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STRUCTURE-ACTIVITY RELATIONSHIPS OF A NOVEL CLASS OF SRC SH2 INHIBITORS

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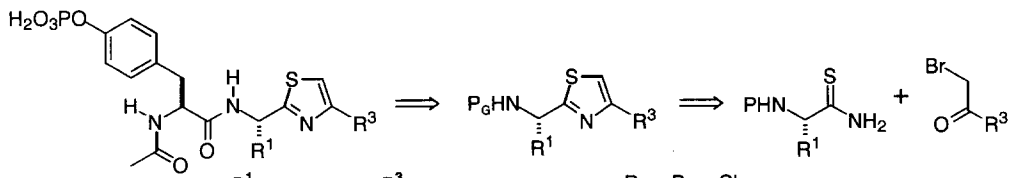
Abstract: The structure-activity relationships (SAR) of a novel class of Src SH2 inhibitors are described. Variation at the pY+1 and pY+3 side chain positions using 2,4- and 2,5-substituted thiazoles and 1,2,4-oxadiazoles as scaffolds resulted in inhibitors that bound as well as the standard tetrapeptide Ac-pYEEI-NH_2.

Introduction: As a part of our efforts toward developing signal transduction inhibitors into therapeutic drugs, we have been interested in applications involving inhibitors of the Src homology 2 (SH2) domain[1] of the tyrosine kinase $pp60^{c\text{-}Src}$, which has been implicated as a potential target[2,3] for therapeutic intervention for both osteoporosis[4-6] and breast cancer.[7] In the preceding communication,[8] we reported on the structure-based design and synthesis of a novel class of Src SH2 inhibitors, represented by the 2,4-substituted thiazole **1b** (Figure 1). This class of inhibitors was designed based on structural studies (X-ray, NMR) of the preferred tetrapeptide sequence pTyr-Glu-Glu-Ile (pYEEI) bound to both the Src and Lck SH2 domains.[9-11] The heterocycle ring was incorporated as a replacement scaffold that would appropriately deliver the pY and pY+3 side chains into their respective pockets while gaining a favorable interaction with the hydrophobic surface resulting from Tyr βD5.[11] It was envisioned that the ready availability of enantiopure amino acids could be exploited for the synthesis of nonracemic heterocycles with diverse pY+1 (R^1) and pY+3 (R^3) side chain substitution. Subsequent structural analysis of thiazole **1b** (IC_{50} = 26 μM) in both Src and Lck SH2 provided a new frame of reference for analog design.[8] This communication describes our initial results based on thiazole **1b**, leading to Src SH2 inhibitors with binding affinities equivalent to that of the standard tetrapeptide, Ac-pYEEI-NH_2.

Figure 1



	R^1	R^3
1a	H	CH_2Chx
1b	$CH_2CH_2CO_2H$	CH_2Chx
1c	$CH_2CH_2CO_2H$	$CH_2CH(CH_3)_2$
1d	$CH_2CH_2CO_2H$	$(CH_2)_2CH(CH_3)_2$

