Targeting transcription-coupled DNA supercoiling for discovery of bacterial DNA gyrase inhibitors

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Antibiotic production has been stagnant in the recent decade in comparison to the 30-years before that. The emergence of multidrug resistant bacteria is of mounting importance and a universal problem. The stagnation of antibiotic production, in part, is due to the lack of innovation in creating highly specific screening tools.

Bacterial DNA gyrase is a type II topoisomerase that introduces negative supercoils into DNA, an essential mechanism for bacteria but absent from eukaryotes, making it an ideal target for identifying antibacterial compounds. In this study, we have constructed an E. coli strain FL#1181 that contains a pair of divergently coupled P_{gyrA} and $P_{T7A1/O4}$ promoters controlling the luc and lacZ genes at the attTn7 site of the E. coli chromosome. Since transcription-coupled DNA supercoiling (TCDS) provided by a strong IPTG-inducible promoter, such as the T7A1/O4 promoter ($P_{T7A1/O4}$), is capable of potently inhibiting the divergently coupled, supercoiling-sensitive gyrA promoter (P_{gyrA}), our results showed that DNA gyrase inhibitors greatly “enhanced” the expression of the firefly luciferase under the control of the divergently coupled, supercoiling-sensitive P_{gyrA}. As a result, the luminescence generated from the firefly luciferase was significantly increased. This unique property of FL#1181 was used to screen a library containing ~30 different DNA-binding compounds.