AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Imidazo[1,2-*a*]Pyridine-3-Carboxamides Are Active Antimicrobial Agents against *Mycobacterium avium* Infection *In Vivo*

Garrett C. Moraski,[a] Yong Cheng,[b] Sanghyun Cho,[c] Jeffrey W. Cramer,[d] Alexander Godfrey,[e] Thierry Masquelin,[e] Scott G. Franzblau,[c] Marvin J. Miller,[f] Jeffery Schorey[b]

Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana, USA[a]; Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana, USA[b]; Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois, USA[c]; Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA[d]; Discovery Chemistry, Eli Lilly and Company, Indianapolis, Indiana, USA[e]; Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana, USA[f]

A panel of six imidazo[1,2-*a*]pyridine-3-carboxamides (IAPs) were shown to have low-micromolar activity against *Mycobacterium avium* strains. Compound ND-10885 (compound 2) showed significant activity in the lung, spleen, and liver in a mouse *M. avium* infection model. A combined regimen consisting of ND-10885 (compound 2) and rifampin was additive in its anti-*M. avium* activity in the lung. Our data indicate that IAPs represent a new class of antibiotics that are active against *M. avium* and could potentially serve as an effective addition to a combined treatment regimen.

The incidence of nontuberculous mycobacteria (NTM) infections has been increasing in the United States (1, 2). *Mycobacterium avium* complex (MAC), which consists of *M. avium* and *M. intracellulare*, is an important cause of pulmonary disease in individuals with underlying lung diseases, such as cystic fibrosis and chronic obstructive pulmonary disease, and is an opportunistic pathogen in immunocompromised patients (3, 4). Among the NTM species isolated from U.S. patients, 80% are classified as MAC (5). MAC is ubiquitous within the environment and is found in soil, treated or untreated water, house plumbing systems, and animals (6). MAC infection is difficult to treat and has been shown to be resistant to many of the clinically used antituberculosis agents (7, 8). We previously disclosed a novel family of compounds, imidazo[1,2-*a*]pyridine-3-carboxamides (IAPs), with potent activity against *Mycobacterium tuberculosis* (9–12). The mechanism of action and anti-*M. tuberculosis in vivo* efficacy of this exciting new class has been documented by us and other groups (9, 13–16). Through a hit-to-lead optimization effort aided by the Lilly TB Drug Discovery Initiative (LTBDDI), additional IAP compounds (1 to 6) were generated and found to have encouraging *in vivo* pharmacokinetics (PK). Herein, we describe the activity of these latest analogs against *M. avium* both *in vitro* and *in vivo*.

Activity of selected compounds *in vitro*. Six compounds having diverse PKs were selected and synthesized according to our published methods (9–11). Experimental data and information on all previously uncharacterized compounds (1, 2, and 6) can be found in the supplemental material. MIC studies were performed using a resazurin-based colorimetric assay and CFU quantification, as described previously (17). Screening the IAPs against *M. avium* strains 101 and 2151 using standard protocols indicated that they had moderate potency (Table 1). The activities of these compounds against *M. avium* (2.6 to 27.8 μM, two strains) were limited relative to *M. tuberculosis* (see Table S1 in the supplemental material) but comparable to positive controls of clarithromycin and azithromycin (1.4 and 13.4 μM, respectively). These compounds also had a good therapeutic window when screened against Vero cells (9) (Table S1).

TABLE 1 Screening of compounds 1 to 6 against two strains of *M. avium*

Compound	Molecular mass (kDa)	cLogP[a]	MIC (μM)	
			MAC 101 (serotype 1)	MAC 2151 (serotype 2)
ND-9873 (1)	363.34	4.6	2.8	27.5
ND-10885 (2)	321.38	3.6	1.6	15.6
ND-9758 (3)	389.43	5.8	1.28	2.57
ND-9759 (4)	405.88	6.4	0.31	1.2
ND-9903 (5)	425.41	6.4	>23.5	>23.5
ND-10890 (6)	382.44	3.4	2.6	13.1
Rifampin	822.95	6.04	0.077	0.158
Ethambutol	204.31	0.12	48.9	>48.9
Clarithromycin	747.95	2.82	1.4	1.4
Azithromycin	748.88	2.28	13.4	>13.4

[a] cLogP, log of partition coefficient of a compound, was calculated by using ChemBioDraw Ultra 14 (PerkinElmer).

