Supplementary Material

Identification of Potent Catalytic Inhibitors of Human DNA Topoisomerase II by Structure-based Virtual Screening

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Table of contents

1. Figure S1

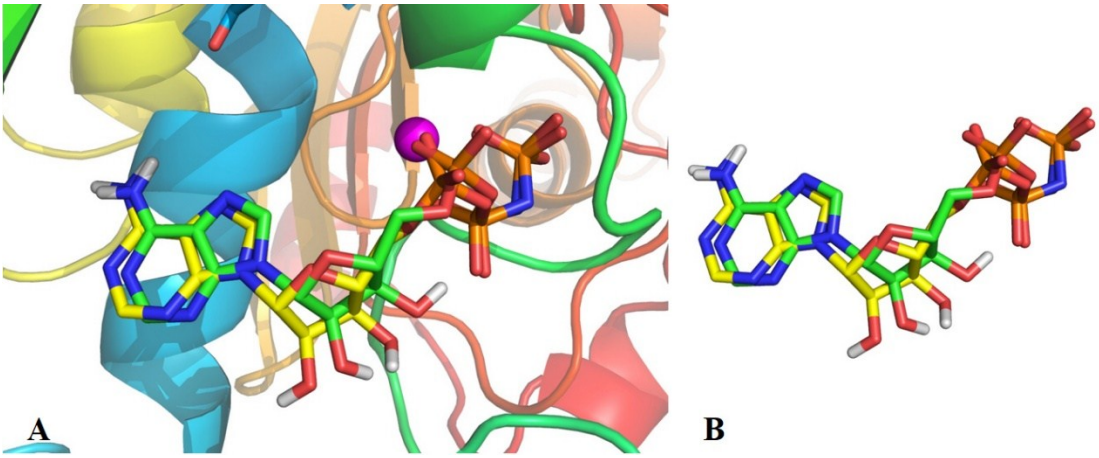


Figure S1. Superimposition of the binding conformation (Yellow) and experimental structures (Green) of **ADPNP** in the active site of ATPase. The figure was generated using PyMol (http://www.pymol.org/)

2. Docking Methods

Molecular docking. The crystal structure of Top2 in complex with ADPNP was obtained from protein database bank (PDB ID: 1ZXM)[1] and prepared for docking using the protein preparation tool in Discovery Studio 3.0.[2] During this process, the ligands and waters were removed and hydrogens were added to the structure. Staged minimization was performed with default setting. The docking studies were carried out using GOLD 5.0.[3] Binding site was defined as whole residues within an 10 Å radius subset encompassing the ADPNP. Conformations were generated by genetic algorithm. GoldScore, ChemSore, ASP and ChemPLP were used as scoring functions to evaluated the binding mode. The best conformation was chosen to analyze the ligand–protein interaction. The image representing the best pose was prepared using PyMOL.

3. Experimental protocols of biological assays

Top2-mediated supercoiled pBR322 relaxation assay. DNA Top2 inhibitory activity of the compounds was measured using Topoisomerase II Drug Screening Kit (TopoGEN, Inc.).[7] The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM $MgCl_2$, 5 mM dithiothreitol, 30 μg/mL bovine serum albumin (BSA), 2

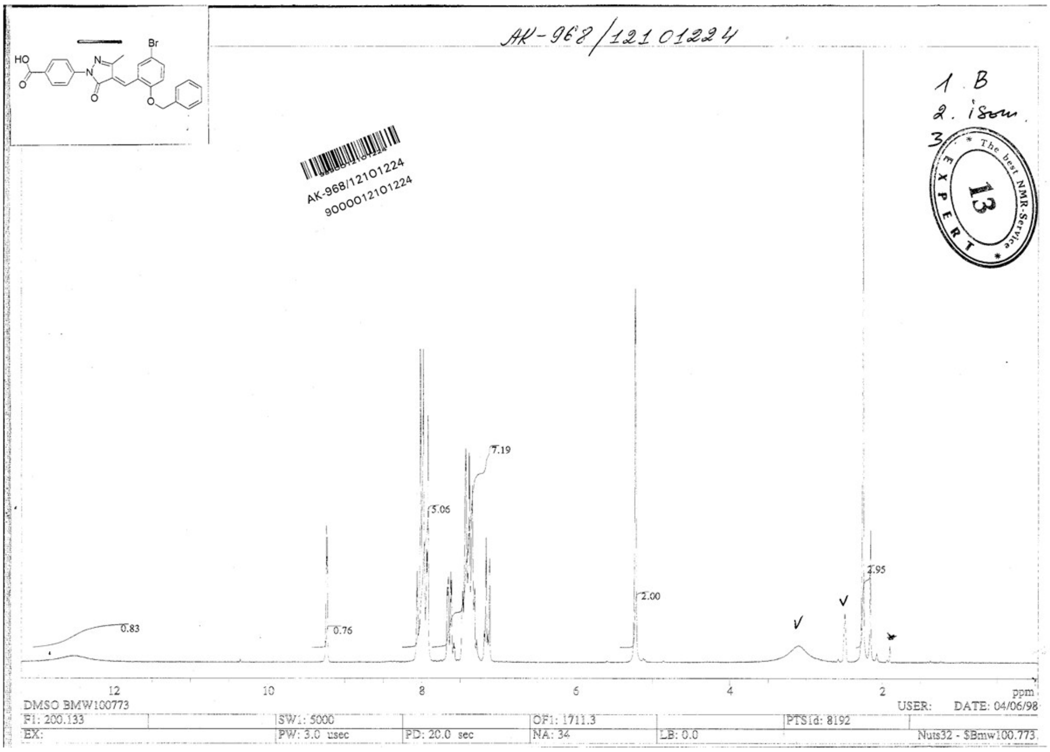
mM ATP, pBR322 plasmid DNA (0.25 μg), the indicated drug concentrations (1% DMSO), and 0.8 unit of Top2 (TopoGEN, Inc.) in a final volume of 20 μL. Reaction mixtures were incubated for 30 min at 37 °C and stopped by addition of 2 μL 10% SDS. After that, 2 μL 10 × gel loading buffer (0.25% bromophenol blue, 50% glycerol) was added. The reaction products were analyzed on 1% agarose gel at 8 V/cm for 1 h with TAE (Tris-acetate-EDTA) as the running buffer. Gels were stained with ethidium bromide (0.5 μg/mL) for 60 min. The DNA band was visualized over UV light and photographed with Gel Doc Ez imager (Bio-Rad Laboratories Ltd.).

