Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 529–532

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND BIOLOGICAL EVALUATION OF α,α-DIFLUOROBENZYLPHOSPHONIC ACID DERIVATIVES AS SMALL MOLECULAR INHIBITORS OF PROTEIN-TYROSINE PHOSPHATASE 1B

Tsutomu Yokomatsu,[a,*] Tetsuo Murano,[a] Ikuko Umesue,[b] Shinji Soeda,[b] Hiroshi Shimeno,[b,*] and Shiroshi Shibuya[a]

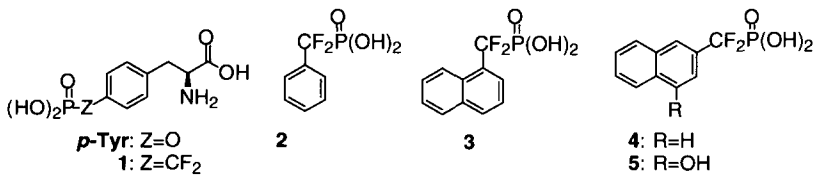
[a]School of Pharmacy, Tokyo University of Pharmacy & Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan
[b]Faculty of Pharmaceutical Science, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

Received 13 November 1998; accepted 28 December 1998

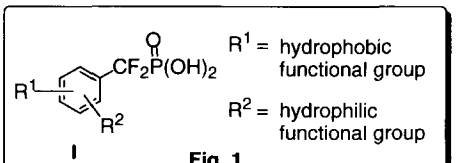
Abstract: A series of α,α-difluorobenzylphosphonic acids having a hydrophobic functional group were prepared *via* the Stille coupling reaction from halogenated α,α-difluorobenzylphosphonates. Evaluation of inhibitory activity toward protein tyrosine phosphatase (PTP 1B) revealed that the ethynyl, phenylethynyl and (*E*)-styryl groups on the benzene nuclei increased the inhibitory activity of α,α-difluorobenzylphosphonic acid. Inhibitory activities significantly increased upon introducing both (*E*)-styryl and bis-methylsulfonamide functional groups onto the benzene nuclei of α,α-difluorobenzylphosphonic acid.

Keywords: enzyme inhibitors; phosphonic acids and derivs; isosteres; mimetics; substituent effects

Protein tyrosine phosphatases (PTPs) catalyze the dephosphorylation process of the phosphotyrosine residue (*p*-Tyr) in proteins and play a regulatory role in many cellular processes such as cell proliferation and differentiation.[1] PTPs are involved in a variety of disease states as positive signal transducers.[2] Because of their potential value as therapeutic agents, reports describing the design and synthesis of PTP inhibitors have been recently accumulating.[3,4] Peptides containing (phosphonodifluoromethyl)phenylalanine (F_2Pmp) **1**, a non-hydrolyzable phosphotyrosine mimic, were shown to have significant inhibitory activity with Ki in the nanomolar region toward PTPs.[3c] Although the peptidyl inhibitors are useful in determining the properties of PTPs such as enzyme-substrate interaction, their utility as therapeutics is limited. Therefore, recent studies have focused on the preparation of non-peptidyl aromatic derivatives having a difluoromethylenephosphonate moiety, which act as a PTP inhibitor without a peptidyl framework.[5,6] Burke *et al.* found that, while difluorobenzylphosphonic acid **2** had no inhibition potency for PTP 1B, some naphthalene derivatives **3-5** bearing a difluoromethylenephosphonate moiety could bind to the catalytic site of PTPs and inhibit the enzyme activities competitively.[5] However these inhibitors were approx. 1000-fold less potent than the peptidyl inhibitors, and their poor specificity for PTP 1B remained to be improved.



X-ray crystallographic analysis of the complex of PTP 1B with **4** revealed that, together with importance of the fluorine atom in binding, the B ring of the naphthalene participated in hydrophobic interaction with aromatic amino acid residues in the active sites.[7] Moreover, the phenolic hydroxyl group introduced at 4-position of the naphthalene nuclei was found to enhance its binding affinity for the catalytic site of the enzyme *via* the interaction with Tyr 46 and Lys 120.[7]



While recent studies have focused on the modification of the naphthalene derivatives **3-5**,[8,9] we presumed that the inhibition potential of **2** would increase upon modification to difluorobenzylphosphonates of type **I** (Fig.

