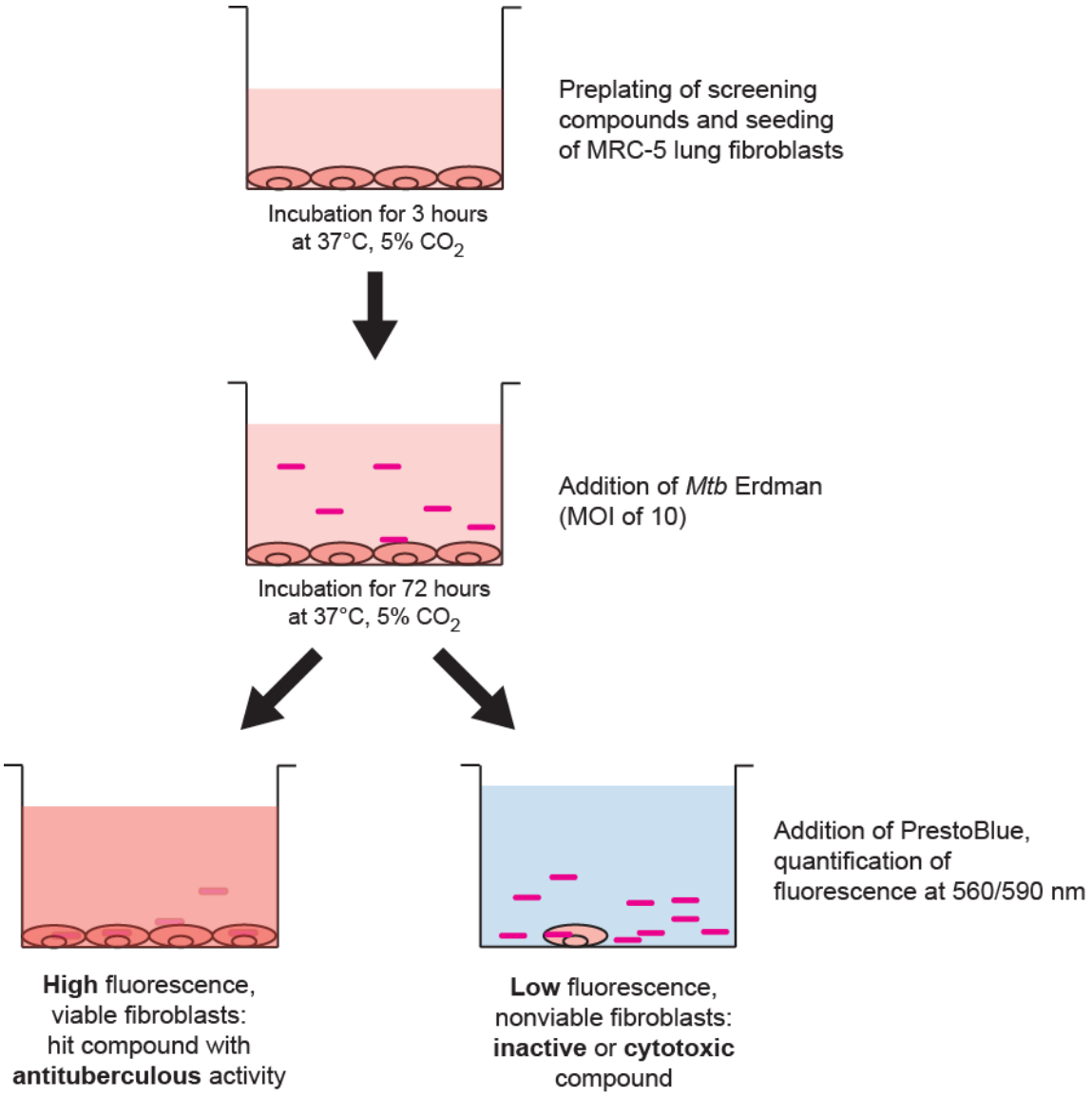
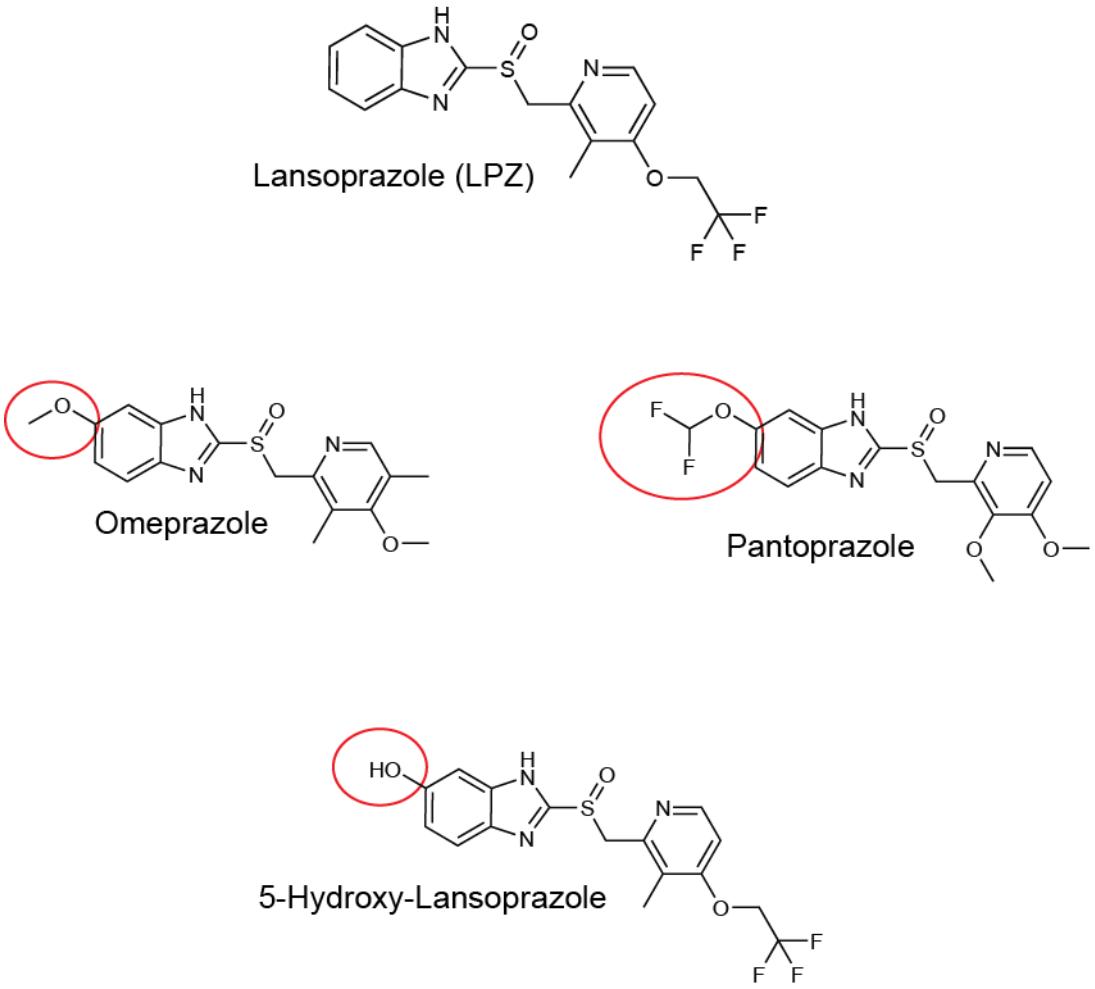
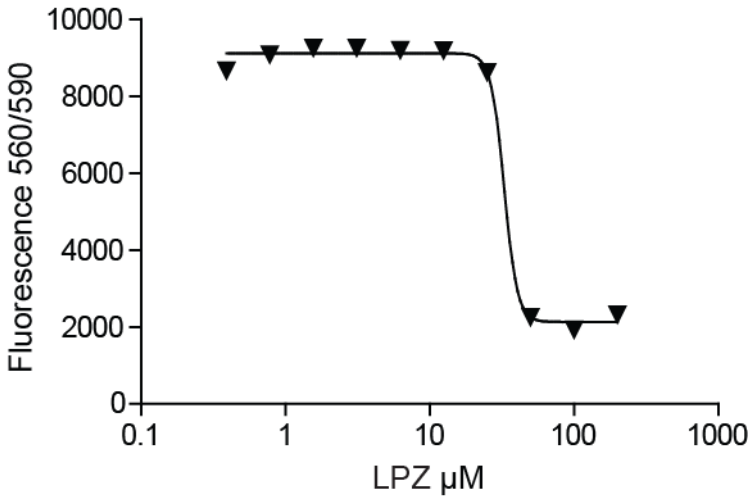


