Supplementary Methods, Datasets, Table and Figures

Inhibiting *Mycobacterium tuberculosis* DosRST signaling by targeting response regulator DNA binding and sensor kinase heme

Huiqing Zheng[1], John T. Williams[1], Bilal Aleiwi[2], Edmund Ellsworth[2], and Robert B. Abramovitch[1*]

[1]Department of Microbiology and Molecular Genetics, [2]Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan, 48824

[*]*Corresponding Author*

Robert B. Abramovitch

E-mail: abramov5@msu.edu

Phone: (517) 884-5416

Fax: (517) 353-8957

Supplemental Methods

Real time-PCR assays. The vitamin C and NO assays were performed as previously described[1]. Briefly, Mtb grown in tissue culture flasks, laying flat to promote an aerated environment, were seeded at an OD_{600} of 0.6 were pretreated with 80 μM HC104A, HC106A or a DMSO control for 24 h, and induced with 50 μM DETA-NONOate or 20 mM vitamin C for 2 h. For the HC106A resistance assays, CDC1551 was transformed with the empty replicating plasmid pVV16 or the plasmid expressing *dosS* from the strong *hsp60* promoter (pVV16-DosS), and treated with 20 μM HC106A for 6 d. Total bacterial RNA was isolated and differential gene expression of DosR-regulated genes, including *hspX* and *tgs1*, was quantified. The experiment was performed in three technical replicates and error bars represent the s.d from the mean. The experiment was repeated twice with similar results. To examine Mtb gene expression in macrophages, murine bone-marrow derived macrophages were isolated as previously described [2] and seeded in T75 vented, tissue culture flasks. Macrophages were infected with CDC1551 with multiplicity of infection ratio of 1:20 as previously described [2]. After infection, the flasks were treated with 40 μM HC104A or HC106A or DMSO for 48 h, with three individual flasks for each treatment. Total bacterial RNA was isolated after treatment, and the transcripts of DosR-controlled genes (*hspX* and *tgs1*) were quantified in RT-PCR. The experiment was conducted with three biological replicates. The error bar represents the s.d. of the biological replicates.

TAG biosynthesis. The lipid labelling and TAG TLCs were performed as previously described [1]. Briefly, CDC1551 was cultured at an initial OD_{600} of 0.1 and radiolabeled with 8 μCi of [1,2-^{14}C] sodium acetate in T25 vented tissue culture flasks. The cultures were treated with 40 μM HC104A, HC106A or DMSO for 6 d at 37°C. CDC1551 (Δ*dosR*) and *dosRS* complement strains were also examined. Total lipids were extracted and ^{14}C incorporation was determined by

scintillation counting. 20,000 c.p.m. of total lipids were analyzed by TLC using silica gel 60 aluminum sheets (EMD Millipore). To determine TAG accumulation, the lipids were developed in hexane-diethyl ether-acetic acid (80:20:1; vol/vol/vol) solvent system. The TLC was exposed to a phosphor screen for 3 d, and imaged on a Typhoon imager and TAG was quantified using ImageJ software [3]. The experiment was repeated twice with similar results, and the error bar represents the s.d. of two biological replicates.

NRP survival assays. Survival during NRP was examined using the hypoxic shift down assays as previously described [1, 4]. Briefly, CDC1551 cells were treated with 40 μM HC104A, HC106A, MSU-39446 or DMSO control in a 24-well plate (1 mL/well). CDC1551 (Δ*dosR*) and *dosRS* complement, overexpressor strains (*dosS, dosS*(G117L), *dosT* or *dosT*(G177L)*)* were also examined. Plates were incubated in an anaerobic chamber (BD GasPak) for 12 d. It took 48 h for cultures to become anaerobic, as monitored by a methylene blue control. Bacterial CFUs were numerated on 7H10 agar plates following incubation. The experiment was repeated twice with similar results.

DosR protein purification. DosR full length protein was purified as previously described [5]. Briefly, the *dosR* gene (Rv3133c) was cloned into pET15b (Novagen Darmstadt, Germany) using the primer set: forward primer 5'-TTTCATATGGTGGTAAAGGTCTTCTTGGTCGATGAC-3'; reverse primer 5'-TTTGGATCCTCATGGTCCATCACCGGGTGG-3'. The His_6-DosR protein was expressed in *E. coli* BL21(DE3) strain. The culture was grown to OD_{600} 0.5-06, and induced with 1 mM IPTG for 6.5 h at 29°C. The cell pellet was suspended in lysis buffer (20 mM Tris-HCl, pH 8.0, 10% glycerol, 500 mM NaCl, 0.5 mg/ml lysozyme and 0.1 mg/ml PMSF), and incubated at 37°C for 30 min. The soluble fraction of lysate was collected after centrifugation and applied to a TALON metal affinity Co^{2+} column (Clontech). The column was washed twice

with washing buffer (20 mM Tris-HCl, pH 8.0, 10% glycerol, 500 mM NaCl) without imidazole, then with 20 mM imidazole. The protein was eluted with the same buffer containing 300 mM imidazole. The fractions with the most DosR protein (as determined by SDS-PAGE) were pooled together for dialysis in 25 mM Tris-HCl, pH 8.0. The final protein concentration was determined using a Qubit kit (Invitrogen).

Electrophoretic mobility assay. The assay is fluorescence-based using 6-carboxyfluorescein (6FAM) labeled 385 bp probe from the *hspX* promoter. In designing the primer set, 6FAM was added to the 5' ends of forward and reverse primers. The *hspX* probe was synthesized via PCR using the primer set: forward primer 5'-6FAM-CAACTGCACCGCGCTCTTGATG-3'; reverse primer 5'-6FAM-CATCTCGTCTTCCAGCCGCATCAAC-3'. The probe was purified by Qiagen PCR purification kit. The DosR protein was pre-phosphorylated in 10 μL of phosphorylation buffer (40 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 50 mM lithium potassium acetyl phosphate), and incubated at room temperature for 30 min. The protein was then transferred to binding buffer in a final volume of 20 μL (final concentration, 25 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 20 mM KCl, 6 mM $MgCl_2$, 10 nM probe, 1 μg poly-dI-dC (Sigma Aldrich)), and treated with HC104A or an equal volume of DMSO or virstatin (Santa Cruz Biotech). Two different assays were performed. Firstly, different DosR protein concentrations from 0.5 μM to 4 μM were treated with 40 μM. Second, dose response assays were performed with 2 μM DosR treated with different concentrations of HC104A or virstatin from 1 μM to 80 μM. After incubating on ice for 30 min, the reactions were terminated by adding 1 μL 80% glycerol, and loaded on a native 5% Tris/Borate/EDTA (TBE) polyacrylamide gel. The gel was run at 50 V, for 5-6 h at 4°C in 1X TBE buffer, and was imaged using a Typhoon scanner with appropriate filters that can detect florescence at excitation = 495 nm, emission = 520 nm. Binding of the unbound probe was

quantified using ImageJ [3]. The assay was repeated at least twice with similar results. The error bar represents the s.d. of two biological replicates.

Autophosphorylation assay. The DosS autophosphorylation assay was performed as previously described [1]. Recombinant DosS protein was treated with 10 μM, 20 μM or 40 μM of HC104A, or HC106A. DMSO and 40 μM HC103A were also included as positive and negative controls, respectively.

