Substituted diphenyl ethers as a novel chemotherapeutic platform against *Burkholderia pseudomallei*

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Identification of a novel class of anti-
Burkholderia compounds is key in
addressing antimicrobial resistance to current therapies as well as naturally
occurring resistance. The FabI enoyl-ACP reductase in Burkholderia is an
underexploited target that presents an opportunity for development of a new
class of inhibitors. A library of substituted diphenyl ethers were used to identify
fabI1 specific inhibitors for assessment in B. pseudomallei ex vivo and murine
efficacy models. Active FabI1 inhibitors were identified in a two-stage format
consisting of percent inhibition screening and minimum inhibitory concentration
(MIC) determination by microbroth dilution method. Each compound was
evaluated against the B. pseudomallei 1026b (efflux-proficient) and Bp400
(efflux-compromised) strains. In vitro screening identified candidate substituted
diphenyl ethers that exhibited MIC less than 1μg/ml and enzyme kinetic assays
were used to assess potency and specificity against FabI1 enzyme. These
compounds demonstrated activity in a Burkholderia ex vivo efficacy model and
two demonstrated efficacy in an acute B. pseudomallei mouse infection model.
This work establishes substituted diphenyl ethers as a suitable platform for
development of novel anti-Burkholderia compounds that can be used for
treatment of melioidosis.
Burkholderia pseudomallei is a gram-negative bacteria endemic to Southeast Asia and Northern Australia and is the causative agent of melioidosis. This disease is difficult to manage and often fatal in humans if not aggressively and appropriately treated (1-5). While there are current effective treatment options for Burkholderia infections, there has been increasing concern regarding drug resistant and natural resistant strains that render the current treatment regimen ineffective and negatively impact the ability to treat and manage the disease (2, 5-7). Also, mounting evidence indicates B. pseudomallei as an emerging pathogen in other areas of the world and demand for treatment of melioidosis will increase as a result (8-12). These reasons along with the ability of Burkholderia to be easily spread via aerosol and ease of dissemination provide the need to develop novel compounds that inhibit underexploited drug targets.

The enoyl-ACP reductase enzyme FabI1 (encoded on chromosome 1) involved in fatty acid elongation in the bacterial fatty acid biosynthesis type II (FASII) pathway is an underexploited drug target in Burkholderia. Our own antibacterial discovery program has demonstrated that substituted diphenyl ethers inhibit the FabI enzyme of a variety of organisms leading to in vivo efficacy (13, 14). The ability of this compound class to exhibit broad-spectrum activity and demonstrate efficacy in vivo make it attractive for development of novel Burkholderia chemotherapeutics.

Using our library of substituted diphenyl ethers, our drug discovery efforts have been expanded to assess whether inhibition of the B. pseudomallei enoyl-
ACP reductase FabI1 demonstrates efficacy in *ex vivo* and animal models of infection. To assess the activity of our library of substituted diphenyl ethers we first identified lead inhibitors by whole bacterial screening against *B. thailandensis* strains E264 and Bt38 (E264 Δ[amrAB-oprA] Δ[bpeAB-oprB]) and followed up with MIC determination using *B. pseudomallei* strains 1026b (15) and Bp400 (1026b Δ[amrAB-oprA] Δ[bpeAB-oprB]) (16). We then assessed the most potent FabI1 inhibitors in both the *Burkholderia* *ex vivo* efficacy model and our rapid animal efficacy model. The use of selective FabI1 inhibitors, efflux mutant strains, and the *ex vivo* and animal models of efficacy allow us to directly assess whether or not targeted inhibition of FabI1 could be used as a suitable platform for novel anti-*Burkholderia* therapies.
MATERIALS AND METHODS

Kinetics and inhibition of FabI1. FabI1 and octenoyl-CoA (Oct-CoA) were prepared as described previously (17). Inhibition constants (K_i) were analyzed using the standard equation for uncompetitive inhibition. Initial velocities were determined at a fixed substrate concentration and the data were fit to eq 1 using a value of \( K_m \) (Oct-CoA) = 160 \( \mu \)M.

\[ \frac{v}{v_0} = \frac{(K_m + S)/(K_m + S[1 + I/K_i])}{(1)} \]

The parameters \( v \) and \( v_0 \) are the initial velocities in the presence and absence of inhibitor. The substrate concentration was fixed at 30 \( \mu \)M, and the inhibitor concentration was varied from 0-8000 nM. Data fitting was performed using Grafit 4.0 (Erithacus Software Ltd.).

