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STRUCTURE-BASED DESIGN AND SYNTHESIS OF A NOVEL CLASS OF SRC SH2 INHIBITORS

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Abstract: The structure-based design and synthesis of a novel class of 2,4-disubstituted thiazoles as Src SH2 inhibitors is described. Initial results are presented, including the X-ray and NMR analysis of one thiazole inhibitor bound to Lck and Src SH2.

Introduction: As a part of our efforts toward developing signal transduction inhibitors into therapeutic drugs, we have been interested in applications involving inhibitors of Src homology 2 (SH2) domains.[1] SH2 domains are protein recognition motifs found in a variety of intracellular signaling proteins that preferentially bind phosphotyrosine (pTyr)-containing tetrapeptides. Of particular interest are inhibitors of the SH2 domain of the tyrosine kinase $pp60^{c\text{-}Src}$, which has been implicated as a potential target[2] for therapeutic intervention for both osteoporosis[3-5] and breast cancer.[6] It has been established that the Src-related family members select for the tetrapeptide sequence pTyr-Glu-Glu-Ile (pYEEI)[7] ($K_d \approx 0.1$ μM). Several reports have emerged describing the mimicry of pYEEI by simpler dipeptide inhibitors for both Src and Lck SH2.[8-17] The structure-based design and synthesis of 2,4-disubstituted thiazoles as a novel class of Src SH2 inhibitors and the subsequent structural analysis of one of these inhibitors constitute the subject of this communication.

Design: Previous structural studies[18-20] have provided details at atomic resolution of the binding mode of the pYEEI sequence in both Src and Lck SH2 and serve as the basis of our design efforts.[21] The peptide binding site of the Src SH2 domain is defined by a shallow, solvent-accessible groove that contains well-defined pockets for the pY and pY+3 side chains. It appears that the majority of the important protein-ligand interactions occur within these pockets and thus contribute the most to the binding affinity for the pYEEI sequence (Figure 1). The interaction of the pY+1 amide to the His βD4 backbone carbonyl is the only other readily apparent direct protein-ligand contact.[20] The two Glu residues (pY+1, pY+2) maintain relatively minor interactions with the protein surface and apparently serve merely as a scaffold for the delivery of the pY and pY+3 side chains, yet the replacement of the pY+1 Glu side chain with simple alkyl substitution accounts for a 3-14 fold loss in binding affinity.[8,13] Further examination of the Src SH2 structure reveals a hydrophobic surface resulting from Tyr βD5 in the solvent-exposed space between the pY and pY+3 pockets. The recognition of the presence of this hydrophobic surface prompted us to design scaffolds that would interact better with this Tyr βD5 surface, while appropriately delivering the pY and pY+3 side chains into their respective pockets.[24] The initial design was based on a 1,3-disubstituted phenyl ring (**2**, Figure 2) that fulfilled the aforementioned requirements.[25] Anticipation of the synthetic complexity of this series, especially concerning the stereoselective installation of pY+1 side chains (R^1 in **2**), led us to also consider using a heterocycle scaffold, as in thiazole **3** (Figure 2).

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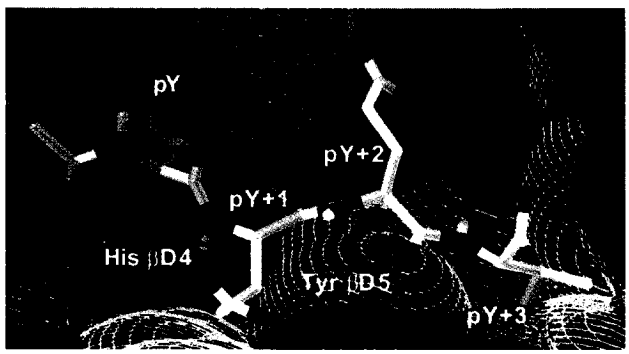
It was envisioned that the wide variety of available enantiopure amino acids could be exploited both as a source of chirality of the pY+1 side chain and for construction of the heterocycle ring. This commercial availability would allow for the preparation of pY+1 Glu-like compounds, which were anticipated to be among the better-binding analogs, as shown with the tetra- and di- peptides.[8,13]



