Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1291–1294

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Discovery of Highly Potent Src $SH_2$ Binders: Structure–Activity Studies and X-ray Structures

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Received 23 October 2001; accepted 19 February 2002

**Abstract**—Optimization of the hydrophobic moiety of caprolactam/thiazepinone based compounds led to the identification of potent Src $SH_2$ binders in two different series incorporating a phosphotyrosine group (RU 81843) or a phosphobenzoic group (RU 79181). The X-ray co-structures with the Src $SH_2$ domain revealed different binding modes for RU 81843 and RU 79181, and an excellent fit between RU81843 and the Src $SH_2$ protein thus explaining its high potency (9 nM, 15-fold more potent than pYEEI reference peptide). 

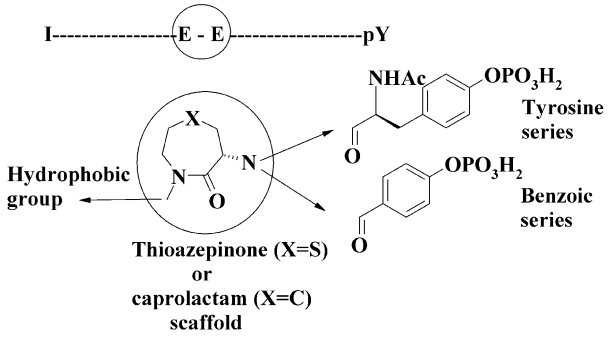
The pp60 Src protein tyrosine kinase[1] has become an attractive target following knock out experiments[2] which indicated impaired osteoclast resorption resulting to osteopetrosis. The Src protein contains several domains, including $SH_2$ (approximately 100 amino acids) and $SH_3$ domains involved in protein–protein interactions.[3] With the aim of finding a drug candidate for the treatment of osteoporosis, we have attempted to inhibit the Src protein via the binding of non-peptidic ligands to the $SH_2$ domain of the Src protein.

Examination of the X-ray structures[4] of the complex between the known pYEEI tetrapeptide ligand and the Src $SH_2$ domain revealed the presence of two major binding pockets, one interacting with the pY and the other with pY + 3 Ile residue. Between these two pockets, the two glutamate EE residues do not make strong interactions with the peptide backbone. From rational drug design, new ligands less peptidic than pYEEI but with a similar binding potency have been recently identified [5–8] and we have also described the identification of the seven-membered ring thioazepinone scaffold as a promising EE surrogate that proved able to deliver its two substituants in the respective pTyr and pY + 3 pockets.[9]

In this paper, we would like to report how we discovered nM Src $SH_2$ binders from two similar seven-membered-ring scaffolds: the thioazepinone (X = S) and caprolactam ($X = CH_2$) scaffolds. These ligands were obtained by optimization of the substituant interacting with the hydrophobic pocket. Moreover, to be as far as possible from a peptidic structure and taking into account some published Src $SH_2$ ligands incorporating the benzoic moiety,[6c] we decided to explore the replacement of the phosphotyrosine with a phosphobenzoic group (Scheme 1).

## Chemistry

The final compounds **4** were prepared using solution-phase or solid-phase parallel synthesis, as shown in Scheme 2. The introduction of the hydrophophic group




**Scheme 1.**

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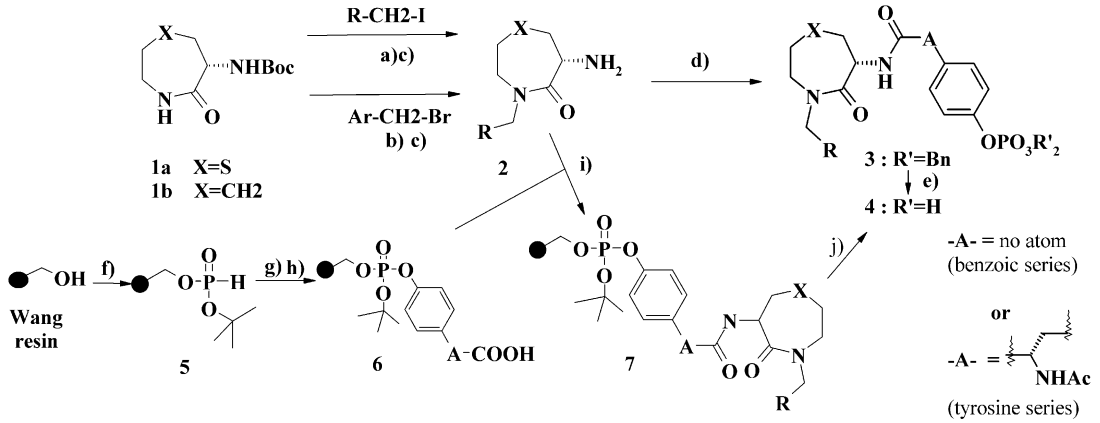