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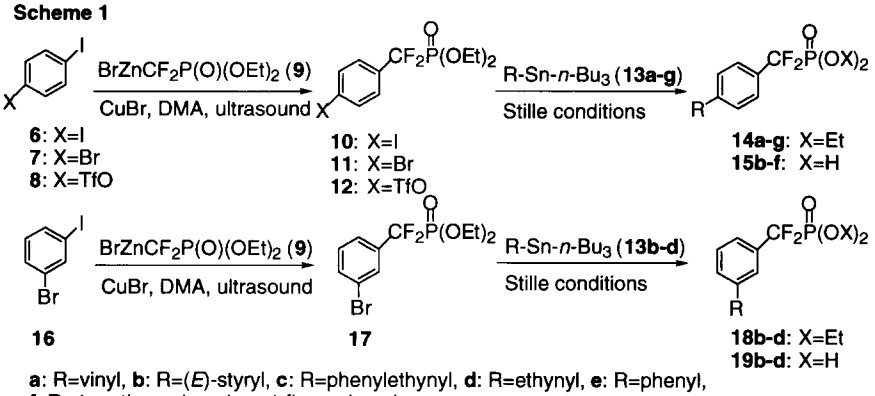
PII: S0960-894X(99)00027-X

1), where a hydrophobic group (R^1) might have the same function as the naphthalene B-ring. The introduced hydrophilic functional group (R^2) may increase the stability of the ligand/enzyme complex by interaction with the Tyr 46 and Lys 120 residues. In this communication, we describe a facile preparation of a series of difluorobenzylphosphonates related to **I** and their inhibitory effects on PTP 1B.

Our first target was to develop a divergent sequence for the synthesis of difluorobenzylephosphonates having a variety of hydrophobic functional groups at either the *para*- or *meta*-position (Scheme 1). Then, halogenated difluorobenzylphosphonates **10**, **11** and **17**, or the corresponding triflate **12** were respectively prepared by the copper bromide-promoted coupling reaction of the zinc reagent **9** and aryl iodides **6-8** and **16** in dimethylacetamide (DMA) according to the method recently developed in our laboratories[10] [Yield: **10**: 52%,[10a] **11**: 81%, **12**: 82%, **17**: 82%].[11] The Stille coupling reaction of *para*-substitued difluorobenzylphosphonates **10**, **11**, and **12** with a tri-*n*-butylvinyltin reagent **13a** was examined under the representative Stille conditions[12,13] to verify the optimal catalytic systems for these reactions. Treatment of iodide **10** with **13a** (1.3 equiv.) in the presence of 2 mol% of bis(triphenylphosphine)palladium(II) chloride ($PdCl_2(PPh_3)_2$) in refluxing acetonitrile[13] for 12 h gave the desired coupling product **14a** in 62% yield. While the bromide **11** unreacted under the conditions, **14a** was obtained in 86% yield upon treatment of **11** with **13a** in the presence of tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) (2 mol%) in acetonitrile under reflux. The triflate **12** proved to be a poor substrate for the Stille coupling reaction, since the yield of **14a** from **12** under the representative conditions with $PdCl_2(PPh_3)_2$ (2 mol%) and LiCl (3.0 equiv.) in refluxing acetonitrile was quite low (31%) and clear separation of **14a** from **12** was not possible. Under the optimized conditions for the Stille coupling reaction with bromide **11**, several series of difluorobenzylphosphonates **14b-g** having a variety of hydrophobic functional groups were obtained from **11** [Yield: **14b**: 68%; **14c**: 81%; **14d**: 71%; **14e**: 65%; **14f**: 59%; **14g**: 30%]. The *meta*-analogues **18b-d** were prepared from the bromide **17** in a similar manner [Yield: **18b**: 69%; **18c**: 80%; **18d**: 54%].[14] The phosphonates **14b-f** and **18b-d** were deprotected in the usual manner (i: TMSBr / CH_2Cl_2, ii: MeOH) to give free acids **15b-f** and **19b-d** in good yield.[15]

Scheme 1



a: R=vinyl, **b**: R=(*E*)-styryl, **c**: R=phenylethynyl, **d**: R=ethynyl, **e**: R=phenyl, **f**: R=4-methoxyphenyl, **g**: 4-fluorophenyl

Table 1 IC_{50} values of aryl(difluoromethylphosphonic acids for the hydrolysis of pNPP with PTP 1B

