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Acquisition of a "Group A"-Selective Src Kinase Inhibitor via a Global Targeting Strategy

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Protein tyrosine kinases (PTKs) catalyze the phosphorylation of tyrosine residues in proteins and synthetic peptides. PTKs are commonly segregated into subgroups on the basis of sequence homology, such as the Src subfamily, which is comprised of Blk, Fgr, Fyn, Hck, Lck, Lyn, Yes, Yrk, and Src. The latter are multidomain enzymes containing SH1 (catalytic core), SH2 (binds to phosphotyrosine-containing sequences), and SH3 (binds to proline-rich sequences) domains.[1] As expected for a highly homologous group of enzymes, the acquisition of inhibitors that selectively target individual Src PTKs has proven quite difficult. Selective agents not only have potential clinical implications but also are essential for elucidating the biological roles of their enzyme targets. For example, Lck and Fyn are the Src kinase family participants in the signaling pathways that drive T cell activation.[2] Although it had been presumed that these enzymes perform similar functions, it is now evident that these presumptions were mistaken and simply a consequence of experimental methods.[2] A selective inhibitor for either of these PTKs could resolve many of the unanswered questions concerning the biochemical contributions of Fyn and/or Lck to T cell signaling. We report herein a combinatorial library/multidomain targeting approach that has resulted in the identification of one of the most potent peptide-derived inhibitors ever devised for a PTK.

Although the overall structural organization as well as the SH1, SH2, and SH3 domains are very similar among all Src kinase family members, specific differences do exist that appear to have global structural ramifications. A recent study demonstrated that the relative orientation of the Lck SH2−SH3 domain pair differs from that of its counterpart in Fyn.[3] Furthermore, disparities in the linker between the SH1 and SH2 domains appear to be responsible for relative domain displacements, as well.[4] Indeed, the latter differences in SH2 linker sequences correlate with the division of the Src kinase family into two separate subgroups (Group A: Fyn, Fgr, Src, and Yes; Group B: Blk, Hck, Lck, and Lyn).[4] This suggests that it might be possible to differentiate between Group A (e.g., Fyn) and Group B (e.g., Lck) PTKs on the basis of differences in the spatial orientation of the SH domains. In short, inhibitors that simultaneously associate with two SH domains could conceivably exhibit a high degree of selectivity. Although Src kinase multidomain-directed "bivalent inhibitors" have been previously described, these species were composed of a low affinity active-site-directed peptide (IC_{50} = 1.6 mM) coupled to a higher affinity SH2 domain-targeted sequence (K_D = 1.3 μM) in order to create a significantly better inhibitor (IC_{50} = 13 μM).[5] We reasoned that a more ideal strategy, in terms of acquiring a selective inhibitor, would employ two "energetically balanced" ligands (Figure 1) that display approximately equal affinities for their respective domains. Under these circumstances, the enzyme affinity of the coupled bivalent inhibitor should significantly exceed that of the individual components alone.[6]

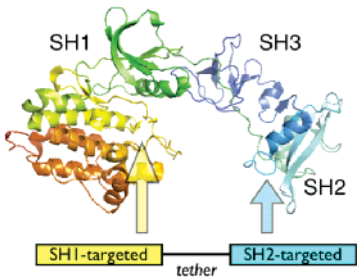

Figure 1. Two-domain-targeting inhibitor for the Src kinase family. The individual SH1- and SH2-targeting sequences are designed to have approximately equal affinities for their respective domains.

Table 1. Src Kinase Inhibitors

inhibitor structure	IC_{50} (μM)
1 (Ac)EEEIFGEFDap[a](Ac)−$NH(CH_2)_2SH$	380 ± 80
4 (Ba)EEEIFGEFDap(Ac)−$NH(CH_2)_2SH$	32 ± 3
7 (Ba)EEEIFGEFDap(Hna)−$NH(CH_2)_2SH$	1.9 ± 0.3
11 (Ba)EEEIFGEFDap(Hna)−βAla−pYEEIE−$NH(CH_2)_2SH$	3.8 ± 0.2
12 (Ba)EEEIFGEFDap(Hna)−βAla_2−pYEEIE−$NH(CH_2)_2SH$	0.24 ± 0.04
13 (Ba)EEEIFGEFDap(Hna)−βAla_3−pYEEIE−$NH(CH_2)_2SH$	0.04 ± 0.01[b]
14 (Ba)EEEIFGEFDap(Hna)−βAla_4−pYEEIE−$NH(CH_2)_2SH$	0.06 ± 0.01
15 (Ba)EEEIFGEFDap(Hna)−βAla_5−pYEEIE−$NH(CH_2)_2SH$	0.16 ± 0.02
16 (Ba)EEEIFGEFDap(Hna)−βAla_6−pYEEIE−$NH(CH_2)_2SH$	0.21 ± 0.07

[a] (L)-2,3-Diaminopropionic acid. [b] K_i = 26 ± 4 nM.

