EXPERIMENTAL SECTION

General Procedures. All common solvents and chemicals were used as purchased without further purification. The progress of all reactions was monitored on Aldrich precoated silica gel plates (with fluorescence indicator UV254) using ethyl acetate/n-hexane or methanol/dichloromethane as solvent system, and by a Waters UPLC-MS with model number C10UPB090A. Column chromatography was performed with Aldrich silica gel 60 (230−400 mesh ASTM) with the solvent mixtures specified in the corresponding experiment. Purity of all final compounds was 95% or higher. Spots were visualized by irradiation with ultraviolet light (254 nm). Proton (1H) NMR spectra were recorded on Bruker Avance 500 or 300 MHz using solvents as indicated in the experimental section. Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for ^{1}H).

5-(Thiazol-2-yl)picolinonitrile (3) A mixture of 5-bromopicolinonitrile (3.6 g, 20.0 mmol), 2-(tributylstannyl)thiazole (10.0 g, 30.0 mmol) and $Pd(PPh_3)_2Cl_2$ (0.82 g, 1.0 mmol) in 1,4-dioxane (150 mL) was heated to 110 ^{o}C for 5 hours under N_2 atmosphere. The mixture was concentrated, and purified by silica gel column chromatography eluting with 15% ethyl acetate in petroleum ether to give **3** as a white solid (3.0 g, 82%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.32 (d, J = 2.0 Hz, 1H), 8.56 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 8.18 (d, J = 3.0 Hz, 1H), 8.12 (d, J = 3.0 Hz, 1H), 8.05(d, J = 3.0 Hz, 1H). MS (EI^{+}, *m/z*): 188 $[M+H]^{+}$.

Methyl 5-(thiazol-2-yl)picolinate (4) A mixture of 5-(thiazol-2-yl)picolinonitrile **3** (5.6 g, 0.03 mol) in HCl in 1,4-dioxane (4.0 *M*, 5.0 mL) and methanol (10 mL) was heated at 75 ^{o}C overnight. The mixture was concentrated, and diluted with ethyl acetate (200 mL). The solution

was then washed with saturated $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4, filtered, and concentrated to give **4** as a white solid (4.14 g, 87%). The material was used directly in the next step without further purification. MS (EI^+, *m/z*): 221 $[M+H]^+$.

Methyl 5-(5-bromothiazol-2-yl)picolinate (5) A mixture of methyl 5-(thiazol-2-yl)picolinate **4** (4.4 g, 0.02 mol) and NBS (9.0 g, 0.05 mol) in acetonitrile (120 mL) was heated at 90 ^oC overnight. The mixture was cooled to room temperature, diluted with water (200 mL), and extracted with ethyl acetate (150 mL x 3). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give **5** as a pale yellow solid (4.66 g, 78%). ^1H NMR (500 MHz, DMSO-d_6): δ 9.22 (d, J = 2.0 Hz, 1H), 8.46 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 8.17 (m, 2H), 3.92 (s, 3H). MS (EI^+, *m/z*): 300 $[M+H]^+$.

7-Propyl-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole (7) A mixture of 7-bromo-1H-indazole **6** (1.97 g, 0.01 mol) and TFA (0.2 mL) was dissolved in 3,4-dihydro-2H-pyran (50 mL) and heated at 90 ^oC for 4 hours, and cooled to room temperature. Then Et_3N (0.5 mL) was added. The mixture was concentrated, and purified by silica gel column chromatography eluting with 3% ethyl acetate in petroleum ether to give 7-bromo-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole as a pale yellow oil (2.56 g, 91%). The material was used directly in the next step without further purification. MS (EI^+, *m/z*): 281 $[M+H]^+$.

A mixture of 7-bromo-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole (2.80 g, 0.01 mol), *n*-propylboronic acid (1.70 g, 0.02 mol), $Pd(dppf)Cl_2$ (0.82 g, 0.1 mmol), a solution of Na_2CO_3 (2.12 g, 0.02 mol) in water (20 mL) and 1,4-dioxane (60 mL) was heated at 110 ^oC overnight. The mixture was concentrated, and purified by silica gel column chromatography eluting with

2% ethyl acetate in petroleum ether to give **7** as yellow oil (1.42 g, 58%). MS (EI^+, *m/z*): 245 $[M+H]^+$.

