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NONPEPTIDIC SH2 INHIBITORS OF THE TYROSINE KINASE ZAP-70

Chi B. Vu,* Evelyn G. Corpuz, Selvaluxmi G. Pradeepan, Shelia Violette, Catherine Bartlett, and Tomi K. Sawyer

ARIAD Pharmaceuticals, Inc.

26 Landsdowne Street, Cambridge MA 02139-4234 , U.S.A.

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Abstract: The synthesis of a series of 1,2,4-oxadiazole analogs is discussed along with their ZAP-70 SH2 inhibitory activity. The tyrosine moiety in the original series has been replaced with nonpeptidic functional groups without a substantial loss of binding affinity.

The tyrosine kinase ZAP-70 plays a critical role in T-cell activation because of the ability of its Src homology-2 (SH2) domains[1] to mediate various intracellular processes. The tandem SH2 domains of ZAP-70 presumably bind to the intracellular, doubly phosphorylated ζ chain of the immunoreceptor tyrosine activated motifs (ITAMs) and thereby activate the kinase domain as well as a number of downstream signaling events leading to T-cell proliferation.[2] Any agents that could selectively bind to either of these SH2 domains would prevent ZAP-70 from triggering the early intracellular cascade and thus might be of utility in immune suppression. We have recently initiated synthetic efforts to identify novel SH2 inhibitors of the tyrosine kinase ZAP-70. With the aid of structural information,[3] we have been able to prepare a series of 1,2,4-oxadiazole analogs as highly effective ZAP-70 SH2 inhibitors.[4] Even though this series of compounds has been designed to be mimetics for the monophosphorylated tetrapeptide sequence found in ZAP-70, many of the best compounds are 200- to 400 fold more potent. These monodentate 1,2,4-oxadiazole analogs, in effect, represent some of the most selective ZAP-70 SH2 inhibitors that have been disclosed to date.[5] A small, representative set of compounds from this series is shown in Figure 1 (compounds **1a-1c**). These compounds all contain a phosphorylated tyrosine moiety, a pY+1 and a pY+3 group (R^1 and R^2 respectively). The 1,2,4-oxadiazole ring forms a spacer which directs the R^2 group into the lipophilic pY+3 pocket. In this paper, we wish to outline the synthesis and biological activity of series of ZAP-70 SH2 inhibitors in which the original tyrosine moiety has been replaced with a number of nonpeptidic functional groups.

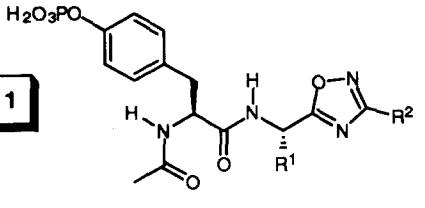

Figure 1

no.	R^1	R^2
1a	CH_3	$CH_2(m,p\text{-}Cl_2)Ph$
1b	CH_3	$CH_2(p\text{-}CF_3)Ph$
1c	CH_2OH	$CH_2(m,p\text{-}Cl_2)Ph$

*Corresponding author's present address: Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142.