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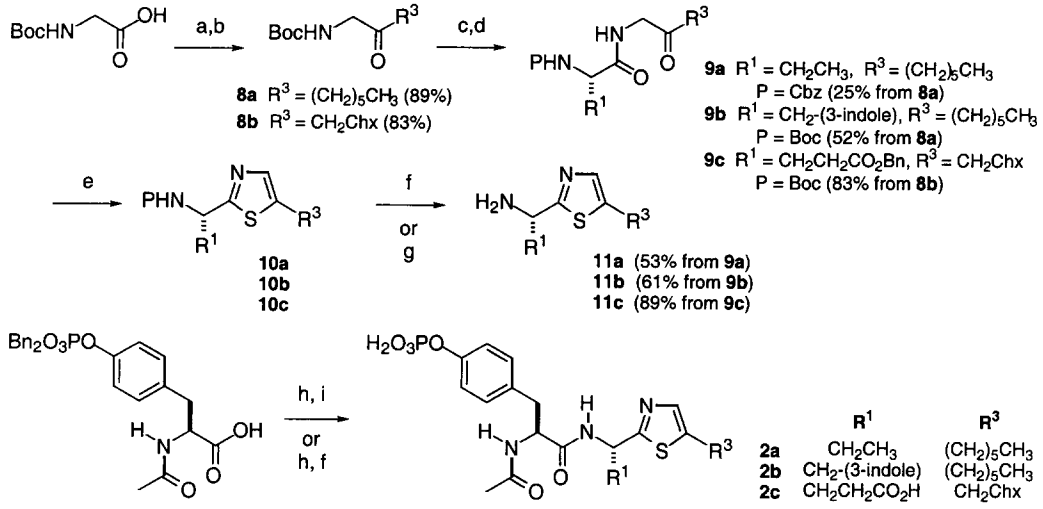
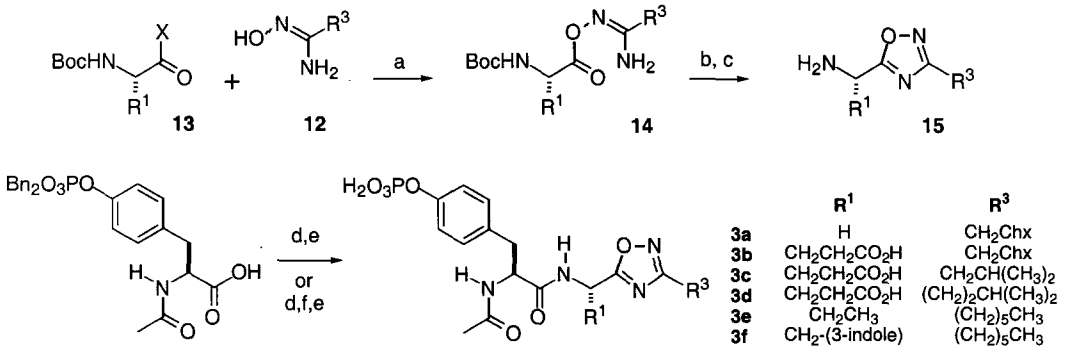
Chemistry: Recent improvements in the Hantzsch synthesis of nonracemic peptide thiazoles from enantiopure amino acids[12,13] led to the choice of the 2,4-substituted thiazole as the initial scaffold. Thiazole targets **1a-1d** (Figure 1) were prepared from amino acid-derived thioamides and α-bromo ketones as described in the previous communication.[8,14]

Due to the somewhat limited availability of diverse α-bromo ketones, either from commercial sources or from short synthetic sequences, an effort was initiated toward using heterocycle scaffolds other than the 2,4-substituted thiazole. We have recently reported on the synthesis of nonracemic 2,4,5-substituted peptide thiazoles from two amino acids and one organometallic reagent.[15] A variation in this synthesis, using Gly as the thiazole ring amino acid, provides access to 2,5-substituted thiazoles, the regioisomers of the Hantzsch 2,4-substituted thiazoles. The R^3 side chain is derived from an organometallic reagent, thus providing a complementary method toward disubstituted thiazoles. The synthesis of 2,5-substituted thiazoles toward targets **2a-2c** is shown in Scheme 1. Boc-Gly-OH was converted into the corresponding Weinreb amide using standard CDI conditions. Addition of the appropriate Grignard reagent[16] provided the protected amino ketones **8a** and **8b**. Deprotection of carbamates **8a** and **8b** with 3N HCl gave the corresponding amine salts, which were then coupled with the protected amino acid succinimide derivatives to afford keto amides **9a-9c**. Treatment with Lawesson's reagent effected the cyclization giving thiazoles **10a-10c**. Deprotection, either by catalytic transfer hydrogenation (**10a**),[17] or with TFA (**10b**, **10c**), afforded amines **11a-11c**. Standard coupling of amines **11a-11c** with Ac-Tyr(PO_3Bn_2)-OH followed by deprotection(s) provided 2,5-substituted thiazole targets **2a-2c**.[14]