UV-visible spectroscopy assay. DosS, DosT and mutant proteins were purified and analyzed as previously described [1]. Briefly, 7.5 μM of recombinant DosS protein was deoxygenated with argon gas in a sealed cuvette. The protein was reduced with 400 μM DTN for 20 min. The reaction was then treated with 100 μM HC106A, 400 μM HC104A, 100 μM CORM-2 (tricarbonyldichlororuthenium (II) dimer) or equal volume of DMSO. The UV-visible spectra were recorded for kinetic changes over 2 h. The experiment was repeated at least twice with similar results. DosT was deoxygenated in a hypoxia chamber and treated examined as described for DosS using HC106A.

Kinetic solubility assay. The assay was performed with 7-point (2-fold) dilutions from 200 μM - 3.125 μM for HC106 analogs. Mebendazole, benxarotene and aspirin were also included as controls. The drug dilutions were added to PBS, pH 7.4, with the final DMSO concentration of 1%, and incubated at 37 °C for 2 h. The absorbance at 620 nm was measured for each drug dilution to estimate of the compound solubility. Three replicates were examined for each dilution.

Checkerboard synergy studies. The reporter strain CDC1551 (*hspX::GFP*) was treated with pairs of DosRST inhibitors from 50 μM – 0.08 μM in 96-well plates, including HC101A-HC104A and HC106A. GFP fluorescence and OD_{600} were measured after 6 d incubation. The percentage of fluorescence inhibition (FI) and growth inhibition were calculated for each drug pair, with limited growth inhibition observed. The FI data was utilized for further analysis of interactions using CompuSyn software [6]. The Combination Index (CI) value was calculated for each drug pair according to the Chou-Talalay method, which is based on the Median-Effect equation derived from the Mass-Action Law principle [7, 8]. The resulting CI values provide quantitative determination of drug interactions, including synergism (CI < 1), additive effect (C = 1), and antagonism (C > 1).

Sources of commercial compounds. HC104A was purchased from Asinex, HC106A was purchased from Maybridge, Virstatin was purchased from Cayman Chemicals.

General procedure for urea (4) formation

Formation of 1,2-oxazolidine-5-carbonyl chloride (**2**). To a stirred solution of 1.0 eq. of N-(4-chlorophenyl)-1,2-oxazole-5-carboxamide (**1**) in dry tetrahydrofuran (THF, 0.4 M) under N_2 atmosphere was added oxalyl chloride (1.5 eq.) dropwise over 5-10 min followed by 1 drop of dimethylformamide and the reaction mixture was continued to stir at room temperature. Upon completion, the reaction mixture was concentrated into a residue in *vacuo* and the residue was redissolved in THF and concentrated again (process repeated 3 times) to ensure the removal of excess oxalyl chloride. The crude acyl chloride **2** was used directly in the next step without further purification.

Formation of acyl azide and rearrangement to 5-isocyanato-1,2-oxazolidine (**3**). Crude 1,2-oxazolidine-5-carbonyl chloride **2** was dissolved in THF (0.4 M) and stirred at room temperature under N_2 atmosphere. Trimethylsilyl (TMS) azide (2 eq.) was added dropwise over 5 min and

stirring was continued for two hours and the mixture diluted with ethyl acetate (0.4 M) and quenched with H_2O (0.4 M). The two layers were separated, and the organic layer dried over anhydrous Na_2SO_4 and filtered. The ethyl acetate solvent was swapped into toluene (0.1 M) by the addition of toluene followed by removal of the ethyl acetate in *vacuo*. Care was taken not to concentrate the toluene. The toluene acyl azide solution was heated at reflux conditions under N_2 atmosphere for 4 h to give the desired isocyanate **3,** which was used as a solution in toluene in the next step without further purification.

Formation of urea **4**. The crude solution of 5-isocyanato-1,2-oxazolidine (**3**) in toluene was mixed with different anilines and amines (1.5 eq.) and stirred at room temperature overnight. Isolation of the ureas was completed by diluting the reaction mixture with hexanes, stirring for few hours and filtration of the formed precipitate. The solid material was washed with hexanes and dried under high vacuum.

1,3-bis(1,2-oxazol-5-yl)urea (*MSU-39444*) . ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 2H), 8.44 (d, *J* = 1.9 Hz, 2H), 6.11 (d, *J* = 2.0 Hz, 2H). HRMS (ESI) m/z calculated for $C_7H_6N_4O_3$ [M+H], 195.0513 found 195.0518.

1-(3-chlorophenyl)-3-(1,2-oxazol-5-yl)urea (*MSU-39445*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.41 (s, 1H), 9.14 (s, 1H), 8.40 (d, *J* = 2.0 Hz, 1H), 7.82 – 7.58 (m, 1H), 7.31 (dd, *J* = 4.9, 1.8 Hz, 2H), 7.07 (dt, *J* = 6.4, 2.3 Hz, 1H), 6.06 (d, *J* = 1.9 Hz, 1H). HRMS (ESI) m/z calculated for $C_{10}H_9ClN_3O_2$ [M+H], 238.0378; found 238.0365.

1-(3,4-dichlorophenyl)-3-(1,2-oxazol-5-yl)urea (*MSU-39452*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.50 (s, 1H), 9.23 (s, 1H), 8.51 – 8.28 (m, 1H), 7.86 (d, *J* = 2.5 Hz, 1H), 7.64 – 7.43 (m, 1H), 7.37 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.26 – 5.80 (m, 1H). HRMS (ESI) m/z calculated for $C_{10}H_8Cl_2N_3O_2$ [M+H], 271.9989; found 271.9969.

1-(4-methoxyphenyl)-3-(1,2-oxazol-5-yl)urea (*MSU-39447*). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.83 (s, 1H), 8.43 – 8.29 (m, 1H), 7.36 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.00 (d, *J* =

1.9 Hz, 1H), 3.70 (s, 3H). HRMS (ESI) m/z calculated for $C_{11}H_{12}N_3O_3$ [M+H], 234.0874; found 234.0863.

1-(4-chlorophenyl)-1-methyl-3-(1,2-oxazol-5-yl)urea (*MSU-39451*). ^{1}H NMR (500 MHz, DMSO-d6) δ 10.13 (s, 1H), 8.34 (d, *J* = 1.9 Hz, 1H), 7.56 – 7.41 (m, 2H), 7.41 – 7.22 (m, 2H), 6.04 (d, *J* = 2.0 Hz, 1H), 3.25 (s, 3H). HRMS (ESI) m/z calculated for $C_{11}H_{11}ClN_3O_2$ [M+H], 252.0535; found 252.0596.

5-chloro-N-(1,2-oxazol-5-yl)-2,3-dihydro-1H-indole-1-carboxamide (***MSU-39453)***. ^{1}H NMR (500 MHz, DMSO-d6) δ 10.49 (s, 1H), 8.41 (d, *J* = 1.9 Hz, 1H), 7.84 (d, *J* = 8.7 Hz, 1H), 7.27 (dt, *J* = 2.3, 1.1 Hz, 1H), 7.18 (dt, *J* = 8.8, 1.6 Hz, 1H), 6.14 (d, *J* = 1.9 Hz, 1H), 4.13 (dd, *J* = 9.1, 8.2 Hz, 2H), 3.17 (t, *J* = 8.6 Hz, 2H). HRMS (ESI) m/z calculated for $C_{12}H_{11}ClN_3O_2$ [M+H], 264.0535; found 264.0552.