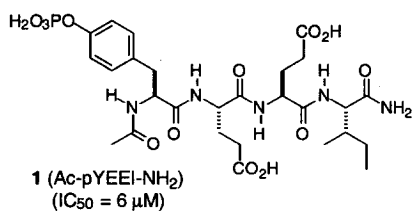
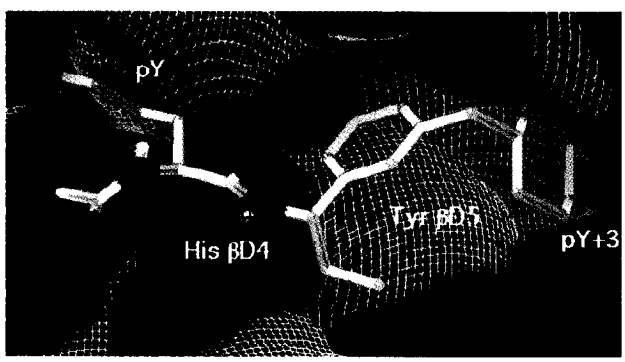


Figure 1. X-ray structure of Ac-pYEEI-OH bound to Lck SH2.



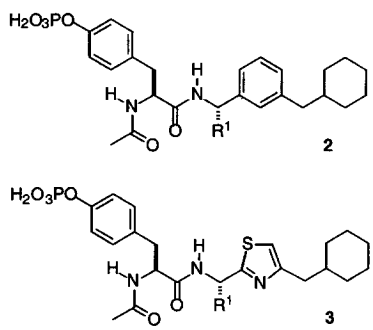
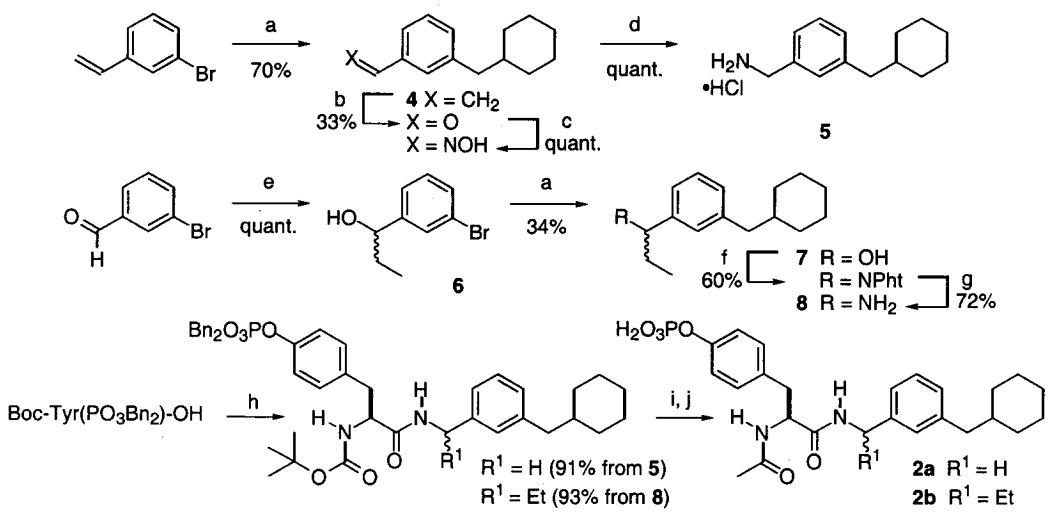


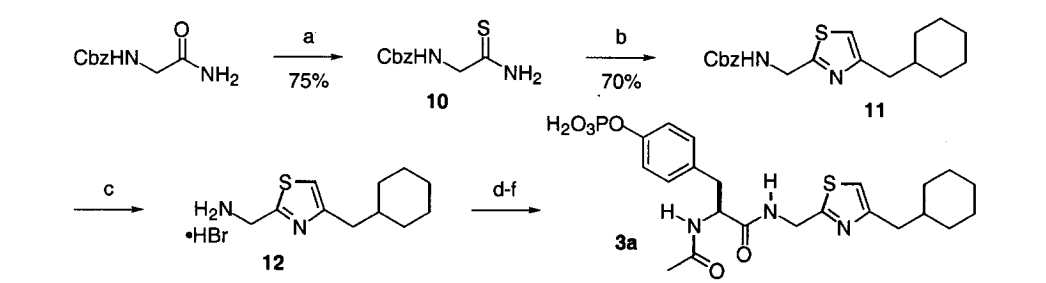
Figure 2. Scaffold **2** (R^1 = Et) docked in Src SH2 model.[21]