Supplementary Figure 1. **Flow diagram of the host cell-based HTS used in this study.** Due to the rapid turnover of PrestoBlue by eukaryotic cells, background fluorescence from bacteria is negligible. A full description of the assay can be found in the methods section. MOI: multiplicity of infection.

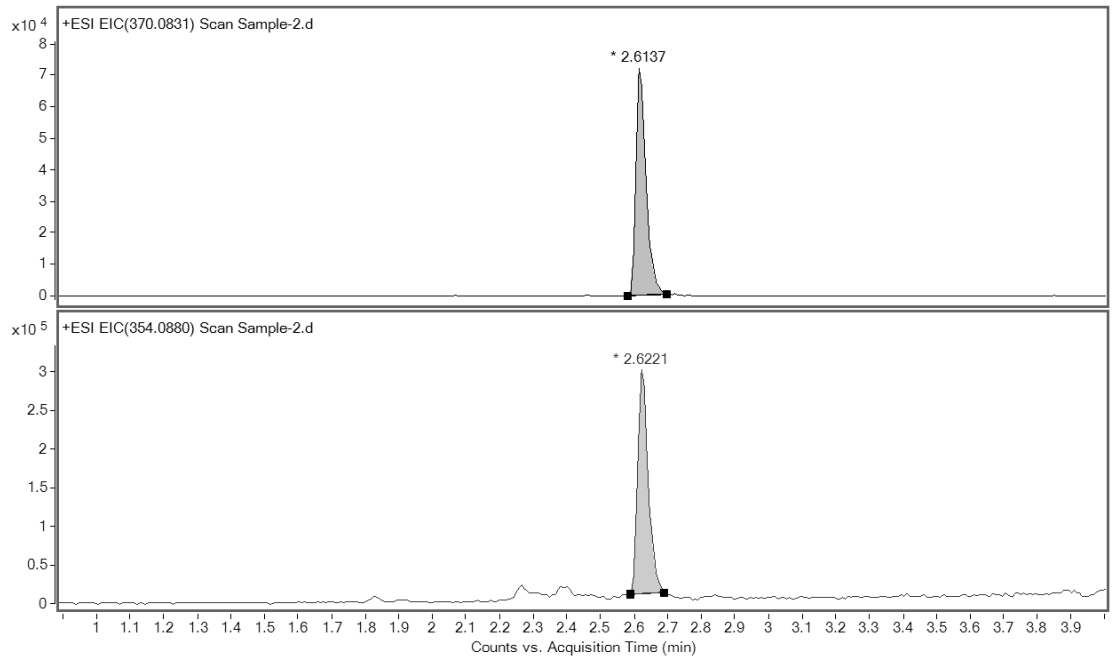


Supplementary Figure 2. **Structures of different proton pump inhibitors and the LPZ metabolite 5-Hydroxy-Lansoprazole**. Molecules carrying substitutions on the benzimidazole ring (red circle) are not active against *Mtb*.

