Inhibition of MptpB phosphatase from *Mycobacterium tuberculosis* impairs mycobacterial survival in macrophages

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**Objectives:** The secreted *Mycobacterium tuberculosis* protein tyrosine phosphatase (MptpB) is a virulence factor for *M. tuberculosis* and contributes to its survival within host macrophages. The aim of this study was to identify potent selective inhibitors of MptpB and to determine the efficacy of these compounds in mycobacterium-infected macrophages.

**Methods:** The inhibitory effect of a small library of compounds on MptpB was first examined in vitro. The efficacy of these compounds was further examined in mycobacterium-infected macrophages.

**Results:** We have identified a new family of double-site isoxazole-based compounds that are potent selective inhibitors of MptpB. Importantly, the inhibitors substantially reduce mycobacterial survival in infected macrophages. In contrast with current anti-tubercular drugs, these MptpB inhibitors do not have bactericidal action but rather, severely impair mycobacterial growth within macrophages. Docking analysis suggests a double-site binding mechanism of inhibition with the isoxazole head in the active site and a salicylate group in a secondary binding pocket that is a unique structural feature of MptpB.

**Conclusions:** These results provide the first evidence that inhibition of phosphatases can be exploited against mycobacterial infections. The cell activity of the inhibitors together with the lack of MptpB human orthologues suggests a strong potential for these compounds to be developed as drug candidates against tuberculosis and promises a new therapeutic strategy to tackle clearance and reduce the persistence of *M. tuberculosis* infection.

Keywords: inhibitors, docking, infection, BCG, tyrosine kinase

**Introduction**

*Mycobacterium tuberculosis* is the causative agent of tuberculosis (TB), a leading killer worldwide that currently infects one-third of the human population. Standard treatment of pulmonary TB uses a combination of different antibiotics that target a number of metabolic processes in mycobacteria resulting in a bactericidal action. The limited effectiveness of current treatments is largely due to their lengthy (>6 months) and complex nature, which leads to poor compliance from patients. The emergence of multidrug-resistant strains and of the virtually untreatable extensively resistant strains has heightened the need for new targets and innovative strategies to tackle TB infections. One such strategy is to target pathogen virulence factors to compromise infection success and persistence. Protein phosphatases are important virulence factors in many infectious diseases caused by *Yersinia pestis*, *Salmonella typhimurium* and *M. tuberculosis*.¹–⁴ Phosphatase inhibitors have been reported against the *Yersinia* YopH,⁵,⁶ but the...
use of phosphatases as drug targets against microbial pathogens has yet to be fully explored. Only recently, promising inhibitors against mycobacterial phosphatases were reported.\textsuperscript{7–10} These compounds show potent inhibitory effects \textit{in vitro}, although their efficacy in cellular systems is still unknown.

The success of \textit{M. tuberculosis} is due in part to its ability to survive and replicate within host macrophage phagosomes. The bacteria manipulate the host defence system\textsuperscript{11} and inhibit the normal process of phagosome maturation into phagolysosomes,\textsuperscript{12} thereby preventing acidification of lysosome contents and bacterial destruction. The molecular mechanisms that allow such survival are still unclear, but manipulation of the host phosphoinositide (PI) metabolism is in part responsible for phagosome maturation arrest.\textsuperscript{13–16} PIs are key components of endosomal membranes and essential for the recruitment of EEA1 and Rab proteins (Rab5 and Rab7) that direct phagosome maturation. Mycobacterial protein tyrosine phosphatase B (MptpB), a secreted phosphatase,\textsuperscript{17} is important in the pathogenesis of \textit{M. tuberculosis}. Mutants lacking \textit{mptpB} are impaired in their ability to survive in both macrophages and guinea pigs.\textsuperscript{3} The biological substrates of MptpB have not yet been defined. We have recently reported that MptpB efficiently dephosphorylates PIs.\textsuperscript{18} The role of MptpB in pathogenesis suggests that chemical inhibition of MptpB could potentially challenge mycobacterial survival and assist in clearing the infection. Furthermore, the lack of human orthologues of MptpB makes this enzyme an attractive new drug target for treatment of TB.