Compound	IC_{50}(μM)	Compound	IC_{50}(μM)
15b	449.9	**19b**	386.2
15c	128.3	**19c**	135.9
15d	77.5[a]	**19d**	19.0[a]
15e	778.9	**2**	NI
15f	451.4	**4**	718.1

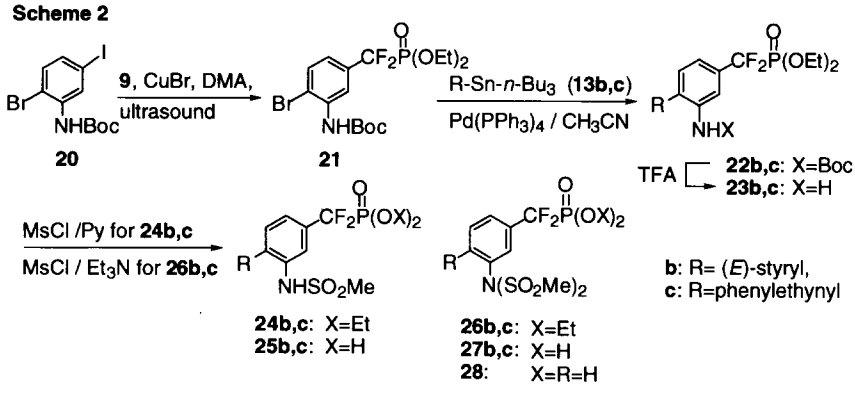
[a] For the data of ammonium salts prepared by the method of Talyor.[8b] [b] No inhibition: a 1.5 fold activation of the control activity was obtained at 1097 μM.

An assessment of the inhibitory potency of **15b-f** and **19b-d** was performed with the IC_{50} values for the PTP 1B-catalyzed hydrolysis of *p*-nitrophenyl phosphate (pNPP).[16] As shown in Table 1, IC_{50} value of **4**[10] as the standard compound was 718.1 μM. The compounds **15b-f** and **19b-d** also had inhibitory potencies, and their IC_{50} values ranged from 778.9 to 19.0 μM. The most potent compounds in this set were difluorobenzylphosphonic acids **15d** (IC_{50} = 77.5 μM) and **19d** (IC_{50} = 19.0 μM) bearing an ethynyl functional group at either the

para- or *meta*-position and are approx. 10 to 38-fold more potent than **4**. Introduction of a phenyl group to the terminal position of the acetylene results in significant decrease of the inhibitory activity; IC_{50} values of **15c** and **19c** were determined to be 128.3 μM and 135.9 μM, respectively. While the IC_{50} values do not significantly decrease, (*E*)-styryl and 4-methoxyphenyl functional groups on the benzene nuclei also seem to be promising functional groups to increase the inhibitory activity of difluorobenzylphosphonic acids.

The strategy at the second phase of the project was focused on introducing both hydrophilic and hydrophobic functional groups onto the benzene nuclei of difluorobenzylphosphonic acids. We chose to introduce either a mono-sulfonamide or a bis-sulfonamide group onto the difluorobenzylphosphonic acids **15b** and **15c**, since these groups proved to function as a good hydrogen bond acceptor in many biological systems.[17] The synthesis and biological evaluation of the polyfunctionalized difluorobenzylphosphonic acids **25b,c** and **27b,c** were then examined (Scheme 2 and Table 2). The cross-coupling reaction of the iodide **20**[18] with the zinc reagent **9** in the presence of CuBr in DMA under sonicated conditions gave the coupling product **21** in 64% yield. Stille coupling reaction of **21** with **13b** and **13c** in refluxing acetonitrile in the presence of $Pd(PPh_3)_4$ (6 mol%) gave (*E*)-styryl derivative **22b** and phenylethynyl derivative **22c** in 96% and 89% yield, respectively. After removal of the *N*-Boc protecting group with trifluoroacetic acid, the resulting amines **23b,c** were divergently manipulated to the mono-sulfonamides **24b,c** and the bis-sulfonamides **26b,c** in the usual manner [Yield: **24b**: 85%; **24c**: 91%; **26b**: 71%; **26c**: 98%]. The ethyl protecting groups were removed using TMSBr in CH_2Cl_2, followed by desilylation with MeOH to give the free acids **25b,c** and **27b,c**. The acids **25b,c** and **27c** were isolated as ammonium salts according to the method of Taylor[8b] for evaluation of the inhibitory activities.