Active site (SH1)-directed peptides of PTKs are generally very poor inhibitors.[7] For example, peptide **1** (Table 1), which is derived from the Src kinase consensus phosphorylation sequence, exhibits an IC_{50} value of 380 ± 80 μM. We previously reported a synthetic strategy that converts weak consensus sequence peptides into higher affinity ligands.[8] We sought to transform **1** into an inhibitory agent whose affinity for the Src kinase active site is similar to that displayed by the SH2 domain targeting sequence Ac-pYEEIE (IC_{50} = 2.0 μM[9]) and then fuse these two sequences together. The consensus peptide **2** was prepared via solid phase peptide synthesis utilizing a disulfide-derivatized TentaGel resin (Scheme 1). The peptide-resin **2** was subsequently distributed, in equal amounts, into individual wells of multiwell synthesis plates. Each well contained one of 720 different carboxylic acids varying in size, shape, electrostatics, and hydrophobicity. Following coupling of the carboxylic acid library to the N-terminus, the peptides were side-chain-deprotected and cleaved from the resin with assay buffer (dithiothreitol) to furnish the primary library **3** (Scheme 1). An ELISA-based screen (Supporting Information) using the prototypical Group A Src kinase furnished the lead derivative possessing a substituted 3-methoxy-4-nitrobenzoic acid (Ba) substituent at the N-terminus. The latter peptide was resynthesized with a C-terminal (L)-2,3-diaminopropionic acid (Dap) moiety (Scheme 1) and its

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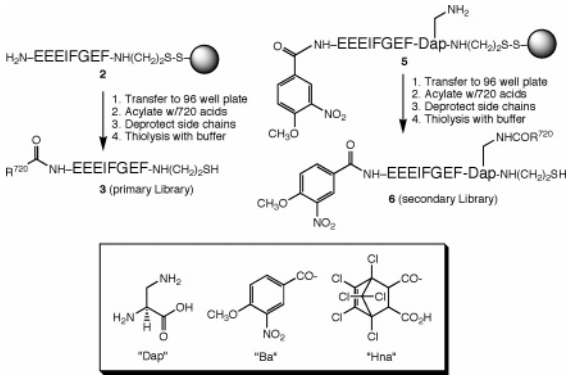
Scheme 1. Two-Step Synthetic Protocol for the Preparation of the Low Micromolar Affinity SH1 Domain-Targeting Inhibitor **7** (Table 1)



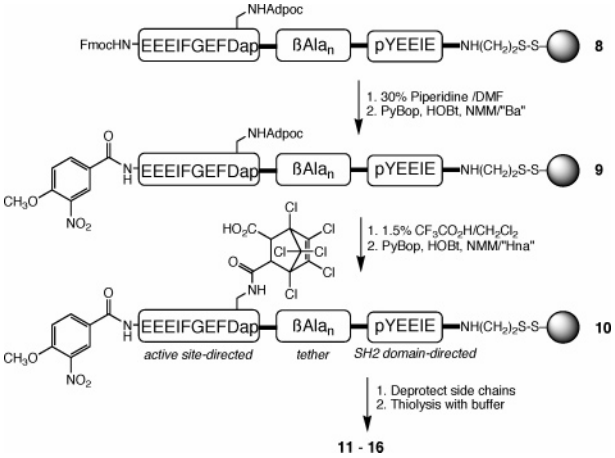
inhibitory efficacy evaluated. Compound **4** is approximately an order of magnitude more potent than the parent peptide **1** (Table 1).

With the Ba-derivatized analogue **4** in hand, a secondary library **6** was prepared which contains the biasing Ba substituent at the N-terminus and molecular diversity incorporated onto the Dap side chain amine at the C-terminus (Scheme 1). The hexachloronorborenyl acid (Hna)-substituted lead **7** exhibits a nearly 200-fold lower IC_{50} (1.9 ± 0.3 μM) than peptide **1** (Table 1). The apparent active site affinity of inhibitor **7** closely approximates the apparent SH2 affinity ($IC_{50} = 2$ μM) of Ac-pYEEIE. The IC_{50} values of **7** are not affected by simultaneous occupancy of the SH2 domain by an SH2 domain ligand (Supporting Information). Interestingly, peptide **7** does exhibit modest selectivity (2−17-fold) in favor of Group A enzymes (Supporting Information).