All six compounds were evaluated for their ability to kill or inhibit *M. avium* replication *in vitro*. Five out of six compounds were bactericidal or bacteriostatic (Fig. 1). Consistent with its MIC, ND-9903 (compound 5) showed no activity against *M. avium* 101 at the highest concentration tested. Separate studies of ND-9758 (compound 3), ND-9759 (compound 4), and ND-

Received 17 March 2016 **Returned for modification** 8 April 2016
Accepted 13 May 2016

Accepted manuscript posted online 23 May 2016

Citation Moraski GC, Cheng Y, Cho S, Cramer JW, Godfrey A, Masquelin T, Franzblau SG, Miller MJ, Schorey J. 2016. Imidazo[1,2-*a*]pyridine-3-carboxamides are active antimicrobial agents against *Mycobacterium avium* infection *in vivo*. Antimicrob Agents Chemother 60:5018–5022. doi:10.1128/AAC.00618-16.

Address correspondence to Jeffery Schorey, schorey.1@nd.edu.

G.C.M. and Y.C. contributed equally to this work.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.00618-16.

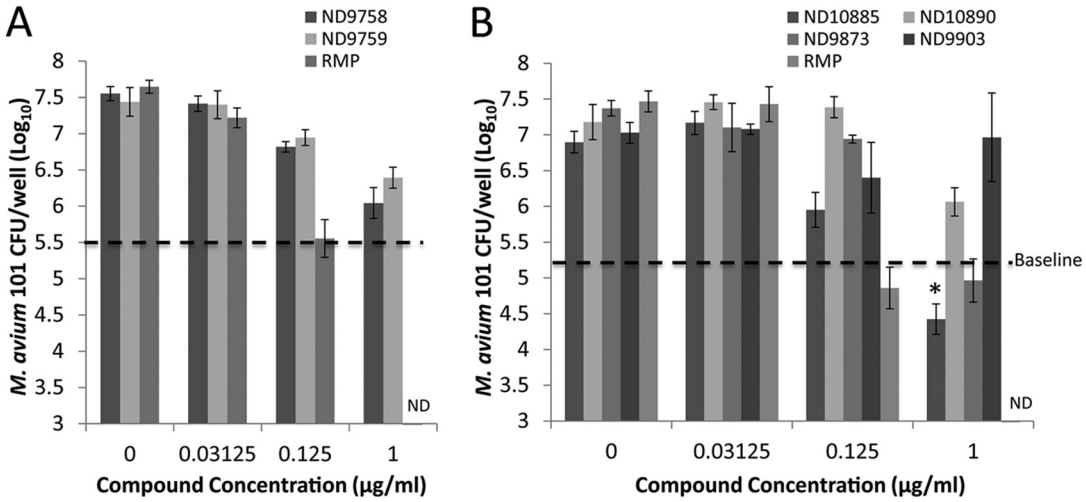


FIG 1 Anti-*M. avium* activities of imidazo[1,2-*a*]pyridine-3-carboxyamides *in vitro*, as measured by CFU counts. *M. avium* was treated with compounds at various concentrations as shown, and then bacterial CFU were determined on Middlebrook 7H10 agar plates. Baseline, *M. avium* CFU at the beginning of treatment. The results are representative of the results from three independent experiments. *, $P < 0.05$ compared to the baseline (one-way analysis of variance [ANOVA] with Tukey posttest). Error bars indicate standard deviations from duplicate infections with or without drug treatment from a single experiment.

10890 (compound 6) at 1.0 μg/ml each indicated that they were bacteriostatic, as the bacterial counts were maintained near those of the original inoculum. ND-10885 (compound 2) and ND-9873 (compound 1) had bactericidal activity against *M. avium* 101 at 1.0 μg/ml, as bacterial numbers decreased by 1 $\log_{10}$ and 0.5 $\log_{10}$, respectively, compared to the original inoculum. Rifampin was the positive control and showed the best bactericidal activity against *M. avium* 101. Except for ND-9903 (compound 9), all compounds showed dose-dependent activity against *M. avium*. ND-10885 (compound 2) also showed activity against various other *M. avium* clinical isolates of different serotypes, although again, it varied 10-fold or more between strains.

