Supplementary Materials:

Materials and Methods:

M. tuberculosis filter culture and metabolite extraction

M. tuberculosis H37Rv was cultured in a biosafety level 3 facility at 37 °C in Middlebrook 7H9 broth (m7H9) or on 7H10 agar (m7H10) (Difco) supplemented with 0.2% glucose, 0.05% glycerol, 0.04% Tyloxapol, 0.5 g/l bovine serum albumin and 0.085% NaCl. M. tuberculosis-laden filters used for MIC determinations and metabolomic profiling were generated as previously described (*21, 22*) and incubated at 37°C for 5 days to reach the midlogarithimic phase of growth. M. tuberculosis-laden filters were transferred onto chemically identical media containing drugs or dimethylsulfoxide vehicle as indicated. MICs were determined by using the same bacterial inoculum size as for metabolomic profiling experiments and identifying the drug concentration needed to suppress visible growth after 14 days of incubation atop drug impregnated agar media. For metabolomic profiling experiments, M. tuberculosis-laden filters were metabolically quenched by plunging filters into a mixture of acetonitrile/methanol/H_2O (40:40:20) precooled to −40°C ; metabolites were extracted by mechanical lysis with 0.1 mm Zirconia beads in a Precellys tissue homogenizer for 3 min (6,500 rpm) twice under continuous cooling at or below 2 °C. Lysates were clarified by centrifugation and then filtered across a 0.22 μm filter. Residual protein content of metabolite extracts (BCA protein assay kit, Thermo Scientific) was determined to normalize samples to cell biomass. All data obtained by metabolomics were average of independent triplicates.

Liquid chromatography-coupled mass spectrometry

Extracted metabolites were separated on a Cogent Diamond Hydride Type C column (Gradient 3) (*23*). The mobile phase consisted of the following: solvent A (ddH_2O with 0.2% acetic acid) and solvent B (acetonitrile with 0.2% acetic acid). The gradient used was as follows: 0–2 min, 85% B; 3–5 min, 80% B; 6–7 min, 75%; 8–9 min, 70% B; 10–11.1 min, 50% B; 11.1–14 min 20% B; 14.1–24 min 5% B followed by a 10 min re-equilibration period at 85% B at a flow rate of 0.4 ml/min. The mass spectrometer used was an Agilent Accurate Mass 6220 TOF coupled to an Agilent 1200 LC system. Dynamic mass axis calibration was achieved by continuous infusion of a reference mass solution using an isocratic pump with a 100:1 splitter. This configuration achieved mass errors of 5 ppm, mass resolution ranging from 10,000 to 25,000 (over m/z 121–955 AMU), and 5 log10 dynamic range. Detected ions were deemed metabolites on the basis of unique accurate mass-retention time identifiers for masses exhibiting the expected distribution of accompanying isotopomers and authentic chemical standards. Metabolite identities were searched for using a mass tolerance of <0.005 Da. Metabolites were quantified using a calibration curve generated with chemical standard spiked into homologous mycobacterial extract to correct for matrix-associated ion suppression effects.

Enzyme assays

FolP1 from M. tuberculosis was cloned, expressed and purified as previously described (*11*). 6-Hydroxymethyl-7,8-dihydropterin pyrophosphate (H_2PtePP) substrate was chemically reduced according to literature methods (*24*) from 6-hydroxymethyl-7,8-pterin pyrophosphate (Schircks Laboratories). FolP1 activity was measured by high performance liquid chromatographic analysis. Reactions contained 50 mM Tris pH 7.5, 5 mM MgCl2, 1 mM

dithiothreitol, 10 μM H_2PtePP (excess), 50 nM FolP1 and varying PABA or PAS. Reactions were initiated by addition of enzyme. Reactions were incubated at 25 °C prior to quenching with 50 mM (final) EDTA. Data were collected using a Phenomenex Luna(2) 2 x 50mm 3μm 100Å C_{18} column on an Agilent 1100 LC-MS instrument. Samples were eluted with a gradient of 95% Solvent A (0.1% formic acid in water), 5% Solvent B (0.1% formic acid in acetonitrile) to 5% A, 95 % B in 10 min (0.3 mL/min). Product formation was determined by comparing peak area of H_2Pte or H_2PtePAS formed (A_{284}) and comparing to a standard curve to determine concentration. Data were fit to the Michaelis-Menten equation to determine k_{cat} and K_M values.

