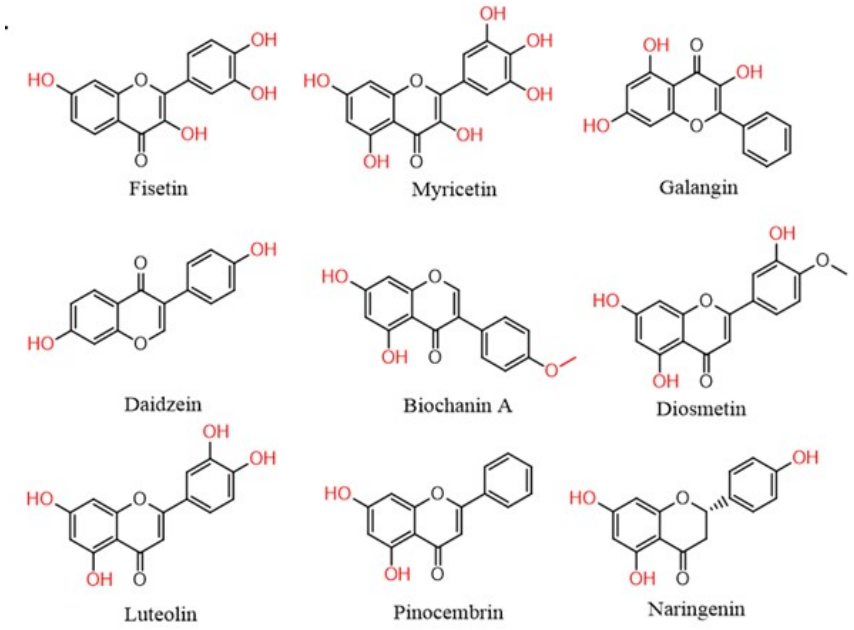
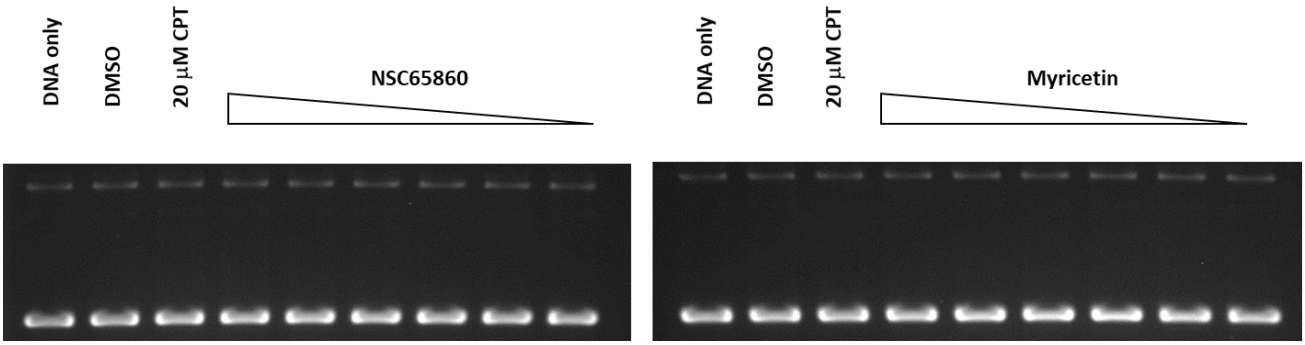
Supplementary Table 1. Effect of NSC65860 treatment on CFU/mL of EKY3/pYES2-TOP1-T718A
Cultures grown in SC-U media supplemented with raffinose were diluted to OD600 = 0.025 with SC-U media with 1% galactose. Two hundred microliters of diluted cultures containing DMSO (1% v/v) or 100 μM NSC65860 were added to individual wells in a 96-well plate. After incubation at 30ºC for the indicated amount of time, 20 μl of cultures were removed for serial dilutions in Phosphate Buffered Saline (PBS) and plated on YPD plates. Viable colonies were counted after incubation at 30ºC for 3 days. The average of results from two technical replicates of a representative experiment is shown in the table here. The average of normalized colony counts from three independent experimental replicates are shown in Figure S4.