Scheme 1. (a) CDI, CH_2Cl_2, 0 °C, 10 min, then DIEA, NH(Me)OMe•HCl, rt, 21 h; (b) R^3MgBr, THF, 0 °C, 10 min, then rt, 4-7 h; (c) 3N HCl, 1:1 dioxane-EtOAc, rt, 17 h-2.5 d; (d) Et_3N, DME, (Cbz-Abu-OSu for **9a**) or (Boc-Trp-OSu for **9b**) or (Boc-Glu(Bn)-OSu for **9c**), rt, 6-32 h; (e) Lawesson's reagent, THF, 67 °C, 2-3 h; (f) for **11a** (and **2c**), HCO_2NH_4, 10% Pd-C, MeOH, 64 °C, 2-6 h; (g) for **11b** and **11c**, TFA, CH_2Cl_2, rt, 1.25 h; 5% $NaHCO_3$, EtOAc; (h) **11a**, **11b** or **11c**, EDC•HCl, HOBT, DIEA, CH_2Cl_2, DMF, 0 °C to rt; (i) 95:5 TFA-H_2O, rt.

In a continued search for alternative heterocyclic scaffolds, we became aware of a report from the Luthman group[18] describing the synthesis of nonracemic 1,2,4-oxadiazoles, again utilizing amino acids as the source of the R^1 side chains. The R^3 side chains are derived from nitriles, many of which are commercially available, after facile conversion into the corresponding amidoximes. Scheme 2 shows the general sequence followed for the preparation of 1,2,4-oxadiazole targets **3a-3f**, corresponding to thiazoles **1a-1d** and **2a-2c**. The nitrile-derived amidoximes **12** were coupled to the appropriate amino acid derivatives **13**, giving the intermediate *O*-acylamidoxime **14**. Cyclization of **14** in refluxing pyridine followed by deprotection gave rise to amine **15**. Standard coupling of amine **15** with Ac-Tyr(PO_3Bn_2)-OH followed by deprotection(s) provided 1,2,4-oxadiazole targets **3a-3f**.[14]



Scheme 2. (a) **13** (X = OSu), DME, rt (alternatively, any Boc-amino acid (**13**, X = OH), CH_2Cl_2, DMF, EDC•HCl, HOBT, DIEA, rt); (b) pyridine, reflux; (c) TFA, CH_2Cl_2, rt; (d) **15**, EDC•HCl, HOBT, DIEA, CH_2Cl_2; (e) 95:5 TFA-H_2O; (f) LiOH•H_2O, THF-H_2O for R^1 = $CH_2CH_2CO_2Bn$ to R^1 = $CH_2CH_2CO_2H$.

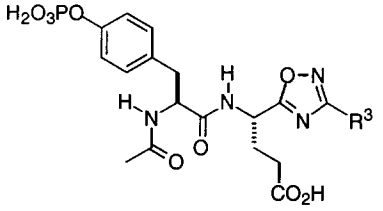
Results and Discussion - Comparison of the heterocyclic scaffolds: With synthetic routes using three heterocyclic scaffolds available, attention was turned to the comparison of Src SH2 binding affinity between these scaffolds. During the synthetic development, it became apparent that the 1,2,4-oxadiazole series provided the most versatility toward analog preparation, and resulted in a shorter, more streamlined synthetic pathway. Thus we were particularly interested in whether the 1,2,4-oxadiazole series bound to Src SH2 with similar affinity as the original 2,4-substituted thiazole series (as in **1b**). Table 1 contains the assay results[19] for the selected thiazole and oxadiazole derivatives (**1a-3f**) prepared for this scaffold comparison. As shown in Table 1, there exists a close correlation in binding affinity between the heterocycle series throughout the range of binding affinities,[20] including the initially-prepared set of 2,4-substituted thiazoles **1a-1d** and 1,2,4-oxadiazoles **3a-3d**. In addition, the binding affinities for the 2,5-substituted thiazoles, **2a** and **2b**, were similar to those for the corresponding 1,2,4-oxadiazoles **3e** and **3f**, which resulted from the subsequent R^1/R^3 scan (vide infra). Finally, 2,5-substituted thiazole **2c**, prepared for the comparison with both the 2,4-substituted thiazole and 1,2,4-oxadiazole series, had a binding affinity that was similar to those shown for **1b** and **3b**.[20]

