Assessment of carbapenems in a mouse model of *Mycobacterium tuberculosis* infection

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Abstract

We present further study of a subset of carbapenems, arising from a previously reported machine learning approach, with regard to their mouse pharmacokinetic profiling and subsequent study in a mouse model of sub-acute *Mycobacterium tuberculosis* infection. Pharmacokinetic metrics for such small molecules were compared to those for meropenem and biapenem, resulting in the selection of two carbapenems to be assessed for their ability to reduce *M. tuberculosis* bacterial loads in the lungs of infected mice. The original syntheses of these two carbapenems were optimized to provide multigram quantities of each compound. One of the two experimental carbapenems, JSF-2204, exhibited efficacy equivalent to that of meropenem, while both were inferior to rifampin. The lessons learned in this study point toward the need to further enhance the pharmacokinetic profiles of experimental carbapenems to positively impact *in vivo* efficacy performance.

Introduction

Tuberculosis represents a global health pandemic caused by *Mycobacterium tuberculosis* infection. It is characterized by approximately ten million new cases and 1.4 million deaths per year [1]. Multi-drug resistant (MDR) cases, defined by resistance to at least isoniazid and rifampin, constituted nearly one-half million cases in 2019 [1]. Although the recent regulatory approvals of bedaquiline and pretomanid have introduced two new tuberculosis drugs, reports of resistance emerging to either drug reinforce the urgency of developing additional new agents to enable regimens comprised entirely of new agents [2,3].

While β-lactams are arguably the most successful class of antibacterials and the carbapenem sub-class, in particular, occupies a significant niche in treating life-threatening, drug-resistant infections, this family of molecules is currently absent from front-line tuberculosis treatments [4]. However, interest in using carbapenems to treat MDR-TB has increased significantly, as...
Assessment of carbapenems

Materials and methods

Synthetic chemistry procedures and spectroscopic data

All reagents were purchased from commercial suppliers and used without further purification unless noted otherwise. All chemical reactions occurring solely in an organic solvent were carried out under an inert atmosphere of argon or nitrogen. Analytical TLC was performed with Merck silica gel 60 F254 plates. Column chromatography was conducted with Teledyne Isco CombiFlash Companion or Rf+ systems. 1H NMR spectra were acquired on Varian Inova 400, 500, and 600 MHz instruments and are listed in parts per million downfield from TMS. LC-MS was performed on an Agilent 1260 HPLC coupled to an Agilent 6120 MS. All synthesized compounds were at least 95% pure as judged by their HPLC trace at 250 nm and were characterized by the expected parent ion(s) in the MS trace.

Synthesis of 4-nitrobenzyl (4R,5S,6S)-6-[(R)-1-hydroxyethyl]-4-methyl-7-oxo-3-(phenethylthio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (compound 2)