FolC from M. tuberculosis was cloned, expressed, and purified as previously described (*25*), modified by the use of unaltered pET-42a vector (Novagen), Factor Xa protease (New England Biolabs) and Factor Xa removal resin (Qiagen), and pGro7 (Takara) chaperones. H_2Pte(X) was enzymatically synthesized: reactions contained 0.5 μM FolP1, 50 mM Tris pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, 20 μM H_2PtePP, and 24 μM pABA or PAS and were allowed to proceed to completion as judged by LC-MS. H_2Pte(X) synthesis reactions were added directly to FolC reaction tubes to initiate the FolC reaction. FolC reactions contained (final concentrations) 1 μM FolC, 0.25 μM FolP1, 50 mM Tris pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, 200 mM KCl, 5% DMSO, 5 mM L-glutamate, 5 mM ATP, and 10 μM H_2Pte(X). Reactions were quenched after 45 min at 25 °C with 50 mM EDTA (final concentration) prior to analysis. Data were collected using a Phenomenex Luna(2) 2 x 50mm 3μm 100Å C_{18} column on an Agilent 1100 LC-MS instrument. Samples were eluted with a gradient of 100% solvent A (0.1 M ammonium acetate in 1% acetonitrile) to 91% solvent A, 9% solvent B (100% acetonitrile) in 20 min (0.3 mL/min) (modified from Montero and Llorente 1993, DOI: 10.1007/BF02272192). H_2Pte(X) consumption was monitored by measuring peak area (A_{284}) and comparing to initial H_2Pte(X) peak area following FolP1 reaction.

FolP1 and FolC were >95% pure as judged by SDS-PAGE.

