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# Discovery of Thioazepinone Ligands for Src SH2: From Non-specific to Specific Binding

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**Abstract**—The structure-based design and synthesis of new thioazepinones as ligands for Src SH2 protein is presented. From benzothioazepinones, ligands with somewhat unspecific binding properties, simpler thioazepinones were designed, the best ones demonstrated nanomolar affinity for Src SH2. A few of these new ligands were crystallized with the protein and demonstrated a specific binding mode with the protein. 

## Introduction

Tyrosine kinases are involved in many pathologies such as cancer, inflammation or osteoporosis and are consequently important targets for the pharmaceutical industry.[1] In particular, Src has been shown to be essential in the process of bone resorption.[2] Src is the member of a family of 10 homologous proteins featuring several functional domains, including SH2 and SH3 domains involved in protein–protein interactions, and a tyrosine kinase catalytic domain.[3] Rescue experiments[4] suggested that the SH2 domain is critical to the bone resorbing activity of Src and therefore we embarked in a program of finding inhibitors of the SH2 domain of Src. The SH2 domain of the Src family specifically recognizes a sequence of tetrapeptide featuring a phosphotyrosine and a lipophilic amino acid at the +1 and +3 positions. In particular, pYEEI, a sequence found on the PDGF receptor upon activation[5] has been shown to specifically recognize Src SH2 domain. The approach that we have used to discover nonpeptidic inhibitors of this tetrapeptide sequence has been typically rational drug design driven from a peptidomimetic modular approach where the pYEEI peptide was viewed as a three-component ligand (phosphotyrosine–central scaffold–hydrophobic). The study of the X-ray structure of pYEEI with Src SH2[6] reveals that the middle glutamate amino acids do not make a strong interaction with the peptide backbone, and therefore, mainly serve the purpose of presenting the phosphotyrosine and the hydrophobic group to their respective pockets. However, the aliphatic chain of the +1 glutamate makes an important hydrophobic interaction with a tyrosine of the protein and we decided to search our library in order to identify potential scaffolds capable of restoring this interaction in a more rigid frame. On the other hand, the +2 glutamate is solvent oriented and molecular modeling suggested that a glycine would be sufficient to present the hydrophobic moiety in the desired position.

## Chemistry and Biology

For the +1 glutamate replacement, we explored by molecular modeling several scaffolds we had at hand in our library. One of them, the benzothioazepinone scaffold found in diltiazem,[7] was chosen first. Initially, a compound where the +1 glutamate had been replaced by a benzothioazepinone was synthesized. Starting benzothioazepinone **1**[8] was condensed with protected L-tyrosine using EDC and BOBt in dichloromethane and the resulting compound **2** was alkylated on the cyclic nitrogen with various substituted derivatives of

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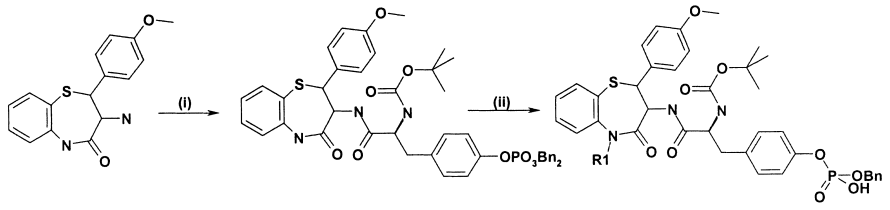



**Scheme 1.** (i) BOC-Tyr-O($PO_3Bn_2$), EDC, HOBt, $CH_2Cl_2$ Hunig's base, DMF (49%); (ii) (1) $R_1Br$, NaH, DMF; (2) NaI, acetone.

bromoacetamide using sodium hydride in DMF with good yields. The resulting benzylphosphate esters were mono deprotected using NaI in acetone to afford the new derivatives **3a**–**k** (Scheme 1).

Table 1 presents the hydrophobic moieties that were explored and the binding affinities of the new derivatives on Src SH2 (determined with an assay relying on fluorescence polarization[9]). The best one, compound **3k**, presented binding affinity comparable to the one of pYEEI in this assay.