A solution of 4-nitrobenzyl (4R,5S,6S)-3-[(diphenylphosphononoxy]-6-[(R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (compound 1; 15.0 g, 25.2 mmol) in dimethylformamide (90 mL) was cooled to 0°C, and N,N-di-i-propylethylamine (9.30 mL, 50.4 mol) was added. The reaction mixture was stirred for 1 h at 0°C. The crude product was purified by flash chromatography using hexane/EtOAc (6:4) to afford the desired product as an off-white solid (10.3 g, 21.3 mmol, 84.5%): 1H NMR (500 MHz, CDCl3) δ 8.21 (d, J = 8.5 Hz, 2), 7.65 (d, J = 8.5 Hz, 2), 7.32 (t, J = 7.4 Hz, 2), 7.24 (dd, J = 13.9, 6.9 Hz, 1), 7.21 (d, J = 7.4 Hz, 2), 5.51 (d, J = 13.8 Hz, 1), 5.24 (d, J = 13.8 Hz, 1), 4.25 (p, J = 6.3 Hz, 1), 4.14 (dd, J = 9.1, 2.2 Hz, 1), 3.36–3.28 (m, 1), 3.26 (dd, J = 6.7, 2.2 Hz, 1), 3.16–3.02 (m, 2), 3.02–2.89 (m, 2), 1.35 (d, J = 6.2 Hz, 3), 1.23 (t, J = 9.3 Hz, 3). One H was unaccounted for and presumably was evidenced by numerous case reports, case series, and reviews describing their use [5–12]. More important to understanding the potential contribution of this class, a phase 2 study (NCT02349841) recently demonstrated substantial early bactericidal activity of a globally marketed injectable carbapenem, meropenem [8] (co-administered with amoxicillin/clavulanate to inhibit the M. tuberculosis β-lactamase, BlaC). The results showed a promising reduction of bacterial load in patients’ sputum as compared to standard four-drug therapy [9]. However, the clinical utility of meropenem remains limited due to the fact that it must be administered as an intravenous infusion every 8 h, which is not conducive to widespread use in the field. In addition, it must be administered with clavulanate for optimal activity. Faropenem is an efficacious inhibitor of M. tuberculosis growth in vitro [13,14], but still may not be potent enough to reach effective concentrations when delivered orally as the sodium salt (even thrice daily) at doses used for other indications [15,16]. Therefore, the availability of more potent carbapenems with improved pharmacokinetics and oral bioavailability clearly will remain an unmet need in combating MDR-TB [14,17]. Our previous publication in this area outlined the use of Bayesian modeling and structure-based design tenets, to design a new set of carbapenems and profile their biological activity [6]. Herein, we report our continued studies to pursue these carbapenems, with a specific focus on evaluating the pharmacokinetic (PK) profile and efficacy of select compounds against M. tuberculosis in mice.
the OH; $^{13}$C NMR (126 MHz, CDCl$_3$) δ 172.8, 160.5, 152.9, 147.6, 143.2, 139.2, 128.8, 128.6, 128.2, 126.9, 123.8, 123.4, 65.9, 65.3, 59.6, 56.1, 43.3, 36.3, 33.0, 21.9, 16.9. LRMS (ESI): Calculated for C$_{15}$H$_{27}$N$_2$O$_6$S (M+H)$^+$ = 483.2; Observed 483.2.

**Sodium (4R,5S,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-(phenethylthio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (JSF-2417)**

To a solution of 4-nitrobenzyl (4R,5S,6S)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-3-(phenethylthio)-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (compound 2; 8.00 g, 16.6 mol) in a mixture of n-butanol (50 mL) and water (50 mL) was added sodium bicarbonate (1.53 g, 18.2 mol). The reaction mixture was subjected to catalytic hydrogenation at 60 psi pressure in a Parr shaker hydrogenation apparatus in the presence of 10% (w/w) palladium on charcoal (1.53 g, 18.2 mol). The reaction mixture was subjected to catalytic hydrogenation at 60 psi pressure in a Parr shaker hydrogenation apparatus in the presence of 10% (w/w) palladium on charcoal (1.53 g, 18.2 mol). The reaction mixture was filtered through a pad of Celite and washed with water (10 mL). The aqueous layer was washed with diethyl ether (25 x 3 mL) and concentrated under reduced pressure. The residue was purified by chromatography on a reverse-phase C18 column using a solvent system of water/acetonitrile to provide the desired compound as a white solid (4.28 g, 11.6 mol, 69.8%):

$^1$H NMR (500 MHz, D$_2$O) δ 7.34 (t, $J = 7.2$ Hz, 2), 7.28 (t, $J = 8.9$ Hz, 3), 4.19 (p, $J = 6.0$ Hz, 1), 3.88 (d, $J = 9.0$ Hz, 1), 3.29 (d, $J = 5.8$ Hz, 1), 3.14–3.01 (m, 2), 2.98–2.82 (m, 3), 1.27 (d, $J = 6.3$ Hz, 3), 1.05 (d, $J = 7.1$ Hz, 3); Two hydrogens were unaccounted for and were presumably the OHs.

$^{13}$C NMR (126 MHz, D$_2$O) δ 176.2, 167.9, 143.6, 139.9, 130.1, 129.1, 128.6, 126.7, 65.1, 58.0, 55.7, 41.9, 35.99, 32.0, 20.2, 15.9. Calculated for C$_{18}$H$_{22}$NO$_4$S (M+H)$^+$ = 348.1; Observed 348.0.

