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Design and Synthesis of Nonpeptidic, Small Molecule Inhibitors for the *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase PtpB

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Abstract

The design and synthesis of new inhibitor analogues for the *Mycobacterium tuberculosis* (*Mtb*) phosphatase PtpB is described. Analogues were synthesized by incorporation of two common and effective phosphate mimetics, the isothiazolidinone (IZD) and the difluoromethylphosphonic acid (DFMP). The basic scaffold of the inhibitor was identified from structure-activity relationships established for a previously published isoxazole inhibitor, while the phosphate mimetics were chosen based on their proven cell permeability and activity when incorporated into previously reported inhibitors for the phosphatase PTP1B. The inhibitory activity of each compound was evaluated, and each was found to have low or submicromolar affinity for PtpB.

Tuberculosis (TB) is a chronic, infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). Each year, nearly 2 million deaths occur out of over 13 million active cases of TB,¹ and current treatment of drug-sensitive strains requires 6-9 months to fully eradicate the infection. New *Mtb* drugs that act on novel targets are needed to shorten treatment time and address the emergence of antibiotic resistance. *Mtb* PtpB is a secreted virulence factor that functions within human macrophages, and chemical interference with PtpB function has been shown to reduce bacterial loads in an *Mtb* macrophage infection model.² PtpB is attractive as a therapeutic target due to its localization outside of the unusually thick mycobacterial cell wall, which is difficult to penetrate and leads to long treatment times for tuberculosis (TB).

We previously reported the development of isoxazole carboxylic acid inhibitor **1** for PtpB, using the substrate activity screening (SAS) method, a novel fragment-based approach for the identification of phosphatase inhibitors (Figure 1).³ Development of the isoxazole inhibitor revealed that *ortho* substituents are preferred for optimal PtpB binding, with the cyclohexyl group providing the most favorable enzyme interactions. In order to compare binding affinity and cell activity, we desired a route to analogueous inhibitors incorporating other commonly used phosphate mimetics.

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A variety of phosphate isosteres have been reported and could be introduced in place of the isoxazole,⁴ but many contain at least two acidic sites and lead to inhibitors with poor cellular permeability.⁵ We therefore chose two phosphate mimetics that have been effectively used in inhibitors of PTP1B, a human phosphatase targeted for diabetes treatment, with good cell permeability and activity, namely the isothiazolidinone (IZD) and difluoromethylphosphonic acid (DFMP) mimetics (Figure 2). The monoanionic IZD⁶ phosphate mimetic was developed by Incyte through structure-based design,^{6g} while the DFMP pharmacophore is a commonly used phosphate mimetic that has been investigated extensively in the literature.⁷ Despite being dianionic, the DFMP isostere has recently been shown by Merck to be cell permeable and orally bioavailable in animals when incorporated into nonpeptidic inhibitors of PTP1B.⁸

Several syntheses have been reported for the IZD isostere.^{6a,6c-e} However, due to considerable steric interactions introduced by the cyclohexyl group *ortho* to the IZD isostere in **2**, implementation of successful cross-coupling methods for installing the isostere posed a significant challenge (Scheme 1).

First, *o*-cyclohexyl phenol **4** was subjected to Suzuki coupling with 4-fluoro-3-trifluoromethyl boronic acid **5** to afford the biaryl phenol **6**. Treatment with triflic anhydride under basic conditions then afforded triflate **7**, which was then coupled with bis(pinacolato)diboron to provide boronic ester **8**.⁹ This coupling step required significant optimization due to the presence of the sterically encumbered *ortho* cyclohexyl group, which has not been previously reported as a partner in Suzuki couplings (Table 1). To determine optimal conditions for boration of hindered triflate **7**, we screened conditions using the biphenyl model substrate **13**. Standard Suzuki conditions for coupling with bis(pinacolato)diboron failed to produce product (entry 1), as did standard Buchwald conditions using cyclohexyl JohnPhos as the ligand (entry 2). Similarly, all attempts to couple with pinacol borane gave no reaction (entries 3-4). Finally, inclusion of the Buchwald ligand SPhos⁹⁻¹⁰ afforded product, albeit in low yield (entry 5). Increasing the temperature to 130 °C decreased the yield, likely due to protodeborylation of the product (entry 6), but switching to the precatalyst Pd₂(dba)₃ in addition to reversed-phase purification of the somewhat unstable boronic ester improved the yield significantly to 66% (entry 7). Upon applying the same conditions to the desired triflate **7**, the pinacol ester **8** was obtained in 69% yield (Scheme 1).