In this study, we present the identification of a new family of potent inhibitors of MptpB, which prevented the intracellular survival of mycobacteria within macrophages, causing a substantial reduction in the bacterial burden 72 h post-infection. These selective inhibitors have a double-site binding mechanism, exploiting both the active site and a secondary pocket that is unique to MptpB. This is the first evidence that inhibition of phosphatases can be exploited against mycobacterial infections and suggests a strong potential for these compounds to be developed as drug candidates against TB.

\textbf{Methods}

\textit{Overexpression and purification of recombinant proteins}

The open reading frame of Rv0153c, encoding MptpB, was amplified from \textit{M. tuberculosis} H37Rv DNA and cloned into a pET28a vector (Novagen) to generate an N-terminal His\textsubscript{6}-tagged expression construct.\textsuperscript{19} This construct was transformed into \textit{Escherichia coli} (DE3) and expression induced at 18°C with 0.5 mM IPTG for 16 h. His-tagged MptpB was purified by nickel affinity chromatography in 50 mM HEPES, 500 mM NaCl, pH 7 buffer. Constructs of PTEN, VHR and DUSP22 in pGEX-4T were a gift from Dr Rafael Pulido (Centro de Investigación Príncipe Felipe, Valencia, Spain). The plasmids were transformed into \textit{E. coli} and protein expression induced at 18°C with 0.5 mM IPTG for 16 h. Purification of glutathione S-transferase (GST)-tagged proteins was done by GST affinity chromatography in 50 mM HEPES, 500 mM NaCl, pH 7 buffer and eluted with 20 mM glutathione. We used \textit{ThBPTP1} (Trypanosoma \textit{brucei} protein tyrosine phosphatase 1) and human LMW-PTP (low-molecular-weight protein tyrosine phosphatase) as negative controls. His-\textit{ThBPTP1} and GST-hLMW-PTP were expressed in \textit{E. coli} BL21 cells and purified by affinity chromatography.\textsuperscript{18} For GST-hLMW-PTP, the GST tag was removed by thrombin cleavage and purified using a Superdex 75 column (Amersham Bioscience) in 50 mM HEPES buffer at pH 7.

\textit{Inhibition assays}

Inhibitors were a gift from Abbott Laboratories (Abbott Park, IL, USA) and BZ3 (3-(3,5-dibromo-4-hydroxy-benzoyl)-2-ethylbenzofuran-6-sulfonicacid-(4-(thiazol-2-ylsulfanyl)-phenyl)-amide) was purchased from Calbiochem. For each experiment, the reaction was conducted in 96-well microplates with 100 μL of reaction mixture containing 3.75 μg of MptpB protein in 50 mM Tris/50 mM BisTris/100 mM sodium acetate buffer pH 6 and the different inhibitors (0–250 μM of compound) and incubated for 10 min at room temperature, followed by the addition of p-nitro phenyl phosphate (pNPP) to a final concentration of 0.2 mM and incubated for a further 5 min. The reaction was quenched by the addition of 0.5 M NaOH to measure absorbance at 405 nm. Production of p-nitro phenol (pNP)\textsuperscript{19} was quantified using a pNP (Sigma) calibration curve (2–2000 μM). Control reactions without enzymes were included to account for the spontaneous hydrolysis of pNPP. Phosphate release was calculated as a percentage of the specific activity and plotted as a function of inhibitor concentration to determine the IC\textsubscript{50}. All assays were performed in triplicate in at least three independent experiments.

