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NMR-BASED DISCOVERY OF PHOSPHOTYROSINE MIMETICS THAT BIND TO THE LCK SH2 DOMAIN

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Abstract: Using an NMR-based screen, a series of novel phosphotyrosine mimetics were discovered that bind to the SH2 domain of Lck. These compounds may serve as useful leads for the design of nonpeptide inhibitors of SH2 domains with improved bioavailability and metabolic stability compared to the natural ligands that contain phosphotyrosine.

Src homology 2 (SH2) domains bind to phosphotyrosine-containing peptides and proteins and mediate critical protein–protein interactions in many signal transduction pathways.[1] Thus, SH2 domains are potential drug targets for intervening in a wide variety of biological processes.[2] Although natural peptide ligands are known that bind to the individual SH2 domains,[3,4] these compounds are unsuitable as drug molecules. In addition to their liabilities as peptides, they all contain a metabolically unstable phosphotyrosine that will limit membrane permeability due to the two negative charges at physiological pH. To overcome the problems associated with the phosphotyrosine moiety, peptides have been synthesized that contain phosphotyrosine mimetics, including phosphonates, difluorophosphonates, dicarboxylic acids, and carboxymethyl-phenylalanine (cmF).[5–7] However, the phosphonate and dicarboxylic acid mimetics still contain two charges, and the cmF replacement exhibits a significant reduction in binding affinity (450-fold) for SH2 domains. Moreover, the number of phosphotyrosine replacements that can be investigated is limited using this strategy, since different synthetic routes must be developed for incorporating each phosphotyrosine analog into the peptide scaffold. Here we describe the discovery of novel phosphotyrosine mimetics using a fragment-based strategy that allows the rapid evaluation of large libraries of potential replacements before synthetic efforts are initiated to incorporate these fragments.

Using conventional screening assays, compound potencies in the low micromolar range are typically required for reliable detection. This precludes the direct evaluation of phosphotyrosine mimetics, as phosphotyrosine itself only binds to the SH2 domains in the millimolar range. However, using an NMR-based screening approach called SAR by NMR™, compounds that bind in the μM to mM range can be reliably identified.[8,9] In addition, the binding site for the ligands can be located from an analysis of the amide

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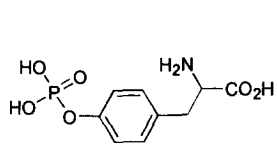
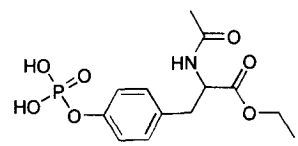
chemical shift changes which occur upon addition of the test compound. Thus, quantitative structure–activity relationships can be established for potential phosphotyrosine mimetics based on their ability to bind to the phosphotyrosine binding site on the SH2 domain.

We applied this NMR-based screening strategy to identify novel phosphotyrosine mimetics for the SH2 domain of Lck. First, the binding affinity of phosphotyrosine for this SH2 domain was determined by analyzing the amide chemical shift changes of uniformly ^{15}N-labelled Lck as a function of increasing concentrations of phosphotyrosine (**1**) and N–acetyl–carboxyethyl–phosphotyrosine (**2**).



1
0.4 mM



2
0.3 mM

Compounds **1** and **2** were found to have K_D values of 0.4 and 0.3 mM, respectively, and induced significant chemical shift changes in the backbone amide resonances of residues F11, K12, N13, L14, R16, D18, A19, E37, S38, S44, F45, S46, I65, and R66 of Lck. We then screened a library of over 3500 compounds for binding to this SH2 domain[10] and identified a number of compounds[11] (Figure 1) which bind to the phosphotyrosine binding site as evidenced by chemical shift changes in these same residues. Several phosphate-containing compounds (e.g., **3** and **6**) were found to bind to the Lck SH2 domain with dissociation constants comparable to phosphotyrosine. In addition, a number of phosphonates (e.g., **4**, **5**, **7**, and **8**), multiply charged aromatic acids (e.g., **9** and **10**), and phthalamate analogs (e.g., **11–14**) were identified which also bind to the phosphotyrosine binding site.