3-Iodo-7-propyl-1H-indazole (9) A mixture of 7-propyl-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole (1.20 g, 5.0 mmol) in TFA (5.0 mL) and dichloromethane (20 mL) was stirred at room temperature for 3 hours, and then diluted with dichloromethane (50 mL). The organic phase was washed sequentially with saturated aqueous Na_2CO_3 solution (80 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4 and concentrated to give 7-Propyl-1H-indazole **8** as a pale yellow oil (0.68 g, 87%), which was taken to the next step without further purification. MS (EI^+, *m/z*): 161 $[M+H]^+$.

A mixture of 7-propyl-1H-indazole **8** (10.7 g, 0.066 mol), I_2 (25.0 g, 0.099 mol) and KOH (18.2 g, 0.132 mol) in DMF (100 mL) was stirred at room temperature for 1 hour. After quenching with water (400 mL), the solution was extracted with ethyl acetate (300 mL x 2). The combined organic phase was washed sequentially with saturated aqueous $Na_2S_2O_3$ solution (200 mL) and brine (300 mL). The solution was then dried over Na_2SO_4 and filtered. The filtrate was concentrated. The residue was purified via silica gel chromatography eluting with 5% ethyl acetate in petroleum ether to give **9** as a brown solid (14.3 g, 75%). ^1H NMR (500 MHz, DMSO-d_6): δ 13.58(s, 1H), 7.21-7.26(m, 2H), 7.13 (d, *J*=7.5 Hz, 1H), 2.85(t, 2H, *J*=7.5 Hz), 1.65-1.68(m, 2H), 0.93(t, 3H, *J*=7.5 Hz). MS (EI^+, *m/z*): 287 $[M+H]^+$.

7-Propyl-1-(tetrahydro-2H-pyran-2-yl)-3-(trimethylstannyl)-1H-indazole (11) To a solution of 3-iodo-7-propyl-1H-indazole **9** (18.3 g, 0.064 mol) in 3,4-dihydro-2H-pyran (200 mL) was

added TFA (0.05 mL). After heating at 90 ^{o}C for 3 hours, the mixture was cooled to room temperature. Triethylamine (5.0 mL) was added to the solution. The resulting mixture was concentrated and purified by silica gel chromatography eluting with 2% ethyl acetate in petroleum ether to give 3-Iodo-7-propyl-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole **10** as a white solid (15.4 g, 65%). MS (EI^+, *m/z*): 287 $[M\text{-}THP+H]^+$.

A mixture of 3-iodo-7-propyl-1-(tetrahydro-2H-pyran-2-yl)-1H-indazole **10** (3.70 g, 0.01 mol), hexamethyldistannane (3.90 g, 0.012 mol) and $Pd(PPh_3)_2Cl_2$ (702 mg, 1.0 mmol) in 1,4-dioxane (70 mL) was heated at 100^{o}C overnight. After cooling down to room temperature, the mixture was concentrated, diluted with water (50 mL), and extracted with ethyl acetate (50 mL x 2). The combined organic phase was washed with birne (50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated to give crude **11** as a pale yellow solid (4.2 g, 100%), which was used immediately. MS (EI^+, *m/z*): 409 $[M+H]^+$.