**Scheme 2.** Solution- and solid-phase synthesis of phosphate Src $SH_2$ ligands **4**. Reagents and conditions: (a) NaH (1.1 equiv) DMF 1 h at 0 °C, then add RX (1.1 equiv), overnight at rt or 4 h at 60 °C (35–65% yield); (b) Ar–$CH_2$–Br (1 equiv), KOH (1.1 equiv), $Bu_4NI$ (0.1 equiv), THF, overnight at rt (70–90% yield); (c) $CH_2Cl_2$/TFA 2:1 2 h at rt; (d) Ar–A–COOH, BOP, DIEA, $CH_2Cl_2$, 3 h at rt; (e) H2, Pd/C, MeOH, overnight; (f) see ref 12; (g) $CCl_4$, DMAP, HO–Ph–A–COOAll, 1 h at rt; (h) $Pd(P(Ph)_3)_4$, DMF/AcOH/NMM (9:1:0.15) 1.5 h; (i) **2**; DIC, HOBt, $CH_2Cl_2$/DMF overnight; (j) $CH_2Cl_2$, 10% TFA, 30 min.

was achieved through an alkylation of the amide function of the starting *N*-Boc protected amino thioazepinone[10] **1a** and caprolactam **1b**, respectively.[10] Two procedures were used depending on the nature of the alkylating agent: benzyl halide or alkyl halide. For the more reactive benzyl halides, an efficient heterogeneous alkylation (KOH in THF)[11] proved to be very convenient for parallel synthesis and led to the desired alkylated amide in good yield (>70% after purification on silica cartridges) under mild conditions. Unfortunately, this procedure was unsuccessful for alkyl halides (including alkyl iodides) so we used the standard NaH method, albeit less convenient for parallel synthesis. Due to the various reactivities of the substituted alkyl halides, we decided to convert all the selected alkyl halides to alkyl iodides and thus the reaction of amide alkylation with NaH was performed in parallel fashion with reasonable yields. After deprotection of the *N*-Boc protecting group with trifluoroacetic acid, the crude amine **2** was coupled directly with benzoic acid dibenzylphosphate or with NAc dibenzyl phosphotyrosine using the standard BOP coupling procedure. The resulting protected phosphate **3**, was finally treated under hydrogenolysis conditions to give the desired free phosphate **4**.

An alternative solid-phase procedure was also developed based on the DIC coupling between the amino scaffold **2** and the carboxylic acid supported phosphate **6**. The resulting compound **7** was finally cleaved off the resin with a rapid TFA treatment to generate the desired phosphate **4**, which was further purified using preparative HPLC (reverse phase). The method of preparation of the phosphate bound compound **6** was performed through the reaction between the phosphite **5**[12] and the phenol moiety of NAc Tyrosine *O*-allyl (or 1-carboxy-4-hydroxy phenyl allyl ester) in the presence of $CCl_4$, as developed recently in our laboratory,[13] followed by an allyl ester deprotection with palladium.

## Results and Discussion

The solution- and solid-phase syntheses led to the preparation of dozens of compounds resulting from the combination of the three fragments: caprolactam or thioazepinone scaffold, phosphobenzoic or phosphotyrosine head group and various hydrophobic residues. A selection of representative compounds is listed in Table 1 and all the purified phosphates were tested in a competition assay (scintillation proximity assay, SPA),[15] with the pYEEI tetrapeptide as a reference (150 nM).

The lactam substituants have been selected with the help of molecular modeling: knowing that the hydrophobic pocket was rather deep, it appeared that a spacer was needed to link the scaffold with a hydrophobic fragment located at the bottom of the pocket. Different spacers (alkyl/benzyl/allyl) bearing aromatic or aliphatic hydrophobic substituents have been chosen.

In the tyrosine series [with A = CH(NHAc)$CH_2$], (entries 1–18), significant modifications of the binding affinity (from μM to nM) were observed depending on the hydrophobic tail, indicating the crucial role of this specific interaction for a potent binding affinity. Thus, a three-carbon atom spacer (entries **1** and **2**) appeared to be the right length with a phenyl hydrophobic residue.

