

THE DEVELOPMENT OF EXO-AFFINITY LABELING AGENTS, INACTIVATORS OF PROTEIN TYROSINE PHOSPHATASE 1B

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ABSTRACT

The phosphorylation of tyrosine residues is one of the most crucial reactions that regulate numerous biological processes. The phosphorylation is controlled by well-balance of opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Malfunction of either class of enzymes results in pathological diseases. Despite an equal importance of both classes of enzymes, the development of inhibitors of PTKs is advanced, while that of PTPs is lagging. Therefore, we provide a mini-review of inhibitors of protein tyrosine phosphatase 1B (PTP1B), an enzyme in PTP superfamily, aiming to gain an attention to the development of PTP inhibitors. PTP1B is a PTP that has been fully characterized and proved as a drug target for type 2 diabetes and obesity treatment. In the past few decades, the development of effective PTP1B inhibitors by pharmaceutical industries has been unsuccessful and remained challenging due to the difficulty in balancing drug properties, i.e. potency, selectivity, and cell permeability of the inhibitors. In this dissertation, we present a novel strategy called “exo-affinity-labeling” to modulate PTP1B activity. An exo-affinity labeling agent covalently inhibits PTP1B in a unique way and this, so called, inactivator is promising of providing the desired drug properties. We design, synthesize, and characterize the

behavior of our inactivators of PTP1B. A short-linker TDZ **8a** and long-linker TDZs **11a**, and **11b** exhibit time- and concentration-dependent loss of PTP1B activity. Mass spectrometry analysis shows that **8a** covalently modifies Cys121. However, the inactivation reaction is second-order with the rate constant (k_{inact}) of $168 \pm 25 \text{ M}^{-1}\text{min}^{-1}$ and we did not observe saturation kinetics in a re-plot of observed pseudo-first order rate constant (k_{obs}) versus concentrations. This suggests that the inactivation by **8a** is not an affinity-labeling agent. The absence of the saturation kinetics is also observed in the inactivation by **11a**. On the other hand, **11b** is the only inactivator that exhibits the saturation kinetics, suggesting the affinity-labeling mechanism. Fitting a curve to a hyperbolic equation for affinity-labeling agent gives a rate constant (k_{inact}) of $4.7 \pm 0.6 \times 10^2 \text{ M}^{-1}\text{min}^{-1}$ and a dissociation constant (K_i) = $17 \pm 4 \text{ }\mu\text{M}$. However, further study is needed to reveal an insight of the inactivation mechanism.