4-(5-(7-Propyl-1H-indazol-3-yl)thiazol-2-yl)picolinic acid (2) A mixture of methyl 5-(5-bromothiazol-2-yl)picolinate **5** (3.0 g, 0.01 mol), $P(furyl)_3$ (0.232 g, 1.0 mmol), 7-propyl-1-(tetrahydro-2H-pyran-2-yl)-3-(trimethylstannyl)-1H-indazole **11** (5.4 g, 0.013 mol) and $Pd(PPh_3)_2Cl_2$ (702 mg, 1.0 mmol) in 1,4-dioxane (100 mL) was heated at 100 ^{o}C overnight. The mixture was concentrated and redissolved in aqueous HCl aq (6.0 M, 100 mL). The mixture was heated at 100 ^{o}C for 5 hours and concentrated. The residue was purified by prep-HPLC to give **2** as off white solid (2.77 g, 76%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.63(s, 1H), 9.15(s, 1H), 8.65(s, 1H), 8.46(dd, *J*=8.0 Hz, *J*=2.0 Hz, 1H), 8.11(d, *J*=8.5 Hz, 1H), 8.03(d, *J*=8.0 Hz, 1H), 7.20-7.26(m, 2H), 2.91(t, *J*=8.0 Hz, 2H), 1.70-1.75(m, 2H), 0.96(t, *J*=7.5 Hz, 3H). MS (EI^+, *m/z*): 364 $[M+H]^+$.

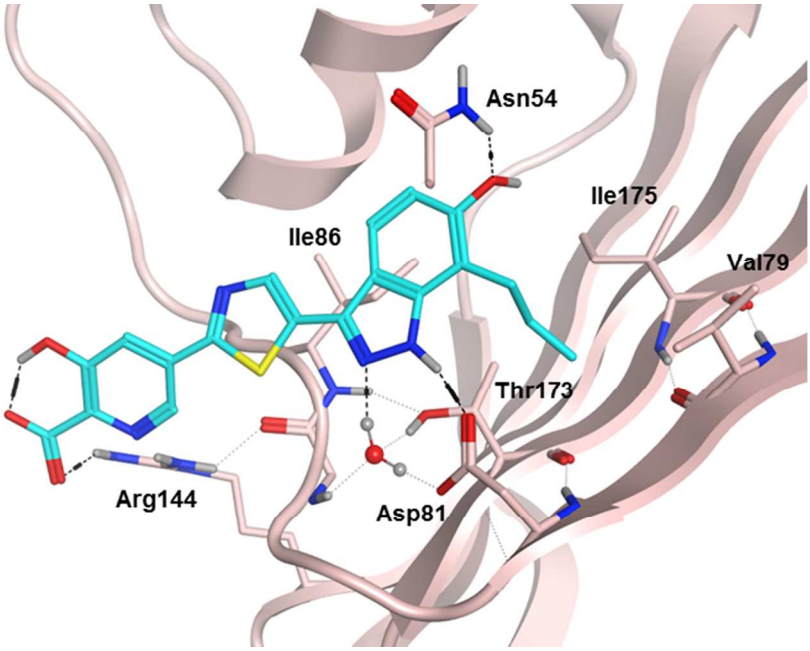
Bacterial GyrB and TopoIV IC50 Determination. Proteins were obtained from Inspiralis Ltd. (Norwich, United Kingdom). *Sa* gyrase was used at final concentration of 7.5 nM in a solution of 40 mM HEPES-KOH pH 7.6, 10 mM magnesium acetate, 10 mM dithreitol, 50 g/L BSA, 500 mM potassium glutamate, 1% DMSO, 100 mM ATP, and 10 nM linear pBR322 DNA. *Sa* topo IV was used at final concentration of 8.5 nM in a solution of 100 mM Tris pH 7.5, 2 mM magnesium chloride, 1 mM dithreitol, 50 g/L BSA, 200 mM potassium glutamate, 1% DMSO, 300 mM ATP, and 10 nM linear pBR322 DNA. Reactions were carried out in a volume of 10 microliters per well. Reactions were initiated with the addition of ATP and incubated at 20°C for 30 minutes. To quantify ADP concentration, reactions were stopped by addition of 10 microliters of Transcreener ADP2 FP assay reagent and fluorescence polarization measurements were made according to the manufacturer's protocol (Bellbrook Labs, Madison, WI).

