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Stereoselectivity of binding of α-(*N*-benzylamino)benzylphosphonic acids to prostatic acid phosphatase

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ABSTRACT

The inhibition effects of enantiomerically pure α-(*N*-benzylamino)benzylphosphonic acids and their derivatives on human prostatic acid phosphatase have been investigated. As expected, (*R*)-α-(*N*-benzylamino)benzylphosphonic acid demonstrated higher affinity for the enzyme than (*S*)-enantiomer. At the same time, (1*R*,2*S*)-phenyl[(1-phenylethyl)amino]methylphosphonic acid was found to be a significantly weaker inhibitor than its (1*S*,2*R*)-analogue. The enantioselectivity has been explained using a molecular modeling approach by computational docking of inhibitors into active center of prostatic acid phosphatase.

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Human prostatic acid phosphatase catalyzes non-specific hydrolysis of low molecular weight phosphate monoesters and the reaction of transphosphorylation.[1] The results of several studies indicate that this enzyme exhibits protein tyrosine phosphatase activity and may be involved in crucial cellular functions.[2] It was established that prostatic acid phosphatase can be responsible for dephosphorylation of the tyrosine residues of human epidermal growth factor receptor-2[3] and has a growth-suppressing effect.[2a] The enzyme is also capable of hydrolyzing lysophosphatidic acid that is involved in multiple activities and detected in various biological fluids.[4] Recent studies have identified human prostatic acid phosphatase as a prognostic factor for patients with intermediate- and high-risk prostate cancer.[5]

With increasing understanding of the physiologic role of prostatic acid phosphatase and its isoforms expressed in prostate and many non-prostatic cells and tissues,[2] there is growing interest in the regulation of activity of this enzyme. Natural and synthetic inhibitors of prostatic acid phosphatase may be useful in a variety of biochemical applications as selective chemical tools for research of enzyme functions in model systems and in living cells. Derivatives of α-benzylaminophosphonic acid have been proved the most effective synthetic inhibitors of human prostatic acid phosphatase.[6,7] Racemic α-(N-benzylamino)benzylphosphonic acid was found to be a powerful inhibitor of human prostatic acid phosphatase, which exhibited an IC_{50} = 4 nM when *O*-phosphotyrosine was the hydrolysis substrate.[6b] The present research was undertaken in order to evaluate the activity of (*R*)- and (*S*)-α-(*N*-benzylamino)benzylphosphonic acids as well as their derivatives with two stereogenic center—(1*R*,2*S*)- and (1*S*,2*R*)-phenyl[(1-phenylethyl)amino]methylphosphonic acids. To elucidate the enzyme–inhibitor complex structure, these compounds have been docked computationally to the active site of human prostatic acid phosphatase.

Individual enantiomers of α-(*N*-benzylamino)benzylphosphonic acids were synthesized according to previously developed synthetic protocols.[8,9] All compounds, in their hydrochloride salt forms (Fig. 1), were evaluated in vitro as inhibitors of human pros-

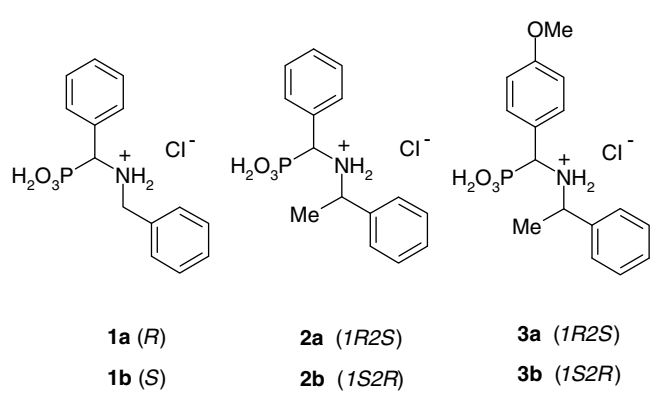

Figure 1. Chemical structures of compounds **1a**,**b**, **2a**,**b**, and **3a**,**b**.

