**Supporting Information for:**

# N-Benzyl-3-sulfonamidopyrrolidines are a New Class of Bacterial DNA Gyrase Inhibitors

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**Methods**

**Species and growth conditions for MICs.** We grew bacteria using the conditions in parenthesis to determine minimum inhibitory concentrations (MICs): *E. coli* strain BW25113 wt or •tolC Keio collection strain [1] (Luria-Bertani [LB] media, 30 °C, 200 rpm shaking, 14 h), *Pseudomonas aeruginosa* strain K (LB, 37 °C, 200 rpm shaking, 16 h), *Salmonella enterica* ATCC 19585 (brain heart infusion [BHI] media, 37 °C, static conditions, 14 h), *Staphylococcus aureus* strain FRI 100 (LB, 37 °C, static conditions, 14 h), Streptococcus pneumoniae ATCC 10813 (BHI, 37 °C, static conditions, 24 h), and *Enterococcus faecalis* strain 1131 (BHI, 37 °C, static conditions, 14 h). We diluted cultures to $5\times10^5$ cells/mL in growth media for the starting inoculum. We dissolved **1-3** in DMSO to a concentration of 100 mM and ciprofloxacin in 1:1 MeOH:0.1 M HCl to a concentration of 10 mM and diluted these stocks for assays.

**Micro- and macro-dilution protocols**. Micro- and macro-dilution protocols were performed according to the NCCLS guidelines [2]. To make a two-fold dilution series for the determination of the MIC using a macrodilution technique, we added compounds to the first culture tube to give the highest concentration and subsequently diluted it in a set of tubes containing inoculated media. The final volume for each culture was 2 mL. We prepared solvent controls and sterility controls using the same concentration of solvent as the tubes with the highest concentration of antibiotic. We

determined the macrodilution MIC endpoints in triplicate by identifying the lowest concentration of compound that completely inhibited growth by visual inspection. We performed microdilution MIC determinations using a format identical to that described for the macrodilution experiments, with the exception that the cultures were prepared in 96-well plates containing a final volume of 100 μL. We measured the absorbance of cultures (λ, 600 nm) to assist in determining the microdilution endpoints. The MIC was chosen as the lowest antibiotic concentration at which we were unable to measure a detectable absorbance. The susceptibility to antibiotics determined by either the macro- or microdilution protocols produced the same MIC values.

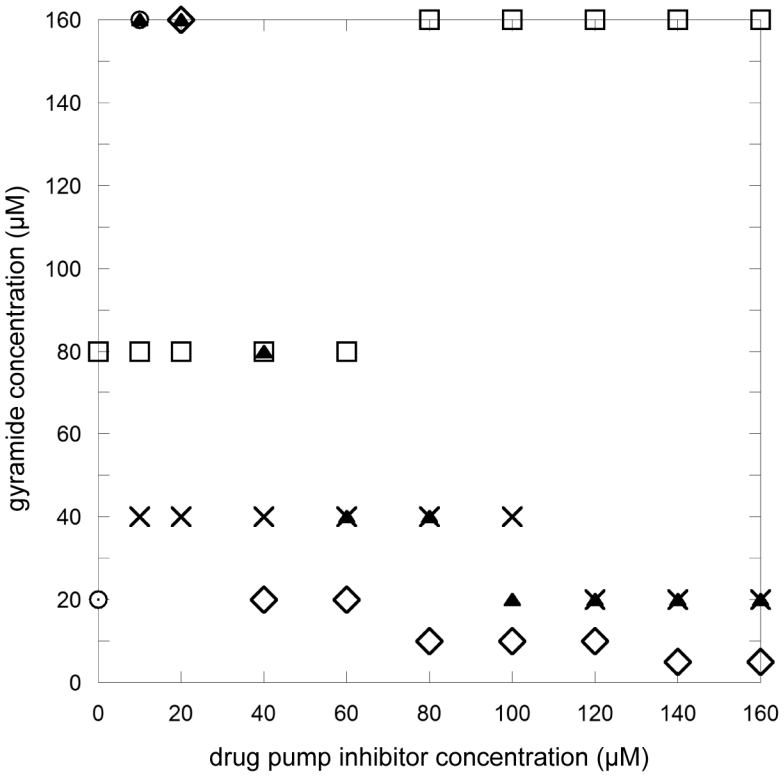


**Figure S1.** Effect of drug pump inhibitors on sensitivity to the gyramides. We treated *S. aureus* and *E. coli* with **1-3** in the presence of the drug pump inhibitors reserpine and MC-207,110, respectively. Represented in the figure: *S. aureus* with **2** and reserpine (□), *S. aureus* with **3** and reserpine (○), *E. coli* with **1** and MC-207,110 (▲), *E. coli* with **2** and MC-207,110 (×), and *E. coli* with **3** and MC-207,110 (◊). The addition of reserpine did not potentiate the activity of gyramides over the DMSO controls; at many concentrations reserpine was antagonistic towards gyramide efficacy. MC-207,110 improved the activity of gyramides against *E. coli*, which is consistent with inhibition of the AcrAB-TolC drug pump.