MIC Determination. Antibacterial activity of all the compounds was demonstrated by the minimum inhibitory concentrations (MIC) of the compounds against various bacteria measured by the broth microdilution assay performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines with modifications described below. [3] Individual colonies were isolated by streaking frozen glycerol stock of the bacterial species being tested onto rich, non-selective, tryptic soy agar containing 5% sheep's blood (TSAB), and incubated at 37°C for 18-24 hrs. *Streptococcus pneumoniae* strain was streaked on TSAB plates and incubated at 37°C with 5% CO_2 for 18-24 hrs. On the day of the assay, primary cultures were started by inoculating 5-10 colonies from the TSAB plates into ~5 mL of Mueller Hinton Broth (MHB) in 14 mL culture tubes and incubated at 37°C with aeration (200 rpm) for ~2 hrs until the OD600 was ≥0.1. Inoculum cultures were prepared by standardizing the primary cultures in MHB so that the final inoculum density was ~10^5 colony forming units per milliliter. 50 µL of the diluted

inoculum cultures was added to 96 well broth microdilution assay plates along with 50 μL of MHB containing compound (concentrations ranging from 32 – 0.03 μg/mL in two-fold dilutions) for a final volume 100 μL per well with a final culture OD600 of approximately 0.001. For *S. pneumoniae*, 5-10 colonies from TSAB plates were resuspended into MHB to an OD600 of ≥0.1. This material was used to prepare inoculum culture as mentioned above. The final DMSO concentration in the assay plates was 2%. Plates were incubated for 18-20 hours at 37°C with aeration (200 rpm). Assay plates containing *S. pneumoniae* were incubated at 37°C with 5% CO_2 for 18-24 hrs. Following incubation, growth was defined as turbidity that could be detected with the naked eye or achieving minimum OD600 of 0.1. MIC values were defined as the lowest concentration producing no visible turbidity.

X-ray Crystal Structure Determination. Loop-deleted 24 kDa construct of *S. aureus* GyrB was used for X-ray crystallography. Protein crystals were grown using the hanging drop method at pH 7.6, followed by soaking of fragments into the crystallization buffer. Soaking times varied from 16 hours to 33 days. Data were collected using either a Rigaku RA-Micro7 HF rotating anode or at the Diamond Light Source. Structures were solved by molecular replacement using PDB entry 1KZN as a search model. Refinement and model building were completed using REFMAC5 and Coot, respectively.

An alternative view of 28/GyrB ATPase co-crystal structure.



Mouse septicemia model: Bacterial Strain: *Staphylococcus aureus* 1118. Study design: Mice received a lethal, intraperitoneal inoculum of a bacterial-mucin suspension and were then dosed with either vehicle (as a negative control), test substance or comparator drugs, including moxifloxacin or novobiocin subcutaneously (SC) or intravenously (IV) twice for one day post-infection. Animal survival was monitored for a period of seven days. Test substance and comparator drug potencies were enumerated by calculating ED_{50} values, i.e., the dose calculated to protect 50% of the lethally infected test mice. Preparation of Bacterial Strain: Bacteria were propagated at 37 °C. *S. aureus* 1118 was grown Mueller Hinton Broth (MHB). MIC testing was performed according to NCCLS guidelines for broth microdilution. Experimental Animal Preparation and Inoculation: A diluted exponential-phase growing overnight culture of *S. aureus* 1118 corresponding to $5x10^7$ CFU in 0.5 ml of sterile saline with 6% hog gastric mucin, was injected intraperitoneally into conscious CD-1 mice. The actual concentration of bacteria was confirmed by determination of viable counts by dilution plating infection inoculum on colisitin naladixic acid agar (CNA) plates with 5% sheep blood. The number of CFU of bacteria was

determined after an incubation of 16 h at 37°C. Administration of Test Substance: Groups of five mice were used for each dose of the test compounds and the vehicle. Each group received subcutaneous (SC) or intravenous (IV) injections of test compounds or vehicle beginning at 1 hour post-infection, and then at 4 hours post-infection for a total of 2 doses. In each trial, there were three or four dose groups per test compound. Sample Collection: No samples were collected. Animal survival was monitored for seven days prior to euthanasia by CO_2. Pharmacological Analysis: Antibiotic efficacy was enumerated by calculating the dosage required to protect 50% of the lethally infected mice (effective dose 50%, ED_{50}). Statistical Analysis: Probit analysis was used to determine ED_{50} values.

