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A gatekeeper residue for inhibitor sensitization of protein tyrosine phosphatases

Anthony C. Bishop* and Elizabeth R. Blair

Department of Chemistry, Amherst College, Amherst, MA 01002, USA

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Abstract—Allele-specific enzyme inhibitors are powerful tools in chemical biology. However, few general approaches for the discovery of such inhibitors have been described. Herein is reported a method for the sensitization of protein tyrosine phosphatases (PTPs) to small-molecule inhibition. It is shown that mutation of an active-site isoleucine to alanine (I219A) sensitizes PTP1B to inhibition by a class of thiophene-based inhibitors. This sensitization strategy succeeds for both 'orthogonal' inhibitors, designed to be incompatible with wild-type PTP active sites, and previously optimized wild-type PTP inhibitors. The finding that the I219A mutation sensitizes phosphatase domains to a variety of compounds suggests that isoleucine 219 may act as a 'gatekeeper' residue that can be widely exploited for the chemical–genetic analysis of PTP function.

Large gene families that encode homologous proteins represent particularly challenging cases for chemical biology. This is, in large part, due to the 'degeneracy' problem; it is difficult to chemically differentiate between active (or allosteric) sites that bear a high degree of structural similarity with one another. One, now well established, method for circumventing the degeneracy problem is through the engineering of protein/small-molecule interfaces—that is, through modification of a protein receptor's binding site, in addition to the complementary modification of a potentially selective small-molecule ligand.[1,2]

One powerful application of protein/small-molecule engineering is the generation of allele-specific enzyme inhibitors. Several critical enzyme families in the eukaryotic proteome, including protein kinases,[3] protein methyltransferases,[4] and phosphoinositide 3-kinases,[5] have been engineered to possess novel inhibitor sensitivity. This engineered sensitivity, not present in related wild-type enzymes, allows for the identification of selective inhibitors from relatively small panels of putative inhibitors. The enzyme-sensitization approach has proven to be particularly effectual for the protein kinases, as members of the kinase family possess a 'gatekeeper' residue that can readily be identified through protein sequence alignments.[6] Targeted mutation of the gatekeeper residue (to alanine or glycine) has led to the creation of a general strategy, termed chemical–genetic analysis, for the study of protein kinase function. Recently, this method has been used to elucidate the in vivo function of an impressive array of yeast kinases (e.g., Cdc28,[6,7] Ime2,[7] Pho85,[8] Fus3,[6] and Apg1[9]), as well as mammalian kinases (e.g., CamKII,[10] v-erbB,[11] and GRK2.[12]).

We have recently described the first example of inhibitor-sensitization for another large and important class of signaling enzymes: the protein tyrosine phosphatases (PTPs).[13,14] The engineering of a prototype phosphatase, the type II diabetes drug target PTP1B, was guided by the following criteria: an amino acid chosen for mutagenesis must be large enough that substitution by a small amino acid creates a novel binding pocket; the mutant PTP must retain catalytic activity; and the residue identified for PTP1B-sensitization must be present in other PTPs, eliminating the need to redesign the PTP/inhibitor interface for each target. Valine 49 and isoleucine 219 (V49 and I219, human PTP1B numbering) both meet these criteria.[13]

In a previous report, we utilized the indolic nitrogen of an oxalylaminoindole PTP inhibitor (compound **1**, Fig. 1a) as a 'hook' to which we attached groups that were designed to be incompatible with wild-type PTP active sites.[13] While this approach did yield a 10-fold selec-

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* Corresponding author. Tel.: +1 413 542 8316; fax: +1 413 542 2735; e-mail: acbishop@amherst.edu