Table 1: Src SH2 binding affinity comparing similarly substituted thiazoles and oxadiazoles.[19]

R^1	R^3	(2,4- thiazole) Cmpd	IC_{50} (μM)	(1,2,4- oxadiazole) Cmpd	IC_{50} (μM)	(2,5- thiazole) Cmpd	IC_{50} (μM)
H	CH_2Chx	**1a**	483	**3a**	896		
$CH_2CH_2CO_2H$	$CH_2CH(CH_3)_2$	**1c**	218	**3c**	166		
$CH_2CH_2CO_2H$	$(CH_2)_2CH(CH_3)_2$	**1d**	76	**3d**	61		
$CH_2CH_2CO_2H$	CH_2Chx	**1b**	26	**3b**	29	**2c**	16
CH_2CH_3	$(CH_2)_5CH_3$			**3e**	25	**2a**	12
CH_2-(3-indole)	$(CH_2)_5CH_3$			**3f**	16	**2b**	14

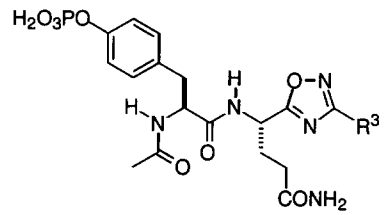
Results and Discussion - pY+1/pY+3 SAR: With results suggesting that the various heterocycles could be interchanged and a facile 1,2,4-oxadiazole analog synthesis, we began to analyze the SAR for various R^1 and R^3 substitution patterns.[21] In the tetrapeptide and dipeptide series, it was shown that pY+1 = Glu (R^1) provided inhibitors with the best binding affinity.[1,22] Table 2 shows the binding affinities for several Glu-derived 1,2,4-oxadiazoles, using **3b** as the new frame of reference. Shorter branched alkyl chains (**3d** and **3c**) resulted in a loss in binding affinity, as did replacement of the cyclohexyl ring (**3b**) with a phenyl ring (**4d**). The *n*-pentyl analog **4c** bound as well as the corresponding CH_2Chx analog **3b**. Compounds **4b** and **4a**, with the slightly longer *n*-hexyl and *n*-heptyl side chains, displayed IC_{50}'s that were 3-4 times better than **3b**, or equivalent to the standard tetrapeptide sequence Ac-pYEEI-NH_2 (IC_{50} = 6 μM). Efforts are currently underway toward obtaining additional structural information that may help explain the improved binding affinities for these *n*-alkyl R^3 analogs.

Table 2: Src SH2 binding affinity for L-Glu-derived 1,2,4-oxadiazoles.[19]



Cmpd	R^3	IC_{50} (μM)
4a	$(CH_2)_6CH_3$	7
4b	$(CH_2)_5CH_3$	8
4c	$(CH_2)_4CH_3$	21
3b	CH_2Chx	29
3d	$(CH_2)_2CH(CH_3)_2$	61
4d	CH_2Ph	92
3c	$CH_2CH(CH_3)_2$	166

Table 3: Src SH2 binding affinity for L-Gln-derived 1,2,4-oxadiazoles.[19]



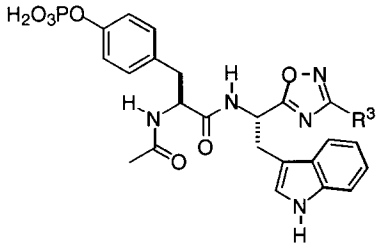
Cmpd	R^3	IC_{50} (μM)
5a	$(CH_2)_5CH_3$	24
5b	CH_2Ph	72
5c	CH_2(1-naphthyl)	135
5d	$(CH_2)_2Ph$	156
5e	CH_2Chx	278
5f	$(CH_2)_2CH(CH_3)_2$	320
5g	$(CH_2)_3CH_3$	384