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tatic acid phosphatase. The employed enzyme substrates were α-naphthyl phosphate and *O*-phosphotyrosine. Enzymatic activities were determined by following the change in absorbance that accompanied the hydrolysis of α-naphthyl phosphate to α-naphthol (λ = 320 nm, $\Delta\varepsilon$ = 2080 M^{-1} cm^{-1}) or *O*-phosphotyrosine to L-tyrosine (λ = 286 nm, $\Delta\varepsilon$ = 760 M^{-1} cm^{-1}).[10] The value of IC_{50} calculated from a dose-dependent curve was the concentration of the tested compound which decreased the enzyme activity to 50%.[11] K_m values of the prostatic acid phosphatase were reported[10a,12] to be 0.14 mM and 1.95 mM for α-naphthyl phosphate and *O*-phosphotyrosine, respectively.

In reaction medium containing α-naphthyl phosphate as substrate, 0.1 μM (*R*)-α-(*N*-benzylamino)benzylphosphonic acid **1a** provided more than 80% reduction of enzyme activity, whereas (*S*)-enantiomer **1b** exhibited no significant effect (Fig. 2). Somewhat unexpectedly, in the cases of phosphonic acids **2a**,**b** and **3a**,**b** with two stereogenic centers only (1*S*,2*R*)-isomers **2b** and **3b** showed strong inhibitory activity toward prostatic acid phosphatase. The enzyme activity was decreased with the increased concentrations of compounds **2b** and **3b.** At the same time, corresponding (1*R*,2*S*)-enantiomers **2a** and **3a** at concentrations from 1 nM to 1 μM showed a weak effect on prostatic acid phosphatase activity. The IC_{50} values with 95% confidence intervals (in brackets) were 31 (29–33) nM, 148 (119–184) nM, and 76 (68–85) nM for compounds **1a**, **2b**, and **3b**, respectively.

Under assay conditions with *O*-phosphotyrosine used as a substrate of human prostatic acid phosphatase, (*R*)-compound **1a** with IC_{50} = 5.0 nM was approximately 40-fold more potent than the (*S*)-enantiomer **1b** (Table 1). Introduction of methyl group into the structure of (*R*)-α-(*N*-benzylamino)benzylphosphonic acid leads to the analogue (1*R*,2*S*)-**2a**, being 240 times less effective inhibitor as compared to the compound (*R*)-**1a.** Compound (1*R*,2*S*)-**3a**, which bears methoxy group in the ortho position of the phenyl ring, has approximately the same activity as (1*R*,2*S*)-**2a.** On the other hand, the inhibitors (1*S*,2*R*)-**2b** and (1*S*,2*R*)-**3b** were 7 and 11 times more potent than the unsubstituted (*S*)-α-(*N*-benzylamino)benzylphosphonic acid **1b** and 45–60 times more effective compared with the (1*R*,2*S*)-analogues **2a** and **3a.** These experimental results emphasize the importance of the stereochemical requirements in the interaction of α-(*N*-benzylamino)benzylphosphonic acids with human prostatic acid phosphatase.

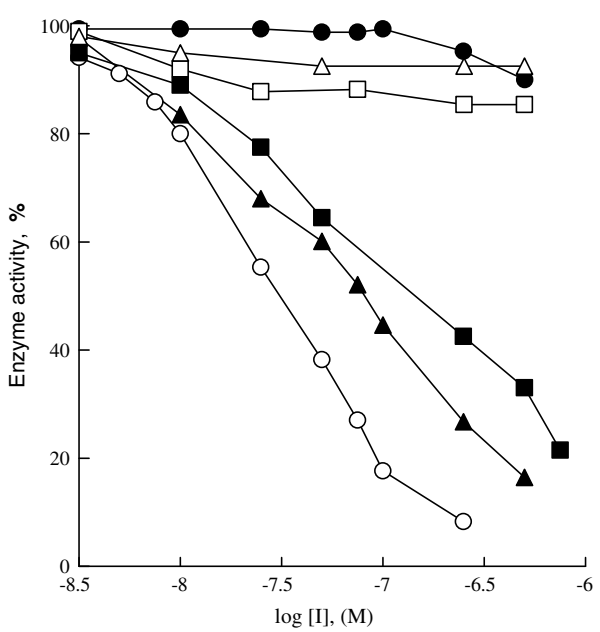

Figure 2. The dose-dependent inhibition of human prostatic acid phosphatase by compounds 1a (○), 1b (●), 2a (□), 2b (■), 3a (△), and 3b (▲). The α-naphthyl phosphate (2 mM) was used as enzyme substrate.