To determine the type of inhibition, different inhibitor concentrations were selected to yield 30% to 75% inhibition for each concentration of pNPP (62.5 μM, 125 μM, 250 μM, 500 μM, 1 mM and 2 mM) in the reactions performed as explained earlier. The phosphate released (\textit{V}) was quantified as explained earlier and plotted in a Lineweaver–Burk plot (1/\textit{V} against 1/[\textit{I}]). All assays were done in triplicate. To estimate the inhibition constants, a range of inhibitor concentrations was used for each assay (0, 3.9, 7.8, 15.6, 31.3, 62.5 and 125 μM) and three specific pNPP concentrations were used, 100, 200 and 300 μM, to determine the phosphate released (\textit{V}). For competitive inhibitors, the Dixon plot was constructed by plotting 1/\textit{V} as a function of [\textit{I}] and inhibition constant (\textit{K}i) determined as the point of interception of each line (different substrate concentrations). For the non-competitive inhibitor (BZ3), the slope of the double reciprocal (Lineweaver–Burk plot) was plotted as a function of [\textit{I}] and the \textit{K}i was determined by the \textit{x} intercept. Control assays for selectivity with human protein phosphatases VHR and DUSP22, and lipid phosphatase PTEN, used 10 μg of protein and 80 μM of inhibitor incubated for 10 min at room temperature, followed by the addition of pNPP to a final concentration of 0.2 mM or PI(3,4,5)P3 (Echelon Bioscience) to a final concentration of 125 μM in the case of PTEN.

\textbf{Cell and bacterial culture conditions}

J774A.1 macrophage cells (ATCC) were cultured in Dulbecco’s modified Eagle’s medium (Sigma) containing glucose (25 mM) and l-glutamine (4 mM) supplemented with 10% heat inactivated fetal bovine serum (FBS, Invitrogen) at 37°C in a humidified atmosphere with 5% CO\textsubscript{2}. \textit{Mycobacterium bovis} Bacille Calmette-Guérin (BCG, Pasteur) was grown at 37°C in Middlebrook 7H9 medium (Difco) containing 0.05% Tween 80 with shaking, or on Middlebrook 7H10 agar medium. Both types of medium were supplemented with 10% oleic acid/albumin/dextrose/catalase enrichment and 0.5% glycerol.

\textbf{Macrophage infections}

J774A.1 cells were seeded in 12- or 6-well culture plates (Corning) at a density of 2×10\textsuperscript{5} or 1×10\textsuperscript{6} cells per well, respectively, and
 incubated overnight. Cells were subsequently washed twice in Dulbecco’s PBS (Sigma) and 1 mL of fresh culture medium added, supplemented with the inhibitors to a final concentration of 80 μM (dissolved in dimethyl sulphoxide, DMSO) or the equivalent volume (8 μL) of DMSO. Frozen aliquots of BCG, in PBS containing 10% glycerol, were thawed, vortexed vigorously with sterile glass beads, washed twice with DMEM supplemented with 10% FBS and used to infect J774A.1 cells at a multiplicity of infection of 1:1. After 4 h of infection, cells were washed four times with Dulbecco’s PBS to remove extracellular bacteria and 1 mL of fresh culture medium added; this was defined as time 0 h. At 24 h, the infected cells were lysed in 0.05% Tween 80 and the number of viable bacteria in each well determined by plating 10-fold serial dilutions on Middlebrook 7H1O agar plates in triplicates. The plates were incubated for 3 weeks at 37°C prior to counting the number of viable bacteria. The numbers of viable macrophages and control bacteria grown in cell culture medium in the presence or absence of inhibitors were also determined by Trypan Blue exclusion or colony counts, respectively. All assays were performed in triplicate in at least three separate experiments.

Results

Identification of MptpB inhibitors

Our approach for identifying MptpB inhibitors utilized a focused library of compounds originally developed to inhibit human protein tyrosine phosphatase 1B (hPTP1B).21,22 The initial set contained six compounds of different chemical scaffold families with various potencies against hPTP1B (Table 1). These compounds were either single-site p-Tyr mimetics (compounds 2–4 and compound 6), that bind to the active site (P1 pocket), or double-site compounds (1 and 5) that target the P1 pocket together with a secondary p-Tyr binding pocket in hPTP1B (P2 pocket).23 From the initial set, only compound 1 showed significant inhibition of MptpB with an IC₅₀ <10 μM, and a Ki of 1.5 μM; the rest were poor inhibitors with IC₅₀ values >90 μM. Compound 1 contains an isoxazole head and a salicylate group, a combination shown before to confer both cell permeability and activity.22,24 Further screens with derivatives of the initial set (11 single-site and 6 double-site compounds) showed that isoxazole-based single-site inhibitors, like compound 6, had no inhibitory effect on MptpB at the concentrations tested [up to 250 μM; Table S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. In contrast, double-site isoxazole-based compounds (compounds 7–12 in Table 1) identified another potent inhibitor of MptpB, compound 7, with a Ki of 3.5 μM. This compound contains the same isoxazole–salicylate groups as compound 1 with an additional nitro group substituent in the salicylate moiety. Interestingly, compound 7 is a poor inhibitor of hPTP1B with a Ki of 63.4 μM (Table 1).

