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NOVEL HYDROXYPHOSPHONATE INHIBITORS OF CD-45 TYROSINE PHOSPHATASE

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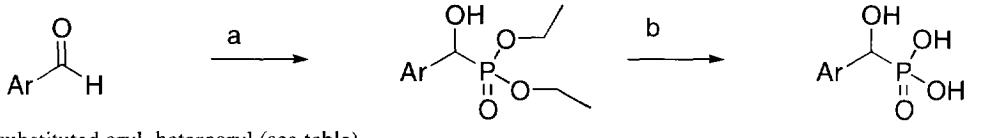
Abstract: CD-45 tyrosine phosphatase [E.C. 3.1.3.48] is an important player in the regulation of cell activation and proliferation in hematopoetic cells. As part of a program in immune response modulation, we prepared the first series of small organic molecule inhibitors of CD-45. The preparation and in vitro screening of these hydroxyphosphonates is described herein. © 1997 Elsevier Science Ltd.

The signal transduction process, mediated by peptide growth factors, that regulates cell activation and proliferation, requires allosteric and covalent modifications of cellular proteins. These crucial structural changes are initiated by phosphoryl transfer reactions, beginning with the phosphorylation of cellular proteins on tyrosine residues by tyrosine kinases.[1] Abundant evidence exists to show that tyrosine phosphorylation and the reverse reaction, catalyzed by phosphatases, are involved in transduction of the T-cell activation signal through the T-cell antigen receptor complex.[2] CD-45 tyrosine phosphatase, present in all hematopoetic cells, has been implicated in the regulation of this signal transduction and the concomitant functional responses.[3] This tyrosine phosphatase enzyme is a particularly interesting target for a novel drug discovery program since no synthetic inhibitor series has been reported. Inhibition studies have been done recently with naturally occurring aporphine alkaloids[4] and suramin, a polyanionic anti-trypanosomiasis drug.[5]

At the outset of this work, no structural information was available for the CD-45 enzyme. In addition, there were no published reports of tyrosine phosphatase inhibition that we were aware of, although tyrosine kinase inhibition strategies had been noted.[6a,b] Therefore, inhibitor design focused on the chemistry taking place at the active site. Lacking structural details, we knew only that the active site must accommodate a phosphorylated tyrosine residue. By analogy to the work in tyrosine kinase inhibition, we decided that compounds that mimic the phosphorylated tyrosine, having a non-cleavable phosphate group, would be a suitable starting point. Our first series of inhibitors featured aryl or heteroaryl α–hydroxyphosphonate derivatives prepared by the route outlined in Scheme 1.

Scheme 1



Ar = substituted aryl, heteroaryl (see table)

Reagents: (a) basic alumina, diethylphosphite[7] (b) TMSBr, methylene chloride[8]

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Compounds prepared in this manner were screened against a Jurkat cell membrane preparation.[9] CD-45 is the major tyrosine phosphatase in these membrane preparations.[3b] The results are expressed as a percentage of total inhibition for the indicated concentrations (Table 1). The compounds showing most promise were carried on to a concentration dependent IC_{50} determination.

The unsubstituted phenyl analog (entry 1), and the two naphthyl derivatives (entries 2,3)[6c] were weakly active, but good activity was observed with 3-nitro-α-hydroxybenzylphosphonate (entry 4). Varying the electronics at the 3-position afforded weak inhibition, with the cyano and chloro groups being slightly superior to amino and methyl groups (entries 5-8); and a phenol substituent eliminated activity (entry 9). Of the 4-substituent variants (entries 10-12), the strongly electron withdrawing 4-nitro substituted derivative (entry 11) was also a potent inhibitor. Several heterocyclic α-hydroxyphosphonates were tested as well. The π-deficient analogs were poor inhibitors (entries 13-16), but a nitro-substituted thiophene derivative (entry 17) exhibited an IC_{50} value of 38 μM. Thus, a CD-45 pharmacophore was emerging: an aryl hydroxyphosphonate having a nitro substituent in the 3-position appeared important for activity. The importance of the α-hydroxy group was verified with the "des-hydroxy" analog shown (entry 24 vs. 4). Addition of an α-methyl substituent was also detrimental to activity (entry 23).

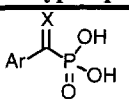
The most beneficial modifications resulted from holding the nitro group in the 3-position and placing additional substituents on the aromatic ring. This strategy led to a dramatic improvement of activity (entries 18-22). As noted with entry 9, however, the presence of a phenolic hydroxy group was detrimental. The best compound of the series (entry 22), with an IC_{50} of 1.2 μM, was substituted with thiocyclohexane at position 6, para to the nitro group.