Although the three-dimensional structures of both inactive and active forms of full-length Src kinases are known, these enzymes have not yet been successfully crystallized bearing bound peptides.[2] Consequently, the relative orientation and distance between individual peptides separately associated with the active site and SH2 domain are not known. With this uncertainty in mind, we decided to synthesize a small library of multidomain-targeting peptides in which the active site-directed compound **7** is linked to its SH2-targeted counterpart, pYEEIE, via a variable length tether. The synthetic protocol illustrated in Scheme 2 furnished the peptide series **11**−**16**, whose members differ only in the number of β-Ala residues comprising the linker region. The length of the β-Ala-containing tether has a profound impact on inhibitory potency. Compound **13** (three β-Ala residues) is the most effective derivative in the bivalent series, exhibiting an IC_{50} of 36 ± 2 nM ($K_i = 26 \pm 4$ nM), approximately 4 orders of magnitude better than the parent peptide **1**.

The bivalent inhibitor **13** displays several intriguing properties. First, as noted above, the potency of the vast majority of peptidic inhibitors of Src and related enzymes is extraordinarily poor. By contrast, compound **13** is, to the best of our knowledge, one of the most potent peptide-based, active site-directed, PTK inhibitors ever described.[10] Second, the β-Ala tether length is extremely short, suggesting that the SH2 and active site regions assume an essentially

Scheme 2. Preparation of Bivalent Inhibitors **11**−**16**



contiguous spatial arrangement when both sites are concurrently occupied. Third, compound **13** simultaneously targets two functionally distinct regions on the Src kinase, whereas simple monovalent ligands can either block enzymatic activity (active site-directed) or signal complex formation (SH2 domain-directed), *but not both*. Finally, **13** exhibits up to a 1600-fold selectivity in favor of Group A [Fyn ($IC_{50} = 53 \pm 4$ nM), Fgr (180 ± 40 nM), Src (36 ± 2 nM), Yes (27 ± 3 nM)] versus Group B [Blk (2.6 ± 0.7 μM), Hck (2.3 ± 0.7 μM), Lck (43 ± 4 μM), Lyn (2.6 ± 0.8 μM)] enzymes. The 900-fold selectivity of **13** for Fyn versus Lck should prove useful in disentangling the overlapping roles played by these enzymes in T cell activation.

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Supporting Information Available: Experimental details of library synthesis, structure, screening, and inhibitor characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Boggon, T. J.; Eck, M. J. *Oncogene* **2004**, *23*, 7918−7927.
(2) Palacios, E. H.; Weiss, A. *Oncogene* **2004**, *23*, 7990−8000.
(3) Hofmann, G.; Schweimer, K.; Kiessling, A.; Hofinger, E.; Bauer, F.; Hoffmann, S.; Rosch, P.; Campbell, I. D.; Werner, J. M.; Sticht, H. *Biochemistry* **2005**, *44*, 13043−13050.
(4) Williams, J. C.; Wierenga, R. K.; Saraste, M. *Trends. Biochem. Sci.* **1998**, *23*, 179−184.
(5) Profit, A. A.; Lee, T. R.; Niu, J.; Lawrence, D. S. *J. Biol. Chem.* **2001**, *276*, 9446−9451.
(6) Jencks, W. P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4046−4050.
(7) Lawrence, D. S.; Niu, J. *Pharmacol. Ther.* **1998**, *77*, 81−114.
(8) Li, H.; Lawrence, D. S. *Chem. Biol.* **2005**, *12*, 905−912 and references therein.
(9) Park, S.-H.; Won, J.; Lee, K.-H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2711−2714.
(10) See two alternative strategies that have delivered potent peptide-based inhibitors for Src kinase family members: (a) Alfaro-Lopez, J.; Yuan, W.; Phan, B. C.; Kamath, J.; Lou, Q.; Lam, K. S.; Hruby, V. J. *J. Med. Chem.* **1998**, *41*, 2252−2260. (b) Parang, K.; Till, J. H.; Ablooglu, A. J.; Kohanski, R. A.; Hubbard, S. R.; Cole, P. A. *Nat. Struct. Biol.* **2001**, *8*, 37−41.

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