Alternatively, several analogues where the tyrosine group had been replaced by another moiety were synthesized as shown in Scheme 2. The resulting new derivatives are presented in Table 2, the best analogue being the one where the tyrosine replacement was a 4-monobenzylphosphate-phenyl acetate (**4e**).

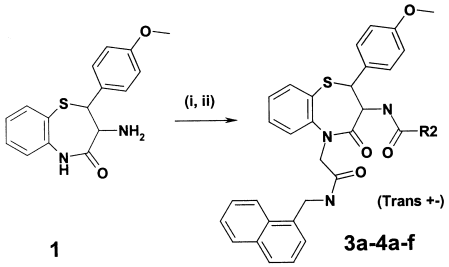
However, after several rounds of optimization around compounds **3k** or **4e** (data not shown), binding affinity could not be further improved. In addition, none of these inhibitors gave rise to successful co-crystallization with the protein. One main problem was solubility of the inhibitors and we began to question the specificity of the binding. This was also suggested by the shape of the binding curves. For instance, while pYEEI showed a straight binding curve with a Hill coefficient[10] around 1, **4e** displayed a steeper curve with a Hill coefficient of 1.75 suggestive of cooperative binding (Fig. 1). In addition, when we performed binding with increasing DMSO concentration which did not affect the binding affinity of pYEEI, the affinity of the nonpeptides was sometimes dramatically decreased (data not shown) which also alluded to the higher lipophilic component of the binding affinity (in agreement with the high log P values calculated for these compounds).

It has been shown from the early work of Gilmer et al.[11] that changing the +1 L-glu of pYEEIE peptide for a D-Glu residue resulted in a 250-fold loss in binding affinity. In the case of **3c**, the four separated isomers gave about the same binding affinity when comparing the pairs varying at +1 position (Table 3, cf. **3c** with **3e** and **3m** with **3n**). This further suggested that the binding displayed some unspecific component (Table 3).

**Table 1.** Variation of the hydrophobic moieties of compound **3**

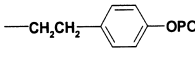
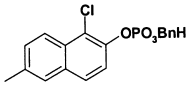
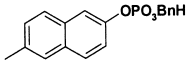
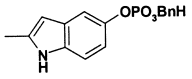
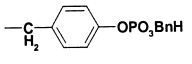
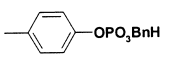
| $R_1$ | Compd | Isomers | $IC_{50}$[a](μM) |
|---|---|---|---|
| pYEEI | — | — | 6.5 |
| $CH_2CONHCH_2$-1-naphthyl | **3a** | *trans*, 2 *isos* | 128 |
| $CH_2CONHCH_2$-1-naphthyl | **3b** | *cis*, 2 *isos* | 118 |
| $CH_2CONH$-1-naphthyl | **3c** | *trans*, *iso* 1 | 189 |
| $CH_2CONHPh$-2-(OMe) | **3d** | *trans*, *iso* 1 | 95 |
| $CH_2CONHPh$-3-(OPh) | **3e** | *trans*, *iso* 1 | 14.2 |
| H | **3f** | *trans*, *iso* 1 | 873 |
| CH(Ph)CONH-1-Naphthyl | **3g** | *trans*, *iso* 1 | 23 |
| $CH_2Ph$ | **3h** | *trans*, *iso* 1 | 46 |
| $CH_2CONHPh$-4-(OPh) | **3i** | *trans*, *iso* 1 | 29 |
| $CH_2Ph$-4-Ph(2′-COOtBu) | **3j** | *trans*, *iso* 1 | 18 |
| $(CH_2)_3$Cyclohexyl | **3k** | *trans*, *iso* 1 | 6.4 |

[a]$IC_{50}$'s of inhibition determined using the FP assay[9] in a medium containing 2% DMSO.




**Scheme 2.** (i) $R_2COOH$, EDC, BOBt, $CH_2Cl_2$, Hunig's base, DMF; (ii) (1) $BrCH_2CONHCH_2$-1-naphthyl, NaH/DMF; (2) NaI/acetone.