1-(6-chloropyridin-3-yl)-3-(1,2-oxazol-5-yl)urea (*MSU-39448).* ^{1}H NMR (500 MHz, DMSO-d6) δ 10.55 (s, 1H), 9.25 (s, 1H), 8.64 – 8.24 (m, 2H), 7.98 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 1H), 6.08 (d, *J* = 18.1 Hz, 1H). HRMS (ESI) m/z calculated for $C_9H_8ClN_4O_2$ [M+H], 239.0331; found 239.0364.

1-(5-chloropyridin-2-yl)-3-(1,2-oxazol-5-yl)urea (*MSU-39450*). ^{1}H NMR (500 MHz, DMSO-d6) δ 11.00 (s, 1H), 9.66 (s, 1H), 8.42 (d, *J* = 1.9 Hz, 1H), 8.34 (d, *J* = 2.6 Hz, 1H), 7.96 – 7.79 (m, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 6.10 (q, *J* = 2.7, 2.2 Hz, 2H). HRMS (ESI) m/z calculated for $C_9H_8ClN_4O_2$ [M+H], 239.0331; found 239.0356.

1-(5-fluoropyridin-2-yl)-3-(1,2-oxazol-5-yl)urea (*MSU-39446*. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.90 (s, 1H), 8.38 (d, *J* = 1.9 Hz, 1H), 7.54 – 7.37 (m, 2H), 7.26 – 7.02 (m, 2H), 6.03 (d, *J* = 2.0 Hz, 1H). HRMS (ESI) m/z calculated for $C_{10}H_9FN_3O_2$ [M+H], 222.0674; found 222.0675).

3-(5-chloropyridin-2-yl)-1-(4-methylphenyl)urea (MSU-41324). ^{1}H NMR (500 MHz, DMSO-d6) δ 9.91 (s, 1H), 9.50 (s, 1H), 8.31 (d, *J* = 2.6 Hz, 1H), 7.85 (dd, *J* = 9.0, 2.7 Hz, 1H), 7.67 (d,

J = 8.9 Hz, 1H), 7.39 – 7.30 (m, 2H). HRMS (ESI) m/z calculated for $C_{12}H_{10}Cl_2N_3O$ [M+H], 282.0196; found 282.0182

1,3-bis(4-chlorophenyl)urea (MSU-41425) ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.83 (s, 1H), 7.53 – 7.39 (m, 2H), 7.39 – 7.16 (m, 2H). HRMS (ESI) m/z calculated for $C_{13}H_{11}Cl_2N_2O$ [M+H], 281.0243; found 281.0258.

1-{[1,1'-biphenyl]-4-yl}-3-(1,2-oxazol-5-yl)urea (*MSU-41443*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.36 (d, *J* = 28.4 Hz, 1H), 9.13 (d, *J* = 45.7 Hz, 1H), 8.40 (d, *J* = 1.9 Hz, 1H), 7.70 – 7.59 (m, 5H), 7.59 – 7.49 (m, 3H), 7.43 (t, *J* = 7.7 Hz, 3H), 7.32 (t, *J* = 7.4 Hz, 1H), 6.06 (d, *J* = 1.9 Hz, 1H). HRMS (ESI) m/z calculated for $C_{16}H_{14}N_3O_2$ [M+H], 280.1081; found 280.1083.

1-(4-tert-butylphenyl)-3-(1,2-oxazol-5-yl)urea (*MSU-41442).* ^{1}H NMR (500 MHz, DMSO-d6) δ 10.15 (s, 1H), 8.77 (s, 1H), 8.38 (d, *J* = 1.9 Hz, 1H), 7.44 – 7.27 (m, 4H), 6.03 (d, *J* = 2.0 Hz, 1H), 1.25 (s, 9H). HRMS (ESI) m/z calculated for $C_{14}H_{18}N_3O_2$ [M+H], 260.1394; found 260.1406.

3-(1,2-oxazol-5-yl)-1-phenylurea (*MSU-33189*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.85 (s, 1H), 8.39 (d, *J* = 1.9 Hz, 1H), 7.54 – 7.37 (m, 2H), 7.35 – 7.21 (m, 2H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.04 (d, *J* = 1.9 Hz, 1H). HRMS (ESI) m/z calculated for $C_{10}H_{10}N_3O_2$ [M+H], 204.0768; found 204.0777.

1-(4-chlorophenyl)-3-(1,2-oxazol-5-yl)urea (***MSU-33231***). ^{1}H NMR (500 MHz, DMSO-d6) δ 10.29 (s, 1H), 9.01 (s, 1H), 8.39 (d, *J* = 1.9 Hz, 1H), 7.56 – 7.41 (m, 2H), 7.41 – 7.27 (m, 2H), 6.05 (d, *J* = 1.9 Hz, 1H). HRMS (ESI) m/z calculated for $C_{10}H_9ClN_3O_2$ [M+H], 238.0378; found 238.0391.

1-benzyl-3-(1,2-oxazol-5-yl)urea (*MSU-41462*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.31 (d, *J* = 2.0 Hz, 1H), 7.43 – 7.07 (m, 3H), 6.92 (s, 1H), 5.94 (d, *J* = 1.9 Hz, 1H), 4.30 (d, *J* = 6.0 Hz, 2H). HRMS (ESI) m/z calculated for $C_{11}H_{12}N_3O_2$ [M+H], 218.0924 found 218.0956.

3-(1,2-oxazol-5-yl)-1-[4-(trifluoromethyl)phenyl]urea (*MSU-41463*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.39 (s, 1H), 9.29 (s, 1H), 8.41 (d, *J* = 1.9 Hz, 1H), 7.67 (d, *J* = 1.0 Hz, 4H), 6.08

(d, *J* = 1.9 Hz, 1H). HRMS (ESI) m/z calculated for $C_{11}H_9F_3N_3O_2$ [M+H], 272.0642; found 272.0653.

1-(4-bromophenyl)-3-(1,2-oxazol-5-yl)urea (*MSU-41464).* ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.29 (s, 1H), 9.01 (s, 1H), 8.39 (d, *J* = 1.9 Hz, 2H), 7.50 – 7.45 (m, 1H), 7.45 – 7.40 (m, 1H), 6.05 (s, 1H). HRMS (ESI) m/z calculated for $C_{10}H_9BrN_3O_2$ [M+H], 281.9873; found 281.9876.

methyl 4-{[(1,2-oxazol-5-yl)carbamoyl]amino}benzoate (*MSU-41465*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.38 (s, 1H), 9.27 (s, 1H), 8.41 (d, *J* = 2.0 Hz, 1H), 8.00 – 7.79 (m, 2H), 7.66 – 7.48 (m, 2H), 6.08 (d, *J* = 1.9 Hz, 1H), 3.81 (s, 3H). HRMS (ESI) m/z calculated for $C_{12}H_{12}N_3O_4$ [M+H], 262.0823; found 262.0827.