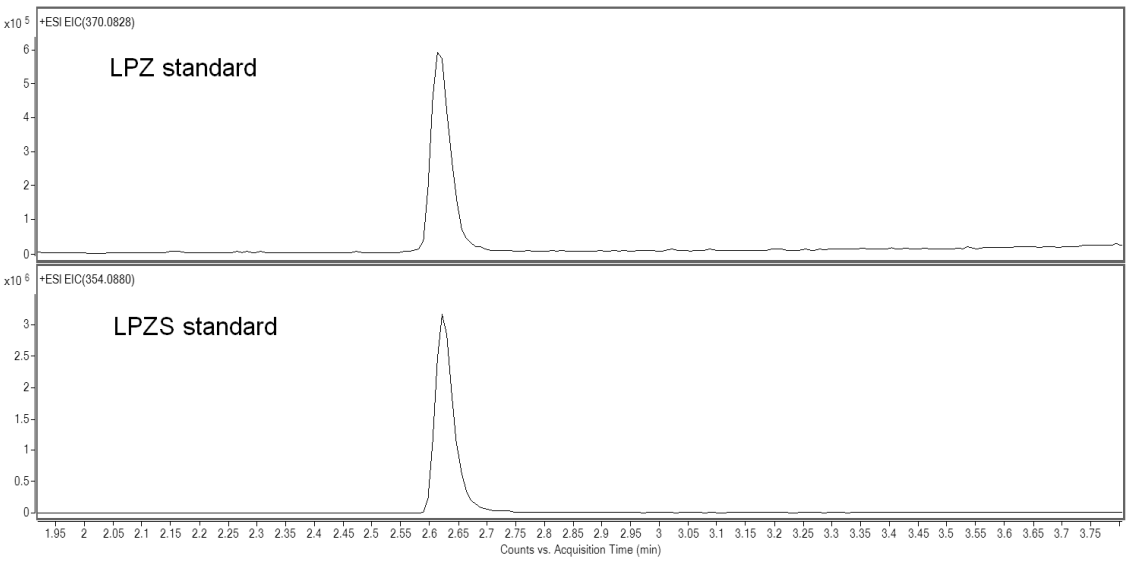


Supplementary Figure 3. Dose-response curve of LPZ against *Mtb* H37Rv in 7H9 broth. Representative example of three individual experiments (mean ± SD of duplicates).