**Table 2.** Variation of the phosphotyrosine moieties of compound **3**

| $R_2$ | Compd | $IC_{50}$[a] (μM) |
|---|---|---|
| pYEEI | — | 6.5 |
| L-Tyr(NBOC)OPO(OBn)(OH) | **3a** | 128 |
|  | **4a** | 339 |
|  | **4b** | 27 |
|  | **4c** | 23 |
|  | **4d** | 32 |
|  | **4e** | 5 |
|  | **4f** | 20 |

[a]$IC_{50}$'s of inhibition determined using the FP assay[9] in a medium containing 2% DMSO.

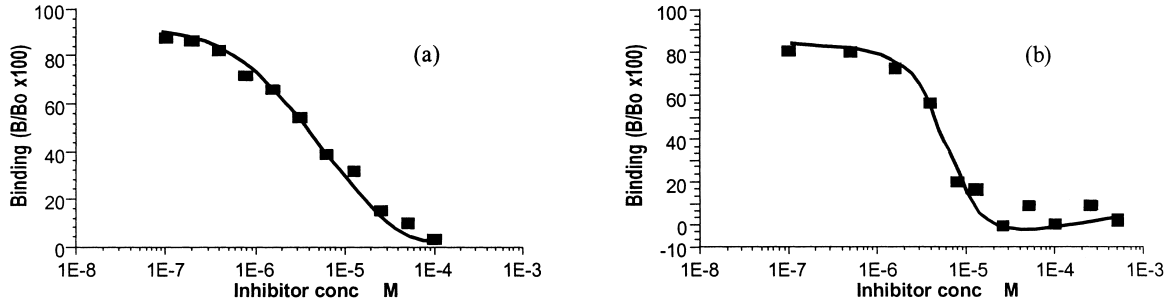


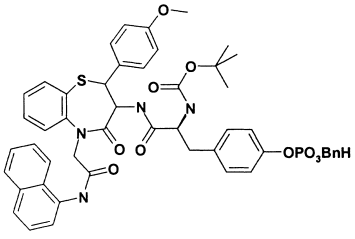

**Figure 1.** Displacement curves of pYEEI from Src SH2 by pYEEI (a) and **4e** (b) using fluorescence polarization assay.

In this series, monobenzylphosphates appeared with better binding affinities than the corresponding free phosphates (i.e., compare **3k** with its deprotected analogue **3o** in Table 4) and molecular modeling suggested that this could arise from an additional beneficial hydrophobic interaction around the phosphotyrosine binding pocket (data not shown). However, the monobenzylphosphate ester of the pYEEI peptide was synthesized and its very poor binding affinity for Src SH2 ($\pm 100$ μM) further suggested that the binding mode of the nonpeptidic inhibitors was substantially different from the peptides.

Molecular modeling suggested that neither of the two aromatic rings on the benzothioazepinone moiety were involved in the binding, so in order to lower the lipophilicity of the compounds, we embarked in the synthesis of scaffolds **5**[12] and **7**[13] devoid of one and the other aromatic rings, respectively. Benzothioazepinones **5** and **6** were prepared from orthofluoronitrobenzene and L- or D-cysteine, respectively, as described[12] while the thioazepinone **7** was prepared via a one-step condensation between L-cysteine and aminochloroethane as reported.[13] From **5**, **6** and **7**, the synthesis of **8**, **9**, **10** and **11** was straightforward (Scheme 3).

In the meantime, because our FP assay was at its detection limits and required high concentrations of compounds giving rise to solubility problems, we developed a more sensitive binding assay using SPA technology.[14] In this assay, the pYEEI reference peptide