3-(1,2-oxazol-5-yl)-1-[4-(phenoxymethyl)phenyl]urea (*MSU-41545).* ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.71 (s, 1H), 8.37 (d, *J* = 1.9 Hz, 1H), 7.57 – 7.14 (m, 5H), 7.11 – 6.69 (m, 2H), 6.02 (d, *J* = 1.9 Hz, 1H), 5.06 (s, 2H).HRMS (ESI) m/z calculated for $C_{17}H_{16}N_3O_3$ [M+H], 310.1187; found 310.1178.

1-cyclopentyl-3-(1,2-oxazol-5-yl)urea (*MSU-41546*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.69 (s, 1H), 8.30 (d, *J* = 1.9 Hz, 1H), 6.42 (d, *J* = 7.2 Hz, 1H), 5.91 (d, *J* = 1.9 Hz, 1H), 3.92 (h, *J* = 6.7 Hz, 1H), 1.83 (dq, *J* = 12.8, 6.6, 6.0 Hz, 3H), 1.73 – 1.46 (m, 5H), 1.46 – 1.13 (m, 3H). HRMS (ESI) m/z calculated for $C_9H_{14}N_3O_2$ [M+H], 196.1081; found 196.1144.

1-cyclohexyl-3-(1,2-oxazol-5-yl)urea (*MSU-42002*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.30 (d, *J* = 1.9 Hz, 1H), 6.34 (d, *J* = 7.8 Hz, 1H), 5.90 (d, *J* = 1.9 Hz, 1H), 3.58 – 3.39 (m, 1H), 1.77 (dt, *J* = 11.1, 3.7 Hz, 1H), 1.64 (dt, *J* = 12.9, 4.1 Hz, 1H), 1.52 (dd, *J* = 10.4, 6.3 Hz, 1H), 1.37 – 0.93 (m, 3H). HRMS (ESI) m/z calculated for $C_{10}H_{16}N_3O_2$ [M+H], 210.1238; found 210.1282.

3-(1,2-oxazol-5-yl)-1-(2-phenylethyl)urea (*MSU-42004*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.07 (s, 2H), 8.31 (d, *J* = 2.0 Hz, 1H), 7.37 – 7.06 (m, 5H), 6.40 (s, 1H), 5.93 (d, *J* = 1.8 Hz, 1H), 3.31 (t, *J* = 7.2 Hz, 2H), 2.74 (t, *J* = 7.2 Hz, 2H). HRMS (ESI) m/z calculated for $C_{12}H_{14}N_3O_2$ [M+H], 232.1081; found 232.1105.

1-(3-bromophenyl)-3-(1,2-oxazol-5-yl)urea (*MSU-42003*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.36 (s, 1H), 9.06 (s, 1H), 8.40 (d, *J* = 1.9 Hz, 1H), 7.83 (t, *J* = 2.0 Hz, 1H), 7.46 – 7.03 (m, 3H), 6.07 (d, *J* = 2.0 Hz, 1H). HRMS (ESI) m/z calculated for $C_{10}H_9BrN_3O_2$ [M+H], 281.9873; found 281.9915.

3-(2-methylpropyl)-1-(1,2-oxazol-5-yl)urea (*MSU-41542*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.20 – 9.49 (m, 1H), 8.30 (d, *J* = 1.9 Hz, 1H), 6.44 (t, *J* = 5.9 Hz, 1H), 5.91 (d, *J* = 1.9 Hz, 1H), 2.93 (t, *J* = 6.3 Hz, 2H), 1.68 (dh, *J* = 13.3, 6.7 Hz, 1H), 0.85 (d, *J* = 6.7 Hz, 6H). HRMS (ESI) m/z calculated for $C_8H_{14}N_3O_2$ [M+Na], 206.0899; found 206.0926.

N-(4-chlorophenyl)-1,2-oxazole-5-carboxamide *(MSU-41422).* Acyl chloride 2 (1 eq.) was dissolved in dichloromethane (0.2 M) and 4-chloroaniline (1.2 eq.) was added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated in *vacuo* and the residue was purified by flash column chromatography. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.41 (d, *J* = 1.8 Hz, 1H), 8.26 (s, 1H), 7.71 – 7.52 (m, 2H), 7.44 – 7.33 (m, 2H), 7.06 (d, *J* = 1.8 Hz, 1H). HRMS (ESI) m/z calculated for $C_{10}H_8ClN_2O_2$ [M+H], 223.0269; found 223.0265.

(*2-(4-chlorophenyl)-N-(1,2-oxazol-5-yl)acetamide (*MSU-39449). A slurry of 4-chlorophenyl acetic acid (0.10 g, 0.60 mmoles) in 2.0 mL of dichloromethane was treated with oxalyl chloride (0.12 mL, 0.9 mmoles) and 1 drop of dimethylformamide. The mixture (with gas evolution) gradually became homogeneous and was stirred for 30 min. The mixture was concentrated in *vacuo*, diluted with 5 mL of dichloromethane and again concentrated in vacuo, the process repeated three times. The resulting residue was again dissolved in dichloromethane (2 mL) and treated with 5-aminoisoxazole (0.030 g., 0.36 mmoles), followed by pyridine (0.48 mL, 0.6 mmoles). The mixture was then allowed to stir overnight. The mixture was then quenched with 1.0 N HCl and extracted with dichloromethane. The organic layers were combined, washed with saturated $KHCO_3$, dried with Na_2SO_4 and concentrated in *vacuo*. Medium pressure liquid

chromatography (SiO_2, 100% dichlormethane to 3% methanol / dichloromethane) to provide a solid (0.023 g). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.41 (s, 1H), 7.39 (d, J = 2.0 Hz, 2H), 7.35 (d, J = 2.0 Hz, 2H), 6.19 (s, 1H), 3.73 (s, 2H). HRMS (ESI) m/z calculated for $C_{11}H_{10}ClN_2O_2$ [M-H], 235.0269; found 235.1989.

Supplemental Methods References

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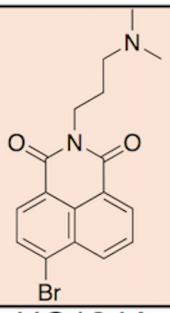
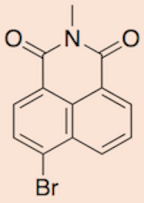
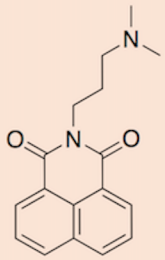
Supplementary Dataset 1. Differential gene expression data of WT Mtb treated with inhibitors

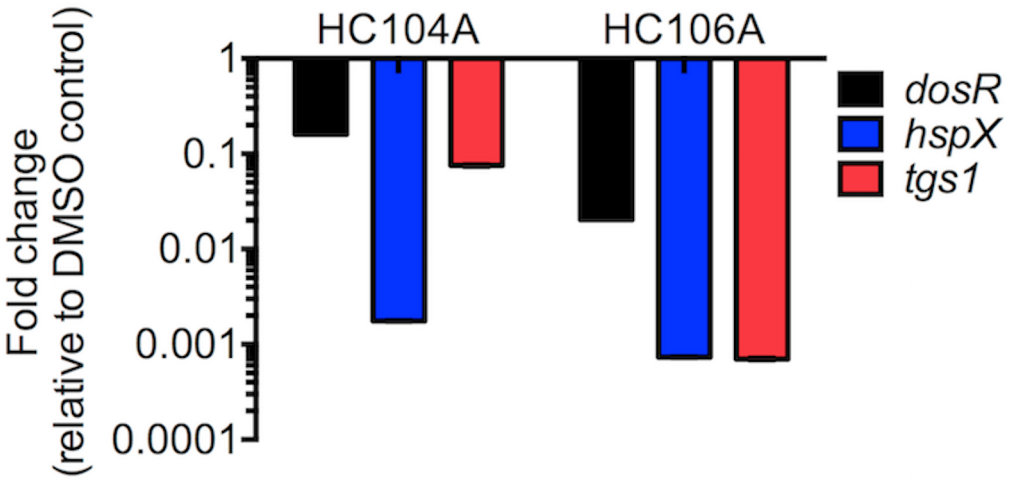
Supplementary Dataset 2. Differential gene expression data of the DosR mutant treated with the inhibitors.