Chemistry: The syntheses of the phenyl scaffold targets **2a** and **2b** are shown in Scheme 1. The synthesis of **2a** (R^1 = H) began with the nickel-catalyzed cross coupling[26] of 3-bromostyrene with cyclohexylmethylmagnesium bromide ($ChxCH_2MgBr$) providing **4** in 70% yield. Ozonolytic cleavage of the vinyl group provided the aldehyde, which was converted into the corresponding oximes. Pd-catalyzed hydrogenation in the presence of HCl gave the desired amine hydrochloride **5** in quantitative yield. Standard EDC•HCl coupling of **5** with Boc-Tyr(PO_3Bn_2)-OH proceeded smoothly in 91% yield. Reaction with aq. TFA served to unmask the amine as well as remove the phosphate protecting groups. Acetylation followed by hydrolytic work up provided **2a** in 70% yield.[27] The synthesis of **2b** (R^1 = Et) utilized a similar Ni-catalyzed cross coupling reaction, but with the racemic bromide **6**. Conversion of alcohol **7** to the corresponding amine utilized the Mitsunobu reaction with phthalimide as the nucleophile. Treatment of the intermediate imide with hydrazine afforded amine **8**. Phosphate **2b** was then prepared according to the same method shown for **2a** and was isolated as a 1:1 mixture of diastereomers.



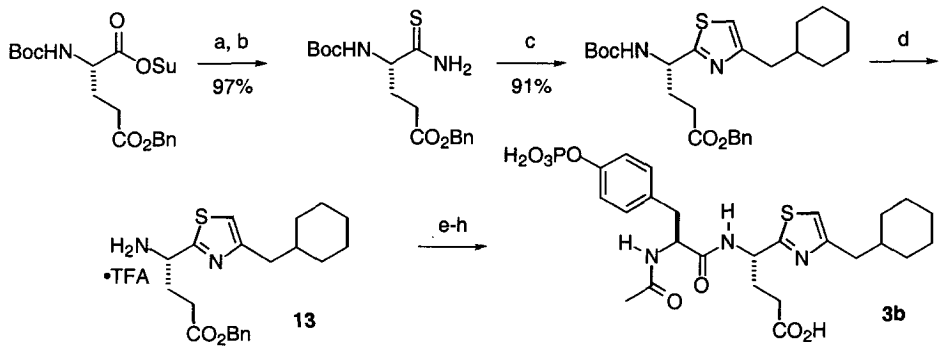
Scheme 1. (a) $ChxCH_2MgBr$, $(dppp)NiCl_2$ (cat), Et_2O, rt, overnight; (b) O_3, CH_2Cl_2, -78 °C, 5 min; Me_2S, rt, overnight; (c) $HONH_2$•HCl, pyridine, 0 °C→rt; (d) H_2 (1 atm), 10% Pd/C, EtOH, HCl, rt, 3 h; (e) EtMgBr, Et_2O, -78 °C→rt; (f) phthalimide, PPh_3, DEAD, THF, 0 °C→rt, overnight; (g) H_2NNH_2•H_2O, 5:1 THF-EtOH, rt, overnight; (h) **5** or **8**, DIEA, CH_2Cl_2, EDC•HCl, HOBT, 0 °C→rt, 3 h; (i) 95:5 TFA-H_2O, rt, 3 h; (j) Ac_2O; DIEA, DMF, 0 °C→rt, 1.5 h.

The amino acid-derived thiazole targets **3a** and **3b** were prepared using the Hantzsch thiazole synthesis. The synthesis of the initial Gly-derived target (**3a**), the thiazole analog of **2a**, is shown in Scheme 2. Bromoketone **9** resulted from the controlled mono-bromination of cyclohexylmethyl ketone.[28] Reaction of bromoketone **9** with Gly-derived thioamide **10** in refluxing EtOH,[29] gave thiazole **11** in 70% yield. Deprotection using 30% HBr in HOAc afforded the amine hydrobromide **12**, which was converted to target **3a** in the same manner as described for **2a**.[27]



Scheme 2. (a) Lawesson's reagent, 2:1 DME-THF, rt, 6 h; (b) $BrCH_2C(O)CH_2Chx$ (**9**), EtOH, 78 °C, 2.5 h; (c) 30% HBr in HOAc, rt, 1h; (d) DIEA, CH_2Cl_2, Boc-Tyr(PO_3Bn_2)-OH, EDC•HCl, HOBT, rt, 3.5 h; (e) 95:5 TFA-H_2O, PhOMe, rt, 2 h; (f) Ac_2O; DIEA, DMF, 0 °C→rt, 3 h.

