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Inhibition of *Yersinia* protein tyrosine phosphatase by phosphonate derivatives of calixarenes

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ABSTRACT

Inhibition of *Yersinia* protein tyrosine phosphatase by calix[4]arene mono-, bis-, and tetrakis(methylenebisphosphonic) acids as well as calix[4]arene and thiacalix[4]arene tetrakis(methylphosphonic) acids have been investigated. The kinetic studies revealed that some compounds in this class are potent competitive inhibitors of *Yersinia* PTP with inhibition constants in the low micromolar range. The binding modes of macrocyclic phosphonate derivatives in the enzyme active center have been explained using computational docking approach. The results obtained indicate that calix[4]arenes are promising scaffolds for the development of inhibitors of *Yersinia* PTP.

Protein tyrosine phosphatases (PTPs) are known to be signal transduction enzymes that catalyze dephosphorylation of phosphotyrosine residues in proteins. The PTP termed YopH is a key outer membrane protein H secreted by pathogenic bacteria *Yersinia*. The species of these bacteria cause several diseases ranging from gastrointestinal syndromes to bubonic plague.[1] In infected cells, YopH can dephosphorylate multiple focal adhesion proteins such as focal adhesion kinase and focal adhesion protein p130Cas to disrupt the signaling pathways and to escape the immune responses.[2] It was established that YopH can also be responsible for dephosphorylation of the phosphotyrosine residues of paxillin, Fyn-binding protein, and other substrates.[3] Natural reservoirs of *Yersinia pestis* still exist[4] and there is a risk of outbreak of diseases or the threat of misuse of the patogenic bacteria for bioterroristic attacks.[5] Because the YopH, one of the most active PTPs known, is an essential virulence factor of the *Yersinia pestis*, there is growing interest in developing inhibitors of this enzyme.

Derivatives of furanyl salicylate compounds,[6] α-ketocarboxylic acid,[7] squaric acid,[7a] hexapeptide,[8] and phosphotyrosyl mimetic-containing tripeptides[9] have been identified as effective inhibitors of the *Yersinia* PTP. Some of vinyl sulfonates and sulfones may serve as mechanism-based enzyme inactivators.[10] Crystal structures were determined for complexes of the YopH with specific small molecule inhibitor, *p*-nitrocatechol sulfate,[11] and with hexapeptide mimetic as enzyme substrate analogue.[8] Many inhibitors of several PTPs contain non-hydrolysable phosphonate fragments, which are known as phosphotyrosine bioisosteres.[9a,12] However, benzylphosphonate,[11] aryloxymethano-, and aryloxyethanophosphonates[13] were found to be weak inhibitors of the *Yersinia* PTP.

Data in the literature indicate that inhibitors with two or more anionic groups show enhanced binding to PTPs when compared to the related monodentate compounds.[7c] We have reported[14] that preorganization of some phosphonate groups on the platform of calix[4]arene, macrocycle with unique structure and properties,[15] may be an efficient strategy for the design of powerful enzyme inhibitors. For example, calix[4]arene bearing two methylenebisphosphonic acid fragments at the wide rim exhibits high affinity for alkaline phosphatase.[14a] Present research was undertaken to evaluate the activity of phosphonate derivatives clustered by a macrocyclic calix[4]arene platform toward *Yersinia* PTP. To elucidate possible enzyme–inhibitor interactions, the functionalized macrocycles have been docked computationally to the active site of this phosphatase.

Calix[4]arene methylenebisphosphonic acids **1**–**3**, calix[4]arene and thiacalix[4]arene methylphosphonic acids **5**, **6**, and model 4-hydroxyphenyl methylenebisphosphonic acid **4** (Fig. 1) were synthesized according to previously developed synthetic procedures.[14,16,17] The compounds obtained were evaluated in vitro as inhibitors of recombinant phosphatase Yop51*.[18] The activities of