Time after 1% galactose addition	DMSO (CFU/mL)	100 μM NSC65860 (CFU/mL)
6 hr	57,000	225,000
24 hr	73,000	248,500

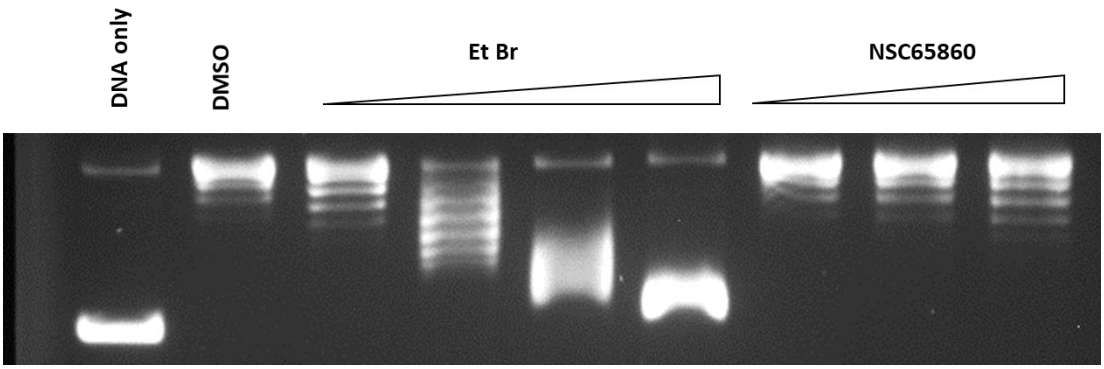
The colony count at time 0 was 168,333 CFU/mL



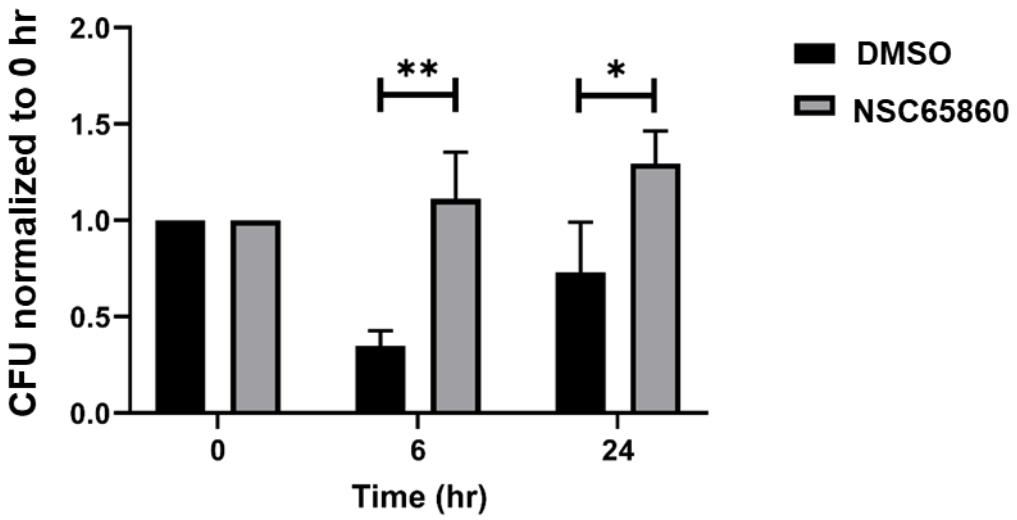
Supplementary Fig. 1. Natural products tested for inhibition of TOP1 relaxation activity



Supplementary Fig. 2. Effect of compounds on electrophoretic mobility of supercoiled plasmid DNA in the absence of enzyme. The buffer, incubation conditions, concentrations of myricetin (50, 25, 12.5, 6.2, 3.1, 1.6 μM) and NSC65860 (5, 2.5, 1.2, 0.62, 0.31, 0.16μM) are identical to those found in Figure 5 and Figure 6.