Progress curve analysis was performed as described previously to identify slow-onset inhibitors of FabI1 (13). Assays were performed by adding enzyme (5 nM) to solutions containing glycerol (8%, v/v), BSA (0.1 mg/v), DMSO (2%, v/v), Oct-CoA (300 \( \mu \)M), NADH (250 \( \mu \)M), NAD\(^+\) (200 \( \mu \)M) and inhibitor (50 nM). Reactions were monitored until the progress curve became linear, indicating that the steady-state had been reached. In this protocol, the low enzyme concentration and high substrate concentration ensure that substrate depletion was minimized so that the progress curves were approximately linear over a period of 30 min in the absence of inhibitors.

Preincubation assays were performed to obtain the true inhibition constants and to determine the preference of slow-onset inhibitors for the
different cofactor-bound forms of FabI1. Enzyme (10 nM) was preincubated in the presence of a fixed concentration of DMSO (2% v/v), NAD$^+$ (20–400 µM), NADH (250 µM) and inhibitors (0–2000 nM) for 5 h at 4°C. After warming to 25°C, assays were initiated by the addition of Oct-CoA (30 µM). The inhibition constant was calculated as previously described (18).

Bacteria and screening and minimum inhibitory concentration determination. B. thailandensis E264 (efflux-proficient) (19), B. thailandensis Bt38 (E264 Δ[amrAB-oprA] Δ[bpeAB-oprB]) (kindly provided by H. P. Schweizer Colorado State University), B. pseudomallei 1026b (efflux-proficient) (15), and B. pseudomallei Bp400 (1026b Δ[amrAB-oprA] Δ[bpeAB-oprB]) (16) were grown to an OD$_{600}$ of ~0.6, frozen at -80°C in 10% glycerol and were used as standard bacterial stocks for these studies. For each evaluation bacteria were prepared fresh by growth from the standard stocks on Luria-Bertani (LB) Agar, Miller (BD) grown at 37°C for 48-72 h. Bacteria recovered from the LB plates were inoculated in 50 mL LB Broth. Broth cultures were then incubated for 24hrs at 37°C passed 1:100 and incubated for an additional 6 h at 37°C. Bacteria were then diluted in to a concentration of 1x10$^4$ colony forming units (CFU)/mL in cation-adjusted Mueller-Hinton broth (caMHB; BD, Franklin Lakes, NJ) and 50 µL of sample was added to each well of the test plate. Substituted diphenyl ethers were screened in a percent inhibition high-throughput fashion against B. thailandensis E264 and B. thailandensis Bt38 in a 96-well plate format to identify lead compounds. All compounds were diluted in caMHB to concentrations of 80
and 40 μg/mL in a 50 μL volume/well. MIC of candidate compounds was determined against *B. pseudomallei* 1026b and *B. pseudomallei* Bp400 by broth microdilution method. For MIC determination, compounds were added to the 96-well plate starting at either 512 μg/mL in the first column and serially diluted 1:2 to column 12 for a final concentration of 0.12 μg/mL caMHB. MIC plates were incubated at 37°C for 18 h at which time 10 μL of Alamar Blue (Invitrogen, Carlsbad, California) was added to each well and plates were incubated for an additional 4 h. Reduction of Alamar Blue was determined by absorbance readings at wavelengths of 570 and 600 nm using a microplate reader (Biotek, Winooski, VT). Percent growth reduction was calculated from spectrophotometric readings over the drug concentration series. MIC was determined as the lowest drug concentration to inhibit visible growth.

**Cytotoxicity assay.** A Vero cell line (African green monkey kidney cells) was used to assess potential cytotoxicity similar to previously described (20). Vero cells were maintained in Leibovitz’s (L15) growth media without phenol and supplemented with heat inactivated Newborn Calf serum and Penicillin-Streptomycin. The cells were grown at 37°C and subcultured weekly. Cell suspensions containing 1.3 X 10^5 cells per ml and resazurin (21) were added to the 96 well plates. The compound was added and 2 fold serial dilutions were completed with the final volume in the wells 100μL. The plates were incubated for 72 hours at 37°C in the dark. The plates were read at 570nm and 600nm in a
plate reader and the LD50 (lethal dose causing 50% loss of cell viability) of the compound was calculated using standard procedures.