Mouse lung infection model: Bacterial Strain: *Streptococcus pneumoniae* 402. Study design: Infection was induced in immunocompetent mice by intranasal inoculation of *Streptococcus pneumoniae* 402. Test substance or comparator drugs, including moxifloxacin or novobiocin were administered subcutaneously (SC) at 1 and 4 hours (BIDx1 day) or at 1, 4, 22 and 26 hours (BIDx2 days) post-infection. At 24 or 48 hours post-infection, mice were humanely euthanized and the lungs of the mice were removed, homogenized, serially diluted, and plated on agar, to quantitate the bacterial burden. The test substance, moxifloxacin and novobiocin were evaluated for their ability to decrease the bacterial burden from the lungs compared with vehicle-treated control mice. Preparation of Bacterial Strain: Bacteria were propagated at 35°C with 5% CO2. *S. pneumoniae* 402 was grown overnight in Todd Hewitt Broth (THB) with 5% horse serum. Following morning, overnight culture was subcultured in THB with 5% horse serum for approximately 3 hours at 35°C with 5% CO2. MIC testing was performed according to NCCLS guidelines for broth microdilution. Preparation of Test Substances: The test substance was dissolved in 5% DMA, 3% Solutol, 92% 100mM Sodium Phosphate Buffer at pH 8.9.

Moxifloxacin was dissolved in sterile water for injection. To dissolve novobiocin, 5% dextrose was used and most concentrated dosing solution was sonicated for approximately 25 minutes until solubilization was complete. A series of dilutions of test compounds were prepared in vehicle used for each compound. Experimental Animal Preparation and Inoculation: A diluted, exponential-phase growing culture of *S. pneumoniae* 402 corresponding to $5x10^6$ CFU in 0.1 ml of sterile saline was inoculated by the intranasal route in CD-1 mice. Just prior to inoculation, mice were anaesthetized briefly with isoflurane. The actual concentration of bacteria was confirmed by determination of viable counts by dilution plating infection inoculum on colisitin naladixic acid agar (CNA) plates with 5% sheep blood. The number of CFU of bacteria was determined after an incubation of 16 h at 37°C. Administration of Test Substances: Groups of five mice were used for each dose of the test compounds and the vehicle control group. Each group received SC injections of test compounds or vehicle at 1 and 4 hours (BIDx1 day) or at 1, 4, 22 and 26 hours (BIDx2 days) post-infection for a total of 2 or 4 doses. In each trial, there were two to three dose groups per test compound. Sample Collection: Twenty hours after the last injection, the mice were euthanized by asphyxiation with CO_2. The lungs were removed aseptically, homogenized in 4 ml of sterile, distilled water and dilution plated on CNA plates with 5% sheep blood to quantify bacterial CFU. The number of CFU of bacteria in lungs was determined after an incubation of 16 h at 37°C (the CFU per milliliter of homogenate). Pharmacological Analysis: The results were expressed as the geometric mean $\log_{10}$ CFU/mL ± the standard deviation. The limit of detection was 10 CFU per mL of lung homogenate. Lungs were considered sterile when no CFU were detected on the agar. The efficacy of the test compounds was assessed by comparing the number of $\log_{10}$ CFU/mL measured in the infected and treated mice with control animals injected with vehicle. The efficacy of test substance was

also compared with the positive comparator antibiotics moxifloxacin and novobiocin. Statistical Analysis: Dose response curves of test compounds were generated for each isolate. Regression lines were generated in Microsoft Excel and used to calculate the dose expected to produce a 3 log_{10} reduction in bacterial count as compared to vehicle-treated group ($ED_{-3log10}$ [mg/kg SC, twice daily (BID)]).

Pharmacokinetics: mice (n of 3) were dosed intravenously with the test article. Plasma samples were collected at time points chosen to provide sufficient coverage of the absorption, distribution, metabolism and excretion phases of the test compound. LC-MS detected bioanalytical analysis was performed on the plasma samples utilizing a standard curve and quality control samples to provide enough precision and accuracy to determine the concentrations of test article for plasma from each time point. Non-compartmental pharmacokinetic analysis of the intravenous bioanalytical data provided area under the curve, clearance, volume of distribution, terminal half-life parameters.