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BENZYLPHOSPHONIC ACID INHIBITORS OF HUMAN PROSTATIC ACID PHOSPHATASE

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Abstract

A series of α–substituted benzylphosphonic acids is described as inhibitors of human prostatic acid phosphatase, an enzyme which has been used as a model to study aryl phosphatases. The most potent inhibitors in this series are 2-trifluoromethylbenzhydrylphosphonic acid (9 μM), and α-(2-phenylethyl)benzylphosphonic acid (14 μM). The structure-activity studies suggest that bulk tolerance beyond the phosphate binding area limits the steric or hydrophobic contribution to inhibitor potency achieved through α-carbon substitution.

The mitogenic action of growth factors such as insulin, epidermal growth factor, and skeletal growth factor involves receptors linked to protein tyrosine phosphorylation and is physiologically antagonized by protein tyrosylphosphatases. [2]

Vanadate, a nonspecific inhibitor of phosphatases, has been shown to potentiate the action of insulin and bone growth[3,4] while fluoride,[4] a specific inhibitor of bone tyrosyl phosphatase, potentiates the action of bone growth factors. Only a few inhibitors of tyrosyl phosphatases are known and are described as potential irreversible inhibitors.[1,5-8] The mechanism-based suicide inhibitors, monofluoro and difluoromethylphenylphosphate.[7,8] have been reported to inhibit both protein tyrosyl phosphatase and prostatic acid phosphatase. While the natural substrate is unknown for most protein phosphatases, it is assumed that a large protein or peptide containing a phosphorylated residue is a likely substrate.[2] Approaches to the design of phosphatase inhibitors based upon phosphonate-containing peptide analogs of substrates have been suggested.[2,5] We wish to report a series of simple benzylphosphonic acids as a prototype of nonpeptide small molecule phosphatase inhibitors of prostatic acid phosphatase, a model enzyme previously used to study aryl or tyrosyl phosphatases.[9]

Chemistry

The synthesis of target phosphonic acids was achieved according to previously published procedures[10-13] utilizing an Arbuzov reaction of substituted benzyl bromides with triethylphosphite. The intermediate esters yielded the target acids upon acid hydrolysis with either refluxing HCl or bromotrimethylsilane. Benzylic carbon substitution, analogues **4-8**, was achieved by alkylation of the benzylphosphonate ester with the appropriate benzyl or

phenethylhalide using n-butyllithium in tetrahydrofuran, followed by hydrolysis of the resulting ester. Other compounds, **2, 3, 9-21, 23-26**, were synthesized by reduction of the corresponding ketones to the benzylic alcohol, brominating with phosphorus tribromide and then refluxing the crude bromides with triethylphosphite.

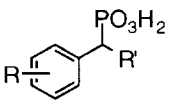
Results and Discussion

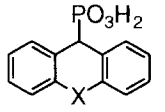
The design of the present series of benzylphosphonates simply involved the replacement of the phenolic oxygen-phosphorus bond (substrate) with a hydrolytically stable phosphonate. We initially investigated substitution of the phenyl ring in a large series of substituted benzylphosphonic acids which led to compound **2** as the only simple benzylphosphonic acid with inhibitory activity below 100 μM. Generally, mono and diethyl esters were noninhibitory. Investigation of a variety of substituents such as hydroxyl, fluoro, methyl, and phenyl at the benzylic carbon yielded the benzhydrylphosphonic acids as the only active structure and that the minimum structural requirement for binding appears to be an acidic moiety combined with an adjacent hydrophobic region. Generally, analogs possessing a diversity of smaller substituents (**7, 9-15, 17, 19, 20**), mainly ortho or meta, all possessed equal or greater potency than the unsubstituted benzhydrylphosphonic acid, suggesting that their main contribution may be steric by inducing a preferred conformation which best interacts with the enzyme. Inhibitor potency increased with the introduction of larger hydrophobic substitution at the α-carbon as in the series **3** (142 μM), **4** (89 μM), **5** (14 μM) and **6** (16 μM), but the plateauing effect on inhibitory potency observed for the phenethyl derivative, **5**, the biphenylmethyl analogue, **6**, and the bis-biphenylmethylphosphonic acid, **21**, and analogs possessing aromatic substitutions in either or both rings (**7, 8, 21**) again suggests the limited hydrophobic binding area adjacent to the phosphate binding site and a bulk tolerant region or space beyond the hydrophobic area.

The rigid analogs (**22, 23** and **26**) displayed little or no inhibition of the enzyme at 100 μM, suggesting that their conformations were not suitable to access the phosphate-binding site. The more flexible analog, **24,** showed a potency slightly greater than **3** while **25** exhibited by far the most potency in the rigid series. It is unlikely that the tricyclic nucleus offered any additional binding since these examples exhibited no potency increase beyond that of the simple 2-methylbenzhydrylphosphonate, **10**.

The most striking aspect of the structure-activity relationship thus far observed for the human prostatic acid phosphatase inhibitors might be the simplicity of requirements for a rather small hydrophobic molecule possessing a strong affinity for the phosphonic acid moiety. The structure-activity relationship thus far identified, describes an enzyme which is rather nonspecific in regards to substrate structures but capable of hydrolzing tyrosyl phosphate.

INHIBITION OF HUMAN PROSTATIC ACID PHOSPHATASE





No.	R	R'	X	IC_{50} μM[a]
1	H	H		>500
2	3-CF_3	H		88
3	H	phenyl		142
4	H	benzyl		89
5	H	phenylethyl		14
6	H	4-phenylbenzyl		16
7	3-CF_3	benzyl		100
8	3-CF_3	4-phenylbenzyl		36
9	2-Cl	phenyl		31
10	2-CH_3	phenyl		38
11	2-CF_3	phenyl		9
12	3-CF_3	phenyl		67
13	3-CH_3	phenyl		14
14	3-Cl	phenyl		15
15	3-NO_2	phenyl		17
16	4-CH_3	phenyl		152
17	4-CF_3	phenyl		41
18	4-Cl	phenyl		143
19	4-OCH_3	phenyl		37
20	4-phenyl	phenyl		67
21	4-phenyl	4-biphenyl		70
22			bond	>500
23			CH=CH	>100
24			CH_2CH_2	94
25			O	22
26			S	>100

[a] Inhibitor conc required for 50% inhibition of the hydrolysis of tyrosine phosphate.

Tyrosyl Phosphatase Assay

Test compounds were incubated with human prostatic protein acid phosphatase and radiolabelled substrate, ^{14}C-phosphotyrosine (NEN-Dupont) plus cold O-phospho-L-tyrosine (10 μM) (Sigma) in a 50 mM sodium acetate buffer (pH 5.5) for 30 minutes at 37 ^{o} C. The reaction was stopped by placing the assay on ice and adding 100 μL aliquot of an inhibitor solution (1.1 mM sodium orthovanadate, 0.55 M sodium fluoride, Sigma). The mixture was passed through an Ag 1-x8 (Bio-Rad) ion exchange column, washed with 2.5 mL of water, the effluent containing ^{14}C-tyrosine was collected, and quantified by scintillation spectroscopy. The concentration of compound necessary to inhibit 50% of the dephosphorylation (IC_{50}) was calculated and is reported as an average of at least duplicate determinations using several concentrations.

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