Importantly, the compounds tested show selectivity when comparing their activities against those of hPTP1B and other tyrosine phosphatases. The best inhibitors of hPTP1B are compounds 2 and 5, with Ki of 2.8 and 1.9 μM, respectively, but they show very low or no activity towards MptpB. On the other hand, compounds 1 and 7 are much better inhibitors for MptpB than for hPTP1B (Table 1). Significantly, substitutions at the isoxazole head or at the linker region of the double-site compounds, found to enhance potency against hPTP1B as seen in compounds 5 and 8,21,22 make these compounds inactive against MptpB. This suggests differences in the mode of binding of the inhibitors towards the human PTP1B and mycobacterial MptpB. None of the compounds tested had significant activity against the control tyrosine-specific phosphatases, TbPTP1 and human LMW-PTP (Table 1).

In addition to the active site inhibitors, we also tested an allosteric inhibitor of hPTP1B, BZ325, which we reported to have an inhibitory effect on TbPTP1.26 This compound exhibited the highest potency against MptpB in vitro, with an IC₅₀ value of 1.2 μM (Table 1). This inhibitory effect was totally unexpected, as MptpB does not contain the same allosteric-binding site as that found in hPTP1B. In fact, both the topology and the three-dimensional structure of MptpB are remarkably different from those of hPTP1B in this region. This suggests that BZ3 exploits an alternative and unknown binding site in MptpB to fulfil its inhibitory effect.

Following the initial screen, we examined the type of inhibition of all the potent inhibitors identified. Kinetic analyses varying the concentrations of both the chromogenic substrate pNPP and the inhibitor clearly demonstrate that compounds 1 and 7 behave as competitive inhibitors of MptpB (Figure 1a and b), where different inhibitor concentrations result in marked changes of the Ki, while the Vmax remained unchanged. Instead, the BZ3 compound behaved as a non-competitive inhibitor as the Vmax values were dependent upon the inhibitor concentration while the Km remained unaltered (Figure 1c). These results indicate that compounds 1 and 7 are likely to bind in the active site.

Inhibitors of MptpB reduce mycobacterial survival in macrophages

Mutants of M. tuberculosis lacking mptpB have reduced virulence3 and hence we hypothesized that chemical inhibition of MptpB may similarly impair intracellular mycobacterial survival. The efficacy of the most potent in vitro inhibitors of MptpB (compound 1 and BZ3) in reducing mycobacterial survival in J774A.1 macrophages was therefore examined. Resting macrophages act as the first line of defence for early clearance of M. tuberculosis with subsequent interferon γ (IFNγ) activation contributing to M. tuberculosis killing; a reduced IFNγ response is associated with M. tuberculosis persistence in the infected host and with disease progression.27 Hence, for this study, the growth of mycobacteria in resting macrophages was examined in order to mimic infection in a susceptible host (where IFNγ activation may be impaired). We selected poor inhibitors of MptpB (compounds 5 and 12) that have an inhibitory effect on hPTP1B as negative controls.

