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PTP1B inhibitors: Synthesis and evaluation of difluoro-methylenephosphonate bioisosteres on a sulfonamide scaffold

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Abstract—We have synthesized and evaluated a series of triaryl sulfonamide-based PTP1B inhibitors in which a difluoro-methylenephosphonate group of a potent lead has been replaced by potential bioisosteric replacements. Several mono- or di-charged compounds (**8a**, **8b**, and **15a**) were shown exhibit inhibitory activity in the low micromolar range, demonstrating the feasibility of using this approach in identifying non-phosphonate *p*Tyr mimetics in a small molecular scaffold. These results also provide a useful indication of the relative effectiveness of these *p*Tyr mimetics.

Recently, there has been tremendous interest in the development of inhibitors of protein tyrosine phosphatase 1B (PTP1B) as therapeutic agents in treating Type II diabetes and obesity.[1] Much of the effort in designing active site directed PTP1B inhibitors has focused on the synthesis of phosphotyrosine (*p*Tyr) mimetics, which serve as non-hydrolyzable replacements for the critical *p*Tyr residue.[2] One of the most effective *p*Tyr mimetics that has been developed is α,α-difluoro-methylenephosphonic acid (DFMP).[3,4] The high affinity has been attributed to the direct hydrogen bonding of fluorines with enzyme active site residues.[5] One limitation of these inhibitors, however, is their poor cell permeability due to the dianionic nature of the phosphonate group at physiological pH, although recent strides have been made in predicting the activity of PTP1B inhibitors.[6] The discovery of effective non-phosphonate *p*Tyr mimetics will facilitate the development of more drug-like PTP1B inhibitors as therapeutic agents.

Because of the electrostatic properties of the enzyme active site,[7] it has proven difficult to develop effective uncharged or monoanionic *p*Tyr mimetics. The replacement of a DFMP moiety in a PTP1B inhibitor by potential bioisosteric replacements generally leads to dramatic decreases in binding ability. Previous efforts have focused on utilizing DFMP-containing high-affinity peptides as display platforms to discover non-phosphonate pTyr mimics, which provide starting points for developing small molecule inhibitors.[8–10]

We have identified previously a series of triaryl sulfonamide based PTP1B inhibitors containing only one single DFMP group.[11] For example, **1a** and **1b** are potent inhibitors of PTP1B with IC_{50} value of 0.074 μM and 0.20 μM, respectively (Fig. 1). It has been shown that these bind to the active site in a competitive manner and also contain optimized substitutions to provide significant interactions beyond the *p*Tyr pocket. Therefore, we sought to use the triaryl sulfonamide as suitable scaffold to investigate non-phosphonate bioisosteric replacements while maintaining reasonable PTP1B inhibitory activity. This paper describes the synthesis and inhibition activities of a series of sulfonamide analogues, where the DFMP group has been replaced with potential *p*Tyr mimics with the focus on identifying minimally charged compounds that retain potency for the PTP1B enzyme.

Keywords: PTP1B; Phosphatase; Bioisostere; Sulfonamide.
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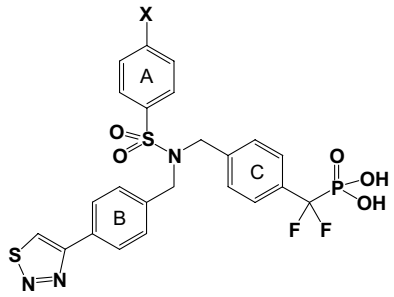


1a, X = OCH_2COOH, IC_{50} = 0.074 μM
1b, X = H, IC_{50} = 0.20 μM

Figure 1. Structures of DFMP sulfonamide leads.