**4-nitrobenzyl (4R,5S,6S)-6-((R)-1-hydroxyethyl)-4-methyl-3-(methylthio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (compound 3)**

To a 500 mL round-bottom flask, 200 mL dimethylformamide was charged, cooled to 0˚C, and 3M sodium methanethiolate (4.20 g, 59.9 mmol), 1,1,3,3-tetramethylguanidine (10.6 mL, 89.7 mmol), and acetic acid (4.3 mL, 72 mmol) were added. The reaction mixture was stirred for 5 min at 0˚C, was then cooled to -40˚C, and a solution of 4-nitrobenzyl (4R,5S,6S)-3-[(diphenylphosphono)oxy]-6-[(R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (32.0 g, 53.9 mmol) in 100 mL dimethylformamide was added. After stirring at -40˚C for 90 min, the reaction mixture was diluted with 500 mL ethyl acetate and washed with water (3 x 250 mL). The organics were washed with saturated aqueous brine solution (1 x 100 mL), dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude product was purified by flash chromatography using hexane/EtOAc (6:4) to afford the desired product as an off-white solid (10.8 g, 27.5 mmol, 51.0%): $^1$H NMR (500 MHz, CDCl$_3$) δ 8.21 (d, $J = 8.1$ Hz, 2), 7.65 (d, $J = 13.8$ Hz, 2), 5.24 (d, $J = 13.8$ Hz, 1), 4.30–4.20 (m, 2), 3.43 (p, $J = 7.6$ Hz, 1), 3.27 (d, $J = 6.9$ Hz, 1), 2.41 (s, 3), 1.78 (br s, 1), 1.37 (d, $J = 6.2$ Hz, 3), 1.27 (d, $J = 7.2$ Hz, 3); Two hydrogens were unaccounted for and were presumably the OHs.

$^{13}$C NMR (500 MHz, D$_2$O) δ 176.2, 167.9, 143.6, 139.9, 130.1, 129.1, 128.6, 126.7, 65.1, 58.0, 55.7, 41.9, 35.99, 32.0, 20.2, 15.9. Calculated for C$_{18}$H$_{22}$NO$_4$S (M+H)$^+$ = 393.1; Observed 393.0.

**Sodium (4R,5S,6S)-6-((R)-1-hydroxyethyl)-4-methyl-3-(methylthio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (JSF-2204)**

To a solution of 4-nitrobenzyl (4R,5S,6S)-6-((R)-1-hydroxyethyl)-4-methyl-3-(methylthio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (compound 3; 6.48 g, 16.5 mmol) in a mixture of n-butanol (50 mL) and water (50 mL) was added sodium bicarbonate (1.52 g, 18.2 mmol). The reaction mixture was subjected to catalytic hydrogenation at 60 psi pressure in a Parr shaker hydrogenation apparatus in the presence of 10% (w/w) palladium on charcoal (3.25 g) for 1 h. After completion of the reaction, the reaction mixture was filtered through a...
pad of Celite and washed with water (10 mL). The aqueous layer was washed with diethyl ether (25 x 3 mL) and concentrated in vacuo. The residue was purified by chromatography on a reverse-phase C18 column using a solvent system of water/acetonitrile. The material was lyophilized to afford the desired product (2.40 g, 8.60 mmol, 52.1%) as a white solid: $^1$H NMR (500 MHz, D2O) $\delta$ 4.20–4.11 (m, 1), 4.07 (dd, $J$ = 8.8, 2.0 Hz, 1), 3.45–3.35 (m, 1), 3.30 (dd, $J$ = 6.3, 2.0 Hz, 1), 2.28 (s, 3), 1.21 (t, $J$ = 9.9 Hz, 3), 1.11 (d, $J$ = 7.2 Hz, 3); Two hydrogens were unaccounted for and were presumably the OHs. $^{13}$C NMR (126 MHz, D2O) $\delta$ 176.0, 168.3, 146.8, 128.1, 65.3, 57.8, 56.0, 41.7, 20.1, 15.9, 13.7. Calculated for C11H16NO4S (M+H)$^+$ = 258.1; Observed 258.0.