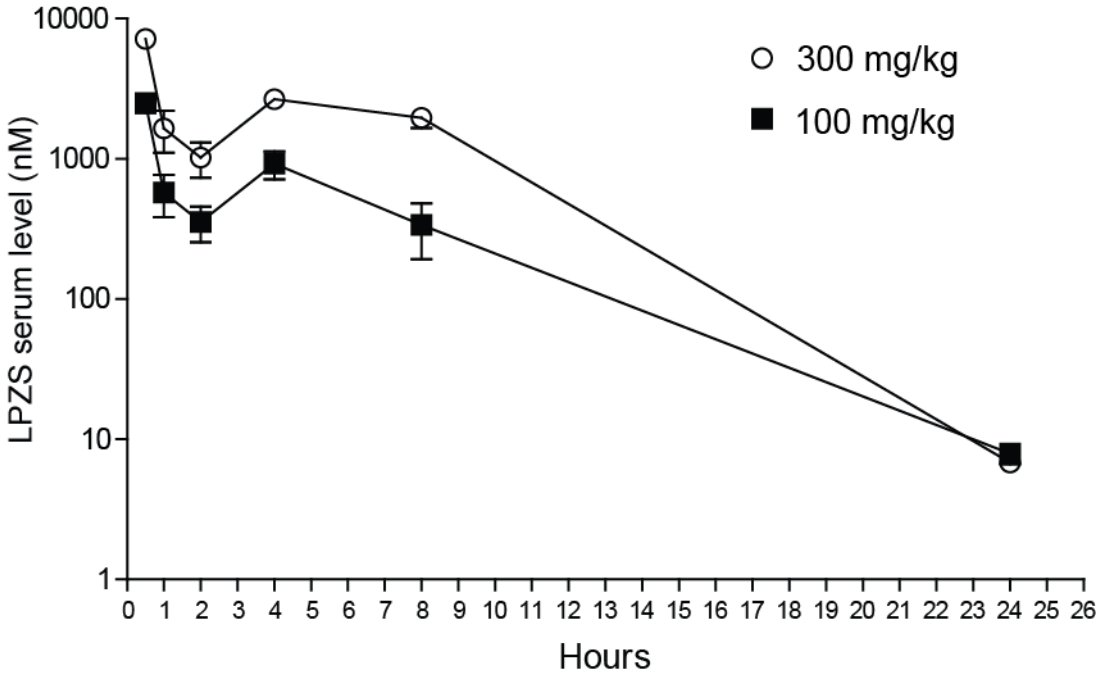
a



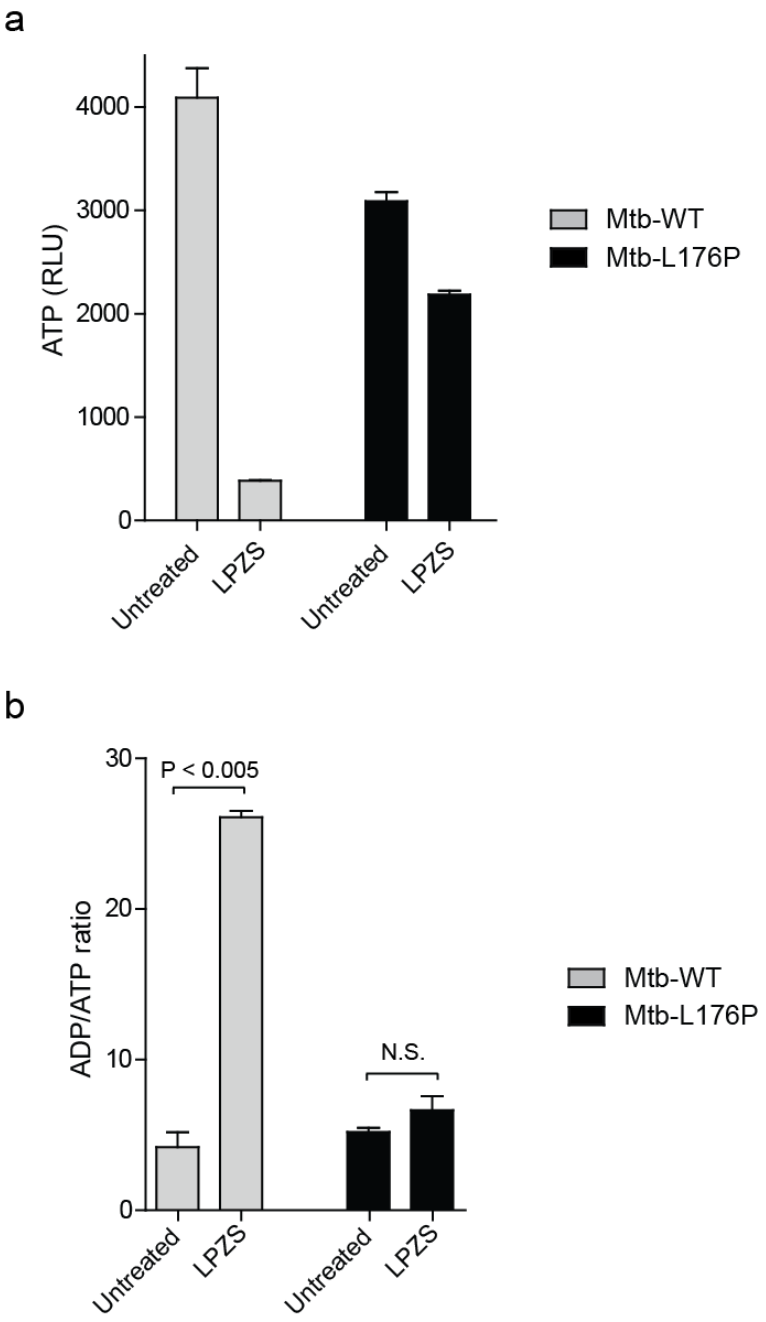
b



Supplementary Figure 4. Extracted Ion Chromatograms (EIC). (a) EIC of *m/z* 354.088 and 370.083 derived from a cell lysate sample of MRC-5 cells treated with LPZ. **(b)** EIC of *m/z* 354.088 and 370.083 corresponding respectively to the LPZ standard and the LPZS standard.

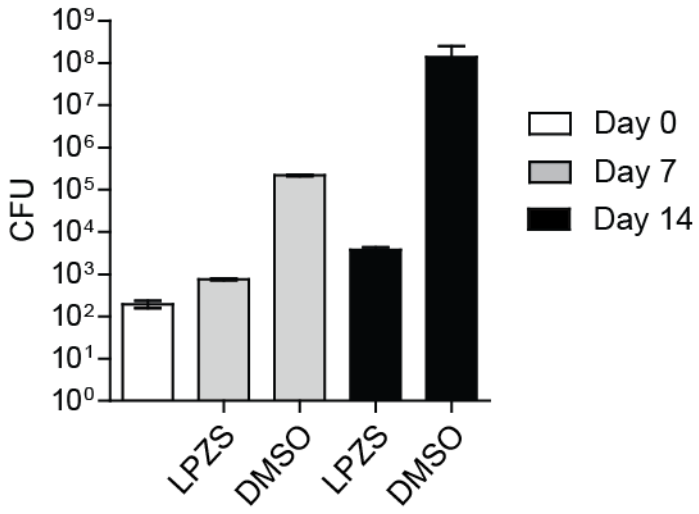


Supplementary Figure 5. *In vivo* pharmacokinetics of LPZS. BALB/c mice (three per group) were given 100 mg/kg or 300 mg/kg of LPZS by oral gavage and serum levels were determined after 0.5, 1, 2, 4, 8 and 24 hours (mean ± SD). LPZS shows non-linear pharmacokinetics with a second peak after approximately 4 hours of administration.

