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α-Ketocarboxylic Acid-Based Inhibitors of Protein Tyrosine Phosphatases

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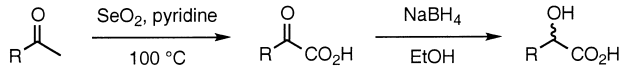
Abstract—A series of aryl α-ketocarboxylic acids was synthesized and investigated as inhibitors for the protein tyrosine phosphatase from *Yersinia enterocolitica*. IC_{50} values for these compounds range from 79 to 2700 μM. Larger aromatic groups, and aromatic groups with high electron density, lead to more potent inhibitors. In general, the related aryl α-hydroxycarboxylic acids show lower activity. © 2001 Elsevier Science Ltd. All rights reserved.

Protein tyrosine phosphatases (PTPases) catalyze the hydrolysis of phosphotyrosine residues during signal transduction. These enzymes, along with the protein tyrosine kinases, play a central role in regulating cell growth, differentiation, and metabolism.[1,2] PTPases have been implicated in a number of disease processes.[3] In particular, PTP1B is a potential therapeutic target for the treatment of type II diabetes.[4] This phosphatase is a negative regulator of insulin-dependent signaling, and likely acts by dephosphorylating the insulin receptor.[5] Thus, there is a significant motivation for developing potent and specific inhibitors for the phosphatases.

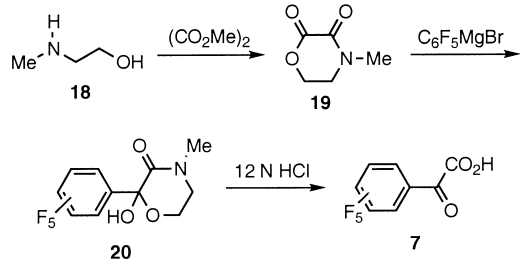
A variety of inhibitors for PTPases has been reported in the literature that incorporate a structural mimic of phosphotyrosine. These include compounds such as cinnamic acids,[6] difluoromethylphosphonates,[7] 4′-*O*-[2-(2-fluoromalonyl)]-tyrosine,[8] 2-(oxalylamino)-benzoic acids,[9] and others.[10] In this paper, we investigate aryl α-ketocarboxylic acids as a new type of phosphotyrosine mimic, and explore their use in the design of inhibitors for the *Yersinia* PTPase.[11] The aryl α-ketocarboxylic acids are designed to make a variety of contacts with residues in the active site of the phosphatase. These contacts include an electrostatic interaction between the carboxylate of the inhibitor and Arg409, a hydrogen bond between the ketone carbonyl group of the inhibitor and Asp356, and hydrophobic interactions between the aromatic ring of the inhibitor and aromatic residues that line the binding pocket.

α-Ketocarboxylic acids **3**, **5**, **9**, and **10** (Table 1) were synthesized using selenium dioxide to oxidize the corresponding methyl ketones as shown in Scheme 1.[12,13] Compound **4** was obtained through benzylic oxidation of homophthalic acid using selenium dioxide in refluxing toluene.[14]



Scheme 1.

The pentafluorophenyl-α-ketocarboxylic acid **7** was prepared as outlined in Scheme 2. The synthesis began with condensation of 2-(aminomethyl)ethanol with dimethyl oxalate to give compound **19**. The ester carbonyl group of **19** reacts with pentafluorophenylmagnesium bromide to give hemiacetal **20**.[15] This compound was then hydrolyzed in strong acid to give ketoacid **7**.



Scheme 2.