Sodium (4R,5S,6S)-6-((R)-1-hydroxyethyl)-4-methyl-3-((3-morpholino-3-oxopropyl)thio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (JSF-2700)

A solution of 4-nitrobenzyl (4R,5S,6S)-3-[(diphenylphosphono)oxy]-6-[(R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (0.66 g, 1.1 mmol) in dimethylformamide (3 mL) was cooled to 0˚C, and N,N-di-i-propylethylamine (0.24 mL, 1.37 mmol) followed by 3-mercapto-1-morpholino propan-1-one (0.240 g, 1.37 mmol) were added. The reaction mixture was stirred for 12 h at 0˚C and was then warmed gradually to rt. EtOAc (5 mL) was then added, and the organic layer was washed with saturated aqueous brine solution (3 x 5 mL). The organics were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography using CH2Cl2/MeOH (9:1) to afford the desired product 4-nitrobenzyl (4R,5S,6S)-6-((R)-1-hydroxyethyl)-4-methyl-3-((3-morpholino-3-oxopropyl)thio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate as a white solid (555 mg, 1.07 mmol, 97.1%): $^1$H NMR (500 MHz, CDCl3) $\delta$ 8.22 (d, $J$ = 8.7 Hz, 2), 7.65 (d, $J$ = 8.7 Hz, 2), 5.50 (d, $J$ = 13.8 Hz, 1), 5.23 (d, $J$ = 13.8 Hz, 1), 4.39–4.15 (m, 2), 3.65 (s, 4), 3.61 (dd, $J$ = 9.5, 4.5 Hz, 2), 3.52 (dt, $J$ = 14.7, 7.3 Hz, 1), 3.46–3.41 (m, 2), 3.28 (dd, $J$ = 6.6, 2.5 Hz, 1), 3.22 (ddd, $J$ = 12.9, 8.4, 6.5 Hz, 1), 3.14 (ddd, $J$ = 12.8, 8.6, 6.4 Hz, 1), 2.77–2.52 (m, 2), 1.36 (d, $J$ = 6.3 Hz, 3), 1.28 (d, $J$ = 7.3 Hz, 3). One H was unaccounted for and presumably was an OH. Calculated for C24H30N3O8S (M+H)$^+$ = 520.2; Observed 520.0.

Biological assay protocols

**Bacterial strains, growth conditions, and drug controls.** *M. tuberculosis* reference strain H37Rv (ATCC 25618) was used in this study. Cultures of this strain were grown in
Middlebrook 7H9 complete broth (Difco) supplemented with 0.5% glycerol, 10% oleic acid-albumin-dextrose-catalase (OADC) and 0.05% Tween-80 as described previously [18] at 37˚C with constant shaking. Organ homogenates were inoculated and M. tuberculosis colony-forming units (CFUs) were grown in Middlebrook selective 7H11 agar plates supplemented with 0.5% glycerol, 10% oleic acid-albumin-dextrose-catalase (OADC) and 0.05% Tween-80 at 37˚C. Rifampin, meropenem, and clavulanate were obtained from Sigma-Aldrich.

**Determination of minimum inhibitory concentration (MIC) against M. tuberculosis.**

For determination of the MIC of drugs against M. tuberculosis H37Rv, the standard microbroth dilution method was used as previously described [19]. Briefly, 10⁵ M. tuberculosis bacilli from exponentially growing culture were inoculated into wells of a 96-well microtiter dish with Middlebrook 7H9 broth without Tween-80 containing a drug at two-fold dilution ranging from 64 – 0.125 μg/mL. β-lactamase inhibitor, such as clavulanate, was not included in the MIC determinations and, therefore, the MIC values reported here were for the drugs alone. The final volume of the bacterial suspension with drug was 150 μL in a 350 μL volume well. Culture broth without drug alone was included as a negative control and with 10⁵ M. tuberculosis bacilli as a positive control. Cultures were incubated at 37˚C in a standing incubator and growth or lack thereof was assessed after 14 d via visual inspection of the size of the bacterial pellet. Sizes of M. tuberculosis pellet at the bottom of the U-shaped wells were recorded. The lowest concentration at which we were unable to observe any pellet were reported as the drug MIC.