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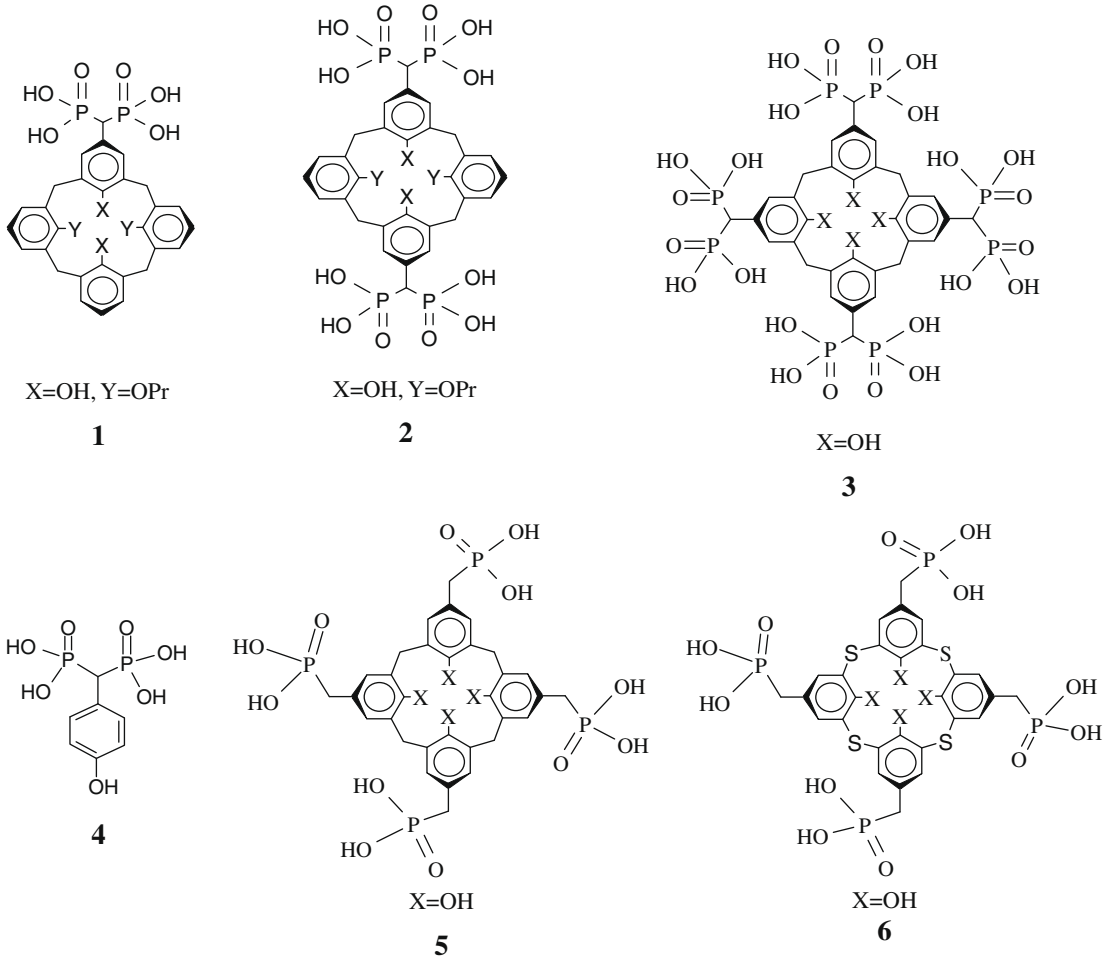

Figure 1. Chemical structures of inhibitors **1–6**. At physiological pH, the inhibitors called as phosphonates exist in $P(O)(OH)O^-$ and partially in $P(O)(O^-)_2$ anionic forms.

the enzyme were assayed by monitoring the rate of hydrolysis of *p*-nitrophenylphosphate used as substrate.[19]

The kinetic analysis revealed that the influence of compounds **1–6** on the activity of *Yersinia* PTP was in agreement with competitive type inhibition. The results correspond to the mechanism that involves the competitive binding of the inhibitor in the active site with formation of enzyme–inhibitor complex. Under assay conditions in the presence of EDTA and dithiothreitol, the Lineweaver–Burk double reciprocal plots were used to determine the values of inhibition constants for phosphorylated calix[4]arenes **2**, **5**, and thiacalix[4]arene **6** (Fig. 2) as well as compounds **1**, **3**, and **4**. The data indicates that the macrocyclic bisphosphonate **1** with K_i = 7.1 μM was approximately 80-fold more potent than the model compound **4** (Table 1). Introduction of two methylenebisphosphonic acid groups into the structure of calix[4]arene leads to the analog **2** being 400 times more effective inhibitor (K_i = 1.4 μM) as compared to the compound **4.** Corresponding tetrakis(methylenebisphosphonate) **3** showed a lower effect on the enzyme activity in comparison with mono- and bis(methylenebisphosphonate) **1** and **2**. Compounds **5** and **6** bearing four methylenephosphonate fragments at the wide rim of the macrocycle showed the highest affinities for the enzyme with inhibition constants in the low micromolar range (0.92 and 0.22 μM, respectively), whereas benzylphosphonate[11] exhibited no significant effect. Calixarene backbones, such as 25,26,27,28-tetrahydroxycalix[4]arene and 25,27-dipropoxy-26,28-dihydroxycalix[4]arene, did not change the enzyme activity at concentrations up to 20 μM. Thus, the higher inhibitory activities of phosphorylated derivatives of calix[4]arenes compared to model compounds can be attributed to synergistic effects of the phosphonate fragments and macrocyclic scaffold involved in the interaction with *Yersinia* PTP.

Although *Yersinia* PTP and mammalian PTPs have very similar catalytic cores, they differ in topology, charge distribution and lipophilicity profile of active surface.[6a] Based on such considerations, bulky macrocyclic phosphonate inhibitors appear to be capable of selective binding to the active center of a particular enzyme. The experiments, which have been carried out with *Yersinia* PTP and human PTPβ[20] show that the inhibitory activity of compound **2** toward *Yersinia* PTP is one order of magnitude higher than toward PTPβ (IC_{50} = 32 ± 7 μM). Noteworthy are more significant variations in IC_{50} values observed among macrocyclic inhibitors of PTPβ. As a result, compound **5** exhibited only modest selectivity for *Yersinia* PTP over PTPβ (IC_{50} = 3.8 ± 0.6 μM). Compound **6** was found to be more potent inhibitor of PTPβ (IC_{50} = 0.13 ± 0.02 μM) and showed an approximately threefold selectivity over *Yersinia* PTP. At the same time, compounds **2**, **5**, and **6** at concentration of 50 μM did not exhibit inhibitory activity against Ser/Thr protein phosphatase, such as human PP1.

To analyze the binding mode of macrocyclic phosphonate inhibitors in the active site of *Yersinia* PTP, computer-simulated docking studies were performed using QXP/FLO+ program.[21] A binding model was constructed automatically on the basis of known X-ray crystal structure of the enzyme in complex with *p*-nitrocatechol sulfate[11] (PDB code: 1PA9). Fulldock+ being the most exact in QXP/FLO+ was used as the method of the further optimization. The ligand was preliminary removed from the binding site of the enzyme, and