Supplemental material

Nanaomycin A selectively inhibits DNMT3B and reactivates silenced tumor suppressor genes in human cancer cells

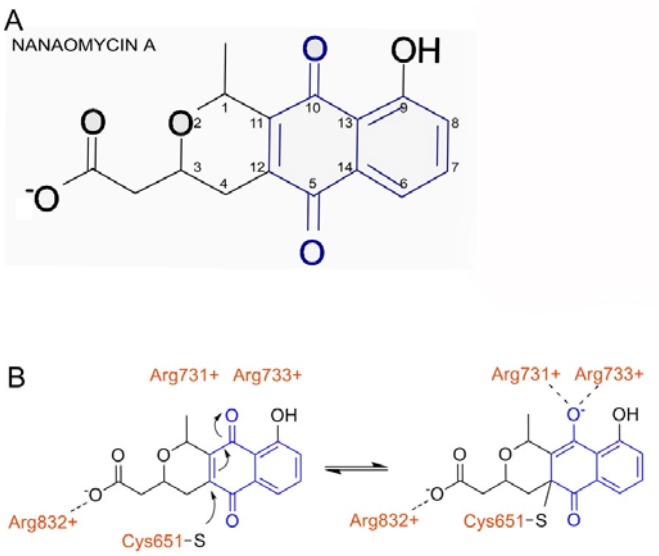
Dirk Kuck [1,2,4] , Thomas Caulfield[3], Frank Lyko[1], Jose L. Medina-Franco[3]

[1]Division of Epigenetics, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 580, 69120 Heidelberg, Germany, [3]Torrey Pines Institute for Molecular Studies, 11350 SW Village Parkway, Port St. Lucie, Florida 34987, USA

[2]Corresponding author phone: +49 6221 42 4731; fax: +49 6221 42 3802; e-mail: d.kuck@dkfz-heidelberg.de.

[1] Division of Epigenetics, Deutsches Krebsforschungszentrum.

[3] Torrey Pines Institute for Molecular Studies.



FigureS1. **(A)** Chemical structure of nanaomycin A showing atom numbering in the central scaffold. **(B)** Proposed mechanism for inhibition of DNMT3B with nanaomycin A. Cys651-S^- initiates a nucleophilic Michael 1,4 addition to the α,β-unsaturated carbonyl system at the β carbon which proceeds on the less steric side. The diene pushes its electron toward the opposite carbonyl atom. Subsequently, an intermediate enolate forms at the carbonyl atom (C-11) and the oxygen atom (O-10). However, the enol isoform of nanaomycin A structure does result; the negative charge located on the oxygen is further stabilized by interaction with positive residues Arg731 and Arg733. This stabilization, plus the covalent addition of the thiol group (Cys651-S^-), blocks the catalytic site DMNT3B.