True to the definition of a versatile scaffold, the triaryl sulfonamides (see Table 1) could be made by a variety of techniques, as shown in Schemes 1 and 2, depending on the availability of the appropriate reagents. Method A (Scheme 1) involved joining the A–B rings first, followed by a reaction with an electrophilic bromobenzyl-C ring protected *p*Tyr mimetic to form the ABC triaryl sulfonamide. Typically, the *p*Tyr mimetic is liberated through either methyl/ethyl ester saponification with base or *tert*-butyl ester cleavage with TFA. In Method B, the B–C rings are first joined by the reaction of a sulfonylchloride A ring with an aminobenzyl C ring protected *p*Tyr mimetic, followed by the reaction with thiadiazolylbenzylbromide and subsequent pTyr mimetic generation with again either base or acid-induced ester cleavage. Method C (Scheme 2) involved forming the B–C ring bonds first through a reduction amination of the C-ring protected *p*Tyr mimetic benzaldehyde with thiadiazolylbenzylamine, followed by the reaction with the A-ring sulfonylchloride to form the triaryl scaffold. The substituted benzylamines, benzylaldehydes, and sulfonylchlorides were either commercially available or formed from straightforward literature procedures. The desired products were isolated as acids by preparative RP-HPLC, their purity assessed by RP-HPLC, and their molecular composition was confirmed by ESI-MS.

The inhibitory activity against PTP1B was evaluated by our previous described method using *O*-methyl fluorescein monophosphate (OMFP) as a substrate.[12] IC_{50} values are shown in Table 1. The compounds are divided into two series. Series I includes analogues which are based on the parent compound **1a**, where X represents oxoacetic acid. In Series II, the compounds are the corresponding analogues based on **1b** lacking the acid (X = H). Of the various DFMP replacements examined, 4 were dianionic at physiologic pH as is DFMP, while 20 contained a single acidic group.

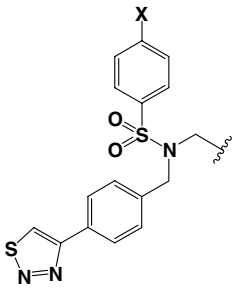
Of the dianionic replacements evaluated, non-fluorinated methylenephosphonate group of **2a** and **2b** are the most similar to DFMP, however, only weak inhibition was obtained ($IC_{50} \sim 110$ μM), indicating the significant role that the fluorine atoms play in DFMP). The observation is consistent with previous reports that the DFMP analogues are 1000-times more potent inhibitors than non-fluorinated analogues[3a] since fluorine atoms are directly involved in hydrogen bonding with the amide bond of the active site residue Phe-182.[5] *O*-Carboxymethyl salicylic acid[13] and 2-(oxalyamino)-benzoic acid[14] represent two types of dianionic *p*Tyr mimetics that have been used in the development of PTP1B inhibitors. Both of these were found to be acceptable when appended to the triaryl sulfonamide scaffold, resulting in low micromolar IC_{50} values for **10a**, **10b**, **15a**, and **15b**. The final dianionic analogue **23a** turned out to be a very weak inhibitor of the enzyme (IC_{50} = 74 μM).

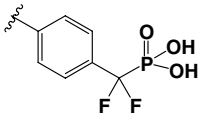
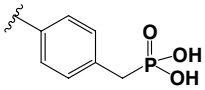
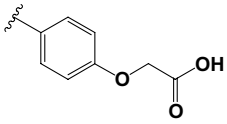
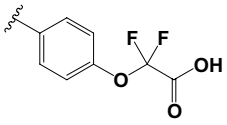
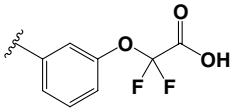
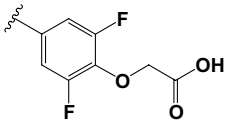
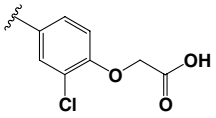
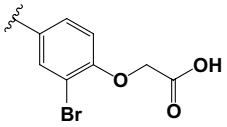
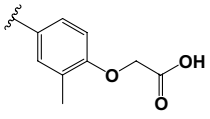
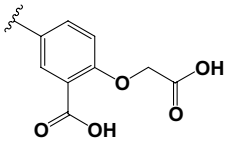
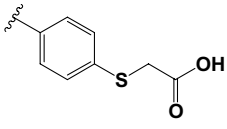
Replacement of the DFMP group with a simple mono-charged oxo-acetic acid gave **3a** and **3b**, which showed IC_{50} values of 59.6 and 54.1 μM, respectively. These results were encouraging considering that the oxo-acetic acid contains only one charge and can be used as a starting point to make additional modification to improve the binding ability. Subsequently, fluorine atoms were introduced in the methylene group to generate **4a** and **4b**, which showed improved affinity, and not surprisingly, the para position of the aromatic group was better than the meta position (**5a** and**5b** are roughly 5-fold worse than **4a** and **4b**). Introduction of substituents into the one or more of the ortho positions of the aryl ring can significantly influence the binding to PTP1B. Thus, the bromo derivatives **8a** and **8b** are remarkably potent relative to the parent ring found in **3a** and **3b**, as we[11] and others have observed.[14] It is likely that bromo substitution in the ortho position of a *p*Tyr mimetic provides a hydrophobic interaction with the enzyme active site. It has been shown from X-ray studies of co-crystal complexes that a hydrophobic pocket exists at the vicinity of the catalytic site.[15] Other substituents such as difluoro (**6a** and **6b**), chloro (**7a** and **7b**), and methyl (**9a** and **9b**) were tolerated, but do not seem to offer any advantage, which is in clear contrast to trends observed by others in the thiophene series of PTP1B inhibitors.[16] We had hoped that the fluorine atoms of **6** would be close enough to interact with PTP1B and compensate for the single charge of the mimetic, but this turned out not to be true. Attempting to capitalize on this ''bromo effect'', we combined the fluorines of **4** with the bromine of **8** to prepare hybrid compounds **21a** and **21b**, but were unable to achieve any synergistic potency; in fact the addition of fluorines to compounds **8a** and **8b** resulted in significantly worse binding to the enzyme.