The binding affinities for the phosphotyrosine analogs shown in Figure 1 correlate well with the potencies observed for peptide-based inhibitors which contain several of these molecular fragments. The 5– to 20–fold loss in binding affinity for phosphonate- vs phosphate-containing ligands (e.g., **4** vs **3** or **7** vs **6**) agrees well with the 6-fold loss in potency observed when phosphonomethyl-phenylalanine was used as a phosphotyrosine replacement in a peptide-based inhibitor.[6] Furthermore, the more than 100-fold loss in potency for phenylacetic acid (**15**) relative to phenylphosphate (**3**) is consistent with the 450-fold loss in potency observed for peptide inhibitors containing carboxymethyl-phenylalanine.[7] Thus, these data indicate that mimetics with binding affinities comparable to phosphotyrosine could yield inhibitors of SH2 domains with potencies comparable to phosphotyrosine-containing inhibitors. Although compounds **3–10** share the common liability of being multiply charged at physiological pH and are therefore not attractive as potential phosphotyrosine replacements, compounds **11–13** contain only a single negative charge and bind to the Lck SH2 domain with only a 3– to 5–fold loss in affinity compared to phosphotyrosine.

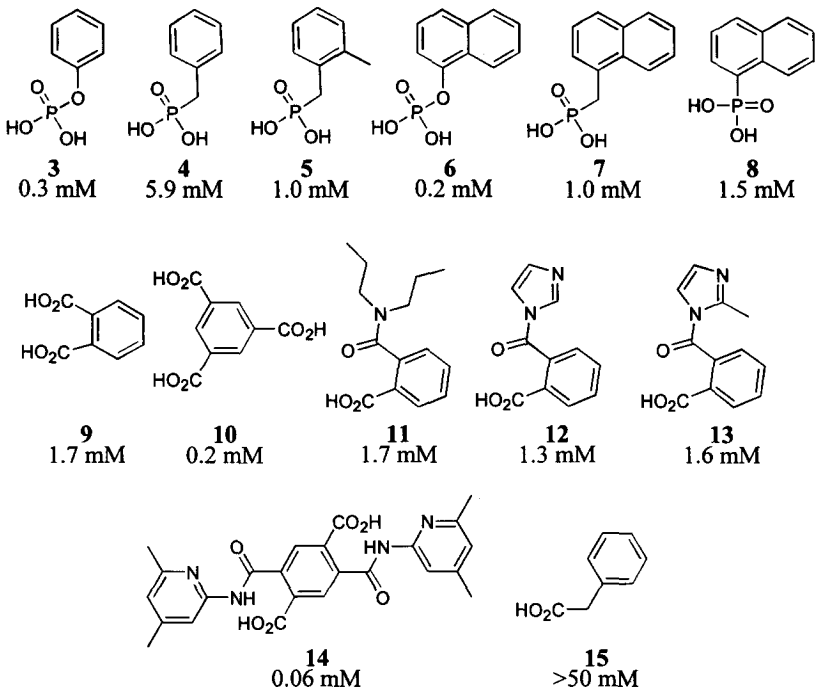


Figure 1. Potential phosphotyrosine mimetics for the Lck SH2 domain identified using an NMR-based screen.

These phthalamate analogs represent novel phosphotyrosine mimetics which may improve the pharmacokinetic properties while maintaining the potency of SH2 domain inhibitors which contain these moieties. Interestingly, bisphthalamates (e.g., **14**) were also identified that bind with significantly higher affinity than phosphotyrosine to the Lck SH2 domain.

In summary, we have used an NMR-based screen to identify novel phosphotyrosine mimetics for the SH2 domain of Lck. The phthalamate analogs that were identified represent novel mimetics which may yield inhibitors equipotent to phosphotyrosine-containing compounds with the advantage of improved pharmacokinetic properties.

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References and Notes

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11. Compounds **1–15** were tested for structural integrity and solubility by analyzing one–dimensional ^{1}H–NMR spectra of the compounds dissolved to 1 mM in D_2O. Structural stability was assessed by analyzing the ^{1}H–NMR spectra after incubating the samples in D_2O at 37 °C for 16 hours. All compounds yielded the expected ^{1}H–NMR resonances and appeared soluble and stable.
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