Pharmacokinetics. Single-dose pharmacokinetics of compounds 1 to 6 were determined in uninfected 8-week-old male BALB/c mice. Mice received by oral gavage a single dose of compound 1 (at 10 and 100 mg/kg), compound 2 (at 100 mg/kg), compound 3 (at 10 mg/kg), compound 4 (at 30 mg/kg), compound 5 (at 100 mg/kg), and compound 6 (at 10 and 100 mg/kg). Compounds were analyzed as previously described (10). Calculated parameters include clearance (CL), area under the concentration-time curve (AUC), half-life ($t_{1/2}$), maximum serum concentration (C_{max}), time to C_{max} (T_{max}), bioavailability (%F), and percent drug fraction unbound in plasma (%*f*plasma).

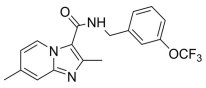
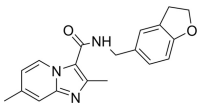
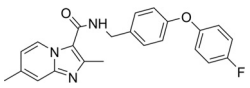
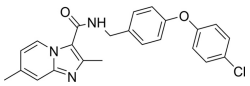
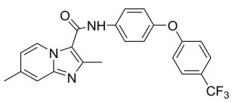
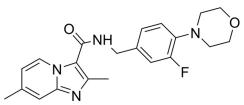
As shown in Table 2, all of these compounds had high plasma exposure at their respective doses, but there was a large range in half-lives observed (from 2.3 to >24 h). In our drug development paradigm, we selected the maximum free drug concentration per dose (fC_{max}) as the most meaningful pharmacokinetic property to use for compound advancement, and ND-10885 (compound 2) and ND-10890 (compound 6) had the highest values (2,795 and 4,232 nM, respectively). The free drug concentrations for those two compounds were high enough above their MICs that they were anticipated to be effective against MAC 101 *in vivo*. The PK parameters for compounds 1, 2, and 6 by intravenous (i.v.) dose can be found in Table S3 in the supplemental material.

Maximum tolerated dose. Five compounds were evaluated in mice to determine their maximum tolerated dose, as previously described (13). Compounds ND-9873 (compound 1) and ND-10885 (compound 2) were tolerated at the highest concentration of 250 mg/kg for 1 week, ND-10890 (compound 6) was well-tolerated at 100 mg/kg for 1 week, and as previously reported, ND-9759 (compound 5) was tolerated at 30 mg/kg for 28 days (13). Mice treated with ND-9758 (compound 3) did not tolerate the drug even at the lowest concentration (30 mg/kg).

Efficacy of ND-10885 in MAC-infected mice. We chose compound ND-10885 (compound 2) for the *in vivo* studies in wild-type BALB/c mice based on its relatively good *in vitro* bactericidal activity against *M. avium*, its PK profile, and its low toxicity in mice. Wild-type BALB/c mice ($n = 3$) were retro-orbitally infected with *M. avium* MAC 101 at a dose of 10^7 CFU in 50 μl of phosphate-buffered saline (PBS) (18). One week after the infection, mice were treated by oral gavage with ND-10885 (compound 2) dissolved in 80% (vol/vol) propylene glycol once daily 6 days a week for 2 weeks. After the final dosing, each mouse was sacrificed, and the mycobacterial burden was determined as described previously (13). The bacterial numbers were quantified by visually counting bacterial colonies. At the beginning of treatment, a group of mice ($n = 3$) were sacrificed to measure the mycobacterial input in the lung, spleen, and liver. As a negative control, a group of mice ($n = 3$) were treated with the vehicle, 80% propylene glycol, only.

ND-10885 (compound 2) significantly inhibited *M. avium* growth in the lungs, spleens, and livers compared to the vehicle-treated *M. avium*-infected mice (Fig. 2). The inhibitory activity of ND-10885 (compound 2) was comparable to that of rifampin in all three organs. In addition, compared to single-compound/drug regimens, mycobacterial counts were lower in all three organs when *M. avium*-infected mice were treated with a combined regimen of ND-10885 (compound 2) and rifampin, although this was statistically significant in the lung only. Compared with baseline mycobacterial counts at the initiation of treatment, the combined

TABLE 2 Pharmacokinetic parameters of compounds 1 to 6[a]

