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Abstract of PhD thesis

Synthesis and activity of phosphonic inhibitors of polyphosphate kinase 2

Phosphonic acids containing the P-C-P motif are structural analogs of pyrophosphate and promising candidates for inhibitors of polyphosphate kinase (PPK) – bacterial transferases involved in various mechanisms in the stationary phase of growth, for example biofilm development, virulence and quorum sensing.

Initially, 23 structurally diverse phosphonic acids were screened against polyphosphate kinases 2 representing three classes of enzymes from different bacteria. Thermal stability tests indicated the most stable proteins. Subsequently, a coupled-enzyme assay and HPLC-based assay were compared to each other, followed by pointing out significant limitations of the first method. IC_{50} was determined for the most active compounds. Four phosphonic ligands of high affinity were chosen as candidates for crystallization with the enzyme. Based on the crystal structures of PPK2-inhibitor complexes, a set of ligands of rationally optimized structures was designed, with modifications involving the aromatic ring and the phosphonic moiety. Accordingly, 14 phosphonic acids were synthesized. The examined structures were extended by a group of phosphinic and polyacidic ligands to provide a series of approximately 50 compounds. The overexpressed and purified enzyme PPK2 from *Cytophaga hutchinsonii*, as well as PPK1 from *Escherichia coli*, was tested. On the basis of the inhibitory activity measured by the HPLC method, differences in the selectivity of both enzymes were indicated and correlated with the distinct architecture of the active center. 8 compounds of micromolar activity were identified. The designed α-aminobisphosphonic acids containing guanidino or amino group in the *meta* position of the ring mimicked well the nucleotide substrate, showing low IC_{50} values. Moreover, good activity of selected polyacid compounds was found, which further increases the potential of structural modifications, and is a promising starting point for the synthesis of selective inhibitors of polyphosphate kinases 1 and 2.