Scheme 2



As shown in Table 2, the introduction of monomethylsulfonamide lowers the IC_{50} by approx. 2.5-fold relative to the parental styryl derivative **15b**. In contrast, there was no significant decrease in the IC_{50} value of phenylethynyl derivative **25c**. Introduction of bismethylsulfonamide was found to be a useful motif increasing the inhibitory potency of both **15b** and **15c** by 2-8 times; the IC_{50} values of the bis-sulfonamide derivatives **27b** and **27c** were 57.9 μM and 88.7 μM, respectively. Therefore these compounds are 8 to 12-fold more potent than the naphthalene derivative **4** (Table 1 versus Table 2). The (*E*)-stryryl and phenylethynyl functionals in **27b** and **27c** are necessary to show the inhibitory activity, since **28**[19] lacking the hydrophobic groups had no inhibitory activity.

Table 2 Comparison of IC_{50} values of **15b,c**, **25b,c** and **27b,c** for the hydrolysis of pNPP with PTP 1B

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
15b	449.9	**15c**	128.3
25b	175.7[a]	**25c**	167.1[a]
27b	57.9	**27c**	88.7[a]

[a] For the data of ammonium salts.

In conclusion, simple modification of α,α-difluorobenzylphosphonic acid by introducing hydrophobic functional groups onto the benzene nuclei resulted in the identification of novel PTP 1B inhibitors **19d** and **27b** without a peptidyl framework. The results clearly show that such modifications open up new opportunity for creating small molecular weight PTP-inhibitors based on difluorobenzylphosphonic acid pharmacophores. However,

the specificities of these inhibitors for the other PTPs and serine/threonine phosphatases remain to be elucidated. When considering the utility as the therapeutic agent, the cell permeability of the PTP inhibitors will be an important subject.

Acknowledgment: This work was supported in part by Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science, Sports and Culture of the Japanese Government.

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11. All new compounds were fully characterized by ^{1}H-, ^{13}C-, ^{19}F-, ^{31}P-NMR, IR and MS analyses.
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14. More than 2 mol% of $Pd(PPh_3)_4$ was necessary to induce good yield.
15. Deprotection of vinyl derivative **14a** under the conditions was problematic due to a concomitant hydrobromination of the vinyl moiety with hydrogen bromide produced during the de-silylation. While the undesired hydrobromination of the ethynyl moiety of **15d** and **19d** was also observed to some extent on standing the crude acids, this was suppressed by converting the free acids to the corresponding ammonium salts immediately.
16. PTP 1B (Upstate Biotech. Inc.) was assayed according to the manufacture's instructions. Briefly, the activity of PTP 1B was assayed at 25 °C in 96-well plates with pNPP as the substrate. The assay mixture contained 5 μL of 40 mM $NiCl_2$ in water, 5 μL of a bovine serum albumin solution (5 mg/mL in water), 5 μL of PTP 1B-agaroses (0.05 units) and 65 μL of 50 mM Tris-HCl buffer (pH 7.0) / 0.1 mM $CaCl_2$ that, if indicated, contained various concentrations of inhibitor, and pre-incubation followed for 15 min. The enzyme reaction was started by the addition of 120 μL of a pNPP solution (1.5 mg/mL in 50 mM Tris-HCl buffer). After an incubation for 30 min, the reaction was stopped by adding 20 μL of a 13% (w/v) K_2HPO_4 solution, and the absorbance at 405 nm was measured. The nonenzymatic hydrolysis of pNPP was corrected by measuring the control without the addition of enzyme. IC_{50} values were determined as the concentrations of compounds that give a 50% of the control enzyme activity. Briefly, experiments were carried out in triplicate at 5 to 8 different inhibitor concentrations. The inhibitor concentrations were plotted as y-axis and the remaining activity (%) as x-axis, and the concentration of inhibitor that give a 50% inhibition was calculated, using the curve-fit equation (CA-Cricket Graph III).
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18. Prepared from commercially available 5-iodoanthranilic acid *via* sequential Sandmeyer reaction ($NaNO_2$, CuBr in *aq.* HBr) and Curtius rearrangement (DPPA in refluxing *tert*-BuOH) in 75% yield for the two-steps.
19. Prepared from *N*-Boc-3-iodoaniline by a similar reaction sequence.