**Burkholderia ex vivo model of efficacy.** Infected RAW 264.7 mouse macrophages (American Type Tissue Collection, Manassas, VA) were used to assess intracellular efficacy of our compounds. RAW cells were cultured in complete medium that consisted of minimal essential medium (Invitrogen), 10% fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine (Invitrogen), 1x nonessential amino acids (Invitrogen), and 0.075% sodium bicarbonate (EMD Science, Gibbstown, NJ). Bacteria were added to 2.5x10^5 RAW cells per well in a 24-well tissue culture plate at a multiplicity of infection of 5 CFU per cell in a 0.5 mL media. The plates were then centrifuged at 2,400 X g for 2 min and placed at 37°C/5% CO2 to incubate for 1 h. Supernatant was then removed; plate washed once with 2 mL PBS and 1 mL of either 0.35 mg/mL kanamycin (Sigma-Aldrich, St. Louis, MO) for wild-type strains or 0.1 mg/mL kanamycin (Sigma-Aldrich) for double efflux mutant strains in complete media. Plates were then incubated for 1 h and washed 2 times with 2 mL PBS.

Selected compounds were then added to each well at either 10, 1, 0.1 μg/mL in complete media in triplicate. Untreated, 1 μg/mL doxycycline (Sigma-Aldrich), and 10 μg/mL ceftazidime were included as controls. Plates were incubated for 18 h and cells observed for signs of infection. Cells were washed 3 times with 2 mL PBS and 1 mL sterile ddH2O added to each well. Each well was thoroughly mixed/scraped to insure complete cell lysis. Lysates were then...
serially diluted 1:10 and inoculum plated from the neat, $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ onto LB agar plates and plates were incubated for 48 h at 37°C. Colonies from each plate was counted and $\log_{10}$ CFU/mL lysate was calculated.

**Acute *B. pseudomallei* mouse model of infection for evaluation of efficacy.**

5-6 week old BALB/c female mice (Charles River Laboratories, Wilmington, MA) were challenged by intranasal infection with 5,000 CFU/mouse *B. pseudomallei* Bp400. Animals were anesthetized with a mixture of 100 mg/kg ketamine and 10 mg/kg xylazine delivered intraperitoneally. The bacteria were diluted to the appropriate concentration in PBS to achieve an inoculum concentration of $2.5\times10^5$ CFU/mL. The inoculum was then delivered in a 20 μL volume dropwise in alternating nostrils. Ceftazidime was formulated for injection in a 0.1 % BSA solution in PBS (pH 7.4) at a concentration of 40 mg/ml. PT52 and PT68 were formulated for injection in a lipid-based delivery system of a combined lipid and surfactant mixture consisting of 40% Captex 200 (Abitec Corp., Janesville, WI): 40% Solutol HS15 (BASF, Mount Olive, NJ): 20% Capmul MCM (Abitec Corp., Janesville, WI). The mixture was gently heated at 50°C to homogenize components. The resulting homogenous isotropic mixture was then diluted with PBS to yield a fine dispersion at a concentration of 40 mg/mL. 200 mg/kg ceftazidime, PT52, or PT68 were delivered intraperitoneally with treatment beginning at time of infection and repeated doses delivered every 12 h. The number of viable bacteria in lung and spleen was determined at 60 h post-exposure by plating serial 10-fold dilutions of homogenates onto LB agar and
incubating for 48 h at 37°C. Bacterial burden was assessed using a one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test with significance determined by a P value < 0.05. Outliers were determined by Grubb’s test.
RESULTS

Substituted diphenyl ethers are potent inhibitors of *Burkholderia*. A library of diphenyl ethers were screened for potency in an established high throughput percent growth inhibition assay and confirmed using the microbroth dilution method. The substituted diphenyl ethers were tested against the efflux mutant strain *B. thailandensis* Bt38 and efflux-proficient strain *B. thailandensis* E264 to assess each candidate as a pump substrate as well as to determine inhibitory activity against whole bacteria. Thirty-two compounds out of the library tested exhibited greater than 50% growth reduction and twenty-two showed greater than 90% growth reduction at 20 μg/mL against *B. thailandensis* Bt38. Only PT52 inhibited greater than 50% growth against *B. thailandensis* E264 at 40 μg/mL. PT51, PT52, PT55, and PT68 were further investigated due to previous SAR studies (17) and to evaluate the effect of A-ring substitutions on efficacy (22).