The benzyl group was also evaluated and substitution at the *para* position of the benzyl group proved to be critical to the binding affinity, with a 1000-fold difference depending on the substituent: a low potency with too short (**3**, **4**) or too bulky substituents (**5**, **6** and **7**) and a higher potency with a *para* propyl group (**8**). A dramatic improvement was observed with a phenyl substituent on the benzyl group (**10** for RU 81843, 9 nMol, 15-fold more potent than the parent pYEEI) and we thus focussed on various biaryl compounds (**11**–**18**). It turned out that the substitution on the second phenyl

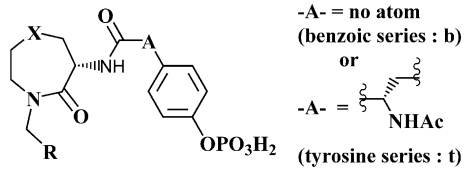
ring was also very sensitive, indicating its close contact with the protein surface (as also shown by the X-ray structure of the complex, Fig. 1). Thus, most of the substituted phenyl groups appeared to be detrimental (dramatically in the *para* position with **14** and **15** at μM level), except when replaced with a thiophene ring (**18**; 3 nM).

As a general rule, we observed that the compounds with the caprolactam scaffold (X = $CH_2$) are 10-fold more potent than those obtained with the thioazepinone scaffold (X = S), (**10** vs **11** and **12** vs **13**), indicating significant contribution of the scaffold itself to the binding.

In the benzoic series also (with A = no atom), a series of compounds has been prepared. However, in this case, we were unsuccessful at improving μM activity. It is interesting to note some SAR similarities between the two series. The best substituent is the biphenyl moiety in both cases (entry **10** and **27**), but with a 100-fold difference in potency. It seems that in the benzoic series, the potency levels off at the μM level.

In the course of this project, we obtained several X-ray structures of Src $SH_2$/ligand complexes from Src $SH_2$/citrate crystals, using soaking methodology.[16] Among them, the structures of **10** (RU 81843, tyrosine series) and **21** (RU79181 benzoic series) have been solved (Figs 1 and 2).

**Table 1.** Binding affinity of Src $SH_2$ ligands




| Entry | A[a] | X = | $CH_2R$ | $IC_{50}$ (nM)[b] |
|---|---|---|---|---|
| 1 | t | C | $(CH_2)_3Ph$ | 290 |
| 2 | t | C | $CH_2$–HC=CH–Ph(*trans*) | 280 |
| 3 | t | C | $CH_2$Ph–3OMe | 1700 |
| 4 | t | C | $CH_2$Ph–3,5$CF_3$ | 1800 |
| 5 | t | C | $CH_2$Ph–4*t*Bu | 2300 |
| 6 | t | C | $CH_2$Ph–4c hexyl | 2040 |
| 7 | t | C | $CH_2$Ph–4Bu | 7800 |
| 8 | t | C | $CH_2$Ph–4Pr | 150 |
| 9 | t | C | $CH_2$–Ph–4COOMe | 58 |
| 10 | t | C | $CH_2$–Ph–4Ph | 9 |
| 11 | t | S | $CH_2$–Ph–4Ph | 87 |
| 12 | t | C | $CH_2$Ph Ph 2′ CN | 26 |
| 13 | t | S | $CH_2$Ph Ph 2′ CN | 315 |
| 14 | t | C | $CH_2$Ph Ph 4′ Me | 2160 |
| 15 | t | C | $CH_2$–Ph–4Ph4′ Cl | 1300 |
| 16 | t | C | $CH_2$–Ph–4Ph2′ 4′ diF | 334 |
| 17 | t | C | $CH_2$–Ph–4 (1naphtyl) | 143 |
| 18 | t | C | $CH_2$–Ph–4 (2thienyl) | 3 |
| 19 | b | C | $(CH_2)_3Ph$ | 4500 |
| 20 | b | C | $CH_2$–HC=CH–Ph(*trans*) | 2300 |
| 21 | b | S | $CH_2$–HC=CH–Ph(*trans*) | 2800 |
| 22 | b | C | $CH_2$–Ph–4CO Ph | 3000 |
| 23 | b | S | $CH_2$–Ph–4CO Ph | 18,500 |
| 24 | b | S | $CH_2$–Ph–3,5 $CF_3$ | 9800 |
| 25 | b | C | $CH_2$–Ph–3,5 $CF_3$ | 10,400 |
| 26 | b | S | $CH_2$–Ph–4Ph | 11,900 |
| 27 | b | C | $CH_2$–Ph–4Ph | 1500 |
| 28 | b | S | $CH_2$–Ph–4Ph 2′ CN | 2460 |
| 29 | b | C | $CH_2$–Ph Ph 2′ CN | 2110 |
| 30 | b | C | $CH_2$–Ph–4Ph 4′ Me | 15,200 |

[a]b, for benzoic series; t, for tyrosine series.
[b]SPA binding assay:[15] reference peptide pYEEI: 150 nM.