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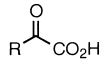
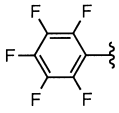
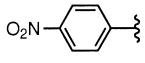
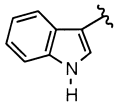
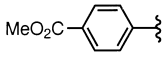
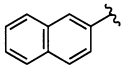
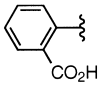
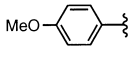
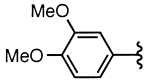
Racemic α-hydroxycarboxylic acids **14**, **16**, and **17** (Table 2) were prepared by reducing the corresponding α-ketoacids with sodium borohydride in ethanol (Scheme 1). Compounds **1**, **2**, **6**, **8**, **11**–**13**, and **15** were obtained from commercial sources.

We have explored a variety of α-ketocarboxylic acids as inhibitors of the *Yersinia* PTPase, as shown in Table 1. The enzyme was assayed at 25 °C by monitoring the hydrolysis of *p*-nitrophenyl phosphate by UV spectrophotometry at 420 nm. Assay solutions contained 100 mM acetate buffer at pH 5.5, 1 mM EDTA, and 10% DMSO. IC_{50} determinations were performed with a fixed substrate concentration of 2.5 mM, which corresponds to the K_M value under the assay conditions.

Compounds with an unsubstituted phenyl ring (**1**) or with electron withdrawing groups in the *para* position (**2** and **3**) are poor inhibitors with IC_{50} values in the low millimolar range. In other classes of inhibitors such as the 2-(oxalylamino)-benzoic acids, incorporation of an *o*-carboxy group often leads to a significant increase in activity.[9,10d] However, compound **4**, which incorporates an *o*-carboxy group, has an IC_{50} value that is only half the value for the unsubstituted inhibitor, and it is much less potent than the corresponding oxalylamino derivative.[9] Compound **5** contains two methoxy substituents and is also a millimolar inhibitor. By comparison, compound **10**, which has a single methoxy group in the *para* position, is more than 10 times as potent. It is likely that the *meta*-methoxy substituent in **5** lowers the activity of this compound due to steric hindrance.[16]

Table 1. Inhibition of the *Yersinia* PTPase by α-ketocarboxylic acids[a]



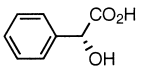
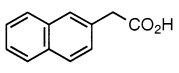
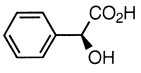
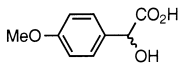
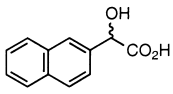
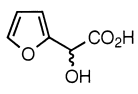
No.	R	IC_{50} (μM)	No.	R	IC_{50} (μM)
1		2700	**7**		790
2		2700	**8**		390
3		2300	**9**		190
4		1500	**10**		150
5		1700	**11**		79[b]
6		950			

[a]Average of two measurements. The error in these values is approximately ±10%.
[b]K_i value.

Increasing the size and hydrophobicity of the aromatic ring of the inhibitors (**7**–**9**) leads to better activity. The naphthalene-based α-ketocarboxylic acid (**9**) is a reasonable inhibitor with an IC_{50} value of 190 μM. Interestingly, increasing the electron density of the aromatic ring by appending an electron donating methoxy substituent at the *para* position as in compound **10**, or by incorporating a heteroatom within the aromatic ring as in compounds **6**, **8**, and **11** leads to a significant increase in activity. The furan-based inhibitor (**11**) is the most potent of the α-ketocarboxylic acids that we have investigated. It is more than 30 times more active than the simple phenyl substituted compound (**1**), and Lineweaver–Burke analysis confirms that it is a reversible competitive inhibitor. There are several plausible explanations for these results. First, the electron-rich aromatic ring of these inhibitors may form more favorable stacking interactions with nearby residues in the active site. A second interpretation invokes a hydrogen bond between the ketone carbonyl group of the inhibitors and Asp356. Compounds **10** and **11** both have resonance structures that significantly increase the electron density on the ketone carbonyl (Fig. 1). These resonance interactions would strengthen the putative hydrogen bond between inhibitor and the enzyme, and thus lead to an increased enthalpy of binding. Finally, release of a strongly bound water of solvation from this carbonyl group upon binding to the enzyme could provide a further entropic driving force.

