Old Target, New Approach: Developing Pterin-like Small Molecules as Inhibitors of the Bacterial Folate Pathway

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Folate biosynthesis is an essential bacterial pathway that is absent in higher animals, and it has been an effective target of antibacterial agents for over 70 years. The sulfonamide drugs inhibit a key enzyme in the pathway, dihydropteroate synthase (DHPS), by acting as non-productive substrate analogs of *p*-aminobenzoic acid (*p*ABA). However, the flexible *p*ABA binding site is structurally susceptible to resistance mutations, and the sulfonamides are rapidly becoming therapeutically ineffective. In contrast, the binding site of the second DHPS substrate, pterin-pyrophosphate, is buried in a conserved pocket that is less likely to tolerate mutations. The overall goal of the project is to generate new classes of DHPS inhibitors that engage this pocket, and have the potential of being developed into novel therapeutic agents that still target folate synthesis but which avoid the problems of resistance. To generate effective inhibitors of any enzyme, it is crucial to understand the structure and mechanism of its active site. This information is largely absent for DHPS, and understanding how DHPS performs catalysis at the molecular level is a central goal of the project. To achieve this, small molecules are also being designed and synthesized to probe the DHPS catalytic mechanism, and these can also be evaluated as DHPS inhibitors. Promising inhibitor scaffolds will also be tested for their potential as anti-microbials by direct screening of select organisms. The more potent inhibitors will be used to create derivative libraries for further screening and evaluation. The combined use of state-of-the-art drug discovery software, library synthesis, high-throughput screening, biochemistry and X-ray crystallography is a central feature of the project. Our general goal is to develop new broad-spectrum anti-infective agents. However, we are also focused on developing specific therapies for the Category A biowarfare agents *B. anthracis*, *Y. pestis* and *F. tularensis*, as well as for pathogenic protozoa and fungi.