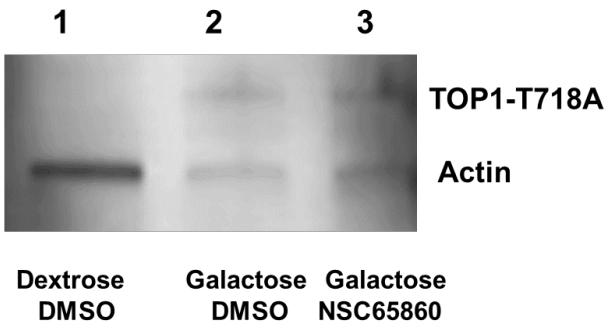


Supplementary Fig. 3. Inhibition of human TOP1 by NSC65860 is not due to DNA intercalation. For each reaction, 200 ng of negatively supercoiled plasmid DNA was first incubated with 1 U of human TOP1 for 30 min at 37ºC to fully relax the supercoiled DNA. This was followed by addition of another 0.3 U of human TOP1 and 0.5 µl of DMSO or compound solution to the 20 µl reaction. After further incubation of 30 min at 37ºC, the reactions were stopped with SDS stop solution for agarose gel electrophoresis. Ethidium bromide at 0.5, 1.25, 2.5 and 5 µM was used as control to demonstrate the effect of an DNA intercalator on the final human TOP1 reaction products. NSC65860 was tested at 1.25, 2.5 and 5 µM.



Supplementary Fig. 4. Effect of NSC65860 treatment on normalized viable colony counts of EKY3 induced for expression of TOP1-T718A. EKY3/pYES2-TOP1-T718A was induced with 1% galactose for expression of TOP1-T718A in the presence of 1% DMSO control or 100 μM NSC65860. Viable colony counts were determined at 6 hr and 24 hr following galactose addition by serial dilution and plating on YPD agar plates. The CFU/mL values were normalized to the CFU/mL values at time 0 before the addition of galactose. The average and standard deviation of results from three independent experiments are shown here. Statistical significance of observed differences in viable colony formation from NSC65860 treatment was determined by using the two-way t-test for two independent means. *: $p < 0.05$; **: $p < 0.01$

Supplementary Fig. 5. NSC65860 has no significant effect on growth of EKY3/pYES2-TOP1in media with dextrose. EKY3/pYES2-TOP1 was cultured in SC-U media containing 1% dextrose with DMSO control or up to 100 µM NSC65860 present.



Supplementary Fig. 6. Western blot analysis of TOP1-T718A expression in EKY3/pYES2-TOP1-T718A cultures. Following overnight growth in SC-U media supplemented with 1% raffinose, culture of EKY3/pYES2-TOP1-T718A were diluted into 1 mL of SC-U media with either 1% dextrose or 1% galactose. The cultures were treated with either 1% DMSO or 100 μM NSC65860 and grown at 30ºC for 6 hrs. Cells were collected by centrifugation and washed with 500 μL deionized water, then re-suspended in a volume of 0.1 M NaOH that achieved an equal OD600 value of 3. Following incubation for 10 min at room temperature, the cell pellets were re-collected from 40 μL of the suspension and boiled for 5 minutes in the same volume of a sample buffer consisting of 60 mM Tris-HCl (pH 6.8), 4% 2-mercaptoethanol, 5% glycerol, 2% SDS, and 0.0025% bromophenol blue. Samples were loaded and run in a 7.5% SDS-PAGE. After transferring separated proteins to a nitrocellulose membrane, the membrane was blocked with 5% Bovine Serum Albumin (BSA) at room temperature for 1 hour, then incubated with a 1:1000 (v/v) solution of mouse anti-HTOP1 antibodies (Developmental Studies Hybridoma Bank, Cat. No. CPTC-Top1-2) and mouse anti-Actin antibodies (Developmental Studies Hybridoma Bank, Cat. No. JLA-20) in 1X TBST buffer at 4°C overnight. The membrane was washed three times for 5 min each in 1X TBST buffer, and then a 1:5000 (v/v) solution of horse-radish peroxidase (HRP) conjugated mouse IgG kappa binding protein (Santa Cruz Biotechnology Cat. No. sc-516102) in 1% BSA solution in 1X TBST buffer was incubated with the membrane at room temperature for one hour. After thorough washing and rinsing of the membrane in 1X TBST buffer, SuperSignal™ West Pico PLUS Chemiluminescent Substrate from Thermo Scientific™ was used to visualize the protein bands.