The road less travelled: taming phosphatases

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Phosphatases are important in signal transduction, bacterial pathogenesis and several human diseases. So far, however, it is their opposite numbers, the kinases, that have received more attention from chemists. Recent progress in inhibitor development offers hope that new probes of cellular processes, and perhaps novel therapeutic agents, may soon become available.

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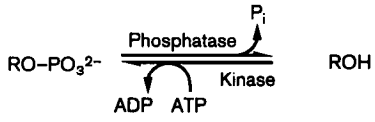
Energy utilization, signal transduction, transcription, translation, DNA replication, and phospholipid metabolism are just a few of the varied biological processes that rely on phosphoryl-group transfer reactions. Kinases and phosphatases are two classes of enzymes that exemplify the biochemistry of phosphoryl-group transfer. Kinases catalyze the synthesis of phosphate esters from ATP and a phosphoryl-group acceptor, while phosphatases catalyze the hydrolysis of phosphate esters:



(1)

Because of their importance in cell transformation, protein kinases have attracted the lion's share of attention from researchers seeking new methods to attack neoplastic disorders. But phosphatases, especially those that act on protein phosphates, may be equally useful targets in the search for new therapeutics. Phosphatases are essential for the growth or virulence of a number of pathogenic organisms, such as bacteria of the *Salmonella* and *Yersinia* families (including those responsible for bubonic plague) and viruses such as *Vaccinia* and *Variola* (which cause pox illnesses, such as smallpox) [1]. The serine/threonine phosphatase calcineurin is the target of immunosuppressants such as cyclosporin A and FK506 [2]. In addition, the importance of phosphatases in signal transduction and cell metabolism suggests that phosphatases may be appropriate therapeutic targets for disorders as diverse as diabetes and cancer.

As attention becomes focused on phosphatases as potential targets, the question of how to design specific inhibitors arises. From a mechanistic and structural standpoint, phosphatases are an enormously diverse group of enzymes [3]. If we add the equally diverse family of phosphodiesterases to the list of enzymes that might be useful targets, it becomes clear that new strategies for inhibitor design are needed. This is a huge and largely unexplored area. In this review, we summarize some of the most recent work on the rational design and development of phosphatase inhibitors and suggest possible new directions for the development of additional types of inhibitors for both phosphatases and phosphodiesterases.

Motifs for phosphatase inhibition

The development of new phosphatase inhibitors would be facilitated if we could identify generic inhibitory 'motifs' that would be a suitable starting point for studying structure–activity relationships or to guide the design of a combinatorial chemistry library. For long-standing problems, such as the development of protease inhibitors, medicinal chemists have a veritable arsenal of such motifs available in the form of affinity reagents, mechanism-based inhibitors, and transition-state analogs. Unfortunately, few such inhibitory motifs are available for phosphatases. To complicate matters, many phosphatases interact largely, or even solely, with the phosphoryl portion of the phosphate ester. For example, alkaline phosphatase binds inorganic phosphate more tightly than it does phosphate monoesters. Indeed, inorganic oxoanions such as vanadate, arsenate, tungstate and molybdate are potent nonselective inhibitors of almost all phosphatases [4,5]. Unfortunately, compounds such as these afford little inspiration for the design of new inhibitor motifs that might ultimately serve as the basis for drug design.

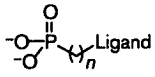
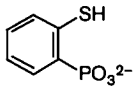
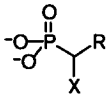
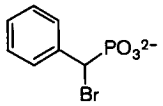
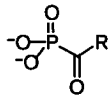
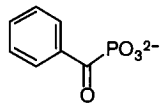
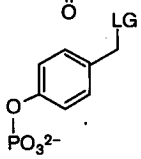
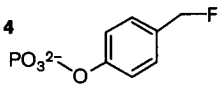
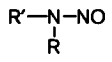
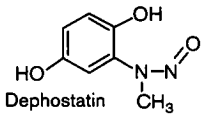
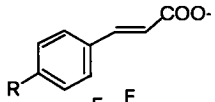
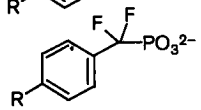
Despite this generally unsatisfactory state of affairs, some inroads into the development of phosphatase inhibitors have been made over the past few years. Figure 1 outlines some newly discovered motifs for phosphatase inhibition. Building combinatorial libraries of small molecules or peptidomimetics around these motifs should yield inhibitors of even higher potency and selectivity. Moreover, a number of these inhibitor types are already sufficiently developed to function as useful research tools.