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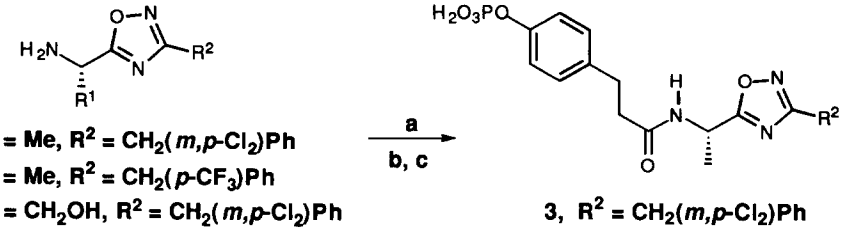
As discussed in the previous paper,[4] compounds **1a-1c** have been prepared by coupling the corresponding amine derivative **2a-2c** with Ac-Tyr(OPO_3Bzl_2)-OH using EDC/HOBT in the presence of Hunig's base. This is followed by treatment with 95% TFA, 5% H_2O in order to unmask the phosphate group. As shown in Table 1, compounds **1a-1c** show low micromolar binding affinity toward the tyrosine kinase ZAP-70. A high level of selectivity over other SH2-containing proteins such as Syk and Src is also been achieved with this series. The N-acetyl group of the tyrosine portion presumably provides a critical interaction with an Arg residue that forms cation-π interactions with the pTyr phenyl ring. This is present at the N-terminus as well as the C-terminus of the tandem SH2 ZAP-70. Interestingly, this same interaction is also present among other SH2 targets such as Src or Grb2. In Src SH2, monophosphorylated tetrapeptide sequences[6] and other effective peptidomimetics[7] all contain an acetyl N-cap. For Grb2, essentially all of the most effective SH2 inhibitors that have been reported to date contain this minimal N-acetyl tyrosine portion.[8] Compound **3** is prepared first to see how much influence this N cap has on the binding affinity toward the tandem ZAP-70. The 1,2,4-oxadiazole amine derivative **2a**[4] is coupled with 3-(4-hydroxyphenyl)propionic acid using EDC/HOBT in the presence of Hunig's base (Scheme 1). This is followed by a standard phosphorylation procedure.[9] As shown in Table 1, compound **3**, with an IC_{50} of 198 μM, is substantially less active against ZAP-70.[10]

Scheme 1



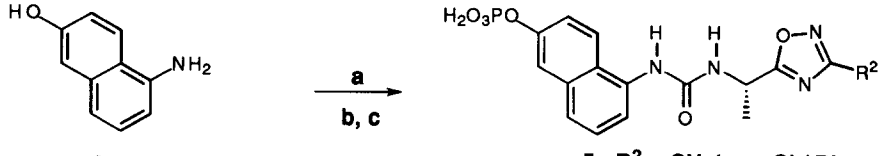
2a, R^1 = Me, R^2 = CH_2(*m,p*-Cl_2)Ph
2b, R^1 = Me, R^2 = CH_2(*p*-CF_3)Ph
2c, R^1 = CH_2OH, R^2 = CH_2(*m,p*-Cl_2)Ph

3, R^2 = CH_2(*m,p*-Cl_2)Ph

Reagents and conditions: (a) **2a**, EDC/HOBT/Hunig's base, 3-(4-hydroxyphenyl)propionic acid, CH_2Cl_2, DMF, rt (88%). (b) $(BnO)_2POH$, CCl_4, Hunig's base, DMAP (cat.), CH_3CN (98%). (c) 95% TFA, 5% H_2O.

In previous Src SH2 work, a number of urea derivatives have been found to be reasonable replacements for the tyrosine group.[11] One of the more effective urea replacements is the one deriving from 5-amino-2-naphthol. We have since installed this urea group onto our current ZAP SH2 inhibitors. As shown in Scheme 2, 5-amino-2-naphthol (**4**) is reacted with triphosgene and then with the 1,2,4-oxadiazole amine **2a**. The resulting urea derivative is then subjected to the standard phosphorylation conditions in order to afford compound **5** (Table 1, IC_{50} of 40 μM against ZAP-70). This urea derivative is about 7-fold less active than the corresponding Ac-pTyr derivative **1a**. This is consistent with the previous Src SH2 work where a similar 7-fold loss of binding affinity has also been observed.[11]

Scheme 2

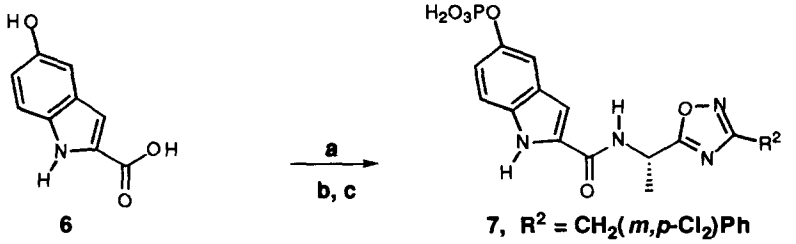


4

5, R^2 = CH_2(*m,p*-Cl_2)Ph

Reagents and conditions: (a) Triphosgene , CH_2Cl_2, Hunig's base, **2a**, rt (65%). (b) $(BnO)_2POH$, CCl_4, Hunig's base, DMAP (cat.), CH_3CN (96%). (c) 95% TFA, 5% H_2O.