Compound	Structure	Parameter	*In vivo* PK	PK calculation
ND-9873		AUC (ng · h/ml)	27,768	-fC_{max} (100 mg/kg, p.o.) = 652 nM
		p.o. dose (mg/kg of body wt)	10	
		$t_{1/2}$ (h)	2.91	
		F%	51	
		C_{max} (ng/ml)	10,294	
		T_{max} (h)	1.0	
		CL (ml/min/kg)	20	
		*f*plasma	0.023	
ND-10885		AUC (ng · h/ml)	51,249.7	-fC_{max} (100 mg/kg, p.o.) = 2,795 nM
		p.o. dose (mg/kg of body wt)	100	
		$t_{1/2}$ (h)	2.35	
		F%	44.3	
		C_{max} (ng/ml)	19,530	
		T_{max} (h)	0.333	
		CL (ml/min/kg)	4.25	
		*f*plasma	0.046	
ND-9758		AUC (ng · h/ml)	11,000	ND
		p.o. dose (mg/kg of body wt)	13.2	
		$t_{1/2}$ (h)	10	
		F%	ND	
		C_{max} (ng/ml)	1,160	
		T_{max} (h)	0.50	
		CL (ml/min/kg)	ND	
		*f*plasma	0.003	
ND-9759		AUC (ng · h/ml)	22,200	-fC_{max} (30 mg/kg, p.o.) = 7 nM
		p.o. dose (mg/kg of body wt)	30	
		$t_{1/2}$ (h)	20.2	
		C_{max} (ng/ml)	2,900	
		F%	ND	
		T_{max} (h)	1.00	
		CL (ml/min/kg)	ND	
		*f*plasma	0.001	
ND-9903		AUC (ng · h/ml)	594,000	-fC_{max} (100 mg/kg, p.o.) = 133 nM
		p.o. dose (mg/kg of body wt)	100	
		$t_{1/2}$ (h)	>24	
		F%	ND	
		C_{max} (ng/ml)	34,900	
		T_{max} (h)	12	
		CL (ml/min/kg)	ND	
		*f*plasma	0.0016	
ND-10890		AUC (ng · h/ml)	32,800	-fC_{max} (10 mg/kg, p.o.) = 1,232 nM; -fC_{max} (100 mg/kg, p.o.) = 4,232 nM
		p.o. dose (mg/kg of body wt)	10	
		$t_{1/2}$ (h)	3.96	
		F%	46.3	
		C_{max} (ng/ml)	11,500	
		T_{max} (h)	0.250	
		CL (ml/min/kg)	2.32	
		*f*plasma	0.039	

[a] p.o., *per os*; ND, not determined.

regimen showed bactericidal activity, with the CFU($\log_{10}$) count decreasing 1.5-fold in the lung.

In conclusion, we show that IAPs are active against *M. avium* clinical isolates. One compound, ND-10885 (compound 2), has significant activity against *M. avium* in mice, reducing bacterial burden in the lung, spleen, and liver compared to the untreated group. Most interestingly, ND-10885 (compound 2) has activity comparable to that of rifampin, a critical component in the combined regimen used to treat MAC infections (8), and a combined regimen consisting of ND-10885 (compound 2) and rifampin showed enhanced bactericidal activity in the lung. Our data suggest that IAPs should be pursued as a new class of