Upon developing conditions to successfully prepare the hindered boronic ester **8**, it was then necessary to carry out Suzuki cross-coupling with heterocycle **9**. As shown in Table 2, to achieve a successful cross-coupling the reaction parameters required careful optimization, including choice of precatalyst, additive, and reaction temperature. The cross-coupling conditions used to prepare boronic ester **8** also afforded product **10**, but in low yield (15%), likely due to competitive formation of ring opened byproducts from either heterocycle **9** or product **10**, as determined by NMR analysis (entry 1). We observed similar decomposition byproducts when using an amination precatalyst developed by Hartwig (entries 2-3), which was designed to increase the rate of oxidative addition, a step that is often rate-limiting in the Suzuki catalytic cycle.¹¹ Ultimately, we found that use of Pd(OAc)₂ as the precatalyst with inclusion of water enabled formation of product at reduced temperatures, which minimized the formation of undesired ring-opened byproducts (entry 4). Increasing the amount of water and the reaction time ultimately gave the coupled product in 69% yield (entry 5). Compound **10** was then reduced with sodium borohydride, followed by oxidation to sulfone **12** with *m*-CPBA. Final TFA deprotection then gave the desired IZD inhibitor **2** in racemic form.

Based on previous studies of IZD inhibitors for PTP1B,^{6a,6c-g} we reasoned that *Mtb* PtpB should bind one IZD enantiomer preferentially, and we therefore separated the two enantiomers **17** and **18** by subjecting compound **12** to chiral preparatory chromatography (Scheme 2),¹² followed by the same TFA treatment described above to remove the *tert*-butyl protecting group from compounds **15** and **16**. Compounds **15** and **16** were found to have >99% ee and >99% purity after separation and isolation.

To complete the PtpB inhibitor panel and provide further comparison of phosphate mimetics in terms of PtpB binding affinity, we also developed a route to DFMP analogue **3**. Several synthetic approaches have been reported for installation of the DFMP isostere and could be used in the synthesis of the DFMP compound.¹³ We chose a relatively mild and straightforward cross coupling approach between aryl iodide **20** and a commercially available diethyl phosphonate,^{13c} because this strategy is compatible with a variety of functional groups and enables late stage incorporation of the DFMP warhead (Scheme 3).

Synthesis of DFMP analogue **3** began by coupling bis(neoglycolato)diboron with triflate intermediate **7** used in the synthesis of the IZD inhibitor (Scheme 3). This was followed by iodination with *in situ*-generated ICl to form the aryl iodide **20**, then copper and zinc-mediated coupling with diethyl(bromodifluoromethyl)phosphonate to afford the aryl phosphonate **21**. Final deprotection then afforded the desired DFMP inhibitor **3**.

Following isolation, each compound was assayed versus *Mtb* PtpB (Table 3). To rule out non-specific aggregation-based enzyme inhibition,¹⁴ the detergent Triton X-100 was included in all assays, and Hill slopes were measured and found to be close to -1.0 for all compounds (see ESI). Racemic IZD inhibitor **2** was found to have a K_i of 3.7 μM versus PtpB, which is only modestly weaker than isoxazole scaffold **1**, while the enantiomerically pure IZD compounds **17** and **18** were found to have K_i values of 2.4 and 8.0 μM , respectively.¹⁵ The chiral discrimination found for these IZD enantiomers is more modest than for IZD inhibitors of PTP1B.^{6e,6g} DFMP compound **3** was found to have a K_i of 0.69 μM versus PtpB, and represents the most potent inhibitor in the series.¹⁶

The results of this study demonstrate that the isoxazole, IZD, and DFMP pharmacophores can each be incorporated into effective inhibitors of *Mtb* PtpB. These inhibitors represent a useful set of tool compounds for further dissection of the biochemical role of PtpB in TB infection, because inhibitors are expected to have different physicochemical properties, which will impact important factors that contribute to compound efficacy such as cell permeability, protein binding, oral bioavailability, and clearance rates.

In conclusion, two new, nonpeptidic inhibitor analogues for *Mtb* PtpB have been identified. Each of these compounds is based on the optimal scaffold previously identified using the SAS method,³ a novel fragment-based approach for phosphatase inhibitor development. Inhibitors **2** and **3** incorporate the IZD and DFMP phosphate mimetics, respectively, in place of the isoxazole carboxylic acid warhead developed for compound **1**. These mimetics were chosen based on previous incorporation into inhibitors of the phosphatase PTP1B, an enzyme targeted for diabetes treatment. The IZD and DFMP analogues show comparable inhibitory activity to isoxazole inhibitor **1** in terms of binding affinity, and they are expected to vary in terms of cell permeability and activity. The entire panel of inhibitor analogues is therefore currently being evaluated in TB-infected cells and future work will include evaluation in animal infection models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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15. Note that the absolute configuration of each compound has not been established; repeated attempts at crystallization failed to produce crystals sufficient for X-Ray analysis, likely due to the inability of these compounds to pack with the necessary co-solvents.
16. For examples of other previously reported PtpB inhibitors, see:(a) Tan L, Wu H, Yang P, Kalesh K, Zhang X, Hu M, Srinivasan R, Yao S. *Org Lett* 2009;11:5102–5105. [PubMed: 19852491] (b) Noeren-Mueller A, Wilk W, Saxena K, Schwalbe H, Kaiser M, Waldmann H. *Angew Chem, Int Ed Engl* 2008;47:5973–5977. [PubMed: 18604796] (c) Correa IR Jr, Noeren-Mueller A, Ambrosi HD, Jakupovic S, Saxena K, Schwalbe H, Kaiser M, Waldmann H. *Chem--Asian J* 2007;2:1109–1126. [PubMed: 17685373]