**Resistant mutant screening and isolation.** We spread freshly saturated cultures of *E. coli* BW25113 •*tolC* on 1.5% LB agar infused with 50 μM of **1**. We plated cells from 40 parallel cultures (0.5 mL, each) that were started from an initial cell density of $10^4$ cells/mL. Plates were incubated at 37 °C in a static incubator for 48 h before counting resistant colonies. We determined the population size plated by enumerating colony forming units in triplicate from cultures grown in parallel with those plated on selective media. These cultures were diluted with 0.1% phosphate buffered peptone water (e.g. 0.1% peptone in phosphate buffer) in a 10-fold dilution series and spread on non-selective 1.5% agar LB plates. We determined population density after 12-16 h by counting plates with dilutions providing between 20-200 colonies. The resistant mutation rate was estimated using the Ma-Sandri-Sarkar Maximum Likelihood

Estimator (MSS-MLE) method through the web tool developed by Hall and coworkers [3].

**Isolation criteria.** We isolated one resistant colony from each plate harboring resistant mutants and grew them in non-selective and selective media. This protocol made it possible to establish that resistance to **1** was a heritable trait and not an adaptive response. We added 20% glycerol to cultures of isolates and stored them at -80 °C.

**Genomic and Sanger sequencing**

**Genomic sequencing equipment and analysis.** We performed full genome sequencing of *E. coli* BW25113 and two isolated mutants that were resistant to **1** using an Illumina GA IIx sequencer and read lengths of 75 base pairs at the Genomic Resources Lab at the University of Wisconsin Biotechnology Center. Short reads were aligned to the annotated reference genome for *E. coli* MG1655. We analyzed short nucleotide polymorphisms and deletions between the wildtype BW25113 and the resistant isolates to identify the single nucleotide changes that conferred resistance to **1**.

**Sanger sequencing of *gyrA* and *gyrB*.** To identify the mutation in *gyrA or gyrB* for the remaining 33 mutants that we isolated, we purified genomic DNA from each isolate using the Epicentre MasterPure™ Complete DNA Purification kit. We performed PCR amplification and sequencing of the genes and submitted samples to the DNA sequencing facility in the University of Wisconsin Biotechnology Center. We analyzed sequencing data using SeqMan Pro (DNASTAR Lasergene suite 8.1.2) to identify

nucleotide changes to *gyrA or gyrB*. A complete list of all mutations found to confer resistance to **1** is given in Table S1.

**Table S1.** A table of mutations identified in GyrA and GyrB in mutants of *E. coli* BW25113 •*tolC* that were resistant to **1**. We isolated 35 independent mutants resistant to gyramide A; all isolates carried one nucleotide mutation located in either the *gyrA* gene or *gyrB* gene that results in an amino acid change. The codon positions are labeled with either an A (*gyrA*) or B (*gyrB*) to signify the gene in which we identified the mutation. We found a total of sixteen different nucleotide mutations that conferred resistance to **1**, which was consistent with the rate at which we observed spontaneous mutants.

| Codon | Wildtype | Mutations |
|---|---|---|
| A34 | Leu | Gln |
| A35 | Pro | Thr |
| A45 | His | Gln, Tyr |
| A96 | Phe | Leu |
| A97 | Ser | Leu, Pro |
| A98 | Leu | Gln, Pro |
| A169 | Asn | Lys |
| A170 | Gly | Cys |
| A172 | Ser | Pro |
| A173 | Gly | Cys |
| A267 | Gln | Lys |
| A335 | Val | Ala |
| B508 | Thr | Met |

***E. coli* DNA gyrase supercoiling activity assay**

**Materials, assay conditions.** We performed in vitro *E. coli* DNA gyrase supercoiling activity assays by incubating 1 unit of DNA Gyrase (New England Biolabs) with 0.5 μg of relaxed pUC19 plasmid (New England Biolabs) in 30 μL of reaction buffer. The reaction buffer contained 35 mM Tris-HCl (pH 7.5), 24 mM KCl, 4 mM $MgCl_2$, 2 mM dithiothreitol, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/mL BSA, and 6.5% glycerol. All reactions contained a final concentration of 3.3% DMSO with different concentrations of gyramides **1-3**. We incubated reactions at 37 °C for 30 min

and immediately quenched reactions by adding SDS and 6X blue/orange loading dye (Promega) to a final concentration of 0.2% SDS and 1X loading dye.

**Gel image processing.** We separated DNA supercoiling reaction mixtures on a 0.8% agarose gel with 1X TBE at a constant voltage at 65 V for 3 h. We stained gels with ethidium bromide, imaged them using a Computar H6Z0812 camera with FOTO/Analyst® PC Image software, and performed band densitometry with ImageJ to quantitate the intensity of each supercoiled band on the gel for the different concentrations of inhibitor. We performed non-linear curve fitting of this data using the open source library SciPy for the Python programming language in order to determine the concentration of inhibitor required to inhibit 50% of the production of the fully supercoiled state [4].