Supplementary Dataset 3. Complete gene expression tables for transcriptional profiling experiments.

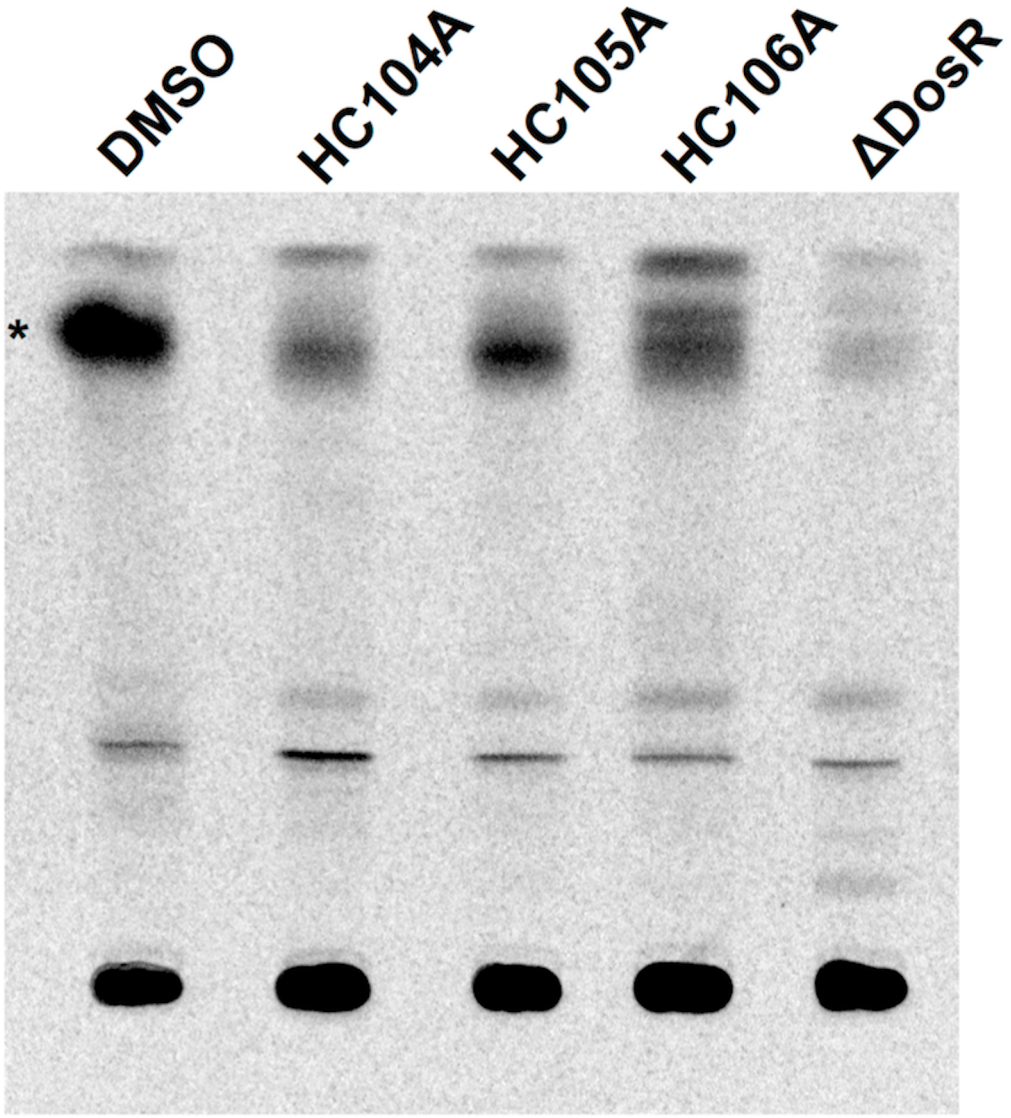
Supplemental Table 1 and Figures 1-9

Compound			
ID#	HC104A	HC104G	HC104B
MW (g/mole)	361.2	290.1	282.3
EC_{50} (μM)	9.8	43.8	>200
Ligand Efficiency	0.34	0.36	0.26
CLogP (cLogD at pH 7.4)	3.0 (0.55)	2.3 (2.4)	2.9 (-0.24)
Druglikeness	4.4	0.55	6.2

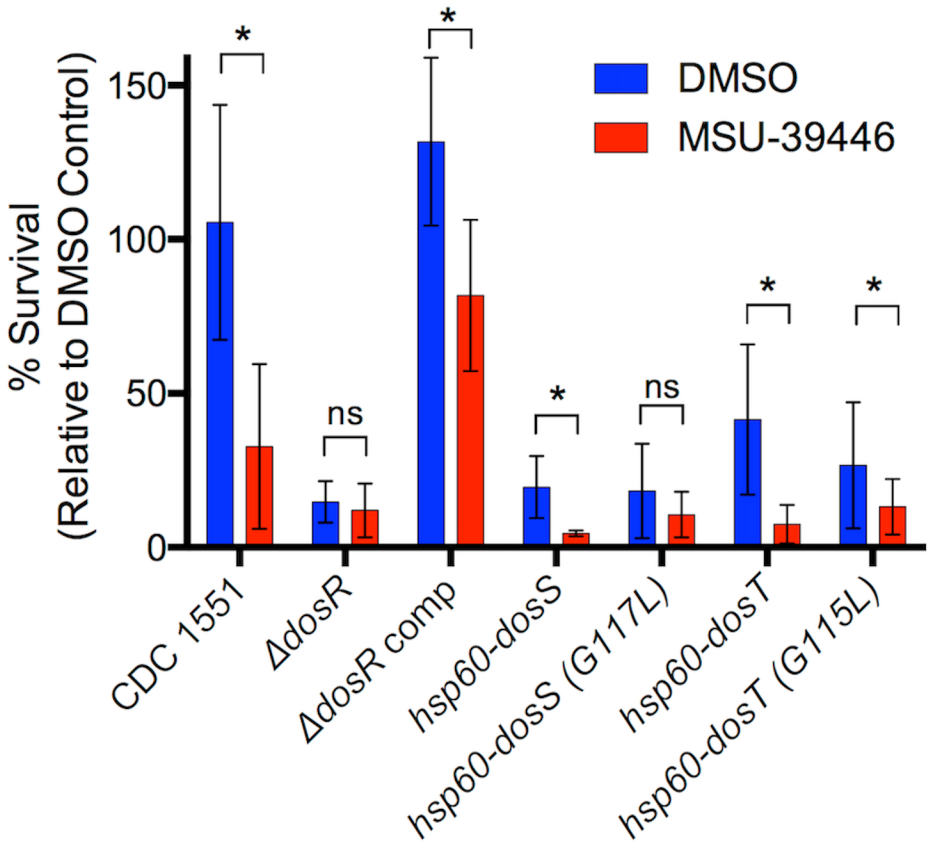
Supplemental Table 1. Structure and properties of HC104 series. Catalog structure activity relationship study performed for HC104 analogs with different R-groups. The reporter strain CDC1551 (hspX'::GFP) was treated across doses of each analog from 200 μM to 0.328 μM. The EC50 values of fluorescence inhibition calculated for each analog to determine their potency. The other chemical properties of the analogs are also included. Compounds with a positive druglikeness score contain fragments that are frequently present in known drugs.