With **10**, multiple and very close interactions can be observed which clearly explained its nM potency. The two most striking features in the X-ray structure are that: (1) The second phenyl ring of the biphenyl is going very deeply in the pocket (much more than the Ileu residue of pYEEI) and fits perfectly with the hydrophobic surface of the protein (in yellow). There are 15 distances shorter than 4 Å between the biphenyl moiety and the protein surface. (2) The caprolactam scaffold itself has a great influence on the binding affinity, lying very nicely on the surface generated by the tyrosine 61 residue (hydrophobic contact).

It also interacts with a structural water molecule via the carbonyl of the lactam group and finally H-bonding with a carbonyl of the protein backbone (H60) with the initial amino group of the aminocaprolactam. Besides these interactions, the contribution of the phosphotyrosine itself with the pY binding pocket is very similar to that of pY of pYEEI (not shown).

Also information rich is the X-ray structure of benzoic compound **21**. When superimposed with **10**, the two

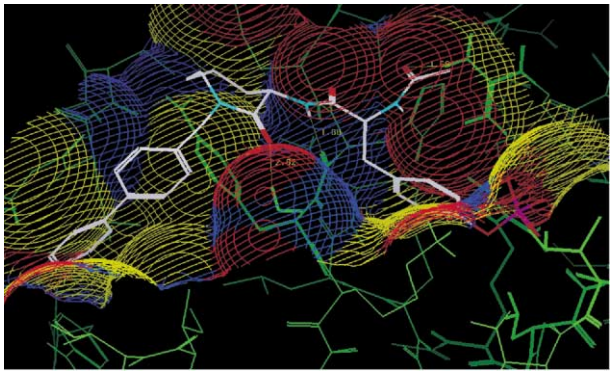


**Figure 1.** X-Ray structure of **10** (RU 81843) in Src $SH_2$ (QXP software:[14] yellow: hydrophobic, blue and red: H bond acceptor and donor).

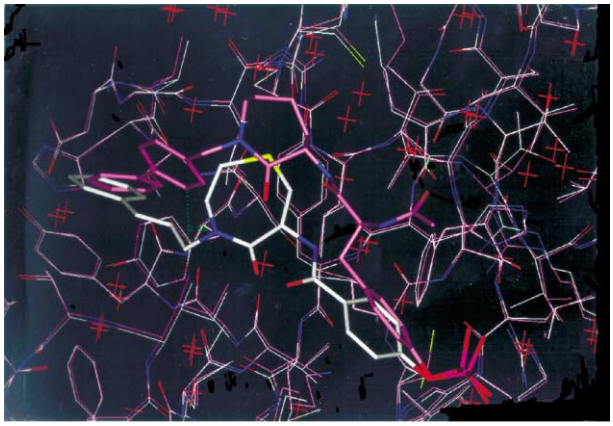


**Figure 2.** Superimposition of X-ray structures of **10** (RU 81843 pink) and **21** (RU 79181, white) within Src $SH_2$.

major Src $SH_2$ binding pockets are filled similarly, with a good superposition between the phosphate groups on one side and the phenyl groups at the bottom of the hydrophobic pocket on the other side (Fig. 2). As a consequence, with two carbon less, **21** adopts a 'short-cut' binding mode. However, here again, the seven-membered ring scaffold appears to be well designed since it is still lying on the surface generated by the tyrosine 61 residue.The structural water molecule (which was interacting with the lactam ring of **10**) is now displaced and replaced directly by the carbonyl of the lactam ring of **21**, interacting with the amide nitrogen of K62. This should be entropically favorable.

However, despite all the positive interactions, benzoic **21** is 300-fold less active than tyrosine **10**. One possible explanation could be that the additional NAc interaction present with **10** contributes significantly to the binding (the carbonyl of Nac interacts with Arg 14). In this case, the benzoic group is not a potent surrogate of the tyrosine group for the Src $SH_2$ protein.

In conclusion, we identified compound **10** RU 81843 as one of the most potent $SH_2$ binder known to date. Its caprolactam scaffold is able to efficiently deliver the biphenyl and pY substituents in the respective binding pockets, whilst also favorably interacting with the protein.

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