Difluoromethylphosphonates and fluoromalonates are two of the most successful phosphotyrosine mimics that have been developed to date.[7,8] In terms of potency, the α-ketocarboxylic acids compare favorably to these other two classes of inhibitors. For example, the simple phenyl difluoromethylphosphonate (**21**) has a K_i value against PTP1B of 2.5 mM,[17] while the corresponding α-ketocarboxylic acid (**1**) has an IC_{50} value of 2.7 mM against the *Yersinia* PTPase (Fig. 2). Furthermore, the naphthalene-derived difluoromethylphosphonate (**22**), fluoromalonate (**23**), and difluoromethylcarboxylate (**24**) all have inhibition constants or IC_{50} values against PTP1B that are similar to, or higher than, the value for the corresponding naphthalene-derived α-ketocarboxylic acid (**9**) against the *Yersinia* PTPase.[17,18]

Table 2. Inhibition of the *Yersinia* PTPase by α-hydroxycarboxylic acids and compound **15**[a]

No.	Compound	IC_{50} (μM)	No.	Compound	IC_{50} (μM)
12		5500	**15**		3500
13		4900	**16**		5700
14		3300	**17**		27

[a]Average of two measurements. The error in these values is approximately ±10%.

Although the inhibition constants for the two different enzymes are not directly comparable, data from the literature suggest that a particular inhibitor is likely to have a similar inhibition constant against PTP1B and the *Yersinia* PTPase. For example, suramin, which is a reversible competitive inhibitor, has K_i values of 4 and 1.3 μM against PTP1B and the *Yersinia* PTPase, respectively.[19] In addition, the K_M values for *p*-nitrophenyl phosphate with the two enzymes are similar. Finally, it is known that these two PTPases have a close similarity in their secondary and tertiary structures.[19]

We have also measured the activity of several α-hydroxycarboxylic acids against the *Yersinia* PTPase (Table 2). In all but one case, reduction of the α-ketocarboxylic acid to the corresponding α-hydroxycarboxylic acid leads to a dramatic decrease in activity. Comparison of compounds **9** and **15** shows that removing the ketone altogether from the inhibitor also leads to a significant decrease in potency.

The one exception to these observations is the furan-based α-hydroxycarboxylic acid **17**. This compound has an IC_{50} of 27 μM, and it is almost 3 times more potent than the corresponding ketoacid **11**. We have explored the possibility that compound **17** is a time-dependent irreversible inhibitor of the phosphatase. Figure 3 shows how this inhibitor could bind in the active site of the enzyme and form a hydrogen bond between the hydroxyl group on the inhibitor and Asp356. The inhibitor could then fragment to release a water molecule and generate an oxonium ion in the active site. This type of fragmentation is analogous to the Lewis acid promoted conversion of 2-hydroxymethylfuran into the corresponding 2-alkoxymethylfuran.[20] The oxonium ion intermediate could then act as an electrophilic trap for a nearby nucleophilic residue on the enzyme, such as Cys403.

To investigate the possibility of time-dependent inhibition, we incubated the phosphatase for up to 2 h with saturating concentrations of inhibitor **17**. Following the incubation, the enzyme and inhibitor were diluted to a point where the concentration of **17** was well below its inhibition constant, and the enzyme was assayed for activity. Comparison of these results with control experiments that were performed in the absence of inhibitor demonstrated that compound **17** is not a time-dependent inhibitor. Thus, we do not believe that the mechanism shown in Figure 3 is responsible for the enhanced activity of this compound. Currently, we do not have an adequate explanation for why α-hydroxycarboxylic acid **17** is more potent than the corresponding ketoacid **11**, while all other α-hydroxycarboxylic acids in the series (**12**–**14** and **16**) are significantly less potent than their analogous α-ketoacids.