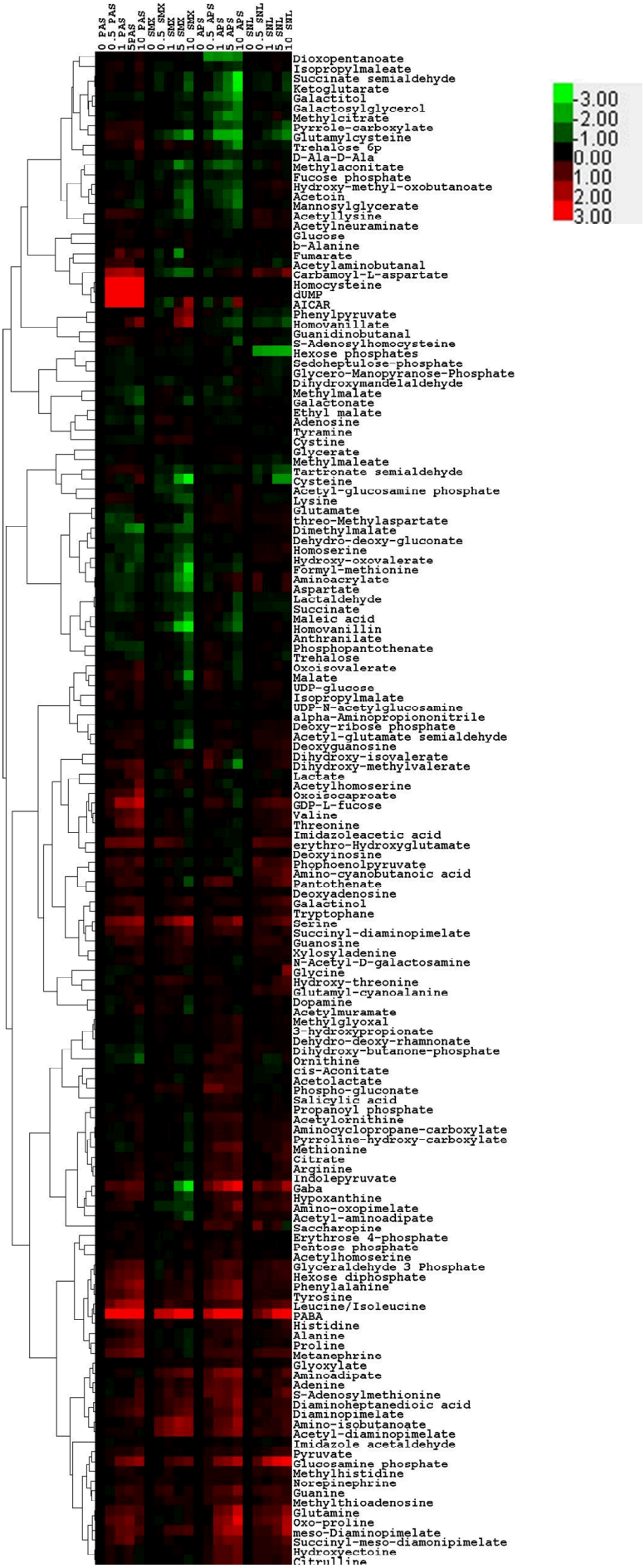
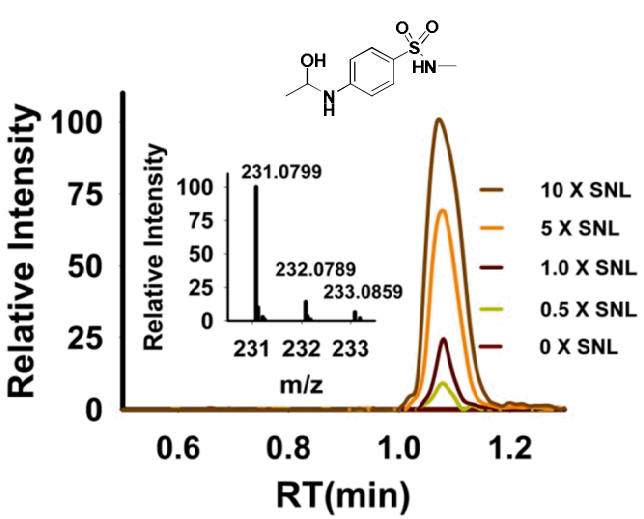