Our results show that in the absence of the inhibitors (DMSO treated), intracellular growth of the mycobacteria (BCG)
### Table 1. Inhibition data for compounds tested against MptpB

<table>
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<th>Compound</th>
<th>Structure</th>
<th>IC₅₀ (µM)</th>
<th>Ki (µM)</th>
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Continued
Allosteric hPTP1B inhibitor

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| Table 1. | Continued |
|-----------------|-----------------|-----------------|-----------------|
| **Compound** | **Structure** | **IC_{50} (μM)** | **K_{i} (μM)** |
| 11              |                | MptpB | TbPTP1 | hLMW-PTP | MptpB | hPTP1B |
| ![Structure](image1) | 124            | >250  | >250   | ND      | 33.6  |
| 12              |                | 250   | >250   | >250   | ND    | 15.1  |

Allosteric hPTP1B inhibitor

BZ3

<table>
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<tr>
<th>Structure</th>
<th>IC_{50} (μM)</th>
<th>K_{i} (μM)</th>
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<td><img src="image2" alt="Structure" /></td>
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<td>10 (ref 26)</td>
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ND, not determined. The initial screen consisted of compounds 1–6 belonging to different chemical scaffold families. The compounds were tested on MptpB and control tyrosine-specific protein phosphatases TbPTP1, hLMW-PTP and hPTP1B. Double-site binders were further explored for inhibition of MptpB, using compounds 7–12. In addition, the commercial allosteric inhibitor of hPTP1B, BZ3, was tested on MptpB and control phosphatases. The calculated IC_{50} and K_{i} values are shown in the table (see the Methods section for details).

Increased 3–5-fold after 72 h of infection (Figure 2a and b). However, when treated with 80 μM of compound 1, intracellular mycobacterial growth was substantially reduced, with up to a 64% decrease in the bacterial burden of macrophages compared with control cells (DMSO treated) after 72 h of infection (Figure 2a and b). Treatment with BZ3 at the same concentration produced a reduction of up to 34% in the bacterial burden. In contrast, treatment of macrophages with compound 5 or 12, specific for hPTP1B, had no effect on the bacterial growth compared with the control cells. Furthermore, reduction in mycobacterial growth is dose-dependent as observed for both compounds 1 and BZ3 (Figure 2c). At 20 μM concentration, compound 1 shows a more dramatic effect on the bacterial burden (42% reduction) compared with that of BZ3 (15% reduction), with compound 1 causing >90% reduction at 160 μM, while BZ3 treatment causes a 60% reduction at the same concentration.

Importantly, mycobacteria grew equally well extracellularly, in cell culture medium (DMEM) in the absence and presence of any of the inhibitors (Figure 2a and b, insets) demonstrating a lack of bactericidal activity of these compounds. Control experiments showed that the number of viable macrophages was unaffected by the presence of DMSO or the various inhibitors in the culture medium (Figure 2d). These findings are consistent with compound 1 assisting bacterial clearance, presumably by impairing the role of MptpB in mycobacterial survival. We believe this to be the result of direct inhibition of MptpB as compounds 5 and 12 that inhibit hPTP1B but not MptpB show no effect on mycobacterial survival. Additional supporting evidence comes from the fact that compound 1 shows high selectivity against other potentially related human phosphatases. Inhibition tests using dual-specificity phosphatases (VHR, DUSP22), tyrosine-specific phosphatases (hLMW-PTP) or lipid phosphatases such as PTEN show no significant loss of their phosphatase activity when treated with compound 1 (Table 2). In contrast, we found that BZ3 exhibits considerable inhibition of the human phosphatases tested, with only 8% to 24% activity remaining for DUSP22, VHR and PTEN. This lower selectivity and broader spectrum of phosphatase targets may explain in part the less accentuated reduction in mycobacterial growth observed for this compound. It is noted that while disruption of mptpB has previously been reported to reduce mycobacterial survival in activated macrophages, mycobacterial survival in resting macrophages was unaffected. It is possible that the apparent disparity in our findings relates to the use of the more virulent M. tuberculosis Erdman strain rather than BCG, and/or a greater multiplicity of infection (1:10, macrophage:bacteria) in that study.