MIC values were determined for the efflux-compromised *B. pseudomallei* Bp400 mutant strain and efflux-proficient strain *B. pseudomallei* 1026b. PT52, PT55, and PT68 had MIC values less than 1 μg/mL against *B. pseudomallei* Bp400 (Table 1). Evaluation of PT51, PT52, PT55, and PT68 against the efflux-proficient strain *B. pseudomallei* 1026b resulted in increased MIC values due to drug efflux (Table 1). While the majority of substituted diphenyl ethers demonstrated potency against *Burkholderia*, they were also readily effluxed.
The cytotoxicity of each compound was evaluated using a standard Vero cell assay (Table 1). Each diphenyl ether compound exhibited LD$_{50}$ values 100-fold greater than MIC, which is consistent with our previous reports for compounds in this drug series (20). There was no toxicity observed in the *in vivo* RAW264.7 macrophage assay for the clinical drugs ceftazidime and doxycycline, which is consistent with published reports (23).

**Diphenyl ethers with whole bacteria activity have potency against FabI1 enoyl-ACP reductase enzyme.**

Based on the analysis of MIC values, PT51, PT52, PT55 and PT68 were selected as lead compounds and their binding affinities and mode of inhibition were determined against FabI1 (Table 2). Compounds PT51 and PT55 were rapid reversible inhibitors displayed moderate binding affinity to FabI1 with IC$_{50}$ values of 2699 ± 287 nM and 695 ± 132 nM.

The addition of a fluoro substituent to the A-ring (PT51 vs. PT55) resulted in only a four-fold improvement in the binding affinity. However, replacement of the fluoro substituent with a larger functional group such as a chlorine (PT52) or a propenyl group (PT68), dramatically enhanced binding affinity to the FabI1-NAD$^+$ binary complex with $K_1$ values of 3.69 ± 0.35 nM and 6.12 ± 0.31 nM, respectively. Progress curve analysis also indicated that compounds PT52 and PT68 were time-dependent inhibitors, displaying a slow-onset inhibition mechanism against FabI1. It is not unusual for some inhibitors to exhibit this kinetic behavior since triclosan and selected diphenyl ethers were shown to be
slow-onset inhibitors of FabI1 (17). Preincubation experiments allowed us to evaluate the dependence of enzyme inhibition on NADH or NAD\(^{+}\), which provides the true thermodynamic affinity of the inhibitor for E-NAD\(^{+}\) (K\(_1\)) and E-NADH (K\(_2\)). For both PT52 and PT68, the dependence of K\(_i\) on [NAD\(^{+}\)] was best described by the equation that includes the inhibitor binding to both E-NAD\(^{+}\) and E-NADH forms of FabI1, albeit based on the K\(_1\) and K\(_2\) values of these two compounds there is a 100-fold preference for E-NAD\(^{+}\).

Substituted diphenyl ethers demonstrate efficacy in a *Burkholderia* ex vivo model of efficacy and in the *Burkholderia* rapid animal model of efficacy. PT51, PT52, PT55 and PT68 were further evaluated in a *Burkholderia* ex vivo model of efficacy based on their whole bacterial activity. Evaluation of activity in the ex vivo model was conducted against *B. pseudomallei* 1026b at 10 \(\mu g/mL\) and at multiple concentrations against *B. pseudomallei* Bp400 (Figure 1AB). PT51, PT52, PT55 and PT68 all inhibited the growth of the efflux pump mutant *B. pseudomallei* Bp400 >90\% at 10\(\mu g/ml\), and growth of *B. pseudomallei* 1026b was not inhibited by PT51, PT52, PT55, and PT68.