**Inhibitor-induced DNA damage assay**

**Assay conditions, gel analysis, linearized DNA control.** We performed inhibitor-dependent DNA cleavage in the presence of DNA gyrase under the same conditions as the supercoiling assay, except we incubated 5 units of DNA gyrase with supercoiled pUC19 in the absence of ATP for 1 h. Supercoiled pUC19 was purified from *E. coli* using a Qiagen® plasmid midi kit. We quenched reactions by adding SDS and proteinase K (Epicentre) to a final concentration of 0.2% SDS and 0.1 mg/mL proteinase K and incubated at 37 °C for 30 min. We added 6X blue/orange loading dye to a 1x final concentration and loaded samples on a 0.8% agarose gel in 1x TBE. We observed the

presence of dsDNA breaks induced by the addition of inhibitor by comparison to a linearized DNA control. We made the linearized DNA control by restriction digest of supercoiled pUC19 with BamHI (New England Biolabs) under standard conditions.

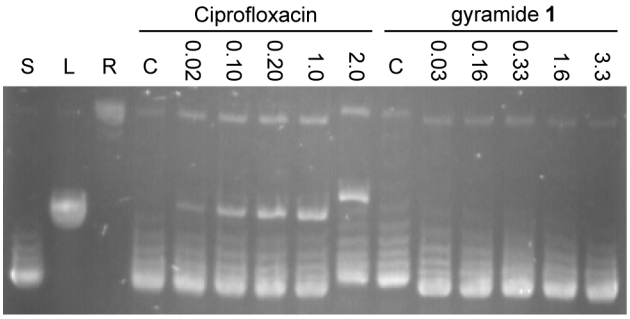



**Figure S2.** Effect of ciprofloxacin and gyramide **1** on stabilization of the dsDNA cleaved DNA gyrase intermediate. Supercoiled ('S'), linear ('L'), and relaxed ('R') pUC19 controls are labeled above the lanes. Concentrations of ciprofloxacin and gyramide **1** are shown (in μM); the label 'C' indicates the lane for the solvent control. The addition of ciprofloxacin stabilized the dsDNA cleaved state of DNA gyrase, which corresponds to the presence of linearized pUC19 in the gel. Gyramide **1** treatment did not generate linearized pUC19 product.

**Affinity reagents synthesis.** We prepared affinity reagents in an effort to pull-down the protein target of **1**. The point of attachment to the resin was guided by the structure-activity relationship information gained in our previous study [5]. Analogs were prepared with *p*-iodosulfonamides, which enabled subsequent coupling to Boc-protected propargyl amine (Figure S3). Compounds (*R*)-**6** and (*S*)-**6** were prepared in direct analogy to previously reported methods, albeit from two different starting

compounds (*R*)-**4** and (*S*)-**5**, respectively, based on availability. Alkylation of Boc-protected sulfonamide **8** using alcohol **7** under Mistunobu conditions provided **9** in high yield. We removed both Boc groups using HCl and introduced the sidechain by reductive amination of *p*-isobutylbenzaldehyde. Sulfonamides (*R*)-**6**, (*S*)-**6**, and **10** were each used in cross-coupling reactions with Boc-protected propargylamine. These intermediates were deprotected and attached to two types of Affygel resin functionalized as NHS esters. Affi-Gel 10 NHS ester resin was transferred as a slurry in isopropanol into a 1.5 mL tube. The slurry was centrifuged and the desired amount was confirmed. Solvent exchange with DMF, centrifuge, remove supernatant, add DMF. Repeat five times. Last exchange use 1% DIPEA in DMF. All exchanges were done at 4 °C. Dissolve up **(*R*)-11a**, **(*S*)-11a**, **12a** in DMF. Agitate resin to make a uniform suspension and add 15-20umol of **(*R*)-11a**, **(*S*)-11a**, **12a** per mL of resin. Agitate in 1.5 mL tube on shaker at 4 °C overnight. Quench by doing solvent exchange with 10% propanolamine in DMF. Repeat three times.

We used these three affinity reagents in a series of pull-down experiments in which resin decorated with (*R*)-**6** was expected to bind to the protein target and the reagents derived from (*S*)-**6** and **10** would serve as negative controls. Unfortunately, we were unable to detect the selective binding of a protein—as judged by Coomassie staining—to warrant isolation and sequencing (Figure S4).