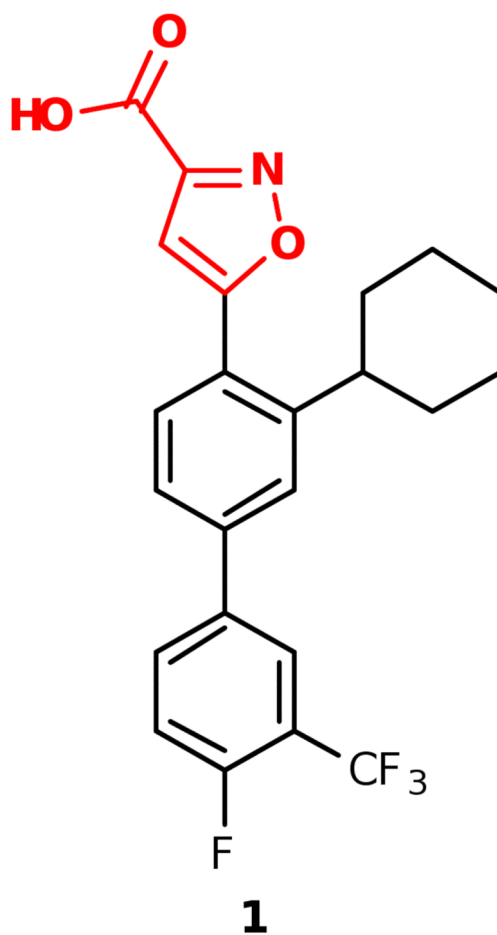


Figure 1.
Previously reported isoxazole PtpB inhibitor 1.

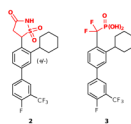
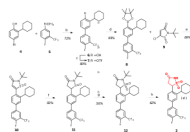
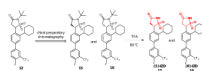


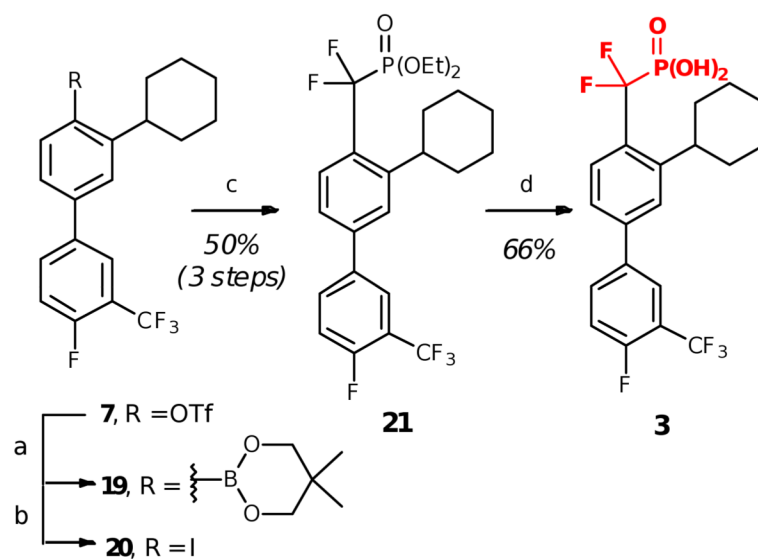
Figure 2.
New PtpB inhibitor analogues incorporating the IZD and DFMP phosphate mimetics.

**Scheme 1.**

Synthesis of IZD inhibitor **2**. Reagents and conditions: (a) Pd(OAc)₂, KF, CyJohnPhos, THF, 50 °C; (b) Tf₂O, Et₃N, CH₂Cl₂, -78 °C; (c) Pd₂(dba)₃·CHCl₃, K₃PO₄, SPhos, B₂pin₂, toluene, 100 °C; (d) Pd(OAc)₂, K₃PO₄, SPhos, cat. H₂O, THF, 60 °C; (e) NaBH₄, MeOH, 0 °C; (f) *m*-CPBA, CH₂Cl₂, 0 °C to rt; (g) *d*-TFA, H₂O, 80 °C.