When situated in these triaryl sulfonamide scaffolds, a bromine and carboxylic acid appear equally tolerated by PTP1B. Thus, in both the oxoacetic acid series represented by compounds **8** and **10**, and the 2-(oxalyamino)-benzoic acid series represented by compounds **15** and **16b**, the activities across the same A-ring series are almost identical. The use of mono-charged pTyr mimetics (i.e., bromo analogues) would presumably have greater beneficial effect on their cell permeability and perhaps make up for the large increase in molecular weight associated with the bromine atom.

Substituting sulfur for oxygen of the oxoacetic acid resulted in decreased PTP1B activity (IC_{50}s for **11a**

Table 1. In vitro activity of *p*Tyr mimics against PTP1B[a]



Compound	*p*Tyr mimetics	Synthesis method	X	IC_{50} (μM)
1a		A	OCH_2CO_2H	0.074
1b			H	0.20
2a		C	OCH_2CO_2H	96.8
2b			H	123
3a		A	OCH_2CO_2H	59.6
3b			H	54.1
4a		A	OCH_2CO_2H	29.1
4b			H	97.1
5a		A	OCH_2CO_2H	230
5b			H	198
6a		A	OCH_2CO_2H	19.9
6b			H	137
7a		A	OCH_2CO_2H	57.7
7b			H	53.8
8a		A	OCH_2CO_2H	4.1
8b			H	7.9
9a		A	OCH_2CO_2H	76.2
9b			H	80.7
10a		A	OCH_2CO_2H	4.4
10b			H	12.2
11a		A	OCH_2CO_2H	114
11b			H	115

(*continued on next page*)

Table 1 (*continued*)