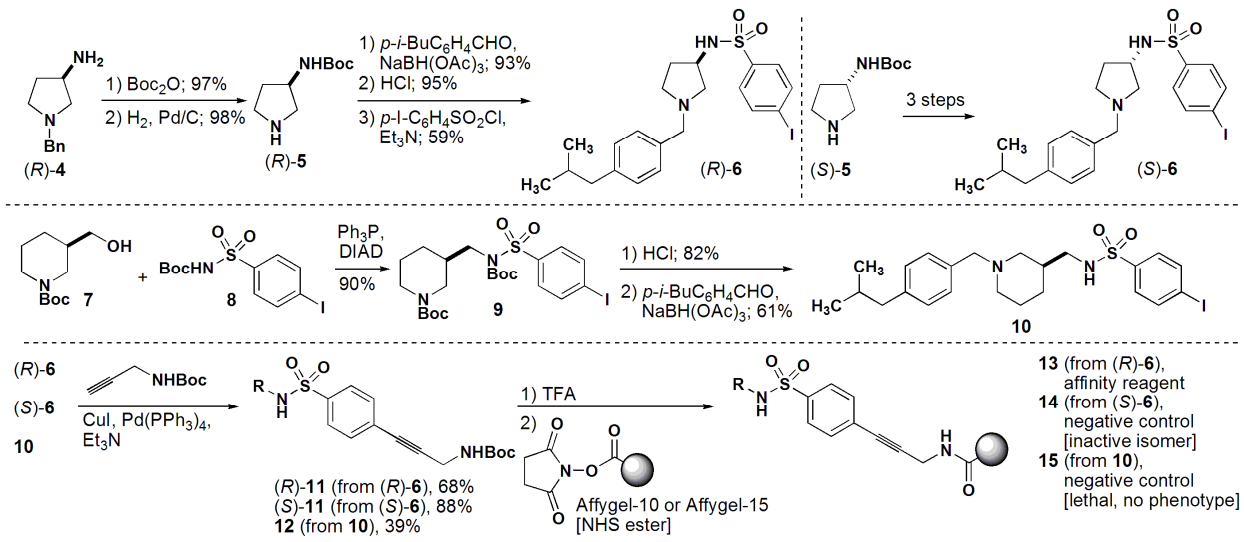
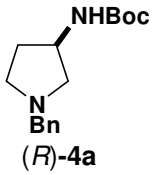
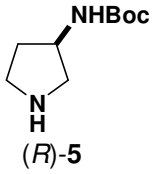



**Figure S3.** Synthetic scheme for **13**, **14**, and **15**.




(*R*)-**4a**

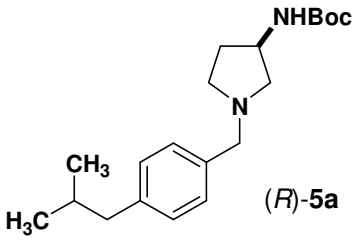
***(R)-tert*-butyl 1-benzylpyrrolidin-3-ylcarbamate ((*R*)-4a).** To a solution of Boc anhydride (2.72 g, 12.48 mmol) in 20 mL of THF was added triethylamine (1.898 mL, 13.62 mmol) and **(*R*)-4** (1.96 mL, 11.35 mmol). The reaction mixture was stirred under argon for 2 h at room temperature. The THF was removed *in vacuo*. The resultant oil was dissolved in 20 mL EtOAc and washed with 15 mL of 1 M NaOH. The organic layer was washed with 3 x 15 mL of $H_2O$. The organic layer was collected, dried with $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography (33:66 to 50:50 EtOAc/hexanes) to afford 3.047 g of product (97%).




(*R*)-**5**

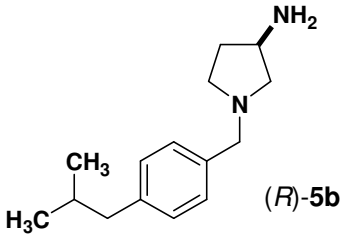
***(R)-tert*-butyl pyrrolidin-3-ylcarbamate ((*R*)-5).** To a solution of **(*R*)-4a** (3.048 g, 11.03 mmol) in 20 mL of ethanol was added 10% Pd/C (0.310 g). The reaction mixture was

stirred under $H_2$ at room temperature overnight. The mixture was filtered through Celite using EtOAc and concentrated *in vacuo* to afford 2.013 g of product (98%).



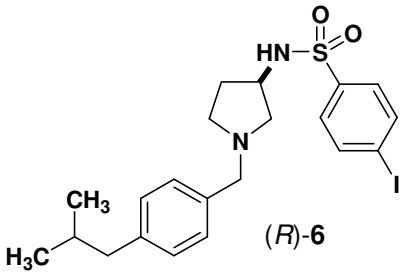
(*R*)-**5a**

***(R)-tert*-butyl 1-(4-isobutylbenzyl)pyrrolidin-3-ylcarbamate ((*R*)-5a).** Compound **5** (0.512 g, 2.75 mmol) was dissolved in 7 mL of DCM followed by the addition of $NaBH(OAc)_3$ (0.874 g, 4.12 mmol) and iso-butyl benzaldehyde (0.491 g, 3.02 mmol). The reaction mixture was stirred under argon at room temperature overnight. The mixture was washed with 10 mL 10% NaOH and the layers were separated. The aqueous layer was extracted with 3 x 15 mL of DCM. The organic layers were combined, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash chromatography (33:66 to 50:50 EtOAc/hexanes) to afford 0.852 g of product (93%).