**Evaluation of drug pharmacokinetics and efficacy in mice.**

All studies in mice were undertaken in accordance with a protocol approved by the Johns Hopkins Animal Care and Use Committee (MO17M279). For determination of level of a compound in plasma, 5–6 week old, female BALB/c mice (Jackson Laboratory) were used. Five experimental carbapenems were evaluated and two commercially available carbapenems, meropenem and biapenem, were included as controls. A single dose of 25 mg/kg was injected subcutaneously into the dorsal flank of the hind limb using a 100 μL bolus per mouse, three mice per compound. At 0.25, 0.50, 1, and 3 h following compound administration, blood was collected from tail vein into heparin vials and plasma was separated via centrifugation at 2,000 xg for 5 min. The concentration of each compound in the plasma samples was determined using a commercially available service with BioDuro (BioDuro, San Diego) relying on LC-MS quantification. A subacute mouse model of M. tuberculosis H37Rv was used to evaluate the efficacies of experimental carbapenems. Female 5–6 weeks old BALB/c mice (Jackson Laboratory) were used. An exponentially growing culture of M. tuberculosis H37Rv was used to prepare an inoculating suspension of 10 mL at an optical density (A₆₀₀nm) of 0.1 by diluting in Middlebrook 7H9 broth and was aerosolized to infect mice. Sixty mice were infected at once in a Glas-Col aerosol chamber using the following cycle: 15 min of preheat, 30 min of nebulization of M. tuberculosis H37Rv suspension, 30 min of cloud decay and 15 min of heat and UV decontamination. Five mice were sacrificed 24 h after infection, lungs were resected, homogenized in sterile 1x PBS, and dilutions were inoculated onto complete Middlebrook 7H10 agar plates and incubated at 37˚C for four weeks at which time M. tuberculosis CFUs were enumerated. Similarly, lung M. tuberculosis CFUs were enumerated from 5 mice at 2 weeks post infection to determine CFU lung burden at the time of treatment initiation. At this time, mice were randomly sorted into five groups, 10 per group. The first group, labeled ‘untreated’ was a control group that did not receive any treatment. The second group, a positive control for tuberculosis drug, received 10 mg/kg rifampin, once daily by oral gavage. The third group received twice daily dose of 400 mg/kg meropenem (a direct comparator drug of the carbapenem class) supplemented with 75 mg/kg β-lactamase inhibitor clavulanate by subcutaneous injection. Two experimental groups were comprised of mice that received twice daily dose of either the carbapenem JSF-2204 or
JSF-2417, 400 mg/kg, also supplemented with 75 mg/kg β-lactamase inhibitor clavulanate by subcutaneous injection. Daily refers to 7 d a week. Five mice per group were sacrificed at one and three weeks following start of treatment and M. tuberculosis burden in the lungs of mice were determined as described above.

Data analysis

One way ANOVA test was used to analyze data. Pairwise comparison of efficacies of different treatment based on CFU data was performed using a Wilcoxon rank sum test.

Results and discussion

A subset of our previously reported carbapenems (JSF-2196, 2204, 2415, 2417) [6] and newly synthesized analog JSF-2700 (Fig 1), primarily chosen based on their minimum inhibitory concentration (MIC; the minimum concentration to inhibit growth by visual inspection of the bacterial pellet) values against M. tuberculosis H37Rv, were assayed for their PK profile in BALB/c mice with clinical carbapenems (meropenem, biapenem) included for comparison. A single subcutaneous (sc) injection of compound, formulated at 25 mg/kg in water, was administered to mice (group of three per compound) and the plasma concentration of compound was quantified over 3 h. A non-compartmental model was fit to the data (Fig 2 and Table 1). The systemic exposure was quantified as the area under the curve for the 3 h time period (AUC$_{0-3h}$), the maximum plasma concentration ($C_{max}$), the percentage of time above the MIC (%T$>$MIC), and the elimination half-life ($t_{1/2}$). Analysis of the PK data for our carbapenems supported the in vivo efficacy study of JSF-2204. It exhibited the highest AUC$_{0-3h}$ and $C_{max}$ values while demonstrating a %T$>$MIC in the range of 33–100. Secondarily, JSF-2417 was also selected for further study. As compared to JSF-2415, JSF-2417 exhibited greater in vitro potency despite having lower values of AUC$_{0-3h}$ and $C_{max}$ values. JSF-2417 was also chosen over JSF-2700 as it exhibited a greater average plasma concentration at $t = 3$ h (261.5 ng/mL vs. 6.0 ng/mL).