To assess whether PT51, PT52, PT55 and PT68 demonstrated inhibition in a concentration dependent manner, they were further evaluated for activity against *B. pseudomallei* Bp400 in the ex vivo model. Each of the compounds inhibited growth of *B. pseudomallei* Bp400 in a dose dependent fashion (Figure 1B), and the precise 90\% growth reduction values for each compound in the ex vivo model were determined (Table 3). Importantly, PT51, PT52, PT55 and
PT68 had MIC values in the *ex vivo* model comparable to those determined in artificial medium. PT52 and PT68 were tested in the acute *B. pseudomallei* animal model of infection to determine efficacy. These compounds were chosen due to their slow-onset inhibitor characteristics observed in enzyme kinetic assays, in addition to inhibitory activity in the *ex vivo* efficacy model. Mice were challenged intranasally with 5,000 CFU *B. pseudomallei* Bp400. Bacterial burden was assessed at 60 h post infection and treated groups were compared to the untreated control group. PT52 treated mice exhibited a 0.6 Log$_{10}$ CFU/mL reduction (P<0.05) in the lung and a 1.1 Log$_{10}$ CFU/mL reduction (P<0.01) in the spleen (Figure 3AB). PT68 showed reduction only in the spleen at 1.3 Log$_{10}$ CFU/ml (Figure 3B). Mice treated with 200 mg/kg ceftazidime were included as a positive control and was determined to have a bacterial burden at the level of detection in both lung and spleen. These studies substantiated that these substituted diphenyl ethers can subterfuge and gain access to the efflux-deficient bacteria under host conditions and inhibit growth through inhibition of FabI1 (24).

**Discussion**

Discovery of new drug targets and chemotherapeutics is key in managing emerging pathogens such as *B. pseudomallei*. *B. pseudomallei* can be particularly challenging to treat due to intrinsic and emerging antimicrobial resistance and the ability to efflux antibiotics. The enoyl-ACP reductase enzymes, particularly FabI in the FASII pathway, are under-exploited new targets...
for the development of anti-\textit{Burkholderia} drugs. Our compounds were developed around the diphenyl ether pharmacophore and in previous studies have exhibited whole bacteria activity and \textit{in vivo} efficacy against \textit{F. tularensis} Schu4 (13, 14).

In this study we have shown this same class of compounds to be efficacious for \textit{in vitro} and \textit{ex vivo} activity against efflux-compromised \textit{B. pseudomallei} Bp400. Unfortunately, the majority of compounds in this class are efflux pump substrates, and while they are effluxed similar to existing clinically used drugs to treat melioidosis, for instance doxycycline, modifications to existing compounds will be made to address this issue or current compounds will need to be formulated to deliver the higher dose requirement that exceeds the higher MIC value.

Importantly, PT52 and PT68 were shown to significantly reduce bacterial burden in the spleen and only PT52 significantly reduced bacterial burden in the lung when compared to untreated mice. This observation, along with the ability of these compounds to display a low nanomolar binding affinity to Fab1 in \textit{vitro}, suggests that the use of substituted diphenyl ethers to inhibit Fab1 enoyl-ACP reductase in \textit{Burkholderia} can be exploited to control infection. Steady-state kinetic analysis showed that PT52 and PT68 were promising inhibitors due to favorable optimization of thermodynamic parameters, specifically inhibitor binding to the Fab1-NAD$^+$ complex represented by \(K_1\). This final stable ground state was coupled with a slow-onset inhibitor mechanism, which was lacking in compounds PT51 and PT55 that displayed higher \(K_1\) values. The loss of slow-onset inhibitor mechanism of PT51 and PT55 against Fab1 may arise from no A-ring
substituent and the smaller van der Waals radius of fluorine compared with that
of chlorine (PT52), respectively. Also, it has been demonstrated that A-ring
substituents play a key role in hydrophobic interaction between diphenyl ether
inhibitors and FabI from *F. tularensis* (13), which leads to slow onset inhibition
and high binding affinity in this system.

By using the diphenyl ethers as tool compounds we were able to show
that inhibition of *B. pseudomallei* FabI1 results in efficacy *in vitro* and in the
animal model of infection. Further optimization of this class, or similar classes of
FabI inhibitors, could open up the possibility for the development of a novel
compound that could be used to treat Melioidosis.

Acknowledgements

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References

16. Mima T, Schweizer HP. 2010. The BpeAB-OprB efflux pump of Burkholderia pseudomallei 1026b does not play a role in quorum sensing, virulence factor
production, or extrusion of aminoglycosides but is a broad-spectrum drug efflux system. Antimicrob Agents Chemother 54:3113-3120.


Tables

Table 1. MIC values of fabI1 inhibitors against wild type and efflux-compromised *B. pseudomallei* strains. MIC values were determined using the microbroth dilution method.