Docking of the active site inhibitors

To better understand the mode of binding and apparent selectivity of the competitive inhibitors, we carried out a computational docking analysis of each compound into the active site of MptpB. Results from both docking methods (GOLD and Glide) were consistent, producing nearly identical docked conformations of the inhibitors. In addition, we found a good correlation between the experimental IC_{50} values and the computational scores from GlideScore (R^2 = 0.85) and the ChemScore (R^2 = 0.84) (Figure 3) (see the Methods section). Both scoring functions confirmed the enzymatic assay results ranking the most active inhibitors, compounds 1 and 7, with high scores [Table S2, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. These results provided an independent validation of the two computational methods against the experimentally determined IC_{50} measurements. Binding of compound 1 involves both electrostatic and hydrophobic interactions: the isoxazole head binds in the active site pocket (P1) of MptpB similar to its binding to hPTP1B.
non-competitive inhibitor as noted by variation in the competitive inhibition of MptpB and (c) BZ3. BZ3 behaves as a competitive inhibition of MptpB, (b) compound 7 (inset) showing competitive inhibition of MptpB and (c) BZ3. BZ3 behaves as a non-competitive inhibitor as noted by variation in the V_{max} at different inhibitor concentrations (inset), while K_{m} remains unchanged.

(Figure 4a and b) and with its carboxylate forming hydrogen bonds with amide groups in the phosphate-binding loop (P-loop) and the side chain of the catalytic Arg166 (Figure 4c). The phenyl group packs against the ring of F161, also in the P-loop. In addition, the salicylate moiety binds in a secondary pocket (P2 pocket) and is placed midway between residues R210 and R63 with interactions between the hydroxyl groups in the inhibitor and the Arg side chains. A similar orientation and binding mode was observed for the related compound 7 [Figure S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)].

Interestingly, this secondary binding pocket in MptpB is diametrically opposed to the P2 binding pocket of compound 1 in hPTP1B (PDB ID 1XB0) (Figure 4a and b). The P2 pocket in MptpB contains several charged residues (R59, E60, R63, R64) and is formed by an insertion (R56–R64), unique to MptpB, that folds as a helical segment (orange in Figure 4a and c). The conformation of the equivalent region in hPTP1B, a disordered loop, is such that it will clash with the inhibitor binding in the same orientation as in MptpB. This region is also unstructured in eukaryotic PTPs, DUSPs and PTEN, explaining the selectivity observed for compound 1.

The presence of F161 in the active site provides a further basis for the specificity of compound 1. This residue marks the divide between the P1 and P2 pockets and restricts the conformational and chemical variations in the linker region between the two head groups. The importance of the linker is highlighted by the lower activity of the cyclopropyl (compound 10) and methyl substituted (compound 11) linkers. The value of the salicylate moiety is supported by the poor binding of the N-substituted derivative (compound 12). More detailed experimental information on the structure of MptpB–inhibitor complexes will be necessary to explain the relevance of the amine (compound 8) or halogen substituted (compound 9) analogues that have modest potency. The proposed binding mode of compound 1 explains both specificity to MptpB, and selectivity when compared with human PTPs and lipid phosphatases (Tables 1 and 2). This suggests the possibility of developing further this compound at the salicylate moiety to increase specificity and retain selectivity.

Discussion

Screening of a focused library of phosphatase inhibitors identified new lead compounds that have a potent inhibitory effect on MptpB. We have shown that compounds 1 and 7 are competitive inhibitors of MptpB (Table 1 and Figure 1), and we propose a mechanism of binding in the active site exploiting a secondary binding pocket that is specific to MptpB (Figure 4 and Figure S1). The novelty of this study resides in the efficacy of the newly identified inhibitors in reducing mycobacterial survival in infected macrophages. Compound 1 caused a substantial reduction in the survival of mycobacteria within resting macrophages, while BZ3, which showed high potency against MptpB in vitro, had a more modest effect. This reduction is dose-dependent and more pronounced for compound 1 than BZ3, particularly at lower concentrations of the inhibitor (20 μM), and reaches >90% reduction at the highest concentration tested (160 μM). In addition, compound 1 appears to exhibit greater selectivity against human protein and lipid phosphatases (VHR, DUSP22 and PTEN) (Table 2).