Table 1
Experimental inhibitory activity (IC50) and docking results (QXP/FLO+) of inhibitors **1a**,**b**, **2a**,**b**, and **3a**,**b** into the active site of human prostatic acid phosphatase

Compound	IC_{50}[a]	ΔGdoc[b] (kJ/mol)	Hbnd[c] (kJ/mol)
1a (*R*)	5.0 nM (8.30 ± 0.04)[d]	−37.1	−30.2
1b (*S*)	198 nM (6.70 ± 0.09)	−35.0	−29.8
2a (1*R*,2*S*)	1210 nM (5.92 ± 0.04)	−33.1	−28.3
2b (1*S*,2*R*)	26 nM (7.59 ± 0.04)	−36.9	−33.9
3a (1*R*,2*S*)	1070 nM (5.97 ± 0.03)	−31.1	−28.0
3b (1*S*,2*R*)	18 nM (7.74 ± 0.09)	−37.6	−33.0

[a] IC_{50} values determined by *O*-phosphotyrosine enzyme assay. Concentration of substrate was 2 mM.
[b] Calculated binding free energy.
[c] Calculated hydrogen bond energy.
[d] Values in brackets express pIC_{50}-s with 95% confidence intervals.

To estimate the binding mode of stereoselective inhibitors to the active site of human prostatic acid phosphatase the computer-simulated docking studies were performed using QXP/FLO+ program.[13] A binding model was constructed automatically by pdb2mod command on the basis of X-ray crystal structure of the enzyme complexed with α-(*N*-benzylamino)benzylphosphonic acid[7] (PDB code: 1ND5). Fulldock+ being the most exact in QXP/FLO+ was used as the method of the further optimization. The ligand was removed from the active site of prostatic acid phosphatase and then the active site was examined with various inhibitors. The study was carried out on the A subunit of the enzyme. The most favorable binding of inhibitor was observed in the case when its phosphoryl residue was in the form of dianion and NH-group was not protonated. Carboxylic group of Asp258 in enzyme active site was in neutral state.

Table 1 shows the results of the docking studies of inhibitors **1a**,**b**, **2a**,**b**, and **3a**,**b**. The binding free energies indicate that (*R*)-enantiomer **1a** exhibits stronger binding affinity for prostatic acid phosphatase than (*S*)-enantiomer **1b** and that compounds (1*S*,2*R*)-**2b** and (1*S*,2*R*)-**3b** bind more tightly to the enzyme than the (1*R*,2*S*)-isomers **2a** and **3a**. The correlation coefficient (r^2) between the calculated free energies of binding and experimental activities, expressed as $\log IC_{50}$, for all inhibitors was 0.85.

Calculated geometry of (*R*)-α-(*N*-benzylamino)benzylphosphonic acid **1a** was compared with the crystallographic pose of the same inhibitor in the active site of human prostatic acid phosphatase.[7] As a result, the positions of ligand atoms of the calculated complex and ligand atoms of X-ray crystallographic structure are similar with an RMSD of 0.93 Å for all atoms of inhibitor submitted in crystallographic data, or 0.48 Å for atoms of oxygen, phosphorus, nitrogen, and α-carbon. In agreement with a previously reported docking study,[14] favorable interactions were found between the oxygens of **1a** phosphonate group and Arg11, Arg15, Arg79, and His257 (Fig. 3). The oxygen of phosphonate group is also hydrogen bonded with amide hydrogen of Asp258. In the calculated structure, phosphorus atom of inhibitor is oriented to NE2 atom of His12 within the distance of 3.13 Å. The phenyl fragments of compound **1a** are involved in interaction with residues of Ile18, Ser175, Phe171, and Trp174.