**Table 3.** Binding of the four isomers of **3c** with Src SH2



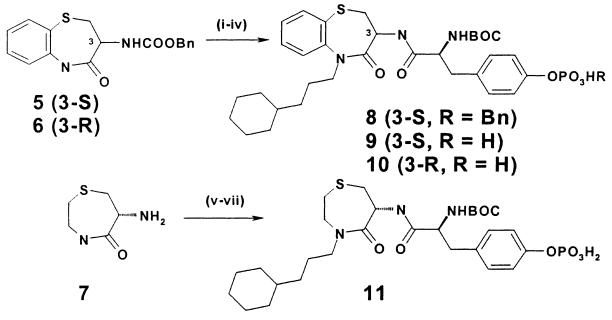

| Isomer | Compd | $IC_{50}$[a] (μM) |
|---|---|---|
| *trans iso* 1 | **3c** | 189 |
| *trans iso* 2 | **3l** | 133 |
| *cis iso* 1 | **3m** | 26 |
| *cis iso* 2 | **3n** | 23 |

[a]$IC_{50}$'s of inhibition determined using the FP assay[9] in a medium containing 2% DMSO.

**Table 4.** Removal of the aromatic rings from compound **3k**

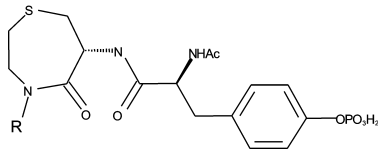
| Compd | $IC_{50}$[a] (μM) | $IC_{50}$[b] (μM) | $IC_{50}$[c] (μM) |
|---|---|---|---|
| pYEEI | 6.5 | 5 | 0.15 |
| **3k** | 6.4 | 28 | >100 |
| **3o** | 20 | 164 | nd |
| **8** | 26 | 87 | 13 |
| **9** | 10 | 11 | 1 |
| **10** | 422 | nd | 18 |
| **11** | 115 | nd | 1.4 |

[a]FP assay using 2% DMSO in the buffer solution.[9]
[b]FP assay using 20% DMSO in the buffer solution.[9]
[c]SPA assay.[14]




**Scheme 3.** (i) $C_6H_{11}(CH_2)_3I$, NaH, DMF (100%); (ii) HClg, AcOH then $NaHCO_3$ (50%); (iii) L- or D-BOC-Tyr-O($PO_3Bn_2$), EDC, HOBt, DMF, $CH_2Cl_2$, Hunig's base, DMF; (iv) NaI/acetone (72%) (**8**) or $H_2$, Pd–C, MeOH (74%) (**9** or **10**). (v) L-BOC-Tyr-O($PO_3Bn_2$), EDC, HOBt, DMF $CH_2Cl_2$, Hunig's base; (vi) $C_6H_{11}(CH_2)_3I$, NaH, DMF (16%); (vii) $H_2$, Pd–C, MeOH (94%).

**Table 5.** Optimization of the hydrophobic moiety on compound




| R | Compd | $IC_{50}$[a] (μM) |
|---|---|---|
| $CH_2COPhNMe_2$ | **12** | 9.3 |
| $CH_2PhOCF_3$ | **13** | 0.4 |
| $CH_2PhCF_3$ | **14** | 1.6 |
| $CH_2PhCOPh$ | **15** | 1.6 |
| $CH_2PhPh$ | **16** | 0.09 |
| $CH_2PhClOCH_2O$ | **17** | 1.2 |
| $CH_2PhCOOMe$ | **18** | 0.13 |
| $CH_2PhPh$-2-CN | **19** | 0.3 |

[a]SPA assay.