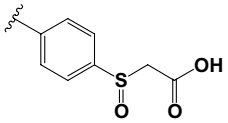
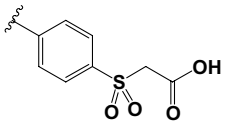
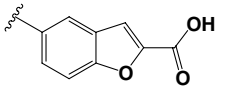
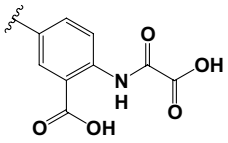
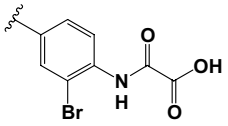
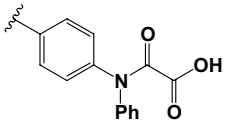
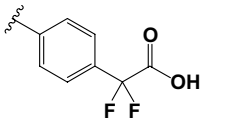
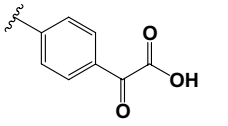
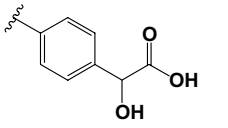
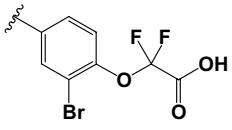
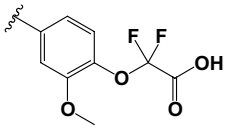
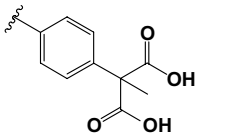
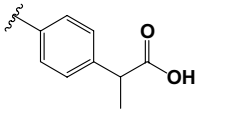
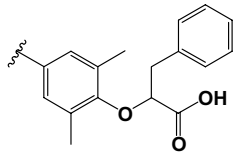
Compound	*p*Tyr mimetics	Synthesis method	X	IC_{50} (μM)
12a		A[b]	OCH_2CO_2H	>250
12b			H	173
13a		A[b]	OCH_2CO_2H	>250
13b			H	232
14a		A	OCH_2CO_2H	29.1
14b			H	63.6
15a		A	OCH_2CO_2H	1.4
15b			H	10.1
16b		A	H	13.8
17a		B	OCH_2CO_2H	>250
17b			H	235
18a		A	OCH_2CO_2H	17.7
18b			H	51.8
19a		A	OCH_2CO_2H	27.6
19b			H	60.3
20a		A[c]	OCH_2CO_2H	22.4
20b			H	>250
21a		A	OCH_2CO_2H	77.9
21b			H	52.1
22a		A	OCH_2CO_2H	>250
22b			H	237
23a		B	OCH_2CO_2H	74.2
24a		B[d]	OCH_2CO_2H	153

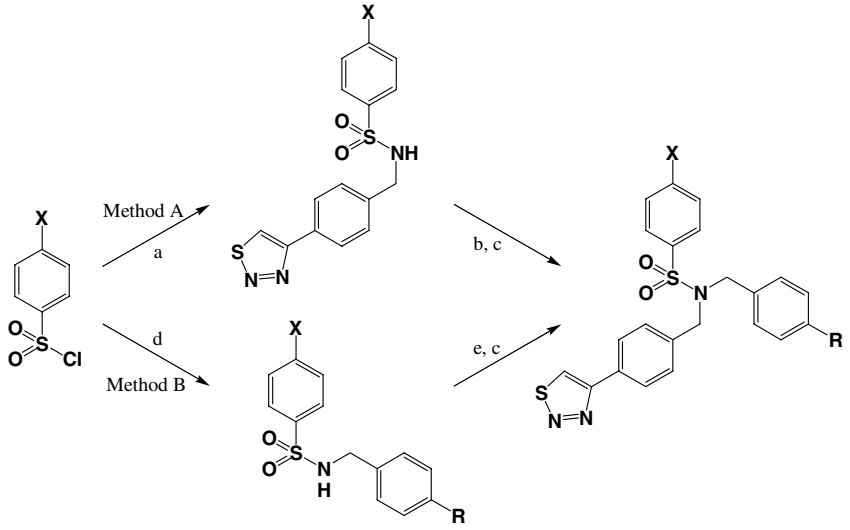
Table 1 (*continued*)

Compound	*p*Tyr mimetics	Synthesis method	X	IC_{50} (μM)
25a		A	OCH_2CO_2H	>250
25b			H	38

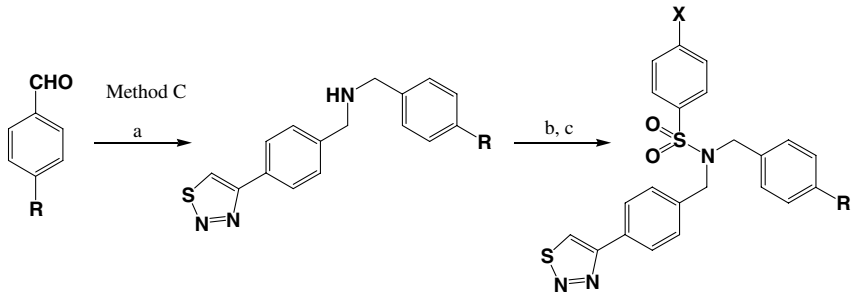
[a] PTP1B binding assays were conducted as previously reported in Ref. 12. Values are means of duplicate experiments, errors are usually within ±10%.
[b] Oxidized from **11a** and **11b** by MCPBA.
[c] Mixture of two isomers.
[d] Formed as byproduct during the synthesis of **23a**.

