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Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 389–391

Discovery of novel PTP1b inhibitors

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Received 3 June 2003; revised 20 October 2003; accepted 31 October 2003

Abstract—A small library of 19 compounds was designed based on unique structural features of PTP1b. Utilizing electrospray ionization mass spectrometry (ESI-MS) to provide binding information about complexes of enzyme and small molecule ligands, two classes of lead compounds were discovered.

Protein tyrosine phosphatases (PTPs) act in opposition with protein tyrosine kinases to control the tyrosine phosphorylation level of proteins. Reversible tyrosine phosphorylation plays an important role in signal transduction and regulation of cell processes such as growth, differentiation, and proliferation.[1] There are two types of PTPs, receptor-like such as CD45 and cytoplasmic forms such as PTP1b.[2] All PTPs have at least one conserved region with their sequence motif of (I/V)HCXAGXXR(S/T)G which defines the catalytic domain.[2] In all PTPs, the Cys residue is conserved.

Several PTPs have been implicated as negative regulators of the insulin signaling pathway; these include PTPα, PTP-LAR, and PTP1b. It has been demonstrated that PTP1b interacts directly with activated insulin receptor and is a key regulator of the insulin signaling pathway.[3] Studies with PTP1b knockout mice have demonstrated that the lack of PTP1b activity resulted in an increased sensitivity to insulin.[4] Regulating PTP1b, therefore, could lead to a safe effective increase in insulin signal for patients who are insulin resistant.

During the dephosphorylation of pTyr on insulin receptor the phosphate group is situated such that it coordinates with main chain amide bonds as well as the Arg-221 sidechain of PTP1b.[2] This orients the phosphorous so that it is susceptible to nucleophilic attack by the active site cysteine. Additional hydrophobic interactions between the aliphatic moiety of Arg-221 and Trp-179 as well as π-stacking interactions between phenyl ring of pTyr and Phe-182 help stabilize the transition state.[3]

Nucleophilic attack of the Cys-215 thiol forms a transition state trigonal bipyramidal PTP1b-Cys-phosphate transition state intermediate (Fig. 1). Dephosphorylation of pTyr is assisted by protonation of the phenolic oxygen of pTyr by Asp-181. Collapse of the transition state results in the release of Tyr and the formation of a thiophosphate ester intermediate.[2] Hydrolysis of the intermediate regenerates the active enzyme.

There has been considerable interest in developing small molecule inhibitors for PTP1b as a treatment for type 2 diabetes. Many of the compounds to date have utilized nonhydrolizable phosphonates, sulfonates, carboxylic acids, and α-keto acids most of which serve as phosphate mimics and coordinate to Arg-221 in the active site.[3,5] We were interested in taking an approach to developing novel lead compounds that was based on some of the structural aspects of PTP1b.

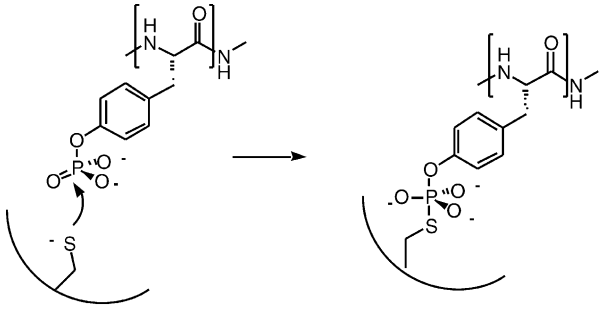

Figure 1. Nucleophilic attack of Cys-215 to form PTP1b-Cys-pTyr transition state.

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doi:10.1016/j.bmcl.2003.10.058

A small library of potential PTP1b inhibitors were designed based on either their potential as transition state mimics or their ability to interact with the active site Cys. Potential small molecule transition state mimics should posses a planar arrangement of oxygens around an electrophilic center similar to that of the transition state intermediate. Additionally, such mimics that could provide additional π-stacking interactions with Phe-182 could potentially be beneficial. Moniliformin and deltic acid are compounds that both have a planar arrangement of oxygens about an electrophilic ring similar to the arrangement of oxygens in the trigonal bipyramidal transition state complex.

Derivatives of these compounds such as **1** and **2** have a planar arrangement of oxygens as well as the potential to provide additional π-stacking interactions. These compounds could, therefore, serve as potential transition state mimics for PTP1b.

Other aspects of PTP1b that can be utilized when designing active site inhibitors are based on the homology of PTP1b and other cysteine proteases. Previous similar strategies have been employed by Ahn et al. and Fu et al.[6] Both classes of proteins have a highly nucleophilic cysteine.[6] There are a number of known classes of small molecule cysteine protease inhibitors such as α-ketoacids, halomethylketones, aldehydes, nitriles, and disulfides that may also be able to interact with Cys-215 of PTP1b.[7]

A library of 19 compounds (Fig. 2), containing differentially substituted aldehydes, boronic acids, nitriles (**3**–**19**), and potential transition state mimics (**1**–**2**) were screened utilizing methodology developed in our laboratory. The library of potential ligands were determined to bind to PTP1b if intact 1:1 complexes were detected by electrospray ionization mass spectrometry (ESI-MS).[8]