The binding mode of phosphonate group of inhibitor **1b** is similar to that observed with enantiomer **1a** (Fig. 3). The hydrogen bonds formed by these compounds with amino acid residues inside the enzyme active site are almost the same. However, benzylamino moiety of less active inhibitor **1b** occupies the lipophilic pocket with an altered orientation without close contact with Ile18 and Ser175.

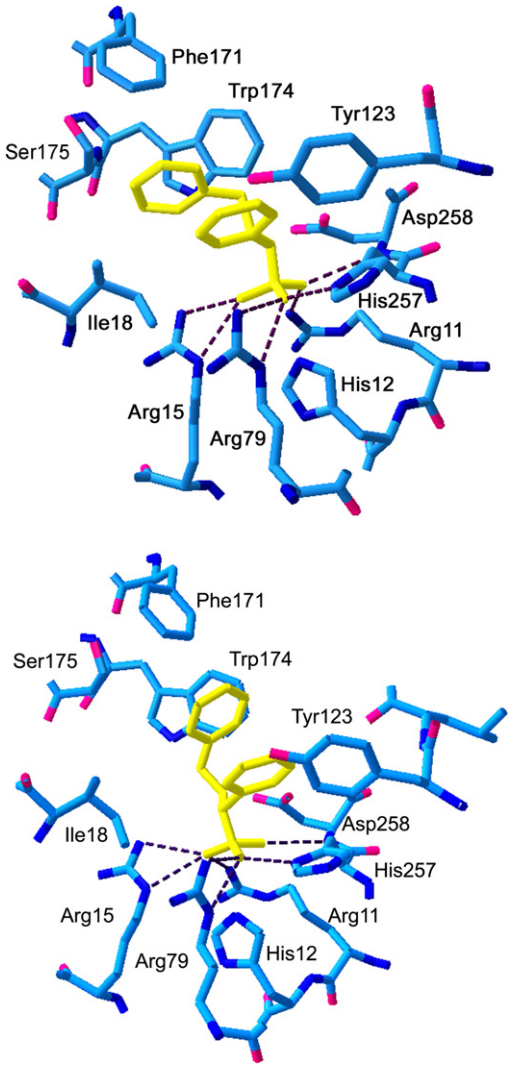

Figure 3. The interacting modes of enantiomers 1a (top) and 1b (bottom) with the adjacent amino acid residues in the active site of human prostatic acid phosphatase obtained after QXP/FLO^{+} calculations. Dotted lines show the hydrogen bonds in which the inhibitor is involved.

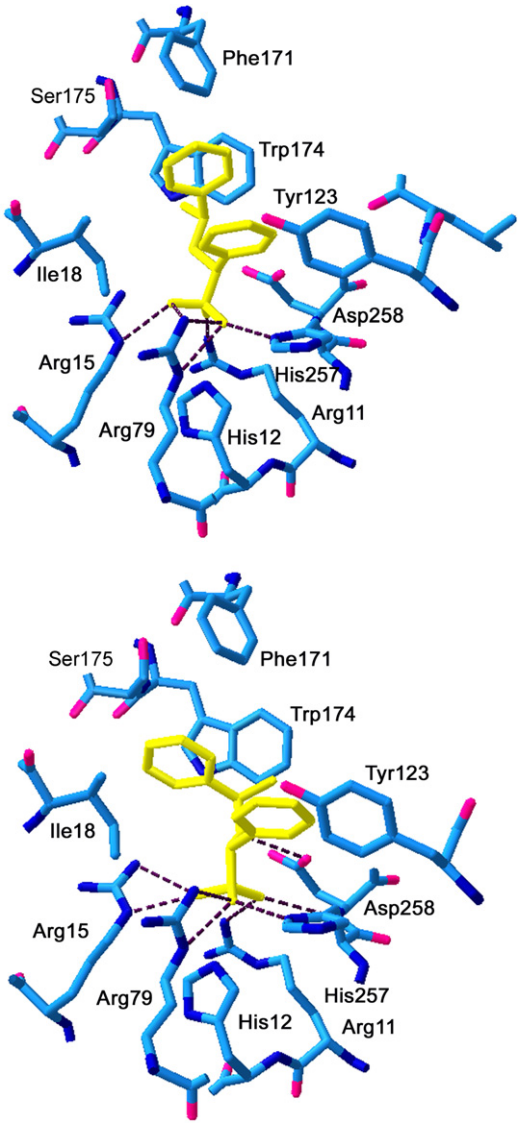

Figure 4. The possible interacting modes of enantiomers **2a** (top) and **2b** (bottom) with the adjacent amino acid residues in the active site of human prostatic acid phosphatase.