The synthesis of the Glu-derived analog (**3b**), shown in Scheme 3, employed the Meyers-modified Hantzsch conditions[30,31] in order to maintain the integrity of the amino acid-derived pY+1 stereocenter. Conversion of amine salt **13** to **3b** proceeded smoothly in the same manner as described for **2a**, with the additional hydrogenation step required for the conversion of the benzyl ester to the corresponding acid.[27]



Scheme 3. (a) NH_4HCO_3, 5:1 CH_2Cl_2-dioxane, rt, 19 h; (b) Lawesson's reagent, 5:1 DME-THF, rt, 5 h; (c) $KHCO_3$, DME, $BrCH_2C(O)CH_2Chx$ (**9**), -15 °C, 1 h, then rt, 2.5 h; TFAA, pyr, -15 °C, 50 min, then rt, 20 min; (d) TFA, CH_2Cl_2, rt, 2.25 h; (e) DIEA, CH_2Cl_2, Boc-Tyr(PO_3Bn_2)-OH, EDC•HCl, HOBT, rt, 4 h; (f) 95:5 TFA-H_2O, PhOMe, rt, 2 h; (g) Ac_2O; DIEA, DMF, 0 °C→rt, 1.3 h; (h) 10% Pd-C, HCO_2NH_4, MeOH, 65 °C, 35 min.

Results: The assay results for targets **2a**, **2b**, **3a**, **3b** and the standard tetrapeptide **1** are shown in Table 1.[32] Compounds **2a** and **3a** (R^1 = H) had IC_{50}'s of 769 and 483 μM, respectively, reflecting only a slight difference between the unsubstituted phenyl and thiazole scaffold analogs. Both of the analogs with R^1 substitution (**2b** and **3b**) displayed improved binding affinity with respect to **2a** and **3a**. The eighteen-fold increase in binding affinity found for thiazole **3b** (vs. up to five-fold for **2b**) can be rationalized by the incorporation of the Glu-like pY+1 substitution. Thiazole **3b** was thus only four-fold less active than the standard tetrapeptide **1** (IC_{50} = 6 μM).[24] The relative potency of thiazole **3b** could be further rationalized following subsequent structural (X-ray, NMR) studies.

Table 1. Src SH2 binding affinity.[32]

Cmpd	IC_{50} (μM)	Cmpd	IC_{50} (μM)
2a	769	**3a**	483
2b (1:1)	306	**3b**	26
		1	6

Structure: Figures 3 and 4 show the X-ray crystal structure[33,34] of thiazole **3b** bound to Lck SH2. The key interactions displayed by the Ac-pY-NH- portion are similar to those found with **1** (Figure 1). Figure 3 highlights the expected interaction of the thiazole scaffold with Tyr βD5 and the delivery of the cyclohexyl ring into the pY+3 pocket. Figure 4 highlights the key crystallographic water molecules in contact with **3b** and the protein backbone. The pY+1 Glu side chain is oriented upward with two water-mediated hydrogen bonds. The thiazole sulfur atom demonstrates a weak water-mediated hydrogen bond with both the NH and C=O of Lys βD6. The thiazole nitrogen atom displays weak water-mediated hydrogen bonds to the pY+1 Glu side chain and to the protein backbone at Cys BG5 (NH) and Gly BG3 (C=O). Noteworthy from Figures 3 and 4 are the improvements suggested for future design strategies. The space between the thiazole ring and the Tyr βD5 surface suggests that this interaction is yet unoptimized, which may be due in part to the awkward trajectory and fit of the cyclohexyl ring in the pY+3 pocket.[35] In addition, the presence of the structural waters (2.9 Å, 3.6 Å) adjacent to the thiazole ring suggests strategies to enhance binding affinity by their displacement.[36]