The PTP1b used for this study was the 37 kD kinase domain expressed in *Escherichia. coli*.[9] Prior to analysis the PTP1b was exchanged from its storage buffer (25 mM HEPES, 3mM DTT, and 0.15 mM NaCl) to 20 mM Tris (pH 7) and used immediately. The protein concentration in each sample was kept constant at 3 μM by diluting with water from stock solutions in Tris. The small molecules were diluted with from stock solutions (10 mM in DMSO) to ca. 50 μM. Prior to analysis all samples were equilibrated at room temperature for 1 h. Samples were introduced into the mass spectrometer via syringe pump at a constant rate of 2.0 μL/min. The orifice potential was set to 65 V, and a sufficient number of scans were collected to achieve a sufficient signal-to-noise ratio.

In this directed library of 19 compounds, three formed complexes with PTP1b as detected by ESI-MS. Aldehydes **6** and **10** formed specific 1:1 complexes with PTP1b. Binding affinities of these ligands were determined based on the assumption that the ionizability of the complexed protein is identical to the uncomplexed. Effective concentrations were calculated as the product

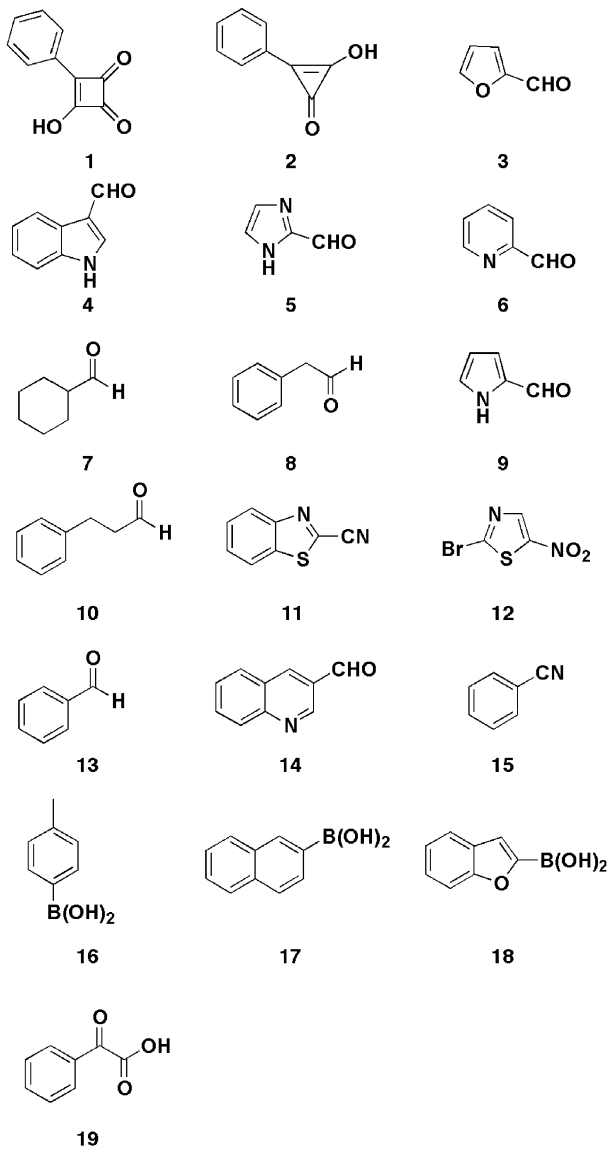

Figure 2. Library of potential inhibitors.

of the initial enzyme concentration and the relative abundance of each of the species present, The K_d was then calculated using standard methodology.[10] The K_d for aldehydes **6** and **10** were 100 and 150 μM, respectfully. Transition state mimics **1** and **2** were analyzed for their ability to bind with PTP1b. No detectable binding was observed for phenylhydroxycyclopropenone (**2**).[12] Compound **1** formed a specific 1:1 interaction with PTP1b as detected by ESI-MS and had a measured K_d of 25 μM.

Compounds **1**, **6**, and **10** were assayed using fluorescein diphosphate as the chromogenic substrate to determine their ability to block function of PTP1b.[11] With both **6** and **10**, the IC_{50} was greater than 300 μM. The inactivity of these aldehydes possibly may be due to a competing formation of thiohemiacetal with the aldehydes and the DTT that was present in great excess in the assay buffer. Compound **1** was assayed using the chromogenic, and its IC_{50} was determined to be 60 μM.

By utilizing ESI-MS to study complexes of potential small molecule ligands with PTP1b, we were able to identify two classes of lead compounds that bound as specific 1:1 complexes with PTP1b from a very small library of 19 compounds. In the case of the transition state mimics, we were able to demonstrate the ability to block the function of PTP1b. There are ongoing efforts to elaborate these ligands into higher affinity compounds.

Acknowledgements

The authors would like to thank Maureen Beresini, Sally Fischer, and Kevin Clark for their efforts in assaying our compounds. We would also like to thank Mark Renz and Mark Ultsch for supplying us with the PTP1b.

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