Competitive inhibitors

Metallophosphatase inhibitors

Approximately half of all phosphatases, as well as a substantial number of phosphodiesterases and triesterases, contain

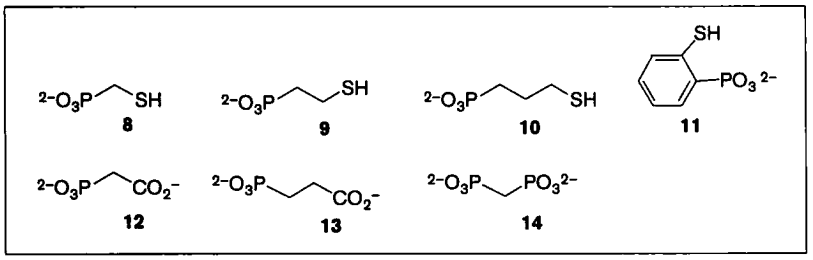
Figure 1

Inhibitor motif	Enzyme(s) target	Specific example	Inhibitor type
	Metallophosphatases	1 	Competitive
	PTPases	2 	Affinity reagent
	PTPases and possibly others	3 	Affinity reagent
	Prostatic acid phosphatase PTPases	4 	Mechanism-based inhibitor
	PTPases and possibly others	5 	Unknown
	PTPases	6 R = Glu-containing peptide	Peptide-based competitive inhibitor
	PTPases	7 R = Glu-containing peptide	Peptide-based competitive inhibitor

Some recently developed motifs for phosphatase inhibitors. X, Cl or Br; LG, leaving group.

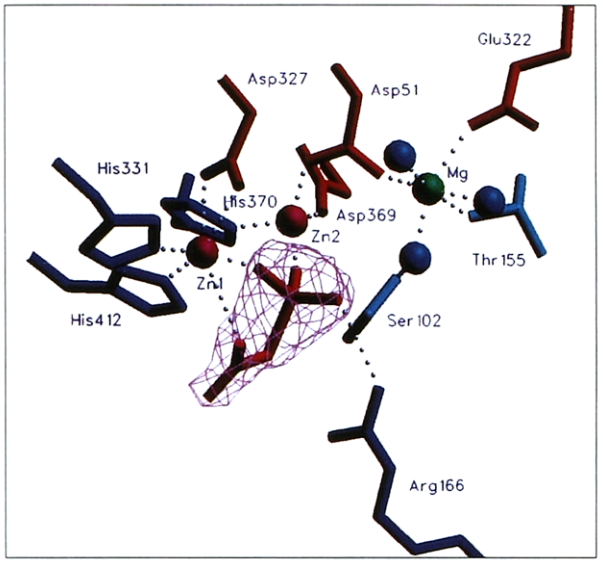
active-site metal ions that are essential for catalysis. Compounds that incorporate an extra metal-binding ligand have been shown to be very effective inhibitors of metalloproteases such as the angiotensin converting enzyme [6]. Surprisingly, until recently no one had designed phosphatase inhibitors that used this strategy, although the naturally occurring phosphatase inhibitor endothall may use this mechanism [7]. Figure 2 shows a number of very simple compounds that inhibit metalloproteases [8]. All these compounds bear a pendant group that is capable of ligating to an active-site metal. These compounds display a great range of potencies. For example the aromatic thiol (compound **11**) is the most potent small molecule inhibitor of alkaline phosphatase that is known, with a k_i of ~200 nM [8]. But compounds such as **12** and **14** have relatively modest, though still significant, inhibitory activity.

Figure 2



Structures of seven very simple metallophosphatase inhibitors.

Figure 3



The crystal structure of the active site of *E. coli* alkaline phosphatase with inhibitor **12** bound. Note that the inhibitor makes three contacts to the active-site metals.

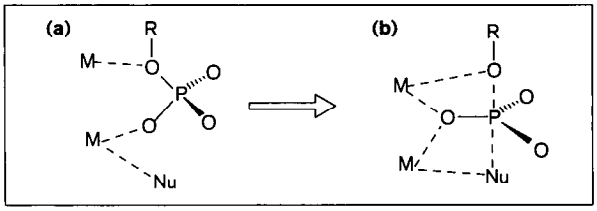
A crystal structure of compound **12** bound to alkaline phosphatase has been determined (T.S.W., J.K.M., B.S., K.M.H. and E.R.K., unpublished observations). In this structure (Figure 3), it can be seen clearly that the pendant group of the inhibitor reaches around to engage in an extra contact to an active-site zinc. In a sense, this inhibitor mimics the binding of a high-energy phosphorane intermediate or transition state, as these species probably make three zinc ion contacts (Figure 4b), while the bound substrate makes only two (Figure 4a).