With the objective of reducing the overall charge of these molecules in order to improve cellular permeability, part of our R^1/R^3 scan strategy involved replacing the $R^1 = CH_2CH_2CO_2H$ (Glu) side chain with several uncharged R^1 side chains. As was demonstrated in the tetrapeptide and dipeptide series, substitution of the Glu side chain with simple alkyl groups resulted in some loss in binding affinity.[1,22] Our initial choice was that of $R^1 = CH_2CH_2CONH_2$ (Gln), since an early comparison between Gln and Glu derivatives (**5b** vs. **4d**) suggested a reasonable correlation in binding affinities (Tables 2 and 3). Unfortunately, it was soon discovered that Gln was not an acceptable R^1 replacement as demonstrated in the significant decrease in binding affinity upon simple variation (Table 3). Analog **5e** was about 10 times less active than the Glu-derivative **3b**. The *n*-hexyl analog, **5a**, however, was only 3 times less active than the corresponding Glu derivative **4b**, again demonstrating that simple *n*-alkyl side chains could be acceptable as R^3 replacements for CH_2Chx.

In our continued efforts toward side chain charge reduction, several analogs in the Trp and Abu series were prepared. As shown in Tables 4 and 5, several analogs (**3f**, **6a**, **7a**, **3e**) were only 2-3 times less active than the corresponding Glu analogs. This combination of better R^1 and R^3 side chains resulted in analogs with binding affinities equivalent to our original lead, **1b**, yet with one less charge unit.

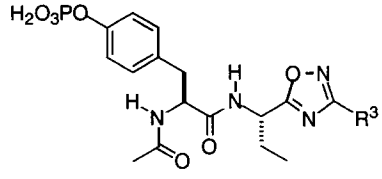
As alluded to previously, the 2,5-substituted thiazoles, **2a** and **2b** (Scheme 1), that corresponded to **3e** and **3f**, were also prepared. As shown in Table 1, **2a** and **2b** were found to have binding affinities that were slightly better than **3e** and **3f**, almost 2 times more active than the original lead **1b**, and with one less charge unit.

Table 4: Src SH2 binding affinity for L-Trp-derived 1,2,4-oxadiazoles.[19]



Cmpd	R^3	IC_{50} (μM)
3f	$(CH_2)_5CH_3$	16
6a	CH_2Ph	25
6b	$(CH_2)_2CH(CH_3)_2$	33
6c	$(CH_2)_6CH_3$	35
6d	$(CH_2)_4CH_3$	36
6e	CH_2Chx	62
6f	$(CH_2)_3CH(CH_3)_2$	89
6g	CH_2(1-naphthyl)	279

Table 5: Src SH2 binding affinity for L-Abu-derived 1,2,4-oxadiazoles.[19]



Cmpd	R^3	IC_{50} (μM)
7a	$(CH_2)_6CH_3$	22
3e	$(CH_2)_5CH_3$	25
7b	$(CH_2)_4CH_3$	65
7c	$(CH_2)_2CH(CH_3)_2$	111

Conclusion: We have described the initial results of variations at R^1, R^3 and heterocycle-type based on our initial lead thiazole **1b**. The 2,4- and 2,5-disubstituted thiazole and 1,2,4-oxadiazole ring appear to be interchangeable as scaffolds for these disubstituted heterocycle analogs.[20] Variation at R^3 has resulted in Src SH2 inhibitors (**4a**, **4b**) that bind as well as the standard tetrapeptide Ac-pYEEI-NH_2. Variation at R^1 has resulted in a reduction of overall charge with only a two fold loss in binding affinity (**3f**, **2a-2c**). Efforts are currently underway at using our 2,4,5-substituted thiazole chemistry[15] toward analogs with improved binding affinity and toward incorporation of pTyr replacements. These results will be reported in due course.

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20. This close correlation suggests that either (a) the R^1/R^3 substituents in Table 1 continue to align the heterocycle ring away from the Tyr βD5 hydrophobic surface as shown in Figure 3 of ref. 8, or (b) the differences in the electronic character of the chosen heterocycles do not significantly affect the subsequent ligand binding affinities.
21. The nitriles chosen for pY+3 were based on a biased selection of commercially available nitriles that could potentially bind in the pY+3 pocket. In addition, several nitriles were chosen based on structural analysis based on the X-ray structure of **1b** (R. S. Bohacek, unpublished results).
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