A variety of α-ketocarboxylic acids and their esters have been used as inhibitors for cysteine proteases.[21] These compounds are able to form a reversible covalent hemithioacetal linkage with the active site cysteine nucleophile of the protease. In addition, Charifson and co-workers have shown that a peptide ligand that incorporates a formylated phosphotyrosine analogue forms a hemithioacetal with Cys188 in the binding pocket of the $pp60^{c\text{-}src}$ SH2 domain.[22] We note that the α-ketocarboxylic acid inhibitors reported here have the potential of forming an analogous hemithioacetal with the active site Cys403 of the *Yersinia* PTPase. However, we observe that inhibitors such as **10** and **11**, which incorporate ketones with lower electrophilicity, show higher activity against the phosphatase. Therefore, we do not believe that these inhibitors are reacting in a reversible covalent fashion with the active site cysteine residue.

In this paper, we have described a series of aryl α-ketocarboxylic acids that inhibit the *Yersinia* PTPase. These

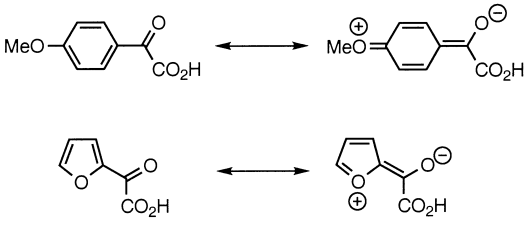


Figure 1. Resonance structures for compounds **10** and **11**.

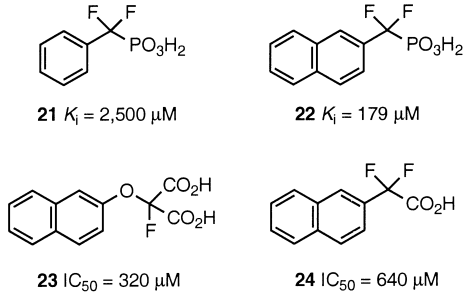


Figure 2. K_i and IC_{50} values for several inhibitors of protein tyrosine phosphatase 1B that have been reported in the literature.[17,18]

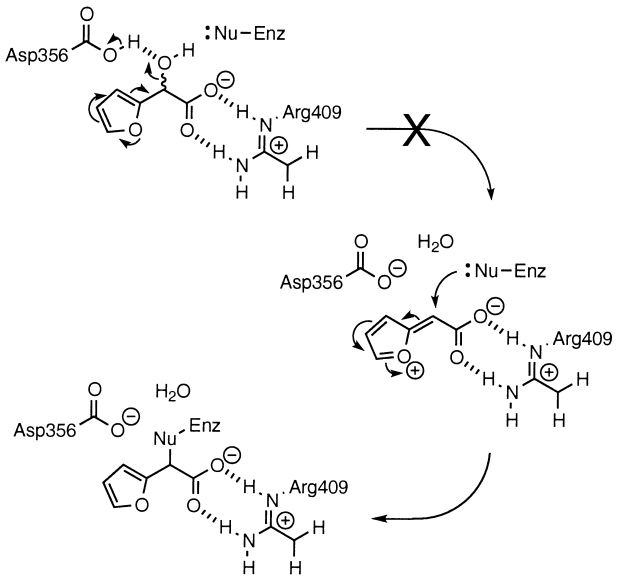


Figure 3. Potential irreversible inhibition of phosphatases by α-hydroxycarboxylic acid inhibitor **17**. The numbering scheme for the active site residues refers to *Yersinia* PTPase.

compounds function as mimics of phosphotyrosine, and are designed to form a variety of specific contacts with residues in the active site of the enzyme. Aryl substituents that increase the electron density at the ketone carbonyl group of the inhibitor increase the activity of the compound. We are currently working to further improve the potency of these inhibitors by extending their interactions with the enzyme into regions beyond the active site. We are also exploring the possibility of using α-ketocarboxylic acids as the basis for designing new ligands for SH2 domains.

Acknowledgement

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