Resting macrophages act as the first line of defence for early clearance of M. tuberculosis in vivo with activation playing an important role in providing host resistance. Hence, the ability of the inhibitors to reduce mycobacterial survival within these cells indicates their potential for clearing infection in a susceptible host (where activation may be impaired) and the role of MptpB as a virulence factor. The growth of extracellular mycobacteria in culture medium was unaffected by the inhibitors and this is consistent with previous observations that, while MptpB...
Table 2. Specific activity remaining after treatment of human protein tyrosine phosphatases (hLMW-PTP, VHR and DUSP22) and lipid phosphatase (PTEN) with 80 μM inhibitor in comparison with MptpB

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<th>Specific activity (%)</th>
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<tr>
<td>MptpB</td>
<td>0</td>
</tr>
<tr>
<td>hLMW-PTP</td>
<td>100</td>
</tr>
<tr>
<td>VHR</td>
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</tr>
<tr>
<td>DUSP22</td>
<td>84</td>
</tr>
<tr>
<td>PTEN</td>
<td>100</td>
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Figure 2. Treatment with MptpB inhibitor compound 1 reduces mycobacterial survival in infected macrophages. (a and b) J774A.1 macrophages were seeded at 2×10³ and 1×10⁵ cells/mL, respectively, infected with BCG in the presence of DMSO (0.8%) with and without an inhibitor at 80 μM [compound 1 (C1), compound 5 (C5), compound 12 (C12) or BZ3], then lysed at 24 and 72 h (a) or 0 and 72 h (b) post-infection and the intracellular bacteria plated for determining cfu. Survival of extracellular bacteria in the culture medium was also determined in parallel after 72 h (insets). Data are expressed as mean cfu for triplicate wells ±SE. Reduction in mycobacterial survival is more prominent for compound 1 than BZ3, especially at lower concentrations of the inhibitors. (d) J774A.1 macrophage viability following growth in the absence (–) of inhibitors, with DMSO (0.8%) and with the various inhibitors (80 μM), determined using Trypan Blue staining.

Figure 3. Correlation between docking functions and experimental inhibition. Correlation between the experimentally determined IC50 values and the scoring functions of ChemScore and GlideScore. Only data for compounds with IC50 values <124 μM were selected for the plot.
Recently, the structure of MptpB in complex with an oxalylamino derivative inhibitor [(oxalylamino-methylene)-thiophene sulphonamide (OMTS)] has been reported. OMTS is a single-site binding inhibitor, unrelated to our double-site binding inhibitors. In the crystallographic complex, a second molecule of OMTS was found bound to a distal site in MptpB. The distal OMTS molecule targets the P2 pocket similarly to the salicylate moiety in our compound 1 (Figure 4d), providing experimental evidence for the role of this specific secondary pocket in MptpB.

The identification in this study of two new inhibitors that bind to a secondary site of MptpB, with unique sequence and conformational features, offers an excellent opportunity to exploit such differences from human phosphatases to develop more potent and selective inhibitors. As MptpB has no human orthologues (hPTP1B with only 6% sequence similarity), it makes this enzyme an excellent candidate for pharmaceutical intervention. Importantly, the inhibitors tested do not have bactericidal action, which in addition to MptpB being an extracellular target, can be advantageous in avoiding evolutionary pressure on the bacteria to develop resistance. Further rational development of the MptpB inhibitors identified in this study could provide a unique and effective means to fight TB and increase the chances of eliminating the pathogen. The cell activity of our inhibitors in infected macrophages is strong proof of the concept that chemical inhibition of MptpB could be used to reduce the survival of mycobacteria and enhance the ability of the innate immune response to clear the infection.

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**Transparency declarations**

B. S. and G. L. were employees of Abbott Laboratories (and now work for Sirtris Pharmaceuticals, Inc. and Ambit Biosciences, respectively) and C. A.-Z. was an employee of Abbott Laboratories (now at UIC). All other authors: none to declare.

**Supplementary data**

Figure S1 and Tables S1 and S2 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).
References