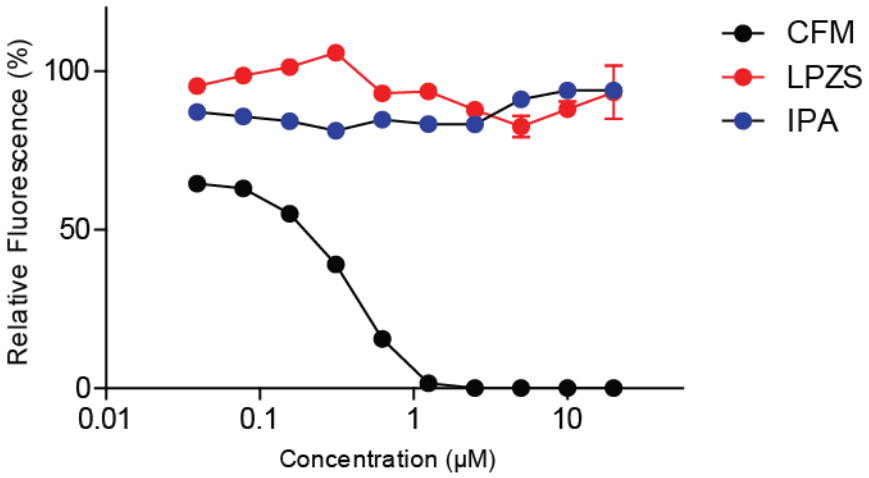


Supplementary Figure 6. ATP depletion and ADP/ATP ratios in wild-type and L176P mutant strain. (a) ATP levels are only slightly affected in the LPZS treated L176P mutant strain. **(b)** The ADP/ATP ratio of LPZS treated wild-type bacteria shifts several fold in comparison to untreated bacteria. A similar effect was not observed for the L176P mutant. LPZS concentration was 5 µM (mean ± SD of three individual experiments; unpaired Student's *t* test was used to compare groups).

a



b



Supplementary Figure 7. CFU counts for LPZS treated bacteria and *Mtb*-18b assays. (a) *Mtb* H37Rv was exposed to LPZS and serial dilutions were plated for CFU counts after 7 and 14 days. The data show that LPZS is a bacteriostatic compound. **(b)** LPZS and an imidazopyridine amide (IPA) compound were tested in the non-replicating, SS18b model using clofazimine (CFM) as a positive control (mean ± SD of two individual experiments).

Supplementary Table 1. Hit list of the Prestwick chemical library screen.

The library contained 1,280 FDA-approved drugs. Shown are hit-compounds with known antimicrobial activity and lansoprazole. The score value is relative to the control rifampicin (value of 1). Score SD represents the standard deviation from measurements of duplicate assay plates.

Catalog-ID	Name	Score	Score SD
Prestw-28	Ethambutol dihydrochloride	1,225814	0,033595
Prestw-1109	Rifabutin	1,189656	0,044552
Prestw-1429	Linezolid	1,188858	0,030995
Prestw-1478	Rifaximin	1,035642	0,001289
Prestw-1157	Rifapentine	1,019149	0,089935
Prestw-127	Isoconazole	0,932132	0,10216
Prestw-1321	Prothionamide	0,923799	0,051977
Prestw-161	Isoniazid	0,918147	0,019877
Prestw-525	Rifampicin	0,911659	0,011737
Prestw-526	Ethionamide	0,878031	0,038328
Prestw-67	Miconazole	0,708215	0,008418
Prestw-1446	Moxifloxacin	0,700677	0,02008
Prestw-787	Merbromin	0,690025	0,082574
Prestw-390	Fusidic acid sodium salt	0,659256	0,103686
Prestw-1072	Lansoprazole	0,633754	0,02461
Prestw-1378	Clarithromycin	0,633549	0,107855
Prestw-1184	Tioconazole	0,602143	0,131729
Prestw-1265	Gatifloxacin	0,59116	0,04248
Prestw-854	Roxithromycin	0,585573	0,002029
Prestw-143	Chlorhexidine	0,563608	0,112897
Prestw-456	Meclocycline sulfosalicylate	0,558025	0,033866
Prestw-753	Demeclocycline hydrochloride	0,540454	0,028363
Prestw-1202	4-aminosalicylic acid	0,504281	0,021594
Prestw-1469	Mercaptopurine	0,49779	0,020288
Prestw-1353	Tylosin	0,484304	0,068005
Prestw-964	Methacycline hydrochloride	0,479672	0,012353
Prestw-1399	Doxycycline hydrochloride	0,467443	0,043805
Prestw-1234	Azithromycin	0,457259	0,002645
Prestw-304	Econazole nitrate	0,435142	0,01197
Prestw-1340	Secnidazole	0,410823	0,08413
Prestw-154	Josamycin	0,389697	0,006481
Prestw-867	Paromomycin sulfate	0,379752	0,006313
Prestw-376	Clofazimine	0,372465	0,012093
Prestw-159	Dihydrostreptomycin sulfate	0,371586	0,033046
Prestw-222	Antimycin A	0,369432	0,009911
Prestw-1241	Bifonazole	0,340554	0,020859
Prestw-389	Ketoconazole	0,315344	0,033782