and **11b** are double those of **3a** and **3b**), presumably because sulfur is a weaker hydrogen binding acceptor compared to an oxygen atom. Further oxidation of sulfur to sulfoxide (**12a** and **12b**) or sulfone (**13a** and **13b**) resulted in the loss of the activity, indicating unfavorable interactions from the sulfoxide or sulfone. It is also worthwhile to note that **14a** and **14b** with the 2-benzofurancarboxylic acid group exhibit similar activity as **3a** and **3b**, indicating that conformational constraint of the acid offers little advantage.

Four *p*Tyr mimetics, difluoroacetic acids **18a** and **18b**, α-keto acids **19a** and **19b**, α-hydroxy acid groups **20a** and **20b**, and propionic acid **24a**, were made with one atom spacing shorter acidic group compared to **3a** and **3a**, and most closely mimic the spacing found in the parent DFMP group of **1a** and **1b**. In the oxoacetic acid Series I, these substitutions resulted in better inhibitors (**18a**, **19a**, and **20a**) than **3a**, but incorporation in Series II led to compounds with similar or poorer activity (**18b**, **19b**, and **20b** worse than **3b**). It has been shown previously that simple biphenyl or naphthalenyl difluoro-car-



Scheme 1. Methods A and B for preparing triaryl sulfonamides. Reagents and conditions: (a) thiadiazolyl-C_6H_4-CH_2NH_2, A-ring sulfonylchloride, CH_2Cl_2, DIEA; (b) $BrCH_2$-C-ring *p*Tyr mimetic, K_2CO_3, CH_3CN, 70 °C; (c) CF_3CO_2H, CH_2Cl_2 or KOH, MeOH, H_2O; (d) H_2NCH_2-C-ring *p*Tyr mimetic, DIEA, CH_2Cl_2; (e) thiadiazolyl-$C_6H_4CH_2Br$, K_2CO_3, CH_3CN, 70 °C.



Scheme 2. Method C for the synthesis of triaryl sulfonamides. Reagents and conditions: (a) thiadiazolyl-C_6H_4-CH_2NH_2, MeOH, $Na(OAc)_3BH$; (b) $XC_6H_4SO_2Cl$, DIEA, CH_2Cl_2; (c) CF_3CO_2H in CH_2Cl_2 or KOH, MeOH, H_2O.

boxylates are weak inhibitors of PTP1B,[10a] while the α-keto acid and α-hydroxy acid groups had been reported as *p*Tyr mimitics.[17]

The use of the benzyl substituted acid head group found in Ertiprotafib [18] afforded compounds **25a** and **25b**, which displayed an unusual selectivity relationship between Series I and II where Series II species **25b** displayed better activity than **25a**. Compound **25b** binds stronger to the enzyme than does **25a**, perhaps indicating a different mode of binding than the other molecules described in this paper.

In conclusion, we have synthesized and evaluated a series of triaryl sulfonamide-based PTP1B inhibitors in which the DFMP group of two lead series has been replaced by potential bioisosteric replacements. Although most of monocharged bioisosteres are not as active as those dianionic *p*Tyr mimetics, *O*-bromophenoxyacetic acid appears to compare favorably with dianionic *o*-carboxymethyl salicylic acid and 2-(oxalyamino)-benzoic acid. Several mono- or di-charged compounds (**8a**, **8b**, and **15a**) were shown to inhibit PTP1B in the low micromolar range, demonstrating the feasibility of using this systematic approach in identifying non-phosphonate *p*Tyr mimetics in a small molecular scaffold. The next step in the optimization of these compounds will be to address their ability to cross a cell wall to where PTP1B resides. In addition, the results from this study might provide an indication of the relative effectiveness of these *p*Tyr mimetics that would be useful in the further design and development of PTP1B and perhaps other[19] protein–tyrosine phosphatase inhibitors.

References and notes

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