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Natural products with protein tyrosine phosphatase inhibitory activity

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ABSTRACT

Protein tyrosine phosphatases (PTPs) play an essential role in maintaining the proper tyrosine phosphorylation state of proteins. Abnormal tyrosine phosphorylation has been implicated in diseases as diverse as type 2 diabetes, cancer, immune disorders and neurological disorders, and thus inhibitors of PTPs have been investigated as potential treatments of these diseases. Natural products are widely regarded to be privileged structures in drug discovery efforts, and are therefore a good starting point for the development of PTP inhibitors. Here we describe reported natural product PTP inhibitors as well as methods to screen for natural product PTP inhibitors using bioassay-guided fractionation. These methods are illustrated using the example of a family of bromotyrosine-derived PTP inhibitors isolated from two marine sponges. We also identify potential pitfalls and false-positives, in particular compounds that are oxidizing agents that react irreversibly with the PTP.

1. Introduction

The phosphorylation state of proteins is one of the most important mechanisms of protein regulation with profound effects on signaling pathways [1]. Phosphorylation of proteins typically occurs on the side-chain hydroxyl group of serine, threonine or tyrosine residues. Protein phosphorylation at tyrosine residues is controlled by the balancing actions of protein tyrosine kinases (PTKs), which phosphorylate proteins at tyrosine residues, and protein tyrosine phosphatases (PTPs), which dephosphorylate proteins at phosphotyrosine residues. Abnormal tyrosine phosphorylation has been implicated in many diseases, and PTPs have been investigated as possible targets for the treatment of type 2 diabetes [2], cancer [3], and immune disorders [4], among others [5].

The sequencing of the human genome has revealed that there are at least 107 genes encoding PTPs [6]. These PTPs are classified into four families, the largest of which are the class I cysteine-based PTPs. This class is subdivided into the "classical" tyrosine-specific PTPs, which are specific for phosphotyrosine residues, and the dual-specificity PTPs, which can dephosphorylate phosphotyrosine as well as phosphoserine and phosphothreonine residues. The "classical" PTPs are further subdivided into the transmembrane, receptor-like PTPs (RPTPs) and the soluble, non-receptor PTPs (NRPTPs). The PTP superfamily shares the active-site sequence motif (H/V)CX_5R(S/T), and the cysteine-based PTPs all share a common catalytic mechanism [7].

In contrast to PTKs, for which commercially successful inhibitors such as imatinib have been developed, there is a lack of potent and specific inhibitors of PTPs. The highly conserved active site of PTPs and the large number of enzymes in this family makes the search for inhibitors that are selective for a particular PTP more challenging. Another challenge is that the active-site cysteine thiol found in cysteine-based PTPs is unusually acidic and is susceptible to oxidation in its deprotonated (thiolate) form [8]. Some of the PTP inhibitors reported in the literature were found to act by irreversibly oxidizing the active-site cysteine residue from a thiolate to a sulfonate moiety through the production of H_2O_2 [9–12], so it is likely that screening libraries of compounds or natural product extracts for inhibitors of PTPs will produce a large number of "hits" that act by an oxidative mechanism. Additionally, compounds with Michael acceptor systems can react covalently with the nucleophilic thiolate of the active-site cysteine as has been observed for some PTP inhibitors [13,14]. Oxidizers and Michael acceptors are often toxic and non-selective and are therefore not likely to be good drug candidates. Thus, the use of catalase to remove H_2O_2 is recommended when screening for PTP inhibitors [15], especially in cases where the inhibitors are suspected to contain chemical functionalities capable of acting as oxidizing agents. Alternatively, the active compounds can be tested against other enzymes containing catalytic cysteine residues such as papain. If the compound also inhibits other cysteine-based enzymes it is a good indication that it might be acting as a non-specific oxidant.

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2. Natural product inhibitors of PTPs

2.1. PTP1B

2.1.1. Role of PTP1B in disease

When insulin binds to the insulin receptor, the tyrosine kinase activity of the insulin receptor is activated leading to autophosphorylation. In its phosphorylated (active) state the insulin receptor functions as a tyrosine kinase and phosphorylates substrates such as IRS-1, setting off a signal transduction cascade that eventually leads to localization of glucose transporters on the outer membrane of muscle and fat cells where they take up glucose from the blood [16]. The insulin receptor is probably the most important substrate for PTP1B, which dephosphorylates the insulin receptor thereby deactivating it and contributing to insulin resistance. A landmark study showed that PTP1B deficient mice had lower blood glucose levels and were more sensitive to insulin than wild-type mice. When these PTP1B mice were fed a high-fat diet they resisted weight gain and remained insulin sensitive, in contrast to the wild-type mice that gained weight and became insulin resistant [17].

2.1.2. Natural product inhibitors of PTP1B

The role that PTP1B plays in insulin signaling has made it an appealing drug target for the treatment of type 2 diabetes, and it has attracted the lion's share of attention in screening efforts looking for natural product PTP inhibitors. This is reflected in the over 300 reported natural product inhibitors of PTP1B, far more than for any other PTP. Natural product inhibitors of PTP1B were the subject of a recent comprehensive review, so this report will not attempt to describe all natural product PTP1B inhibitors and instead refers readers to that review [18]. Of particular note is the large proportion of flavonoids and other phenolics among natural product PTP1B inhibitors.