The phosphonate moieties of (1*R*,2*S*)-inhibitors **2a** and **3a** were anchored to amino acid residues as in case of (*R*)-**1a** but with the exception of Asp258. The phosphonate oxygens of (1*S*,2*R*)-**2b** and (1*S*,2*R*)-**3b** were positioned to make the hydrogen bonds to Arg11, Arg15, Arg79, His257, and NH of Asp258. Furthermore, the presence of methyl group in the structures of **2b** and **3b** provides additional fixation of inhibitors by means of NH group of benzylamino substituent and carboxylic group of Asp258 (Fig. 4). In comparison with (1*S*,2*R*)-compounds **2b** and **3b**, the CH_2Ph groups of weak (1*R*,2*S*)-inhibitors **2a** and **3a** were remote from the residues of Ile18 and Ser175.

Examination of the models also shows that two aromatic rings of (*R*)-**1a** stack favorably with the edge of the CH_2Ph placed over the center of the other phenyl ring. In the (*S*)-**1b** model, the electronegative centers are directly opposed and the aromatic rings are separated more from the Ile18 and Ser175 residues. The intramolecular stacking interaction between the phenyl rings of the (*S*)-**1b** is similar to that of the weak inhibitors (1*R*,2*S*)-**2a** and (1*R*,2*S*)-**3a**.

In conclusion, this study demonstrates the stereoselective effects of enantiomerically pure α-(*N*-benzylamino)benzylphosphonic acids toward human prostatic acid phosphatase. We revealed the higher activity of (*R*)-α-(*N*-benzylamino)benzylphosphonic acid in comparison with (*S*)-enantiomer. On the other hand, (1*S*,2*R*)-phenyl[(1-phenylethyl)amino]methylphosphonic acid was a more active isomer than its (1*R*,2*S*)-analogue. The molecular docking results correspond to activity order (*R*)-**1a** > (*S*)-**1b**, (1*S*,2*R*)-**2b** > (1*R*,2*S*)-**2a**, and (1*S*,2*R*)-**3b** > (1*R*,2*S*)-**3a**, and indicate that stereoselective effects of inhibitors are mainly driven by hydrophobic interactions and the network of hydrogen bonds.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.07.021.

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9. Compounds **1a** and **1b** were prepared by reaction of di[(1*R*,2*S*,5*R*)-menth-2-yl]phosphite or di-endo-bornylphosphite, respectively, with phenylbenzaldimine followed by hydrolysis of corresponding phosphonates. Enantiomeric (1*R*,2*S*)- and (1*S*,2*R*)-phenyl[(1-phenylethyl)amino]methylphosphonic acids **2a**,**b** were obtained in similar manner by the reaction of di[(1*R*,2*S*,5*R*)-menth-2-yl]phosphite with chiral (*S*)- and (*R*)-α-methylbenzylbenzaldimine, respectively. In the case of phosphonic acids **3a**,**b** di[(1*R*,2*S*,5*R*)-menth-2-yl]phosphite was coupled to (*R*)- or (*S*)-α-methylbenzyl-*p*-methoxybenzaldimine. The absolute configurations of enantiomerically pure compounds were determined by comparison of optical rotations with reported values.
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11. In the assay, 2 mM substrate was preincubated for 5 min with varying concentrations of an inhibitor in acetate buffered aqueous solution (pH 5.5) containing 2.5 vol.% of dimethyl sulfoxide at 25 °C. The reaction was started by the addition of the enzyme solution. The total concentration of enzyme was 10 nM. The inhibitory activity was plotted against the log concentration of the inhibitor, and the IC_{50} value was determined from this curve.
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