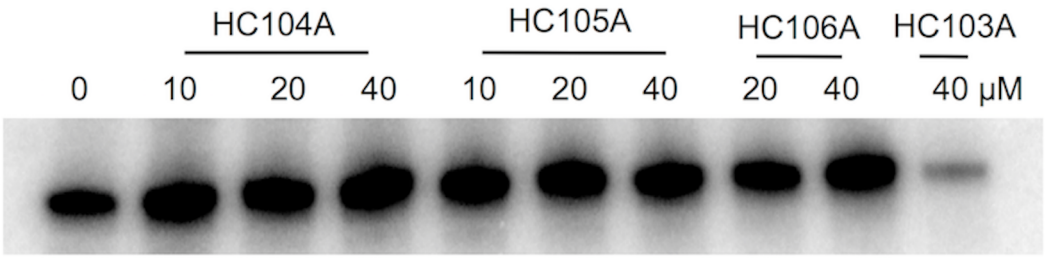
Supplemental Figure 1. Inhibition of the DosR regulon by HC104 and HC106A during hypoxia. Mtb cells were treated with 40 μM compounds for 6 d, and total bacterial RNA was isolated. The DosR regulated genes, dosR, hspX, and tgs1 were quantified in qRT-PCR. The error bars represent the standard derivation of three replicates. The experiment was repeated at least twice with similar results.



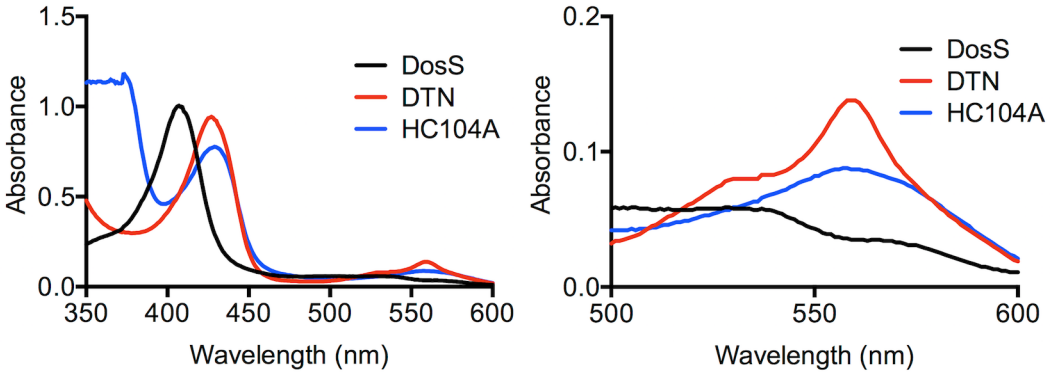
Supplemental Figure 2. TLC of TAG reduction in Mtb treated with HC104A and HC106A. Mtb cells were treated with 40 μM of the compounds and labeled with [1,2-14C] sodium acetate in T25 vented tissue culture flasks for 6 d. Total lipid was isolated and analyzed BY TLC. The experiment was repeated twice with similar results.



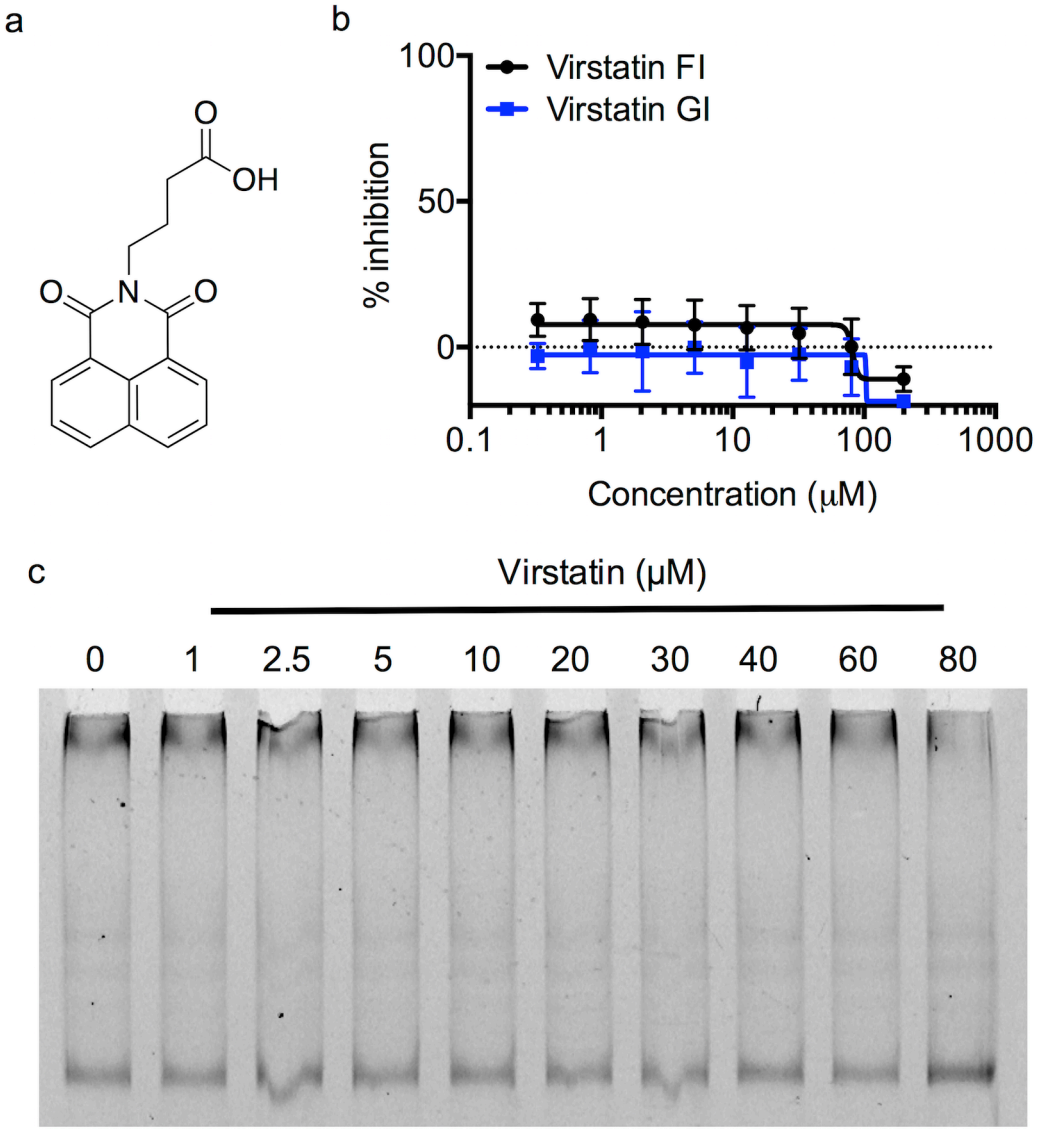
Supplemental Figure 3. Inhibition of Mtb survival by HC106 analog MSU-39446 during NRP in the hypoxic shift-down model. The error bars represent the standard deviation of six biological replicates, combined from two independently conducted experiments. * P value <0.01 based on a t-test.