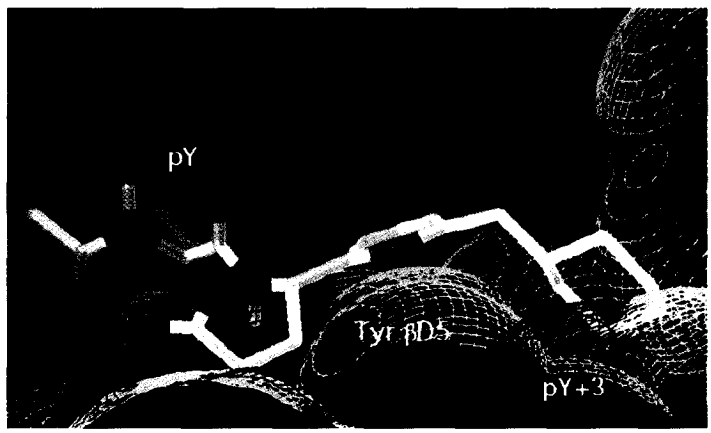


Figure 3. FLO-generated depiction of the X-ray crystal structure of thiazole **3b** bound to Lck SH2 highlighting the Lee and Richards solvent accessible surfaces.

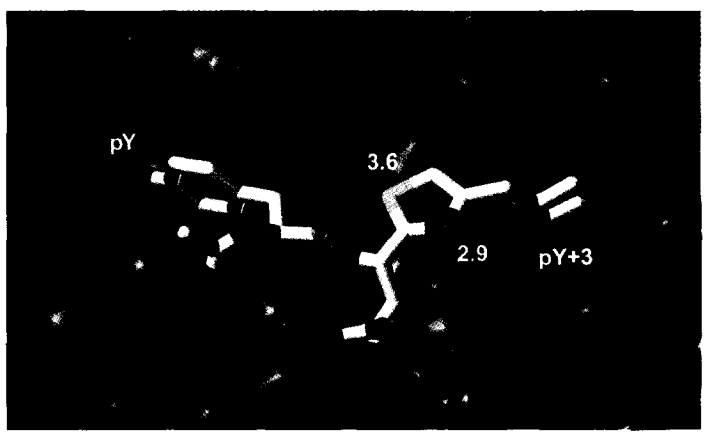


Figure 4. SYBYL-generated depiction of the X-ray crystal structure of thiazole **3b** bound to Lck SH2 highlighting key crystallographic water molecules.

Conclusion: In summary, we have described the structure-based design of an effective mimetic of the tetrapeptide sequence pYEEI for Src SH2. The synthetic potential of the thiazole scaffold provided a starting point for the initiation of structure-activity relationship (SAR) studies of this novel class of Src SH2 inhibitors. These results are detailed in the accompanying communication.[37]

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21. The SH2 binding sites for Lck and Src are nearly identical with only conservative modifications in a few residues. A model of the Src SH2 binding site has been developed using both Src and Lck structures. This model displays the Lee and Richards (ref. 22) accessible surfaces to highlight the areas of hydrophobic and hydrogen bonding interactions as in Figures 1-3. Ligands were examined using the QXP (ref. 23) docking program module of the FLO97 molecular modeling program.
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24. A bridging strategy, using configurationally flexible linkers, was employed by the Glaxo group and resulted in 190- to 860-fold loss in binding affinity in the pYEEIE peptide series (see ref. 13).
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33. **3b** was cocrystallized with wild-type Lck SH2, which was used as a Src SH2 surrogate due to greater success with ligand cocrystallization. The structure was determined by molecular replacement methods and refined using X-PLOR (Brünger, A. *X-PLOR, Version 3.1.* (Yale Univ. Press, New Haven, CT, 1992)). This Lck SH2 crystal structure correlated closely with the Src SH2 NMR structure (ref. 34) as well as that predicted from molecular modeling (ref. 21). In addition, the binding affinity of **3b** in both Lck SH2 and Yes SH2 was equivalent to that reported here for Src SH2.
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35. By definition, the Lee and Richards accessible surface depiction suggests that the optimal interaction would be gained with the thiazole ring situated directly on the yellow TyrβD5 hydrophobic surface.
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