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POTENT INHIBITION OF PROTEIN-TYROSINE PHOSPHATASE-1B USING THE PHOSPHOTYROSYL MIMETIC FLUORO-O-MALONYL TYROSINE (FOMT)

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Abstract: To enhance PTP binding interactions, both inside and outside the pTyr binding pocket, a thioether-cyclized peptide has been designed based on the EGF receptor autophosphorylation sequence ($EGFR_{988-993}$) "Asp-Ala-Asp-Glu-pTyr-Leu", in which the pTyr residue has been replaced by the nonphosphorus-containing pTyr mimetic fluoro-O-malonyltyrosine (FOMT, **2**). The resulting peptide **4** exhibits a K_i value of 170 nM, making it one of the most potent inhibitors of PTP1B yet reported.

Protein-tyrosine phosphatases (PTPs) cooperate with protein-tyrosine kinases (PTKs) as both positive and negative regulators of cytokine and growth factor-induced cellular signal transduction.[1] Because aberrations in such pathways have been associated with a number of diseases, including several cancers, inhibitors of both PTPs and PTKs are of interest as potential new therapeutic agents. One approach toward PTP inhibitor development is the utilization of high affinity peptide substrates as models for inhibitor design. Here note can be taken of the fact that substrate binding involves interactions both inside and outside the phosphotyrosyl (pTyr) binding pocket.[2] In preliminary work focused on binding interactions within the pTyr pocket, replacement of pTyr residues in high affinity PTP substrates, with nonhydrolyzable phosphonic acid-based phosphate mimetics such as phosphonomethyl phenylalanine (Pmp),[3] or its difluoro-analogue F_2Pmp[4] has resulted in potent inhibitors.[5-7] The linear hexamer peptide sequence, "Asp-Ala-Asp-Glu-pTyr-Leu", which corresponds to one of the autophosphorylation sites of the EGF receptor ($EGFR_{988-993}$), and has been shown to be a good substrate for several tyrosine phosphatases, including PTP-1,[6] has proven to be a useful platform in this regard.[7] Using this sequence, substitution of the pTyr residue with the non phosphorus-containing pTyr mimetic O-malonyltyrosine (OMT, **1**)[8] resulted in competitive inhibition with an IC_{50} value of 10 μM. Replacement of the OMT residue in this linear peptide with its fluorine-containing analogue, fluoro-O-malonyltyrosine (FOMT, **2**) produced a 10-fold enancement of affinity (IC_{50} = 1 μM).[9] In a separate study as a further step in the evolution of peptide-based inhibitors, conformationally restricted OMT-containing cyclic peptides examined binding interactions outside the pTyr binding pocket. Here it was found that a thioether-containing cyclic peptide exhibited an 18-fold enhancement in potency (K_i = 0.73 μM) relative to its linear counterpart **3** (K_i = 13 μM).[10]

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The present work, seeks to combine features demonstrated by these previous studies as enhancing binding interactions inside the pTyr binding pocket, with those promoting interaction outside the pocket. In this manner, the FOMT residue, which has a higher affinity within the pTyr pocket, was introduced into the constrained peptide backbone. The resulting peptide **4**[11] exhibits competitive inhibition with a K_i value of 170 nM, making it one of the most potent inhibitors of PTP1B yet reported (Figure 1).[2] The extremely high binding potency of **4** makes it an ideal candidate for co-crystallography with the PTP1B enzyme and X-ray structure elucidation. Such studies could provide valuable information for the design of high affinity nonpeptide ligands.

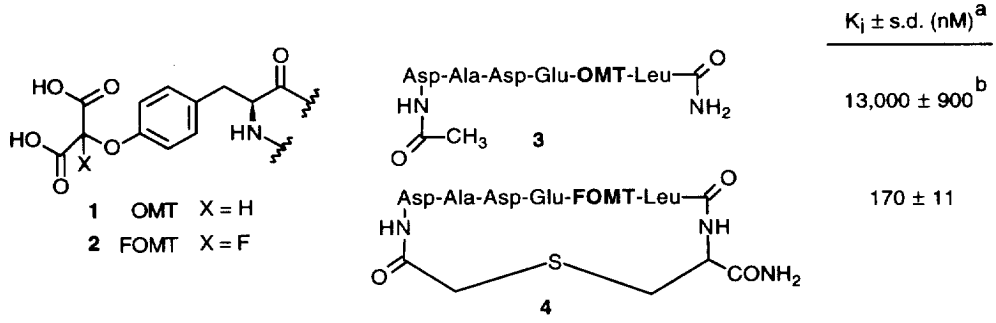

Figure 1. Structures of pTyr mimetics OMT (**1**) and FOMT (**2**) and binding constants of associated peptides. [a]Determined as previously reported.[10] [b]Previously reported.[10]

References and Notes

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11. Cyclic peptide **4** was synthesized according to methodologies used for the preparation of various OMT-containing cyclic peptides,[10] incorporating Fmoc(*L*-FOMT)(O*t*Bu)$_2$-OH using Fmoc chemistry. Peptide **4** was characterized by analytical HPLC, FABMS and amino acid analysis.