Scheme 2.
Isolation of enantiomerically pure IZD inhibitors **17** and **18**.

**Scheme 3.**

Synthesis of DFMP inhibitor **3**. Reagents and conditions: (a) bis(neoglycolato)diboron, K_3PO_4 , SPhos, $Pd_2(dba)_3 \cdot CHCl_3$, toluene, 100 °C; (b) NaI, chloramine-T, 50% aq THF; (c) diethyl(bromodifluoromethyl)phosphonate, Zn^0 , CuBr, DMA; (c) TMSI, $CHCl_3$.

Table 1

Coupling of model biphenyl triflate **13** to afford model biphenyl boronic ester **14**.

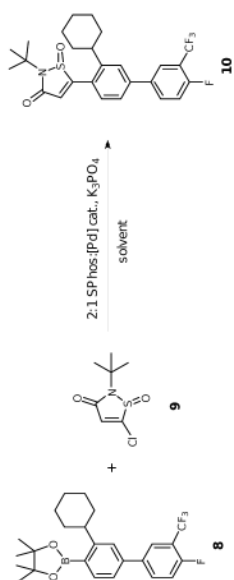


Entry	R	[Pd] cat.	Ligand	Base	Solvent	Temp (°C)	Time (h)	% Yield ^a
1	Bpin	Pd(dppf)Cl ₂	--	KOAc	dioxane	80	26	0%
2	Bpin	Pd(OAc) ₂	CyJohnPhos	KF	toluene	100	17	0%
3	H	Pd(PPh ₃) ₄	--	Et ₃ N	dioxane	80	4	0%
4	H	Pd(dppf)Cl ₂	--	Et ₃ N	dioxane	70	27	0%
5	Bpin	Pd(OAc) ₂	SPhos	K ₃ PO ₄	toluene	100	15	28%
6	Bpin	Pd(OAc) ₂	SPhos	K ₃ PO ₄	toluene	130	16	14%
7	Bpin	Pd ₂ (dba) ₃ ·CHCl ₃	SPhos	K ₃ PO ₄	toluene	110	17	66%

^aYield was determined by mass balance after silica gel column chromatography.

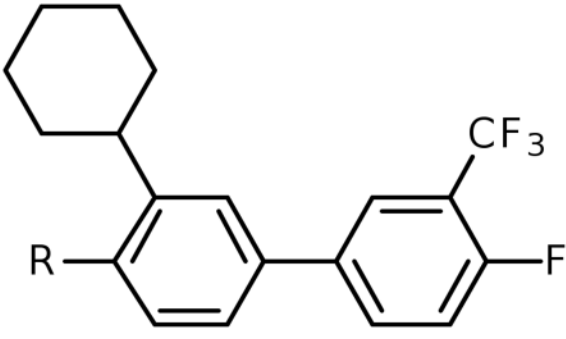
Table 2

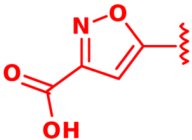
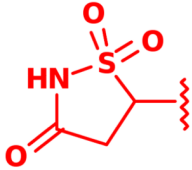
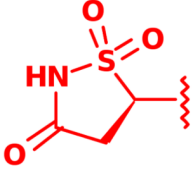
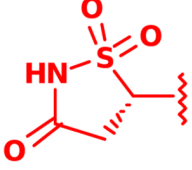
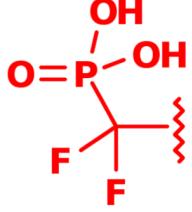
Coupling of biphenyl boronic ester **8** and heterocycle **9** to afford coupled product **10**.



Entry	[Pd] cat.	Solvent	Temp (°C)	Time (h)	% Yield ^a
1	$Pd_2(dba)_3 \cdot CHCl_3$	toluene	100	20	15%
2	$Pd[P(o-tol)_3]_2$	toluene	60	20	15%
3	$Pd[P(o-tol)_3]_2$	toluene	100	23	28%
4	$Pd(OAc)_2$	THF, 1 mol% H_2O	60	20	54%
5	$Pd(OAc)_2$	THF, 10 mol% H_2O	60	24	69%

^aYield was determined by mass balance after silica gel column chromatography.

Table 3Assay of isoxazole inhibitor **1**, IZD inhibitors **2**, **17** and **18**, and DFMP inhibitor **3** versus PtpB.


#	R	K_i (μM) ^a
1		1.3 ± 0.6^b
2		3.7 ± 0.6
17		2.4 ± 0.0 or 8.0 ± 0.4
18		2.4 ± 0.0 or 8.0 ± 0.4
3		0.69 ± 0.21

^a Compounds were assayed in duplicate and repeated in triplicate.^b Assays were run with a new batch of enzyme as compared to previous reports and reproducibly gave a higher K_i value.