Scheme 3



6 **7, $R^2 = CH_2(m,p\text{-}Cl_2)Ph$**

Reagents and conditions: (a) **2a**, EDC/HOBT/Hunig's base, CH_2Cl_2, DMF, rt (80%). (b) $(BnO)_2POH$, CCl_4, Hunig's base, DMAP (cat.), CH_3CN (92%). (c) 95% TFA, 5% H_2O.

One remarkably simple and yet effective replacement for the tyrosine moiety is the one deriving from 5-hydroxy-2-indolecarboxylic acid (**6**). So far, the use of indole as replacement for the tyrosine group has not been prevalent among the current SH2 literature. Nevertheless, this substitution appears to be fairly favorable for some of our Src SH2 inhibitors.[12] The corresponding ZAP-70 SH2 inhibitor bearing this substituted indole is then prepared as outlined in Scheme 3 using standard conditions. As shown in Table 1, compound **7**, with an IC_{50} of 30 μM against ZAP-70, is just about 5-fold less active than the corresponding Ac-pTyr derivarive **1a**. The naphthyl urea and indole derivatives thus represent some reasonable achiral replacements for the tyrosine group. Reasonable selectivity against Syk and Src has also been maintained for both of these substitution patterns.

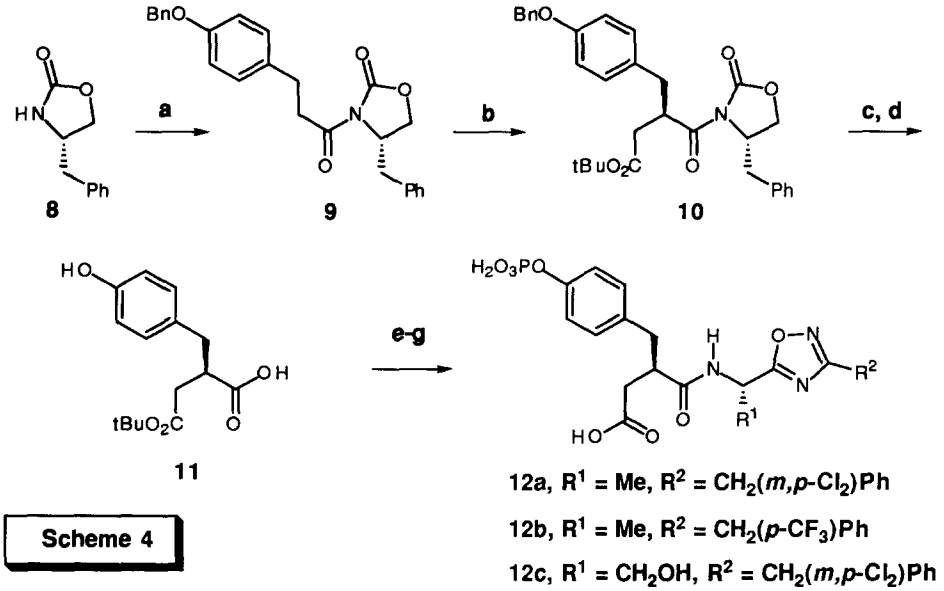
Table 1. SH2 binding of 1,2,4-oxadiazole analogs

no.	IC_{50} (μM) ZAP-70	IC_{50} (μM) Syk	IC_{50} (μM) Src	no.	IC_{50} (μM) ZAP-70	IC_{50} (μM) Syk	IC_{50} (μM) Src
1a	7	>500	210	**7**	30	434	81
1b	6	>500	72	**12a**	18	>500	53
1c	4	>500	75	**12b**	22	>500	102
3	198	>500	459	**12c**	7	>500	54
5	40	310	198	**18**	19	>500	205

All IC_{50}s reported in Table 1 are average of triplicate measurements. Binding assays are carried out using the procedure outlined in reference 13. In all runs, the positive controls are **1b** and the native ζ-1-ITAM peptide (Ac-NQL-pYNELNLRREE-pYDVLD-NH_2). The IC_{50} of the native ζ-1-ITAM peptide has been determined to be 0.038 μM against ZAP-70.