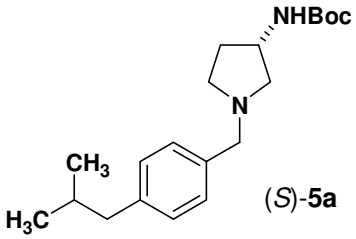
(*R*)-**5b**

***(R)*-1-(4-isobutylbenzyl)pyrrolidin-3-amine ((*R*)-5b).** Compound **(*R*)-5a** (0.611 g, 1.84 mmol) was added to a solution of 4 M HCl in dioxane (6 mL) The reaction mixture was heated to 55 °C and stirred under argon overnight. The reaction mixture was cooled to room temperature and basified with NaOH until the product precipitated out of solution. The mixture was extracted with 3 x 10 mL DCM, dried with $MgSO_4$ and concentrated *in vacuo* to give 0.407 g of product (95%): $^1$H NMR (300 MHz, $CDCl_3$) δ 7.22 (d, *J* = 7.9, 2H), 7.08 (d, *J* = 7.9, 2H), 3.58 (d, *J* = 3.4, 2H), 3.55 – 3.46 (m, 1H), 2.71 (dd, *J* = 8.5, 14.7, 2H), 2.45 (d, *J* = 7.2, 2H), 2.31 (dd, *J* = 4.3, 9.5, 1H), 2.27 – 2.11 (m, 1H), 1.93 – 1.75 (m, 4H), 1.55 – 1.41 (m, 1H), 0.89 (d, *J* = 6.7, 6H); $^{13}$C NMR (300 MHz, $CDCl_3$) δ 140.2, 136.0, 129.3, 129.0, 64.1, 60.5, 53.5, 51.2, 45.4, 35.3, 30.6, 22.7; IR (thin film) 3274, 2953, 2921, 2867 $cm^{-1}$; $[\alpha]_D$ = -6.60 (c 0.89, MeOH) (t = 20.9 °C); HRMS *m/z* $(M+H)^+$ 233.1939, found 233.2018.




(*R*)-**6**

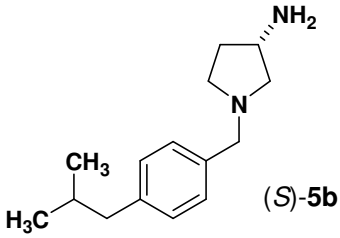
***(R)-N*-(1-(4-isobutylbenzyl)pyrrolidin-3-yl)-4-iodobenzenesulfonamide ((*R*)-6).** Compound ***(R)*-5b** (0.391 g, 1.68 mmol) was dissolved in 6 mL of DCM. This was followed by the addition of 4-iodobenzenesulfonyl chloride (0.560 g, 1.85 mmol), and triethylamine (0.352 mL, 2.52 mmol). The reaction mixture was stirred under argon overnight. The mixture was washed with 10 mL 10% NaOH, extracted with 3 x 15 mL DCM, dried $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography (33:66 to 30:60 EtOAc/hexane) to afford 0.495 g of product (59%): $^1$H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.6, 2H), 7.53 (d, *J* = 8.6, 2H), 7.15 – 7.03 (m, 4H), 4.97 (s, 1H), 3.83 (s, 1H), 3.48 (dd, *J* = 12.6, 28.6, 2H), 2.80 – 2.71 (m, 1H), 2.47 (d, *J* = 7.2, 2H), 2.41 – 2.29 (m, 2H), 2.25 – 2.05 (m, 2H), 1.93 – 1.78 (m, 1H), 1.61 – 1.49 (m, 1H), 0.90 (d, *J* = 6.6, 6H); $^{13}$C NMR (600 MHz, $(CD_3)_2CO$) δ 142.25, 140.53, 138.79, 136.85, 129.28, 129.09, 128.8, 99.4, 60.5, 59.7, 53.1, 52.6, 45.3, 32.3, 30.6, 22.3; IR (thin film) 3254, 2958, 2922, 1569 cm$^{-1}$; $[\alpha]_D$ = 26.5 (c 0.93, MeOH) (t = 20.5 °C), HRMS *m/z* $(M+H)^+$ 499.0838, found 499.0897; mp (95-96.9°C).




(*S*)-**5a**

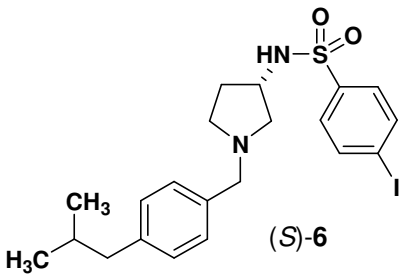
***(S)-tert*-butyl 1-(4-isobutylbenzyl)pyrrolidin-3-ylcarbamate ((*S*)-5a).** ***(S)*-5** (0.358 g, 1.92 mmol) was dissolved in 8 mL of DCM, this was followed by the addition of $NaBH(OAc)_3$ (0.611 g, 2.88 mmol) and iso-butyl benzaldehyde (0.343 g, 2.11 mmol). The reaction mixture was stirred under argon at room temperature overnight. The mixture was washed with 10 mL 10% NaOH and the layers were separated. The aqueous layer was extracted with 3 x 15 mL of DCM. The organic layers were combined, dried with

$MgSO_4$ and concentrated *in vacuo*. The crude product was purified by flash chromatography (33:66 to 50:50 EtOAc/hexanes) to afford 0.598 g, of product (93%).