0.1 PAS
0.5 PAS
1 PAS
5 PAS
10 PAS
0.1 SMX
0.5 SMX
1 SMX
5 SMX
10 SMX
0.1 APS
0.5 APS
1 APS
5 APS
10 APS
0.1 SNL
0.5 SNL
1 SNL
5 SNL
10 SNL
Dioxopentanoate
Isopropylmaleate
Succinate semialdehyde
Ketoglutarate
Galactitol
Galactosylglycerol
Methylcitrate
Pyrrole-carboxylate
Glutamylcysteine
Trehalose 6p
D-Ala-D-Ala
Methylaconitate
Fucose phosphate
Hydroxy-methyl-oxobutanoate
Acetoin
Mannosylglycerate
Acetyllysine
Acetylneuraminate
Glucose
b-Alanine
Fumarate
Acetylaminobutanal
Carbamoyl-L-aspartate
Homocysteine
dUMP
AICAR
Phenylpyruvate
Homovanillate
Guanidinobutanal
S-Adenosylhomocysteine
Hexose phosphates
Sedoheptulose-phosphate
Glycero-Manopyranose-Phosphate
Dihydroxymandelaldehyde
Methylmalate
Galactonate
Ethyl malate
Adenosine
Tyramine
Cystine
Glycerate
Methylmaleate
Tartronate semialdehyde
Cysteine
Acetyl-glucosamine phosphate
Lysine
Glutamate
threo-Methylaspartate
Dimethylmalate
Dehydro-deoxy-gluconate
Homoserine
Hydroxy-oxovalerate
Formyl-methionine
Aminoacrylate
Aspartate
Lactaldehyde
Succinate
Maleic acid
Homovanillin
Anthranilate
Phosphopantothenate
Trehalose
Oxoisovalerate
Malate
UDP-glucose
Isopropylmalate
UDP-N-acetylglucosamine
alpha-Aminopropiononitrile
Deoxy-ribose phosphate
Acetyl-glutamate semialdehyde
Deoxyguanosine
Dihydroxy-isovalerate
Dihydroxy-methylvalerate
Lactate
Acetylhomoserine
Oxoisocaproate
GDP-L-fucose
Valine
Threonine
Imidazoleacetic acid
erythro-Hydroxyglutamate
Deoxyinosine
Phophoenolpyruvate
Amino-cyanobutanoic acid
Pantothenate
Deoxyadenosine
Galactinol
Tryptophane
Serine
Succinyl-diaminopimelate
Guanosine
Xylosyladenine
N-Acetyl-D-galactosamine
Glycine
Hydroxy-threonine
Glutamyl-cyanoalanine
Dopamine
Acetylmuramate
Methylglyoxal
3-hydroxypropionate
Dehydro-deoxy-rhamnonate
Dihydroxy-butanone-phosphate
Ornithine
cis-Aconitate
Acetolactate
Phospho-gluconate
Salicylic acid
Propanoyl phosphate
Acetylornithine
Aminocyclopropane-carboxylate
Pyrroline-hydroxy-carboxylate
Methionine
Citrate
Arginine
Indolepyruvate
Gaba
Hypoxanthine
Amino-oxopimelate
Acetyl-aminoadipate
Saccharopine
Erythrose 4-phosphate
Pentose phosphate
Acetylhomoserine
Glyceraldehyde 3 Phosphate
Hexose diphosphate
Phenylalanine
Tyrosine
Leucine/Isoleucine
PABA
Histidine
Alanine
Proline
Metanephrine
Glyoxylate
Aminoadipate
Adenine
S-Adenosylmethionine
Diaminoheptanedioic acid
Diaminopimelate
Amino-isobutanoate
Acetyl-diaminopimelate
Imidazole acetaldehyde
Pyruvate
Glucosamine phosphate
Methylhistidine
Norepinephrine
Guanine
Methylthioadenosine
Glutamine
Oxo-proline
meso-Diaminopimelate
Succinyl-meso-diamonipimelate
Hydroxyectoine
Citrulline
3.00
2.00
1.00
0.00
1.00
2.00
3.00

Fig. S1. Heatmap representation of 154 intracellular metabolites in M. tuberculosis following treatment with drugs as in **1A**. Numbers denote drug concentration expressed in relation to MIC or, for APS and SNL, molar equivalent of SMX doses used. Color intensities correspond to relative levels are expressed as the log ratio of the normalized signal intensity in drug-treated cells at each concentration to the normalized signal intensity in the drug-free sample ($n \geq 2$ independent experiments). Signal intensities were normalized to cell protein biomass at each concentration.

S2A.