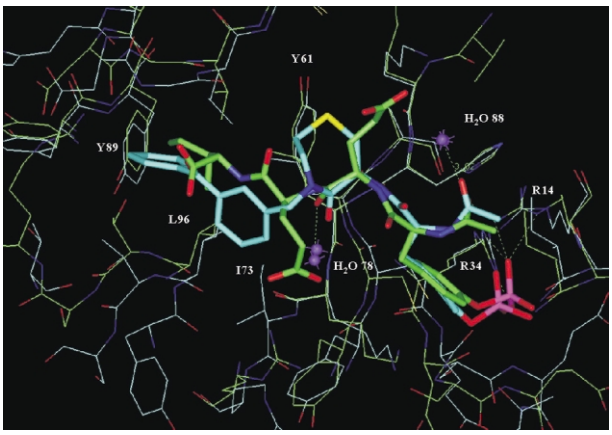



**Figure 2.** X-ray of **15** (turquoise) superimposed with pYEEI.[11]

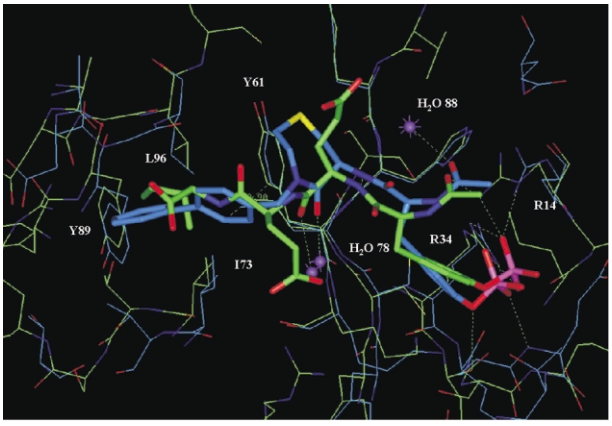



**Figure 3.** X-ray of **16** (blue) superimposed with pYEEI.[11]

gives an $IC_{50}$ about 40-fold lower than in the FP assay, partly because the protein concentration was lowered. The compounds were validated in this assay (Table 4). To our surprise, compound **3k** that displayed a binding affinity comparable to the one of pYEEI in the FP assay, appeared almost inactive in the SPA assay. Compound **8** devoid of the 2-position aromatic ring displayed a slightly lower binding affinity in the FP assay, however its affinity in the SPA binding assay showed a 10-fold improvement suggesting that this assay was better suited to differentiate specific binding from unspecific hydrophobic interactions. In addition, for the first time in these series, the free phosphate **9** demonstrated better binding affinity than the monobenzylphosphate ester (compare **9** and **8** vs **3o** and **3k**) which suggested binding was taking place in the same way as for pYEEI. Also, this new compound now did not present any difference in binding affinity between the 2 and 20% DMSO conditions in the FP assay which also suggested that the lipophilic component of the binding was less important. In addition, Hill coefficients for the binding curves were around 1 suggesting specific displacement (data not shown). Finally, the analogue **10** incorporating a D stereochemistry at the C3 position was synthesized and found about 20-fold less active than the L-analogue **9**, confirming a specific binding mode. Removing the fused aromatic ring afforded compound **11** with approximately the same binding affinity in the SPA binding assay as **9**. These series of lower molecular weight and lipophilicity were chosen for further optimization.

Exploration of the hydrophobic moiety was revisited. The compounds described in Table 5 have been synthesized in an analogous fashion as described above, albeit using solid-phase chemistry.[15] Aside from the *para*-amino substituted aromatic ring which gave rise to lower binding affinity (**12**), the other substituents gave comparable or better $IC_{50}$'s with Src SH2. Best affinity was obtained with the biphenylmethyl substitution, with an $IC_{50}$ of inhibition of 90 nM (**15**).

The binding mode of some of these compounds with Src SH2 was established by X-ray studies. In particular, compounds **15** and **16** (Figs 2 and 3) gave rise to crystals with a 1.8 Å resolution. The comparison of both crystals with pYEEI revealed that the inhibitors indeed bind Src SH2 in the same way as the peptide with main interactions between the phosphate and Arg14 and 34 and in the hydrophobic pocket with Tyr89, Ile73 and Leu96. Both inhibitors are deeper in the pocket and make a closer contact with these amino acids than the +3 Ile of pYEEI. The +1 carbonyl interacts with the protein backbone through a water molecule (78) and the same type of interaction is found in both crystals with the thioazepinone carbonyl. The thioazepinone ring mimics the hydrophobic contact between Y61 and the +1 chain. The better affinity of **16** versus **15** could be accounted for by the additional hydrophobic interaction between the first phenyl ring and Tyr61.

## Conclusion

A family of Src SH2 inhibitors was designed starting from the benzothioazepinone scaffold found in the cardiovascular drug diltiazem. From there, simpler and more specific nanomolar inhibitors were discovered. X-ray analysis established these inhibitors bound to Src SH2 in the predicted mode.

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