(*S*)-**5b**

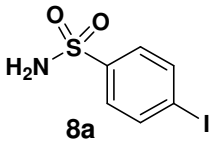
***(S)*-1-(4-isobutylbenzyl)pyrrolidin-3-amine ((*S*)-5b).** Compound ***(S)*-5a** (0.598 g, 1.80 mmol) was added to a solution of 4 M HCl in dioxane (3 mL). The reaction mixture was heated to 55 °C and stirred under argon overnight. The reaction mixture was cooled to room temperature and basified with NaOH until the product precipitated out of solution. The mixture was extracted with 3 x 10 mL DCM, dried with $MgSO_4$ and concentrated *in vacuo* to give 0.395 g of product (94%): $^1$H NMR (300 MHz, $CDCl_3$) δ 7.22 (d, *J* = 8.1, 2H), 7.08 (d, *J* = 8.1, 2H), 3.57 (d, *J* = 4.0, 2H), 3.54 – 3.44 (m, 1H), 2.70 (dd, *J* = 9.1, 17.0, 2H), 2.45 (d, *J* = 7.2, 2H), 2.29 (dd, *J* = 4.3, 9.5, 1H), 2.25 – 2.11 (m, 1H), 1.92 – 1.75 (m, 1H), 1.63 (s, 3H), 1.53 – 1.41 (m, 1H), 0.89 (d, J = 6.6, 6H); $^{13}$C NMR (300 MHz, $CDCl_3$) δ 140.7, 136.5,129.3, 128.9, 64.2, 60.5, 53.5, 51.2, 45.4, 35.4, 30.6, 22.7**;** IR (thin film) 3355, 2952, 2921, 2867 cm$^{-1}$; $[\alpha]_D$ = 6.65 (c 0.87, MeOH) (t = 20.8 °C); HRMS *m/z* $(M+H)^+$ 233.1939, found 233.2003.



(*S*)-**6**

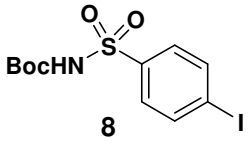
***(S)-N*-(1-(4-isobutylbenzyl)pyrrolidin-3-yl)-4-iodobenzenesulfonamide ((*S*)-6).** Compound ***(S)*-5b** (0.209g, 0.899mmol) was dissolved in 5 mL of DCM. This was followed by the addition of 4-iodobenzenesulfonyl chloride (0.299 g, 0.989 mmol), and triethylamine (0.188 mL, 1.35 mmol). The reaction mixture was stirred under argon overnight. The mixture was washed with 10 mL 10% NaOH, extracted with 3 x 15 mL DCM, dried $MgSO_4$, and concentrated *in vacuo*. The crude product was purified by flash chromatography (25:75 to 50:50 EtOAc/hexane) to afford 0.382 g of product (85%): $^1$H

NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.6, 2H), 7.53 (d, *J* = 8.6, 2H), 7.15 – 7.04 (m, 4H), 5.03 (s, 1H), 3.84 (s, 1H), 3.48 (dd, *J* = 12.7, 28.7, 2H), 2.80 – 2.69 (m, 1H), 2.47 (d, *J* = 7.2, 2H), 2.41 – 2.29 (m, 2H), 2.25 – 2.03 (m, 2H), 1.93 – 1.78 (m, 1H), 1.61 – 1.48 (m, 1H), 0.90 (d, *J* = 6.6, 6H); $^{13}C$ NMR (600 MHz, $(CD_3)_2CO$) δ 142.2, 140.5, 138.8, 136.8, 129.3, 129.1, 128.6, 99.4, 60.5, 59.7, 53.1, 52.7, 45.3, 32.3, 30.6, 22.3; IR (thin film) 3257, 2956, 2921, 1569 $cm^{-1}$; $[\alpha]_D$ = -29.3 (c 0.90, MeOH) (t = 20.2 °C); HRMS *m/z* $(M+H)^+$ 499.0838, found 499.0893; mp (97.1-99.6°C).



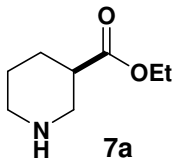
**8a**

**4-iodobenzenesulfonamide (8a).** Compound 4-iodobenzenesulfonyl chloride (0.506 g, 1.67 mmol) was added dropwise to a solution of ammonium hydroxide (4 mL) at room temperature. Once all starting material was added, the reaction mixture was heated to 100°C stirred under argon for 3 h. The reaction mixture was dissolved in 20 mL of EtOAc and washed with 5 x 20 mL of water. The water layers were extracted 3 x 15 mL of EtOAc, the organic layers were dried with $MgSO_4$ and concentrated *in vacuo* to afford 0.408 g of product (86%).