Relative Intensity
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75
50
25
0
0.6
0.8
1.0
1.2
RT(min)
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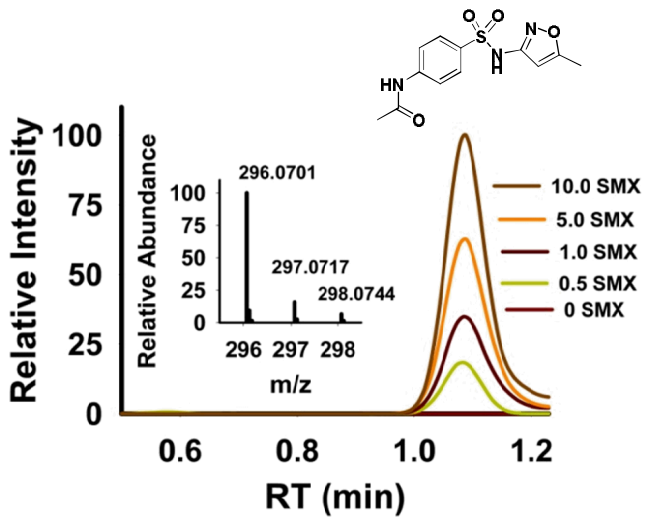
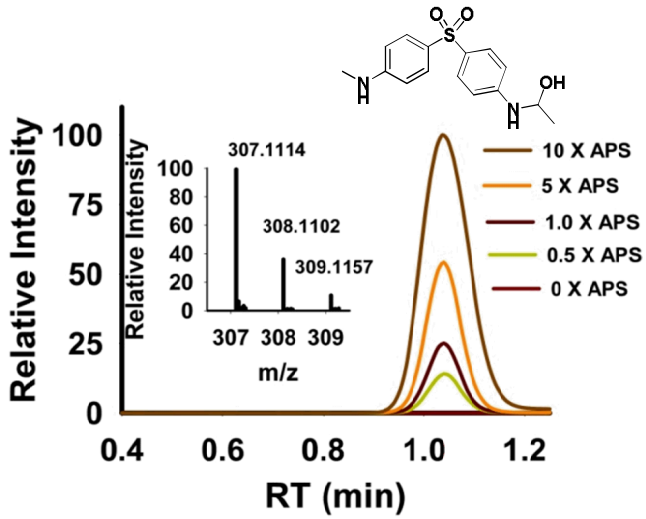
232.0789
233.0859
231 232 233
m/z
10 X SNL
5 X SNL
1.0 X SNL
0.5 X SNL
0 X SNL


Relative Intensity
Relative Abundance
100
75
50
25
0
0.6
0.8
1.0
1.2
RT (min)
296.0701
297.0717
298.0744
296 297 298
m/z
10.0 SMX
5.0 SMX
1.0 SMX
0.5 SMX
0 SMX


Relative Intensity
100
75
50
25
0
0.4
0.6
0.8
1.0
1.2
RT (min)
307.1114
308.1102
309.1157
307 308 309
m/z
10 X APS
5 X APS
1.0 X APS
0.5 X APS
0 X APS

S2B.

Bio-transformed Products	RT. (min)	m/z $(M+H)^+$ obs.	m/z $(M+H)^+$ calc.	error (Δ ppm)
Me-PAS	1.02	168.0658	168.0655	1.79
(Me)2-PAS	1.10	182.0814	182.0812	1.10
PAS-Pte	1.68	329.0995	329.0993	0.61
N-Me-PAS-Pte	1.58	343.1153	343.1149	1.17
PAS-Pte-Glu	9.41	458.1416	458.1419	-0.65
N-Me-Pas-Pte-Glu	9.18	472.1594	472.1575	4.02
N-Acetyl -SMX	1.08	296.0701	296.07	0.33
Hydroxymethyl-(N-Me)-SNL	1.07	231.0799	231.0798	0.43
Hydroxymethyl-(N-Me)-APS	1.04	307.1114	307.1111	0.98

Fig. S2. A. Extracted ion chromatograms and mass spectra corresponding to exemplary inactive biotransformation products of sulfonamide compounds, demonstrating their compound and dose dependence (left, sulfanilamide; center, sulfamethoxazole; right, aminophenylsulfone). **B.** Accurate mass-retention time pairs for specified biotransformation products, confirmed by co-elution with pure standards for Me-PAS, (Me)2-PAS, PAS-Pte.