We proceeded with the scale-up of the synthesis of JSF-2417 to facilitate in vivo efficacy studies. This was a significant challenge as our initial synthetic route to this compound was previously achieved on only a 100 mg scale. Synthesis of the protected carbapenem 2 was carried out by addition of 2-phenylethanethiol to the commercial vinyl phosphate 1 in the presence of N,N-di-i-propylethylamine (DIEA) with a yield of 84% on a 10 g scale (Fig 3). In

![Chemical structures of the carbapenems studied.](https://doi.org/10.1371/journal.pone.0249841.g001)
contrast, our previously reported route [6] utilized di-i-propylamine in DMF and posed purification issues on scales beyond 250 mg. A range of conditions was then explored to achieve removal of the 4-nitrobenzyl moiety via hydrogenation with hydrogen gas in the presence of 10% w/w Pd/C (S1 Table). Conditions utilizing sodium bicarbonate in solvent mixtures such as EtOAc/EtOH/H\textsubscript{2}O afforded the desired product in low yields. A major impurity was observed due to \( \beta \)-lactam ring-opening as verified by LC-MS and \(^1\)H NMR. The hydrogenation reaction was next carried out in a variety of solvent systems in the absence of sodium bicarbonate. Unfortunately, in most of the cases, the reaction required more than 48 h to complete, again leading to \( \beta \)-lactam ring-opening. At 60 psi hydrogen pressure in solvents such as EtOAc, EtOH, or THF, the reaction did not occur to any measurable extent. Ultimately, the catalytic hydrogenation was found to be successful when utilizing a 1:1 mixture of \( n \)-BuOH and water as the solvent system in conjunction with sodium bicarbonate to afford the desired product.

![Plasma concentration versus time curves for the carbapenems post a single 25 mg/kg sc dose.](https://doi.org/10.1371/journal.pone.0249841.g002)

**Fig 2.** Plasma concentration versus time curves for the carbapenems post a single 25 mg/kg sc dose. Each data point represents the mean value and the error bars represent \( \pm \) the standard deviation for 3 mice.

Table 1. Summary of in vitro efficacy and mouse PK data for select carbapenems.

| Cmpd   | MIC (\( \mu \)g/mL) | \( t_{1/2} \) (h) | \%T>MIC | \( C_{\text{max}} \) (ng/mL) | AUC\(_{0-3h}\) (h*ng/mL) |
|--------|---------------------|------------------|--------|----------------------------|--------------------------|
| JSF-2196 | 2.0–4.0             | 0.230            | 17–33  | 20500                      | 9056                     |
| JSF-2204 | 0.25–0.50           | 0.240            | 33–100 | 62950                      | 22970                    |
| JSF-2415 | 4.0–8.0             | 0.241            | 17–33  | 34750                      | 12547                    |
| JSF-2417 | 2.0–4.0             | 0.647            | 8.3–33 | 5190                       | 5741                     |
| JSF-2700 | 2.0–4.0             | 0.258            | 8.3–33 | 17400                      | 5933                     |
| Biapenem | 2.5–5.0             | 0.289            | 33–100 | 55650                      | 63264                    |
| Meropenem | 4.0–8.0             | 0.463            | <8.3   | 1336                       | 613                      |

[https://doi.org/10.1371/journal.pone.0249841.t001](https://doi.org/10.1371/journal.pone.0249841.t001)
compound in 70% yield on a >4 g scale. The scale-up of JSF-2204 began with the synthesis of methyl sulfide 3. Our previous route involved generation of methanethiol, from sodium methyl sulfide and aqueous hydrochloric acid, and its subsequent reaction with phosphate 1 in the presence of DIEA with the overall yield of 3 being 52% on only a 400 mg scale [6]. We then explored other conditions that led to the use of sodium methyl sulfide, acetic acid, and 1,1,3,3-tetramethylguanidine (TMG) in DMF at -40˚C. We prepared ca. 11 g of 3 in a 51% yield. Hydrogenation of the 4-nitrobenzyl ester in 3 under our previously reported [6] conditions (1.1 equiv NaHCO₃, 1 atm H₂(g), THF/EtOH/H₂O (1:1:1), 1 h) only afforded a 23% yield on a 4 g scale. Use of the hydrogenation conditions in the synthesis of JSF-2417 afforded JSF-2204 from intermediate 3 with a 52% yield on a 2 g scale.