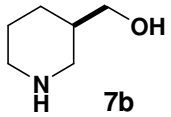
**8**

***tert*-butyl 4-iodophenylsulfonylcarbamate (8).** In a solution of **8a** (0.558 mg, 1.97 mmol) in 5 mL of THF was added DMAP (0.024 g, 0.197 mmol) and triethylamine (0.411mL, 2.95mmol). A solution of boc anhydride (.473 mg, 2.17 mmol) in 3 mL of THF was added to the reaction mixture. The reaction mixture stirred under argon at room temperature for 4 h. The THF was evaporated and the reaction mixture was dissolved with 20mL EtOAc, washed with 10 mL 10% HCl, extracted with 3 x 20 mL EtOAc. The organic layer was collected, dried with $MgSO_4$, and concentrated *in vacuo.* The crude product was purified using column chromatography (20:80 to 33:66 EtOAc/Hexane) to afford 0.608 g product (80%).



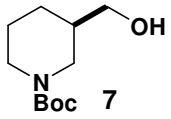

**7a**

***(R)*-ethyl piperidine-3-carboxylate (7a).** In 15 mL of DCM was added (R)-ethyl nipecotate tartaric acid salt (0.537 mg, 1.75 mmol) and an aqueous solution of $Na_2CO_3$ (0.458 g, 3.50 mmol). The mixture was stirred at room temperature for an hour. The mixture was separated with 3 x 15mL of DCM, the organic layer was dried with $MgSO_4$, and concentrated *in vacuo* to afford 0.251 g of product (91%).



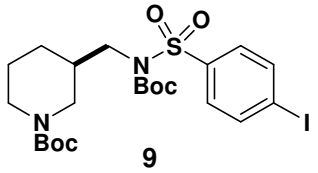

**7b**

***pg. 57 (R)*-piperidin-3-ylmethanol (7b).** In a cooled solution of LAH (0.084 g, 2.21 mmol) in 2 mL of THF flashed with argon was added **7a** (0.174, 1.01 mmol) as a solution in 2 mL of THF. After an additional 2 mL of THF was added, the reaction mixture was stirred and refluxed at 65 °C under argon for 4 h. The reaction mixture was quenched by adding a saturated aqueous solution of $Na_2SO_4$ dropwise, filtered and washed with diethyl ether, dried with $Na_2SO_4$, and concentrated *in vacuo* to afford 0.118 g of product (93%).



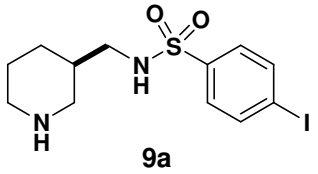

**7**

***(R)-tert*-butyl 3-(hydroxymethyl)piperidine-1-carboxylate (7).** In 1 mL of THF was added **7b** (0.186 g, 1.62 mmol) and a solution of Boc anhydride (0.389 g, 1.78 mmol) in 4mL of THF dropwise. The reaction mixture was stirred under argon at room temperature for 4 h. The reaction mixture was washed with 10 mL saturated $NH_4Cl$, extracted 3 x 10mL of EtOAc, the organic layer was dried with $MgSO_4$, and concentrated *in vacuo.* The crude product was purified using a silica plug (50:50 EtOAc/Hexane) to afford 0.315 g of product (90%).



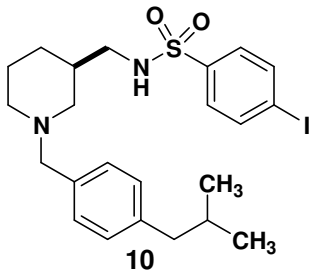

**9**

***(R)-tert*-butyl 3-((*N*-(*tert*-butoxycarbonyl)-4-iodophenylsulfonamido)methyl)piperidine-1-carboxylate (9).** Compound **7 (**0.137g, 0.635mmol), **8** (0.203 g, 0.529 mmol) and triphenyl phosphine (0.166 g, 0.635 mmol) were added to a degassed flask, 2 mL of THF was added along with DIAD (0.125 mL, 0.635 mmol). The reaction mixture was stirred under argon at room temperature for 3 h. The solvent was evaporated from the reaction and then purified using flash chromatography (10:90 to 20:80 EtOAc/Hexane) to afford 0.277 g of product (90%): $^{1}$H NMR (600 MHz, 40°C $CDCl_3$) δ 7.87 (d, *J* = 8.7, 2H), 7.62 (d, *J* = 8.1, 2H), 5.01 – 4.90 (m, 1H), 4.02 (d, *J* = 12.6, 1H), 3.98 – 3.86 (m, 1H), 3.77 – 3.63 (m, 2H), 2.76 (s, 1H), 2.63 – 2.51 (m, 1H), 1.96 (s, 1H), 1.88 – 1.78 (m, 1H), 1.75 – 1.65 (m, 1H), 1.61 (s, 1H), 1.45 (s, 9H), 1.35 (s, 9H); $^{13}$C NMR (600 MHz, 40°C, $CDCl_3$) δ 155.1, 151.3, 140.5, 138.3, 129.7, 101.0, 85.2, 79.8, 50.2, 37.2, 28.8, 28.7, 28.3, 24.9, 22.3, 22.3; IR (thin film) 2932, 1728, 1685, 1569 $cm^{-1}$; $[\alpha]_D$ = -11.8 (c 0.76, MeOH) (t = 22.6 °C); HRMS *m/z* $(M+H)^+$ 581.1104, found 581.1108.