Table 2. Compounds further evaluated for activity against *B. pseudomallei* Bp400 *ex vivo*. Drug concentration that inhibits 90% growth was calculated from linear regression analysis of data obtained at 10, 1 and 0.1 μg/ml.

Table 3. Structure activity relationship studies performed show A-ring substitutions are critical in determining type of inhibition and binding affinity for both E-NAD⁺ (K₁) and E-NADH (K₂).
Figures.

**Figure 1.** RAW264.6 macrophages were infected with *B. pseudomallei* at a multiplicity of infection (MOI) of 5 and treated overnight with either ceftazidime or select substituted diphenyl ethers. *B. pseudomallei* 1026b was treated with 10μg/ml compounds for 18hr, macrophages lysed, and lysate plated on solid media for bacterial enumeration (A). *B. pseudomallei* Bp400 was treated at the same concentration with ceftazidime and at 10, 1, and 0.1mg/ml with substituted diphenyl ethers to observe concentration dependent inhibition. Again macrophages were lysed and lysate plated onto solid media for bacterial enumeration (B).

**Figure 2.** Bacterial burden in mouse lung (A) and spleen (B) at 60 h post infection. The mean of each group was plotted, error bars indicating +/- standard deviation, and dashed line indicates level of detection. Significance was determined by one-way ANOVA and a P-value <0.05. This data is representative of two independent experiments.
### Table 1

*In vitro* Activity of FabI Inhibitors Against *B. pseudomallei*

<table>
<thead>
<tr>
<th>FabI Inhibitor</th>
<th><em>B. pseudomallei</em> 1026b MIC mg/L</th>
<th><em>B. pseudomallei</em> Bp400 MIC mg/L</th>
<th>Vero Cytotoxicity LD&lt;sub&gt;50&lt;/sub&gt; mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT51</td>
<td>2</td>
<td>2</td>
<td>190.2</td>
</tr>
<tr>
<td>PT52</td>
<td>1</td>
<td>0.125</td>
<td>89.8</td>
</tr>
<tr>
<td>PT55</td>
<td>4</td>
<td>0.5</td>
<td>164.9</td>
</tr>
<tr>
<td>PT68</td>
<td>4</td>
<td>0.5</td>
<td>152.9</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.5</td>
<td>0.125</td>
<td>ND</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>2</td>
<td>1</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND – Not determined in this study
Table 2: inhibition of bpFabI-1 by substituted diphenyl ethers

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$K_1$ (nM)</th>
<th>$K_2$ (nM)</th>
<th>Type of Inhibition</th>
</tr>
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<tbody>
<tr>
<td>PT52</td>
<td>3.69±0.35</td>
<td>359±10</td>
<td>Slow-onset</td>
</tr>
<tr>
<td>PT68</td>
<td>6.12±0.31</td>
<td>776±17</td>
<td>Slow-onset</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$IC_{50}$ (nM)</th>
<th>Type of Inhibition</th>
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<tbody>
<tr>
<td>PT51</td>
<td>2699±287</td>
<td>Rapid equilibrium</td>
</tr>
<tr>
<td>PT55</td>
<td>695±132</td>
<td>Rapid equilibrium</td>
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</table>
### Table 3
*Ex vivo Activity of FabI Inhibitors Against *B. pseudomallei* Bp400*

<table>
<thead>
<tr>
<th>FabI Inhibitor</th>
<th>90% Growth Reduction mg/L</th>
<th>FabI Inhibitor</th>
<th>90% Growth Reduction mg/L</th>
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</thead>
<tbody>
<tr>
<td>PT51</td>
<td>6.1 +/- 2.6</td>
<td>PT68</td>
<td>2.0 +/- 2.0</td>
</tr>
<tr>
<td>PT52</td>
<td>2.5 +/- 2.5</td>
<td>Ceftazidime</td>
<td>&gt;10</td>
</tr>
<tr>
<td>PT55</td>
<td>6 +/- 4.3</td>
<td>Doxycycline</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
Figure 2

A

B

Log_{10} CFU/ml

Untreated Control 200 mg/kg Ceftazidime 200 mg/kg PT32 200 mg/kg PT158

Untreated Control 200 mg/kg Ceftazidime 200 mg/kg PT32 200 mg/kg PT158

* *** ** **