Perhaps one of the more closely related compounds to tyrosine would be compound **11**. Here, the carboxyl group would mimic the carbonyl group of the N-acetyl and thus offer the same interactions with the Arg residue that forms cation-π interactions with the pTyr phenyl ring. This type of tyrosine replacement has been used before in some Src SH2 work[14] and Scheme 4 outlines our synthesis of optically active **11** using Evans' chemistry.[15] Commercially available (*S*)-(-)-4-benzyl-2-oxazolidinone is acylated with 3-(4-benzyloxy)phenyl-propionyl chloride[16] to afford compound **9**. This is treated with LDA at -78 °C, followed by quenching with *tert*-butyl bromoacetate to yield compound **10** in high yield and de.[17] The chiral auxiliary is then removed using

BnOH/nBuLi in THF at 0 °C. Subsequent hydrogenation at atmospheric pressure over 10% Pd on C affords **11** in good overall yield. Compound **11** is coupled individually with amines **2a-2c** and then phosphorylated under standard conditions in order to obtain 1,2,4-oxadiazole derivatives **12a-12c**, respectively. As shown in Table 1, this type of tyrosine replacement is acceptable and only a slight loss of ZAP-70 activity is observed.

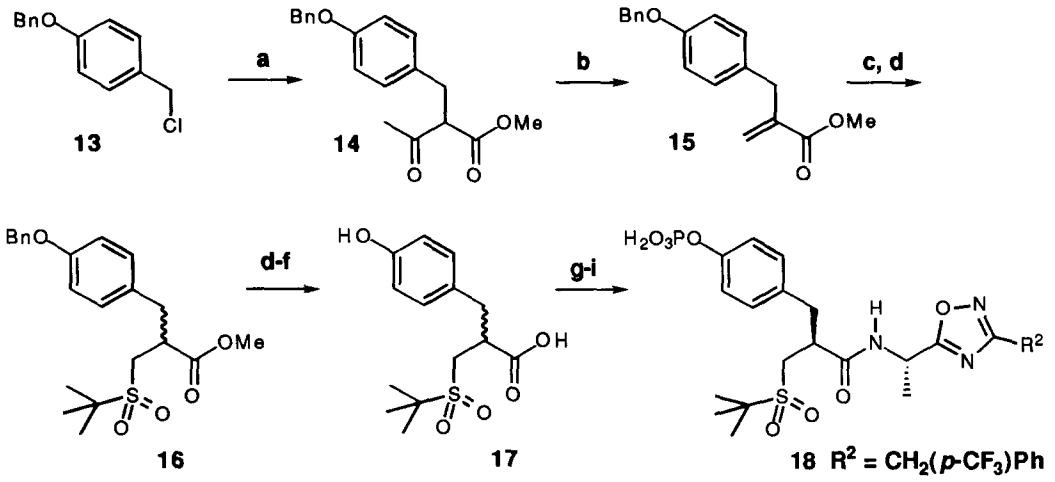


Scheme 4

Reagents and conditions: (a) *n*BuLi, THF, 3-(4-benzyloxy)phenylpropionyl chloride (98%). (b) LDA, THF, -78 °C; *tert*-butyl bromoacetate (85% yield, >99% de). (c) BnOH, *n*BuLi, THF, 0 °C (84%). (d) H_2, 10% Pd on C, rt (96%). (e) EDC, HOBT, Hunig's base, **2a**, **2b**, or **2c** (75-82%). (f) $(BnO)_2POH$, CCl_4, CH_3CN, Hunig's base, DMAP (cat.) (92-96%). (g) 95% TFA, 5% H_2O.