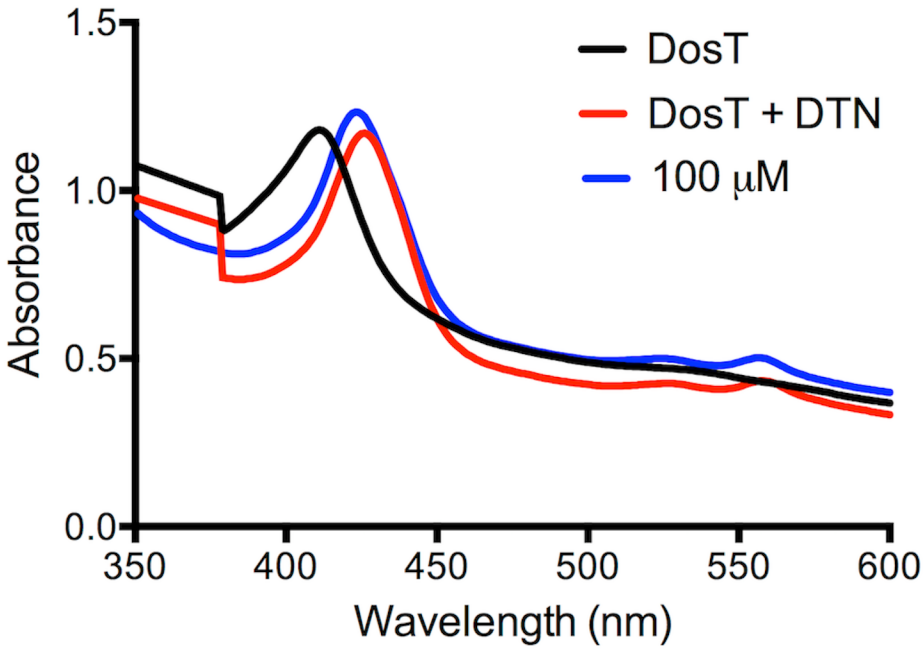
Supplemental Figure 4. Autoradiograph examining the impact of HC104A and HC106A on DosS autophosphorylation. DosS protein was treated with 10 μM, 20 μM or 40 μM of the compounds, with DMSO and HC103A as positive and negative controls, respectively. The results show that HC104A and HC106A have no effect on DosS autophosphorylation.



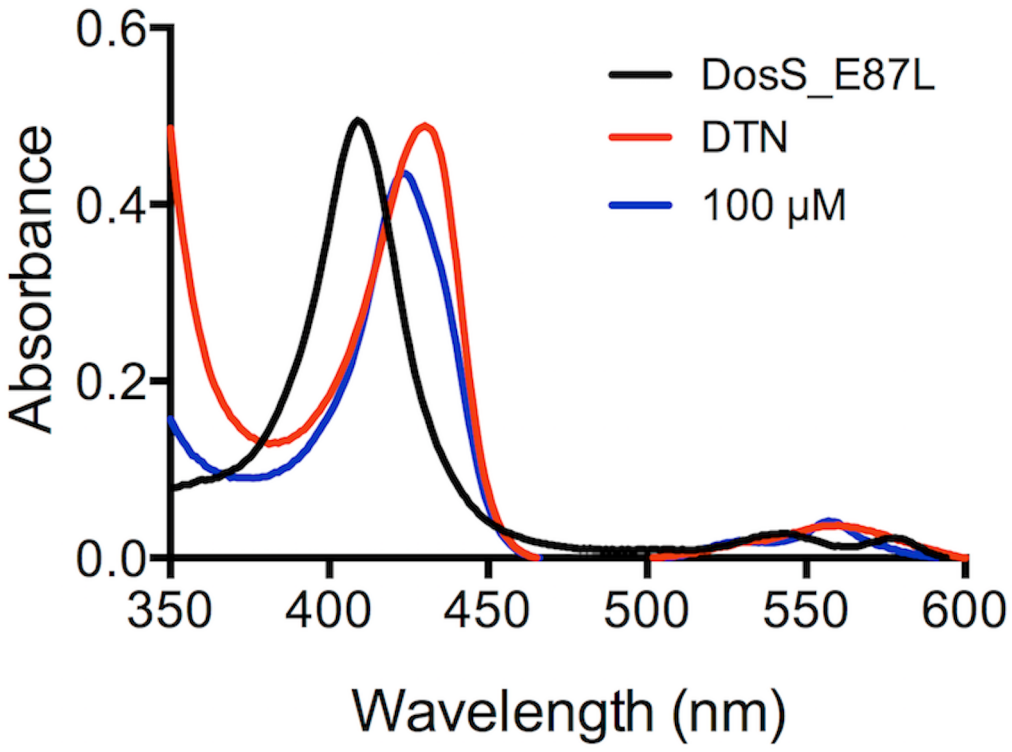
Supplemental Figure 5. Investigation of interaction between HC104A and DosS. WT DosS treated with 400 μM HC104A shows no impact on shifting of the Soret peak in the UV-visible spectroscopy assay. The experiment was repeated at least twice with similar results.