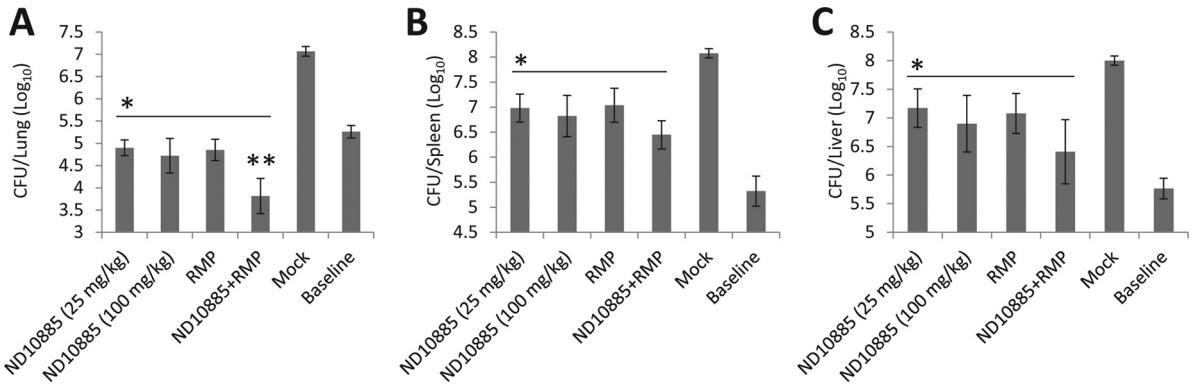


FIG 2 Efficacy of ND-10885 (compound 6) against *M. avium* infection in the BALB/c mouse model. *M. avium*-infected mice were treated with compounds once daily, 6 day per week, for 2 weeks. Bacterial burden in the lung, spleen, and liver was determined. Mock, mice treated with vehicle alone; baseline, bacterial burden at the beginning of treatment; RMP, rifampin. The results are representative of the results from two independent experiments. *, $P < 0.05$ compared to the mock; **, $P < 0.05$ compared to ND10885 (at 100 mg/kg) (one-way ANOVA with Tukey posttest). Error bars indicate standard deviations of *M. tuberculosis* CFU from individual mouse infections ($n = 3$) with or without drug treatment.

compounds to treat infections caused by *M. avium* and perhaps other NTM.

ACKNOWLEDGMENTS

We thank Phil Hipskind of the Eli Lilly TB Drug Discovery Initiative for efforts in the *M. tuberculosis* program. We thank Jennifer DuBois, Lowell Markley, Jed Fisher, Jaroslav Zajicek, Patricia Miller, Ute Mölleman, and Helena Boshoff for meaningful and lasting scientific discussions. We also thank Delphi Chatterjee and Pat Brennan (Colorado State) for the *M. avium* strains used in this study.

The analytical data were obtained in the Mass Spectrometry and Proteomics Facility at the University of Notre Dame (Bill Boggess and Michelle Joyce), which is supported by grant CHE-0741793 from the NSF. This work was supported in part by NIH grant (R01AI054193) and Hsiri Therapeutics.

FUNDING INFORMATION

This work, including the efforts of Jeffery Schorey, was funded by Hsiri Therapeutics. This work, including the efforts of Marvin J. Miller, was funded by HHS | NIH | NIH Office of the Director (OD) (RO1AI054193).