JSF-2417 and JSF-2204 were then evaluated in a mouse model of sub-acute M. tuberculosis infection. Female BALB/c mice were infected with the M. tuberculosis H37Rv strain as to afford a mean bacterial burden of ca. 10⁶ CFUs in the lungs two weeks post-infection. At that point, mice were treated with 400 mg/kg twice daily (bid) sc administration of JSF-2417, JSF-2204, or meropenem; rifampin served as a control with once daily administration orally at 10 mg/kg. Each carbapenem dose was supplemented with 75 mg/kg sodium clavulanate to inhibit the β-lactamase BlaC, in accord with previous studies [20]. The bacterial burden in mouse lungs was assessed in groups of five mice at 1 week and 3 weeks of treatment (Fig 4). After one week of dosing, the bacterial burden increased by ca. 1 log₁₀ in the case of all three carbapenems; rifampin dosing led to a bacteriostatic effect. The bacteriostatic effect of rifampin was also observed after 3 weeks of dosing. Post 1 week of treatment, the bacterial load in the lungs failed to increase with continued dosing of each of the three carbapenems and ultimately each treatment prevented an increase in M. tuberculosis burden as compared to the untreated control. JSF-2204 exhibited similar efficacy as meropenem while JSF-2417 was inferior.

Based on superior %T>MIC, Cₘₐₓ, and AUC₀⁻₃h (Table 1) of JSF-2204 compared to meropenem, this new carbapenem can be expected to exhibit improved efficacy. However, JSF-2204 and meropenem exhibited statistically indistinguishable efficacy in the mouse model (Fig 4). It is possible that JSF-2204 is unable to penetrate the macrophages and other host cells harboring intracellular M. tuberculosis bacilli at the same rate as meropenem thereby failing to achieve sufficiently high local concentrations at these sites. The chemical modification present in JSF-2204 could potentially affect this attribute, eventually producing less than expected efficacy in
The scope of the current study was to identify evolved carbapenems with improvement in in vitro activity and PK attributes over currently recommended carbapenem for treating drug-resistant tuberculosis, e.g., meropenem. JSF-2204 achieved this goal. In the future, we plan to improve the in vivo efficacy of JSF-2204 without sacrificing activity and PK advantages by generating analogs and testing them for in vivo efficacy.

Meropenem, JSF-52417, and JSF-2204 exhibited delayed activity in infected mice as little or no reduction in bacterial burden was observed in the lungs at the culmination of one week of treatment compared to the no-treatment group (Fig 4). Chambers et al. [7] and Kumar et al. [6] have also reported the delayed activity of imipenem and biapenem, respectively, in an acute M. tuberculosis infection model in mice.

The efforts disclosed herein demonstrate that structural modifications can be accommodated at the carbapenem 3-position to modulate PK and pharmacodynamic profiles. Efficacy evaluation of two modified carbapenems in M. tuberculosis infected mice afforded one compound, JSF-2204, that demonstrated statistically equivalent performance to meropenem. The in vivo activity observed for meropenem and our carbapenems to date must still be improved to afford single-agent efficacy data worthy of further translational progression. As in a study published by AstraZeneca, the efficacy shortcomings compared to current front-line drugs support further compound evolution to improve PK [14]. Future efforts will be required to design, synthesize, and biologically profile novel carbapenems to achieve in vivo efficacy superior to meropenem and on par with current antitubercular compounds of clinical relevance.

Supporting information
S1 Table. Hydrogenation conditions for removal of the 4-nitrobenzyl ester in intermediate 2. (DOC)

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Notes. The authors declare that a United States patent (US10,695,322) has been granted, including J.S.F., G.L., and A.K. as inventors, relevant to the carbapenems disclosed herein.
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