2.2. CD45

2.2.1. Role of CD45 in disease

CD45, also known as leukocyte common antigen, is a family of RPTPs consisting of various isoforms that are found in high abundance on the surface of hematopoietic cells. In T cells, CD45 dephosphorylates and activates Lck and Fyn, both members of the Src family of PTKs, which are required for T cell activation in response to antigen binding to the T cell antigen receptor [19]. Consistent with its role as a positive regulator of the immune system, treating mice with a monoclonal antibody raised against the RB isoform of CD45 prevented renal allograft rejection [20]. Inhibiting CD45 is therefore one strategy to suppress T cell activation in order to prevent organ graft rejection and may also be useful in the treatment of autoimmune diseases.

2.2.2. Natural product inhibitors of CD45

The first reported natural product CD45 inhibitor was dephostatin (**1**), which was isolated from a *Streptomyces* sp. and found to have an IC_{50} against CD45 of 7.7 μM [21]. Analogues of dephostatin were later found to have even more potent activity against other PTPs [22]. The alkaloids anonaine (**2**), roemerin (**3**) and nornuciferine (**4**) isolated from *Rollinia ulei* were found to have CD45 inhibitory activity with IC_{50} values of 17, 107 and 5.3 μM, respectively [23]. Phosphatoquinones A (**5**) and B (**6**) isolated from a *Streptomyces* sp. were reported to have CD45 inhibitory activity with IC_{50} values of 28 and 2.9 μM, respectively [24]. Phosphatoquinone B contains a quinone moiety and is approximately 10-fold more potent than phosphatoquinone A, so it is possible that it acts partly through an oxidative mechanism. Dihydrocarolic acid (**7**) and penitricin D (**8**) isolated from *Aspergillus niger* were reported to inhibit CD45 with IC_{50} values of 1.2 μg/mL (6.7 μM) and 2.3 μg/mL (27.4 μM), respectively, and had only weak activity against PTP1B [25]. However, both compounds contain α,β-unsaturated carbonyl moieties and would be expected to be good Michael acceptors. Finally, the natural product purpurin (**9**) was recently reported to inhibit CD45 with an IC_{50} value of 5.97 μM, and inhibited several other PTPs with comparable potency [26]. The structures of **1**–**9** are shown in Fig. 1.

2.3. CDC25

2.3.1. Role of CDC25 in disease

The cell division cycle 25 (CDC25) phosphatases are dual-specificity PTPs with three isoforms in humans: CDC25A, CDC25B and CDC25C. The CDC25 family dephosphorylates and thereby activates cyclin-dependent kinases (Cdks) in a step that is required for cell cycle progression. CDC25A functions primarily at the G1-S transition, while CDC25B and CDC25C are important for the G2-M transition [27]. CDC25s also play an important role in blocking cell cycle progression at the G1-S and G2-M checkpoints in response to DNA damage. When DNA damage is detected, the check point kinases Chk1 and Chk2 phosphorylate and thereby inactivate CDC25s leading to cell cycle arrest [28]. Overexpression of CDC25A and CDC25B has been found in a variety of cancer types and their expression is correlated with poor survival [29], making them an attractive target for the discovery/development of inhibitors [30].

2.3.2. Natural product inhibitors of CDC25s

The first reported natural product inhibitor of a CDC25 phosphatase was dysidiolide (**10**), which was isolated from the sponge

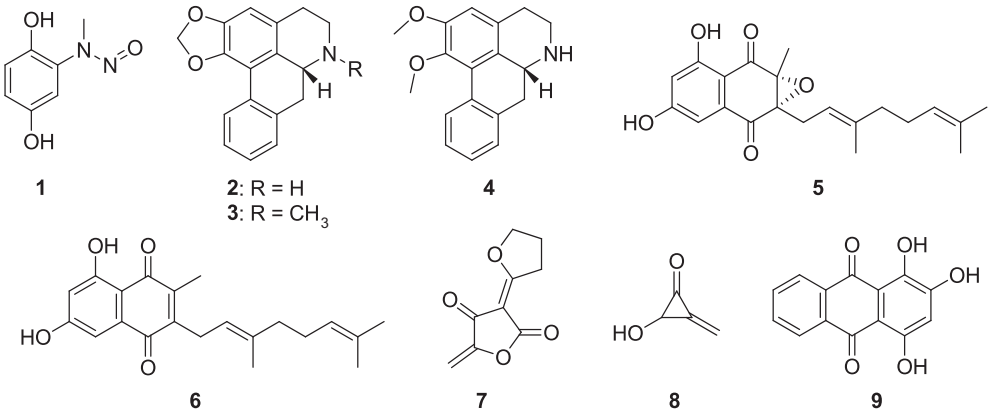

Fig. 1. Natural product CD45 inhibitors.