REFERENCES

1. **O'Brien RJ, Geiter LJ, Snider DE, Jr.** 1987. The epidemiology of nontuberculous mycobacterial diseases in the United States. Results from a national survey. Am Rev Respir Dis **135:**1007–1014.
2. **Adjemian J, Olivier KN, Seitz AE, Holland SM, Prevots DR.** 2012. Prevalence of nontuberculous mycobacterial lung disease in U.S. Medicare beneficiaries. Am J Respir Crit Care Med **185:**881–886. http://dx.doi.org/10.1164/rccm.201111-2016OC.
3. **Maekura R, Okuda Y, Hirotani A, Kitada S, Hiraga T, Yoshimura K, Yano I, Kobayashi K, Ito M.** 2005. Clinical and prognostic importance of serotyping *Mycobacterium avium-Mycobacterium intracellulare* complex isolates in human immunodeficiency virus-negative patients. J Clin Microbiol **43:**3150–3158. http://dx.doi.org/10.1128/JCM.43.7.3150-3158.2005.
4. **Cowman S, Loebinger M.** Nontuberculous mycobacterial pulmonary disease. Clin Pulm Med **22:**8–14.
5. **Prevots DR, Shaw PA, Strickland D, Jackson LA, Raebel MA, Blosky MA, Montes de Oca R, Shea YR, Seitz AE, Holland SM, Olivier KN.** 2010. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. Am J Respir Crit Care Med **182:**970–976. http://dx.doi.org/10.1164/rccm.201002-0310OC.
6. **Halstrom S, Price P, Thomson R.** 2015. Review: environmental mycobacteria as a cause of human infection. Int J Mycobacteriol **4:**81–91. http://dx.doi.org/10.1016/j.ijmyco.2015.03.002.
7. **Wagner D, Young LS.** 2004. Nontuberculous mycobacterial infections: a clinical review. Infection **32:**257–270. http://dx.doi.org/10.1007/s15010-004-4001-4.
8. **Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ, Winthrop K, Mycobacterial Diseases Subcommittee, American Thoracic Society, Infectious Disease Society of America.** 2007. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med **175:**367–416. http://dx.doi.org/10.1164/rccm.200604-571ST.
9. **Moraski GC, Markley LD, Hipskind PA, Boshoff H, Cho S, Franzblau SG, Miller MJ.** 2011. Advent of imidazo[1,2-a]pyridine-3-carboxamides with potent multi- and extended drug resistant antituberculosis activity. ACS Med Chem Lett **2:**466–470. http://dx.doi.org/10.1021/ml200036r.
10. **Moraski GC, Markley LD, Cramer J, Hipskind PA, Boshoff H, Bailey M, Alling T, Ollinger J, Parish T, Miller MJ.** 2013. Advancement of imidazo[1,2-a]pyridines with improved pharmacokinetics and nanomolar activity against *Mycobacterium tuberculosis*. ACS Med Chem Lett **4:**675–679. http://dx.doi.org/10.1021/ml400088y.
11. **Moraski GC, Miller PA, Bailey MA, Ollinger J, Parish T, Boshoff HI, Cho S, Anderson JR, Mulugeta S, Franzblau SG, Miller MJ.** 2015. Putting tuberculosis (TB) to rest: transformation of the sleep aid, Ambien, and "anagrams" generated potent antituberculosis agents. ACS Infect Dis **1:**85–90. http://dx.doi.org/10.1021/id500008t.
12. **Ollinger J, Bailey MA, Moraski GC, Casey A, Florio S, Alling T, Miller MJ, Parish T.** 2013. A dual read-out assay to evaluate the potency of compounds active against *Mycobacterium tuberculosis*. PLoS One **8:**e60531. http://dx.doi.org/10.1371/journal.pone.0060531.
13. **Cheng Y, Moraski GC, Cramer J, Miller MJ, Schorey JS.** 2014. Bactericidal activity of an Imidazo[1, 2-a]pyridine using a mouse *M. tuberculosis* infection model. PLoS One **9:**e87483. http://dx.doi.org/10.1371/journal.pone.0087483.
14. **Abrahams KA, Cox JAG, Spivey VL, Loman NJ, Pallen MJ, Constantinidou C, Fernández R, Alemparte C, Remuiñán MJ, Barros D, Ballell L, Besra GS.** 2012. Identification of novel imidazo[1,2-a]pyridine inhibitors targeting *M. tuberculosis* QcrB. PLoS One **7:**e52951. http://dx.doi.org/10.1371/journal.pone.0052951.
15. **Mak PA, Rao SPS, Ping Tan M, Lin X, Chyba J, Tay J, Ng SH, Tan BH, Cherian J, Duraiswamy J, Bifani P, Lim V, Lee BH, Ling Ma N, Beer D, Thayalan P, Kuhen K, Chatterjee A, Supek F, Glynne R, Zheng J, Boshoff HI, Barry CE, III, Dick T, Pethe K, Camacho LR.** 2012. A high-throughput screen to identify inhibitors of ATP homeostasis in nonreplicating *Mycobacterium tuberculosis*. ACS Chem Biol **7:**1190–1197. http://dx.doi.org/10.1021/cb2004884.
16. **Pethe K, Bifani P, Jang J, Kang S, Park S, Ahn S, Jiricek J, Jung J, Jeon HK, Cechetto J, Christophe T, Lee H, Kempf M, Jackson M, Lenaerts AJ, Pham H, Jones V, Seo MJ, Kim YM, Seo M, Seo JJ, Park D, Ko Y, Choi I, Kim R, Kim SY, Lim S, Yim S-A, Nam J, Kang H, Kwon H, Oh C-T, Cho Y, Jang Y, Kim J, Chua A, Tan BH, Nanjundappa MB, Rao**

SPS, Barnes WS, Wintjens R, Walker JR, Alonso S, Lee S, Kim J, Oh S, Oh T, Nehrbass U, Han S-J, No Z, et al. 2013. Discovery of Q203, a potent clinical candidate for the treatment of tuberculosis. Nat Med **19:** 1157–1160. http://dx.doi.org/10.1038/nm.3262.

17. **Palomino J-C, Martin A, Camacho M, Guerra H, Swings J, Portaels F.** 2002. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother **46:**2720–2722. http://dx.doi.org/10.1128/AAC.46.8.2720-2722.2002.
18. **Yardeni T, Eckhaus M, Morris HD, Huizing M, Hoogstraten-Miller S.** 2011. Retro-orbital injections in mice. Lab Anim (NY) **40:**155–160. http://dx.doi.org/10.1038/laban0511-155.