The successful use of **11** as a replacement for tyrosine is reminiscent of some earlier work on renin inhibitors[18] and HIV proteases.[19] In the renin work, a Boc-Phe group was successfully replaced with a number of nonpeptidic groups including 2-benzyl-3-(*tert*-butylsulfonyl)propionic acid. We were interested in this type of sulfone derivative because of its uncharged nature. Scheme 5 outlines our synthesis of the racemic sulfone derivative **17**. Methyl acetoacetate is first alkylated with 4-benzyloxybenzyl chloride (**13**). The resulting compound **14** is treated with LDA at -78 °C, followed by quenching with paraformaldehyde. Mild thermolysis of the intermediate alcohol (refluxing THF) results in the formation of the α,β-unsaturated derivative **15**. Upon treatment with 2-methyl-2-propanethiol (catalytic NaH), a Michael addition takes place to afford an intermediate sulfide, which in turn, is conveniently oxidized to the corresponding sulfone **16** using OXONE®. The benzyl group is removed by standard hydrogenation over 10% Pd on C. Since this sulfone derivative is sensitive to base hydrolysis, the methyl ester group is removed by heating in strong acid (6 N HCl/glacial HOAc). The resulting acid derivative **17** is coupled with amine **2b** and phosphorylated under standard conditions. Preparative, reverse-phase HPLC is then used to separate the desired diastereomer **18**. As shown in Table 1, compound **18** shows comparable ZAP-70 activity to the carboxyl derivative **12b**.

Scheme 5



Reagents and conditions: (a) NaOMe, methyl acetoacetate (60%). (b) LDA, THF, -78 °C; paraformaldehyde, -78 °C to rt; reflux (56%). (c) 2-Methyl-2-propanethiol, EtOH, cat. NaH . (d) OXONE® (45% from **15**). (e) H_2, 10% Pd on C, EtOAc, rt. (f) 6 N HCl, glacial acetic acid, reflux (75% from **16**). (g) EDC, HOBT, Hunig's base, **2b** (86%). (h) $(BnO)_2POH$, CCl_4, CH_3CN, Hunig's base, DMAP (cat.) (94%). (i) 95% TFA, 5% H_2O. Separation of diastereomers by reverse-phase HPLC.

In summary, we have shown how to replace the tyrosine moiety of the original 1,2,4-oxadiazole series with a number of useful functional groups. Even though these modifications result in a small loss of ZAP-70 binding affinity, they have removed most of the peptidic nature of our inhibitors. The extra lipophilicity imparted by the sulfone group might also be advantageous for our future cellular work where membrane-permeability must be addressed.

In vitro binding assays: Further details of using fluorescence polarization to monitor ligand binding to protein is discussed in reference 13. Compounds were assayed as 1:2 dilutions over a range of concentrations from 1000 μM to 0.04 μM. Results are shown as mean IC_{50} values based on three or more separate experiments. Binding assays were carried out on a Jolly FPM2 96-well plate reader with standard cutoff filters (excitation λ = 485 nm, BP = 22; emmision λ = 530 nm, BP = 30). Compounds were serially diluted in buffer solution (100 mM NaCl, 20 mM phosphate, pH 7.4, 10 mM dithiothreitol, 2% DMSO, 1 mM EDTA, and 0.1% bovine gamma globulin) and then added to 25 nM of tandem ZAP SH2 domain protein, 25 nM Syk SH2 domain protein or 150 nM Src SH2 domain protein premixed with 20 nM of the corresponding fluorescein conjugated peptide. Binding reactions were carried out for 5 minutes at room temperature. The fluoroscein conjugated peptide used to monitor Src SH2 binding was Fluoro-pYpYpYIE-NH_2, the probe used to monitor ZAP tandem SH2 binding was Fluoro-GpYNELNLGRREE-pYDVL-NH_2, and the probe used to monitor Syk binding was Fluoro-ApYTGLSTRN-QETpYETL-NH_2. Complete saturation binding curves were initially carried out with a fixed concentration of peptide and increasing concentrations of protein to determine the K_d of the protein/peptide binding interaction. IC_{50} values were calculated based on the % binding of the fluorescein conjugated peptide protein with compound addition relative vehicle alone control samples.

Protein preparation: Details for preparing tandem ZAP-70 SH2 , tandem Syk SH2, and Src SH2 domain proteins are provided in reference 4.

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