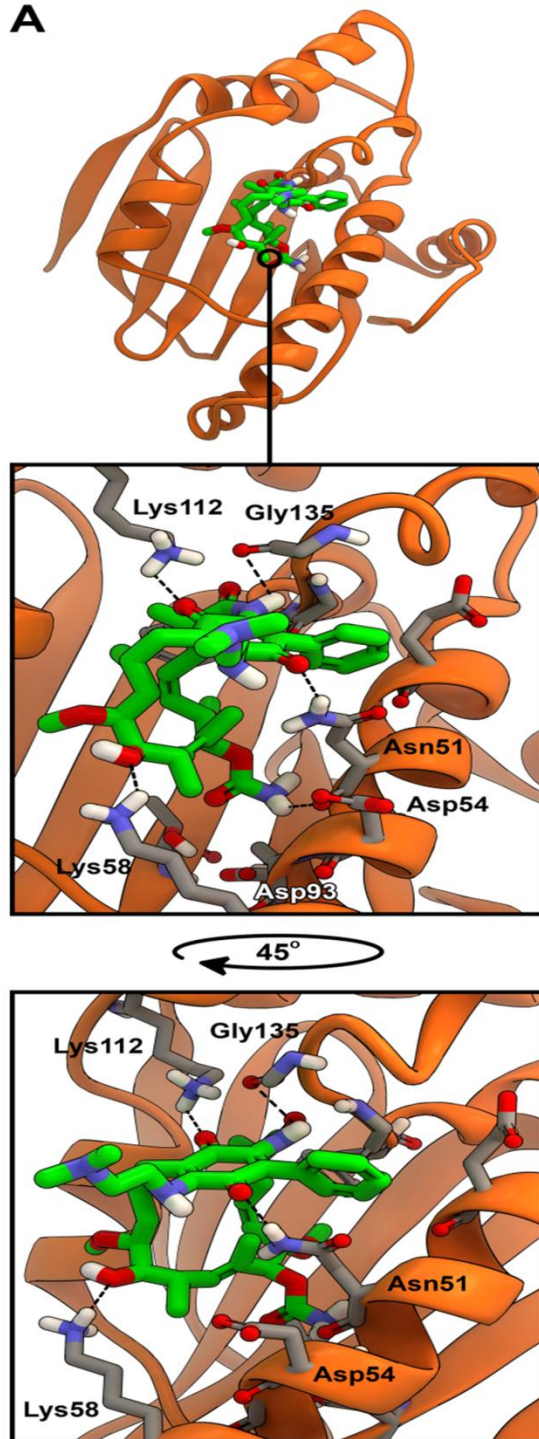


Supplemental Figure 2

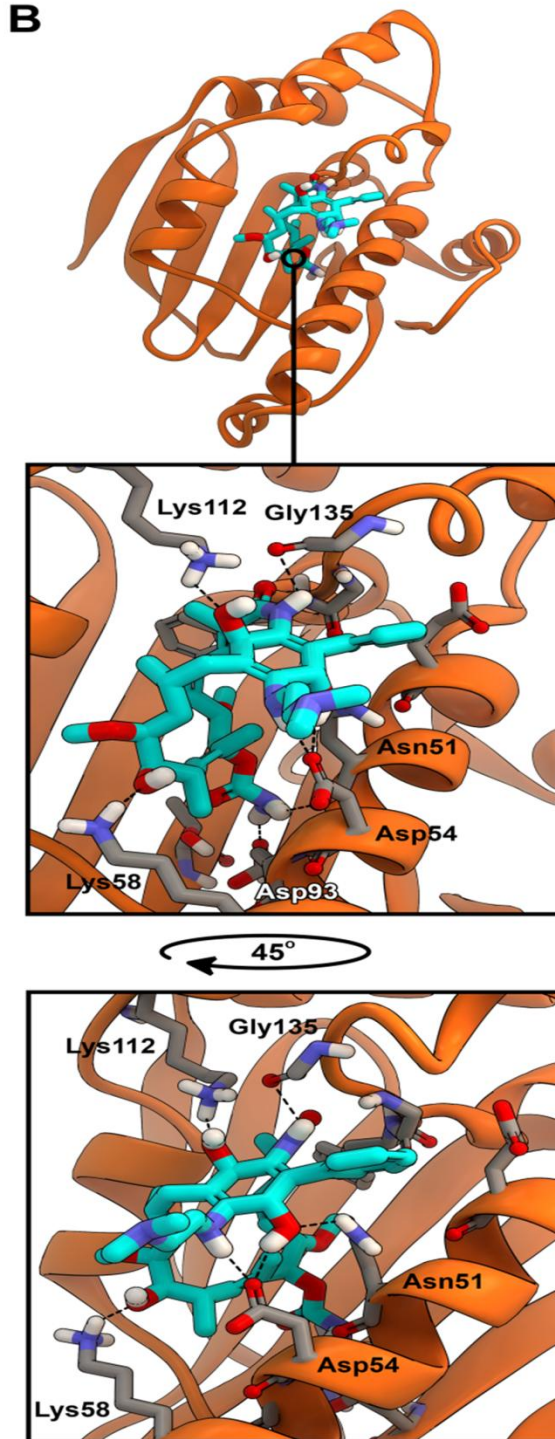


Supplemental Figure 3

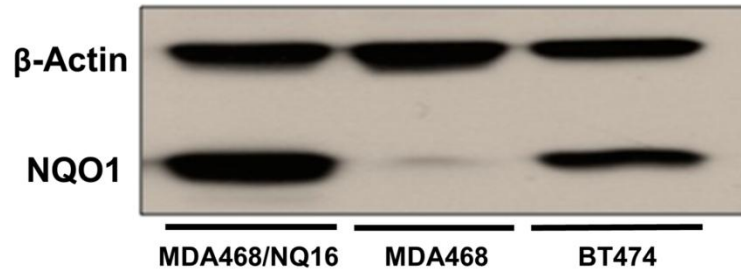
A



B



Supplemental Figure 4



Supplemental Figure 5