This simple notion for inhibitor design can probably be exploited for the design of both potent and selective phosphatase and phosphodiesterase inhibitors. Given the ubiquitous nature of alkaline phosphatase, inhibitors such as compound **11** should be quite useful as tools for studying phenomena associated with protein/small-molecule phosphorylation. In this context, it is worth noting that many of these compounds exhibit surprising selectivity. For example, while compound **11** is a very potent inhibitor of alkaline phosphatase, it is a fairly poor inhibitor of purple acid-phosphatase, another phosphatase that uses a zinc-containing metal dyad.

Phosphotyrosine-phosphatase inhibitors

Compounds **6** and **7** (Figure 1) represent two different competitive inhibitor types for phosphotyrosine phosphatases (PTPases). Inhibitor **6** is based on a cinnamic acid motif that was appended to various peptide-like pieces using combinatorial approaches [9]. The result of this exercise was the development of some fairly potent PTPase inhibitors that showed some level of selectivity even against closely related phosphatases. Compound **7** is based on the difluorophosphonate motif. Inclusion of a phosphonate analog of phosphotyrosine into a suitable peptide yields inhibitors that can be quite potent [10].

Figure 4



The presumed binding mode of **(a)** phosphatase monoesters and **(b)** presumed reaction transition states of metallophosphatases. Nu is an active site nucleophile, or a metal-bound water molecule.

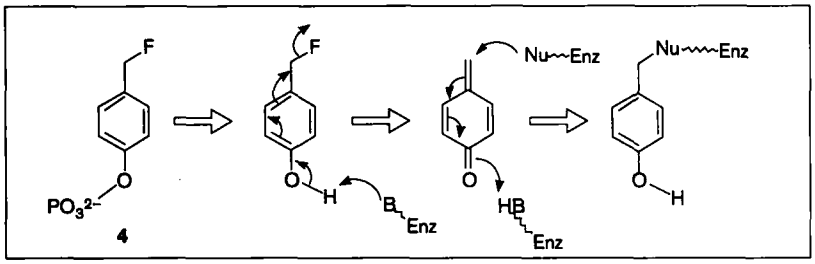
Affinity reagents

Two newly developed classes of affinity reagents are the α-halophosphonates [11] and the α-ketophosphonates (T.S.W., W.P. Taylor, and J. Roestamadji, unpublished observations). To date, little is known about the selectivity and mechanism of action of the α-ketophosphonates, although they appear to be potent in the low micromolar range and to show time-dependent PTPase inactivation. The α-halophosphonates have been studied more extensively, however. These inhibitors appear to be highly specific for PTPases and do not inactivate other phosphatases or even react with strong nucleophiles such as azide, hydroxylamine or thiolates. The α-halophosphanates are easily prepared in three steps from the corresponding aldehydes. Given their selectivity and ease of preparation, these inhibitors show great promise for use in developing a new generation of PTPase inhibitors.

Mechanism-based inhibitors

There is currently only one general type of mechanism-based inhibitor of a phosphatase [12,13]. Compound **4** (Figure 1) is an example of such an inhibitor. These inhibitors are potent against both prostatic acid phosphatase and phosphotyrosine phosphatases. Figure 5 shows the probable mechanism by which these compounds inactivate phosphatases. Cleavage of the phosphate ester leads to the rapid elimination of a fluoride ion to give a quinone methide. This alkylating agent must react with an active site nucleophile prior to its release from the active site. Although some very potent phosphatase inhibitors [14] have been developed using this motif, it seems unlikely that this methodology will be useful for the development of therapeutic agents. This is because nonselective phosphatases are present that would react with these inhibitors before they have an

Figure 5



The probable mechanism for the inactivation of phosphatases by compound **4**. B is an active-site base and Nu is an active-site nucleophile.

opportunity to reach their site of action. These phosphatase inhibitors do, however, represent a useful strategy for the pursuit of phosphodiesterase inhibitors and a ribonuclease A inhibitor has been developed using this motif [15].

Perspectives

In the past five years a number of advances in inhibitor design have opened the doors for the development of a new generation of phosphatase inhibitors. These advances, based largely on the rational design of new molecular motifs, provide starting points for combinatorial chemistry and structure-based design. It seems likely that the next five years will see the advent of selective and potent molecules based on these inhibitory motifs and the application of these compounds to the study of signal transduction pathways and the development of new therapeutic agents.

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