Supplementary Information

Portion mismatch in duplex oligonucleotides as inhibitors of bacterial topoisomerase I

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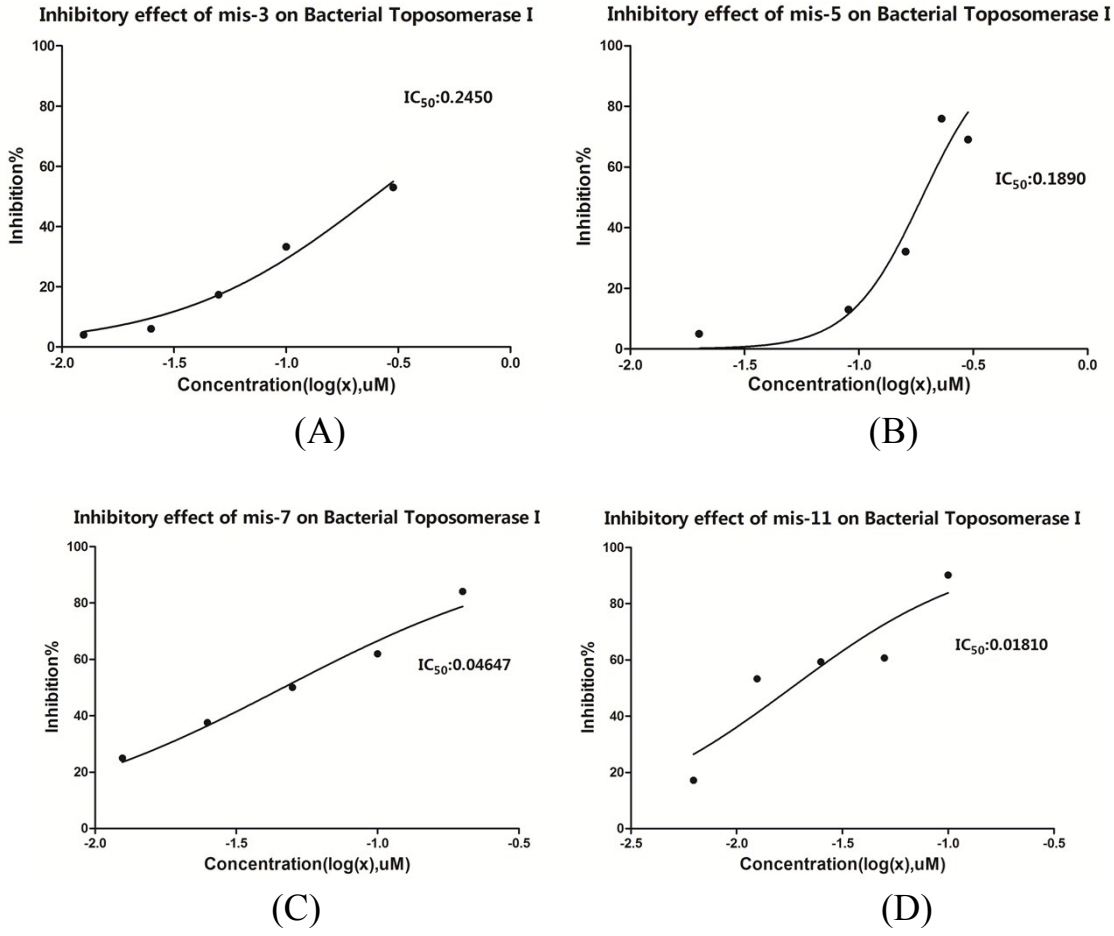

Figure S1. Correlations between concentration of **mis-3** (A), **mis-5** (B), **mis-7** (C), **mis-11** (D) and the corresponding inhibitory effects on btopo I. Percentages of pUC 19 relaxation were defined as the ratio of band density of relaxed DNA over the some of relaxed DNA

plus supercoiled DNA [relaxed DNA/ (relaxed DNA + supercoil DNA)].[1] The DNA bands were quantified using Gel Documentation System (G:Box HR, Syngnene, Cambridge, UK) equipped with Gene Tools Software.

Materials and Methods:

Reagents: Plasmid DNA pUC19 and bacterial topoisomerase I (*E. coli* topoisomerase I) were purchased from New England Biolabs (Ipswich, MA). Eukaryotic topoisomerase I ()were purchased from Takara Bio (Dalian, China).Single strand oligonucleotides were provided by Shanghai Generay Biotech Co.,Ltd (China).

Preparations of 75 bp duplex oligonucleotide: In a solution containing two single strand oligonucleotides, 50 mM NaCl was kept at 90 ℃ for 10 minutes followed by allowing it to cool down to room temperature over a period of 1 hours.

Electrophoretic analysis of duplex oligonucleotide with different mismatch degrees: Each sample was prepared after above method, and then was loaded in a 15% native polyacryamide gel. The polyacryamide gel electrophoresis was run at room temperature, for 3.5 hours (400 V and 15 W). The DNA bands were captured using Gel Documentation System (G:Box HR, Syngnene, Cambridge, UK).

Reactions of Btopo I with pUC 19 and designed duplex oligonucleotide: A mixture containing 50 mM potassium acetate, 20 mM Tris-acetate, 100 μg/ml bovine serum albumin (BSA), 10 mM magnesium acetate, 250 ng pUC 19, 1 U of Btopo I, and each

designed duplex oligonucleotide was prepared respectively and further incubated at 37 ℃ for 30 min. After incubation, the product was analysis by 1% agarose gel electrophoresis. The DNA bands were captured using Gel Documentation System (G:Box HR, Syngnene, Cambridge, UK).

Reactions of eukaryotic topoisomerase I with pUC 19 and designed duplex oligonucleotide: A mixture containing 72 mM potassium chloride, 35 mM Tris-acetate (pH 8.0), 5 mM magnesium chloride, 5 mM DTT, 5 mM spermidine, 0.01% bovine serum albumin (BSA), 250 ng pUC 19, 1 U of eukaryotic topoisomerase I, and each designed duplex oligonucleotide was prepared respectively and further incubated at 37 ℃ for 30 min. After incubation, the product was analysis by 1% agarose gel electrophoresis. The DNA bands were captured using Gel Documentation System (G:Box HR, Syngnene, Cambridge, UK).

References for Supplementary Information:

1. J. P. Laine, P. L. Opresko, F. E. Indig, J. A. Harrigan, C. von Kobbe and V. A. Bohr, *Cancer Res*, 2003, **63**, 7136-7146.