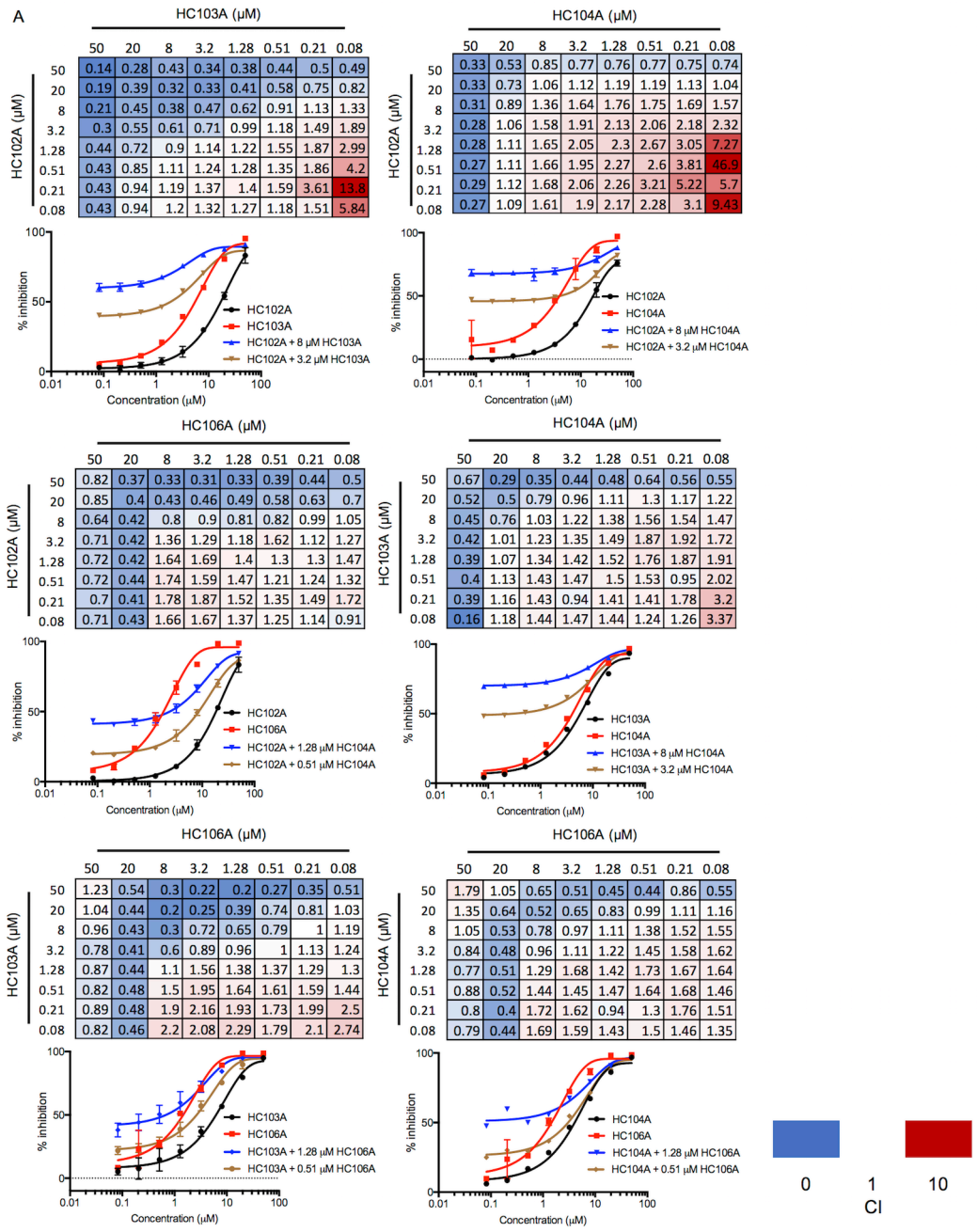
Supplemental Figure 6. The impact of virstatin on DosR DNA-binding and DosRST signaling in Mtb. (a) Chemical structure of virstatin. (b) Dose-response curve of virstatin shows no effect on inhibition of Mtb DosR-driven GFP fluorescence. (c) DosR protein at 2 μM was treated with 9 point dose response of virstatin from 1 μM to 80 μM. The reactions were analyzed on native PAGE gel. The experiment was repeated at least twice with similar results.



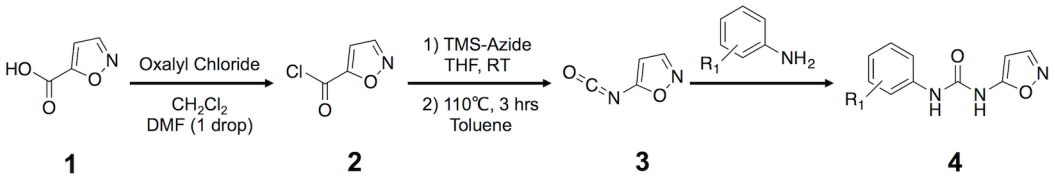
Supplemental Figure 7. Investigating the interaction between HC106A and DosT heme. DosT was treated with 100 μM HC106A after being reduced with DTN. The UV-visible spectra were recorded after each treatment, and a shift to an intermediate position was observed. The experiment was repeated twice with similar results.



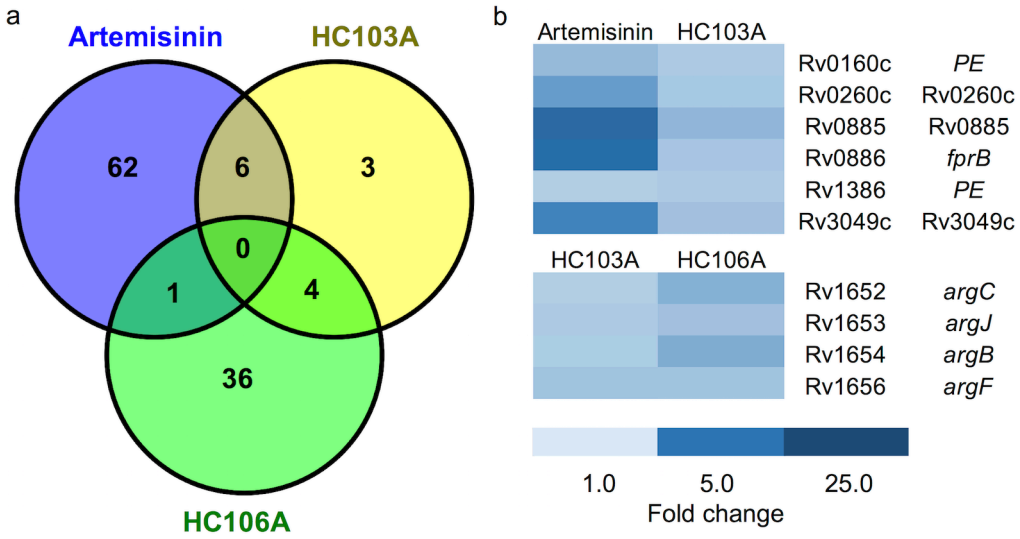
Supplemental Figure 8. Investigating the interaction between HC106A and DosS heme. DosS E87L protein was treated with 100 μM HC106A after being reduced with DTN. The UV-visible spectra were recorded after each treatment, and showed no change on the overall spectrum compared to WT protein. The experiment was repeated at least twice with similar results.



Supplemental Figure 9. Checkerboard assays examining paired interactions of DosRST inhibitors. CDC1551 (hspX'::GFP) was treated with different combination of two compounds from 50 μM to 0.08 μM. GFP fluorescence was measured and used to calculate percentage inhibition. The data were analyzed in the CompuSyn software to determine the combination index (CI) for the panel of each drug combination, including (a) artemisinin and HC104A; (b) HC102A and HC103A; (c) HC102A and HC104A; (d) HC102A and HC106A; (e) HC103A and HC104A; (f) HC103A and HC106A; (g) HC104A and HC106A. Selected dose response curves are presented to illustrate synergistic interactions. The experiment was repeated twice with similar results.



Supplemental Figure 10. Synthetic scheme for the HC106 analogs.



Supplemental Figure 11. Comparison between artemisinin, HC103 and HC106 for interactions. (a) Venn diagram for the downregulated genes (>2-fold; q< 0.05) of CDC1551 (ΔdosR) treated with artemisinin, HC103A, or HC106A. (b) Overlap exists between artemisinin and HC103A or HC103A and HC106A differentially expressed genes (downregulated >2X, q<0.05). The heatmaps represent the commonly downregulated genes between the two compounds.