Supplementary Information

# Identification of DNA primase inhibitors via a combined fragment-based and virtual screening

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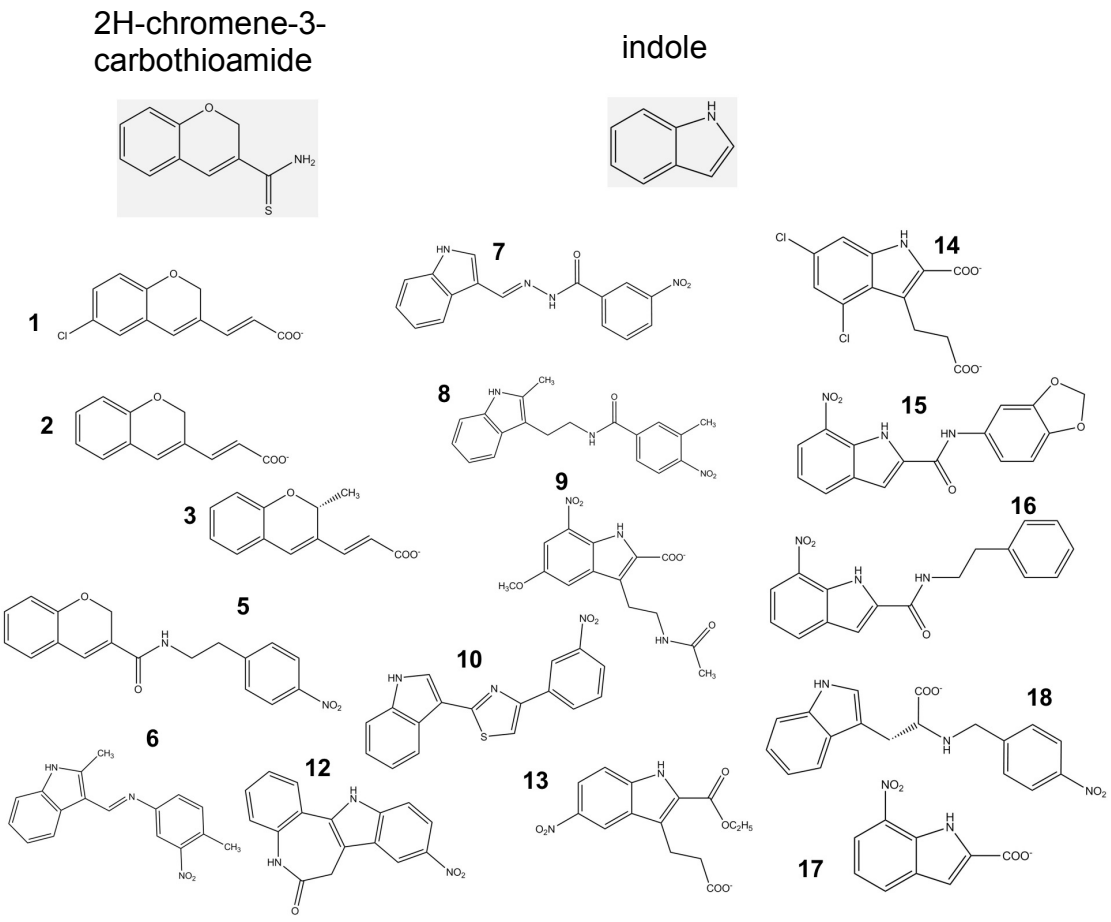


**Figure S1** Chemical structures of 16 small molecules obtained by virtual filtration using the ZINC database[17] and high-throughput docking using AutoDock[18]. The two subsets are based on the scaffolds obtained by STD spectroscopy: 2H-chromene-3-carbothioamide and indole (indicated in grey).

a

| Protein | DNA template | Substrate |
|---|---|---|
| Helicase/Primase Polymerase/trx (Replisome) | Single stranded DNA (M13) | ATP/CTP dNTPs |
| Primase fragment | GGGTCA10 | ATP/CTP |

b

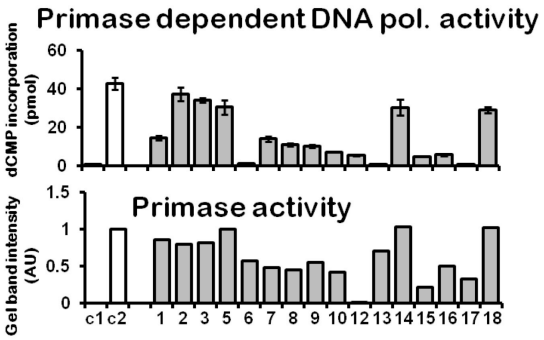


**Figure S2 Inhibitory effect of small molecules on T7 DNA primase activity.** (**a**) Effect of small molecule inhibitors on primase-dependent DNA synthesis mediated by gp5/trx and gene 4 helicase (gp4A). The standard reaction contained the ssM13 DNA template (10 nM), 0.3 mM dATP, dGTP, dCTP and [α-$^{32}$P] dTTP (0.1 mCi), 10 nM gp5/trx, 200 nM monomeric concentrations of gp4B and 250 $\mu$M of small molecule inhibitors. After incubation for 30 min at 37 °C, the level of RNA synthesis was determined based on the amount of [α-$^{32}$P] dTTP incorporated into DNA. (**b**) Effect of small molecule inhibitors on oligonucleotide synthesis by DNA primase. The standard reaction contained the oligonucleotide 5'-GGGTCA$_{10}$-'3 containing the primase recognition sequence, 200 mM [α-$^{32}$P]-CTP and ATP, and 250 $\mu$M small molecule inhibitors in a buffer containing 40 mM Tris-HCl (pH 7.5), 10 mM MnCl2, 10 mM DTT and 50 mM potassium glutamate. The quenched samples were loaded onto 25% polyacrylamide sequencing gel containing 3M urea and visualized using autoradiography.