Prestw-151	Erythromycin	0,304405	0,005892
Prestw-1118	Cefepime hydrochloride	0,304193	0,078126
Prestw-31	Chloramphenicol	0,277552	0,016772
Prestw-1113	Viomycin sulfate	0,267181	0,051968
Prestw-1005	Apramycin	0,264068	0,0378
Prestw-557	Dirithromycin	0,263181	0,00337

Supplementary Table 2. Ratios of LPZ and LPZS

(a) Intracellular ratio of LPZ (*m/z* 370.0834 g/mol) and its metabolite (*m/z* 354.0884 g/mol) determined by electrospray ionisation mass spectrometry (ESI-Q-TOF-MS) over a 48 hour period in MRC-5 cells. (b) LPZ/LPZS ratio determined by ESI-Q-TOF-MS over a 48 hour period in 7H9 broth.

A:

	Replicate 1		Replicate 2		Replicate 3	
	LPZ	LPZS	LPZ	LPZS	LPZ	LPZS
1 hour	1,000	0,0885	1,000	0,0767	1,000	0,091
5 hours	0,360	0,3030	0,281	0,4210	0,311	0,313
24 hours	0,136	0,8240	0,120	0,9100	0,210	0,681
48 hours	0,021	0,9001	0,011	0,9300	0,017	0,811

B:

	Replicate 1		Replicate 2		Replicate 3	
	LPZ	LPZS	LPZ	LPZS	LPZ	LPZS
0 hours	1,000	0,000125	1,000	0,000090	1,000	0,000190
1 hour	0,904	0,004800	0,871	0,009100	0,870	0,006100
5 hours	0,580	0,017000	0,500	0,051000	0,465	0,021000
24 hours	0,089	0,039000	0,081	0,061000	0,051	0,034000
48 hours	0,020	0,045000	0,028	0,054000	0,012	0,061000

Supplementary Table 3. Cytotoxicity of LPZS on human cell lines.

Cytotoxicity of LPZS on THP1 macrophages, human liver hepatoma cells (HuH7-D12), human lung epithelial carcinoma cells (A549) and human embryonic liver cells (HepG2).

	THP-1		HuH7-D12		A549		HepG2	
	TD99	TD50	TD99	TD50	TD99	TD50	TD99	TD50
LPZS (μM)	>100	75	>100	>100	>100	37.5	100	25

Supplementary Table 4. Drug combination studies. LPZS acts additively with first- and second-line anti-TB drugs. ΣFIC: fractional inhibitory concentration indices

Compound	**ΣFIC**	**Outcome**
Rifampicin	1	Additive
Isoniazid	1	Additive
Moxifloxacin	1	Additive
Bedaquiline	1	Additive
BTZ043	0.75	Additive

Supplementary Table 5. The L176P mutation has no effect on imidazopyridine amide (IPA) activity. This stands in contrast to the T313A mutation which confers high level resistance towards IPA-compounds.

	MIC (μM)		
IPA compound	**Wild-type**	**L176P**	**T313A**
GSK2111534A	0.4	0.4	> 25
GSK1829820A	0.2	0.2	> 25
GSK1829736A	0.8	0.8	> 25
Q203	0.006	0.012	> 0.1