Top2-Mediated DNA Cleavage Assay. The same Top2 drug screening kit was used as above, but this time an amount of 8 units of Top2 was added. Proteinase K (200 μg/ml final concentration) was added, and the samples were incubated at 50 °C for 20 min. Then the compounds were removed by phenol extraction Electrophoresis was run in TAE with 0.5 μg/mL ethidium bromide.

In Vitro Cytotoxicity Assay. Three human cancer cell lines, A549, HCT116 and ZR-75-30, which were in the logarithmic phase, were harvested and plated in 96-well microtiter plates at a density of 5×10^3/well and incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h. The cells were exposed to different concentrations of the test compounds for 72 h in three replicates and 0.1% DMSO for control. After that, 20 μL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) solution (5 mg/mL) was added and the plate was incubated for another 4 h. The formazan was dissolved in 100 μL of DMSO. The absorbance (OD) was then read on a WellscanMK-2 microplate reader (Labsystems) at 570 nm. The concentration causing 50% inhibition of cell growth (IC_{50}) was determined by the Logit method.[8-10] All experiments were performed three times.

4. Spectral Data

Compound 8: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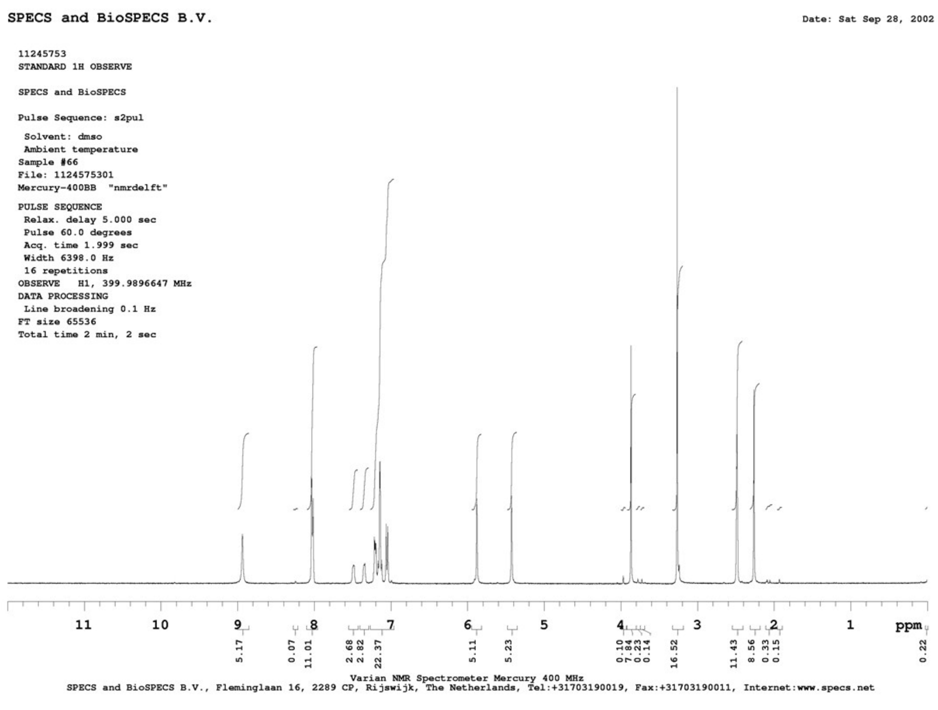
ppm

Compound 20:


SPECS and BioSPECS B.V.
Date: Sat Sep 28, 2002
11245753
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SPECS and BioSPECS
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DATA PROCESSING
Line broadening 0.1 Hz
FT size 65536
Total time 2 min, 2 sec
ppm
Varian NMR Spectrometer Mercury 400 MHz
SPECS and BioSPECS B.V., Fleminglaan 16, 2289 CP, Rijswijk, The Netherlands, Tel:+31703190019, Fax:+31703190011, Internet:www.specs.net

5. References

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