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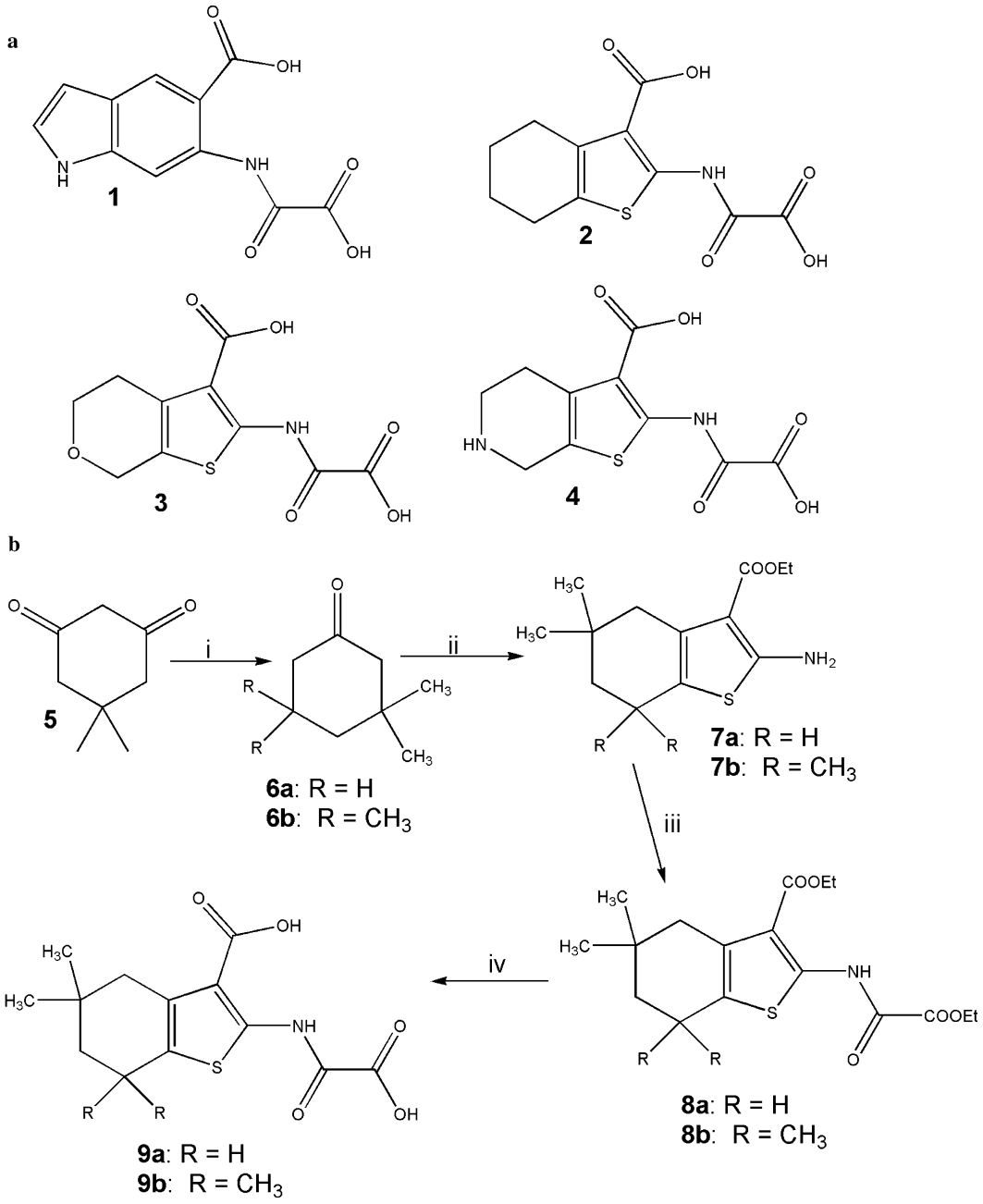

Figure 1. (a) Chemical structures of compounds **1**–**4**. (b) Chemical structures and synthetic schemes for compounds **9a** and **9b**. Reagents and conditions: (i) H_2, AcOH, Pd/C, cat. H_2SO_4; (ii) ethyl cyanoacetate, sulfur, morpholine, EtOH, reflux; (iii) ethyl oxalyl chloride, THF; (iv) NaOH, H_2O, EtOH.

tive inhibitor of a mutated PTP1B (I219A), it suffered from the inherent lack of potency of oxalylaminoindole PTP inhibitors at physiological pH.[13] Moreover, the indole-derivative inhibitors showed little to no selectivity for mutants of valine 49, a position that is particularly attractive for a general PTP inhibitor-sensitization strategy, as it is occupied by valine or isoleucine in 35 of the 37 classical PTP catalytic domains in humans. (Position 219 is occupied by either valine or isoleucine in 28 classical PTP domains.[15])

As a scaffold for a second generation of allele-specific PTP inhibitors, we selected 2-oxalylamino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic acid (compound **2**, Fig. 1a).[16] Previous reports have shown that compound **2** effectively inhibits several PTPs, including PTP1B and its closest homolog, TCPTP, as well as phosphatases that are not especially homologous to PTP1B (e.g., PTPH1).[17–20] The three-dimensional structure of compound **2** bound to a PTP has not been reported; however, a crystal structure of a slightly less potent PTP inhibitor that differs from **2** only by the replacement of a methylene group with an oxygen atom (**3**, Fig. 1a) is known.[16] Surmising that compounds **2** and **3** bind to PTP active sites in identical orientations, we inspected the PTP1B/**3** structure for positions that could