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A new antimicrobial target: the pneumococcal DNA Topoisomerase I

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To figh infectious diseases it is necessary both to prevent the spread of antibiotic resistance and to find new antimicrobial agents against new molecular targets. DNA topology, that regulates essential processes such as transcription and replication, is maintained in bacteria by the activities of DNA topoisomerases. While fluoroquinolones target DNA topoisomerasas of type II, antibiotics efficiently targeting type I topoisomerases have not yet been reported.

Streptococcus pneumoniae has two type II DNA-topoisomerases (DNA-gyrase and DNA topoisomerase IV) and a single type I enzyme (DNA-topoisomerase I, TopA). We cloned the gene encoding topoisomerase I (topA) in Escherichia coli and overexpressed it as a fusion protein with an N-terminal-6His tag. Purified TopA showed nicking-closing activity on negatively-supercoiled plasmid DNA. Two phenanthrenes alkaloids (seconeolitsine and N-methylseconeolitsine), semisynthesized from boldine, inhibited both *in vitro* TopA activity (Figure 1) and pneumococcal growth, at equivalent concentrations (17 µM). This inhibition was observed even in multidrug resistant (including fluoroquinolone-resistant) pneumococcal isolates. Neither compound affected human cell viability. Evidence for *in vivo* TopA targeting by seconeolitsine was provided by the protection of growth inhibition in a *S. pneumoniae* culture in which the enzyme was overproduced. Additionally, hypernegative supercoiling was observed in an internal plasmid after drug treatment (Figure 2). Furthermore, a model of pneumococcal TopA was made based on the crystal structure of E. coli TopA. Docking calculations indicated strong interactions of the alkaloids with the nucleotide-binding site in the protein closed conformation, which correlated with their inhibitory effect.

This study not only identifies two new therapeutic candidates for the treatment of *S. pneumoniae* infections resistant to other antibiotics, but also represents the first successful attempt to show the single pneumococcal type I

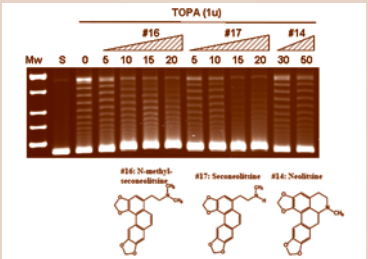

Figure 1. *In vitro* inhibition of TopA by phenanthrene alkaloids

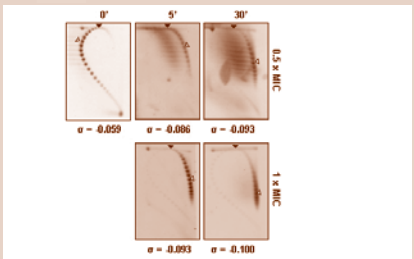

Figure 2. *In vivo* inhibition of TopA by phenanthrene alkaloids