Several recent reports have appeared describing tyrosine phosphatase inhibitors based on the principle of a non-cleavable phosphate, although none has targeted CD-45.[10] In an effort to identify interesting structure-activity relationships, several non-peptide analogs were screened in our assay, and the results included in this report. Table entries 25[10a] and 26[10b] are potent inhibitors of human prostatic acid phosphatase (14 μM and 8.6 nM IC_{50}'s, respectively), and entry 27[10c] is a potent inhibitor of osteoclastic acid phosphatase (1.4 μM IC_{50}). The poor CD-45 inhibitory properties of these compounds reinforces the pharmacophore derived from this work.

In conclusion, this report is the first to identify a series of synthetic inhibitors of CD-45. The active pharmacophore is a 3-nitro-α-hydroxyphenylphosphonate with preliminary results indicating that further investigations of substitution at the 6-position, para to the nitro group, will be a promising approach to increasing activity. An expansion of the preliminary structure-activity relationship indicated here is in progress in parallel with a study of the mechanism of inhibition and an analysis of phosphatase selectivity for compounds in the series. Future work will probably need to address membrane permeability issues that have been observed by others with highly charged phosphonate compounds.[10e] Recent publication of the crystal structure for protein tyrosine phosphatase 1B, which has approximately 40% sequence identity with CD-45,[11a] will be helpful for applying modeling techniques to the optimization of inhibitor-enzyme binding interactions during modifications

targeting improved membrane permeability. An early report concerning the advantageous use of the crystallography data for enhancing phosphatase activity of an aryl phosphonate inhibitor has been published by Burke and co-workers.[11b]

Table 1: Structures and activity data for α-hydroxyphosphonates screened against CD-45 [E.C. 3.1.3.48]



entry	Ar[a]	X	Conc. 1(μM)	%inh	IC_{50} (μM)
1	phenyl	OH,H	1000	39	
2	1-naphthyl	OH,H	1000	53	
3	2-naphthyl	OH,H	500	59	
4	3-NO_2-phenyl	OH,H	1000	93	10
5	3-CN-phenyl	OH,H	1000	49	
6	3-NH_2-phenyl	OH,H	500	23	
7	3-Cl-phenyl	OH,H	500	49	
8	3-CH_3-phenyl	OH,H	500	27	
9	3-OH-phenyl	OH,H	500	0	
10	4-OCH_3-phenyl	OH,H	500	42	
11	4-NO_2-phenyl	OH,H	1000	98	28
12	4-SO_2CH_3-phenyl	OH,H	500	41	
13	4-pyridyl	OH,H	500	0	
14	2-pyridyl	OH,H	500	2	
15	3-pyridyl	OH,H	500	13	
16	4-quinolinyl	OH,H	500	58	
17	2-NO_2-4-thienyl	OH,H	500	93	38
18	4-OH-3-NO_2-phenyl	OH,H	1000	8	
19	3-NO_2, 6-OH-phenyl	OH,H	500	75	
20	3- NO_2-6-Cl-phenyl	OH,H	500	81	20
21	5-NO_2-2-N_3-phenyl	OH,H	500	97	8
22	3-NO_2-6-S-C_6H_{11}-phenyl	OH,H	500	99	1.2
23	3-NO_2-phenyl	OH,CH_3	1000	97	58
24	3-NO_2-phenyl	H,H	500	17	
25	3-CH_3-phenyl	phenyl	500	8	
26	1-naphthyl	benzylamino	250	23	
27	1-naphthyl	bis-benzoyl	500	8	

[a]All compounds, except entry 18, were prepared from commercially available aldehydes. The aldehyde used for entry 18 was prepared from 2-chloro-5-nitrobenzaldehyde by chloride displacement with sodium azide in DMF.

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9. Biological Methods: Jurkat (JEG-6) cells were sonicated in hypotonic lysis buffer (25 mM Tris HCl, pH 7.5, 25 mM sucrose, 0.1 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, 1 mM Peflabloc). The nuclei were removed by centrifugation at 1500 x *g* for 5 min. The membranes were sedimented at 10,000 x *g* for 60 min. The resulting pellet was resuspended in lysis buffer and frozen at -80 °C until used.

The enzyme assay was done in a total volume of 0.50 mL, containing buffer (100 mM sodium acetate, pH 6.0), substrate (100 μM O-phospho-L-tyrosine containing 40-50 x 10^3 dpm of ^{14}C-L-tyrosine phosphate) and the enzyme preparation (normally less than 40% conversion of substrate to product in the controls). After 90 min at 37 °C, the samples were put on ice and 100 μL quench buffer (1.1 mM sodium orthovanadate and 0.55 μM sodium fluoride in assay buffer) was added. The samples were eluted through a 25 mm column containing AG-1X8 resin and washed with 2 mL H_2O. The combined eluates were counted in a scintillation counter.

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