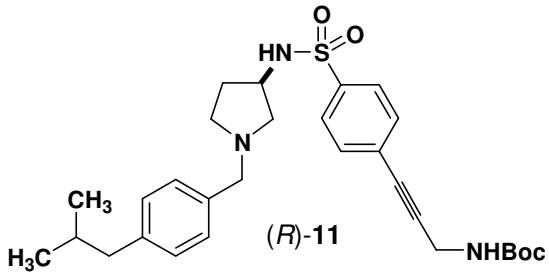

**9a**

***(R)*-4-iodo-*N*-(piperidin-3-ylmethyl)benzenesulfonamide (9a). 9** was added to a solution of 4 M HCl in dioxane. The reaction mixture was stirred under argon at room temperature overnight. After stirring overnight, more HCl was added to the reaction mixture and stirred overnight again. Once the reaction was complete, the solution was basified with NaOH, extracted with 5 x 15 mL of DCM. The organic layer was dried with $MgSO_4$, and concentrated *in vacuo* (82%).




10

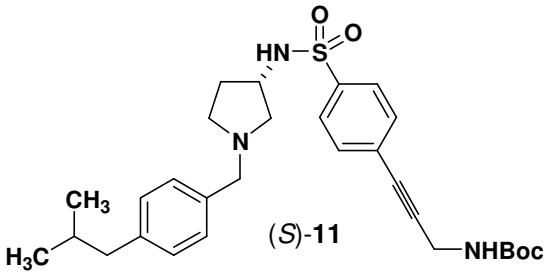
***(R)-N*-((1-(4-isobutylbenzyl)piperidin-3-yl(methyl)-4-iodobenzenesulfonamide (10).** To a solution of **9a** (0.150 g, 0.394 mmol) in 2 mL of DCM, was added iso-butyl benzaldehyde (0.070 g, 0.434 mmol) and $NaBH(OAc)_3$ (0.125 g, 0.591 mmol). The reaction mixture was stirred under argon at room temperature overnight. The reaction mixture was separated with DCM, washed with 10% NaOH and extracted 3 x 10 mL DCM. The organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The crude product was purified using a silica gel plug (25:75 EtOAc/Hexane to pure EtOAc) to afford the product 0.126 g (61%): $^1$H NMR (400 MHz, $CDCl_3$) δ δ 7.83 (d, *J* = 8.6, 2H), 7.51 (d, *J* = 8.6, 2H), 7.15 (d, *J* = 8.0, 2H), 7.08 (d, *J* = 8.0, 2H), 5.49 – 5.17 (m, 1H), 3.39 (q, *J* = 13.1, 2H), 2.96 – 2.77 (m, 2H), 2.60 (d, *J* = 10.0, 1H), 2.56 – 2.49 (m, 1H), 2.46 (d, *J* = 7.2, 2H), 2.15 (s, 1H), 1.91 – 1.80 (m, 2H), 1.78 – 1.59 (m, 3H), 1.55 – 1.44 (m, 1H), 1.18 – 1.01 (m, 1H), 0.90 (d, J = 6.6, 6H); $^{13}$C NMR (600 MHz, $CDCl_3$) δ 140.9, 140.0, 138.6, 135.5, 129.4, 129.2, 128.3, 100.1, 63.5, 57.4, 54.3, 47.6, 45.4, 35.4, 30.6, 28.6, 24.4, 22.8; IR (thin film) 3276, 2925, 1720, 1614 $cm^{-1}$; $[\alpha]_D$ = -4.88 (c 0.82, MeOH) (t = 20.3 °C); HRMS *m/z* $(M+H)^+$ 527.1151, found 527.1132.




(*R*)-11

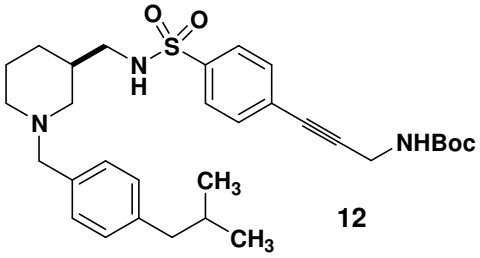
**((*R*)-11)**. To a solution of ***(R)*-6** (0.050 g, 0.100 mmol) in 2 mL DMF was added Boc protected-propargyl amine (0.023g, 0.15mmol), $Pd(PPh_3)_4$ (0.006g, 0.005mmol), copper iodide (0.002 g, 0.01 mmol) and triethylamine (0.0418 mL, 0.300 mmol). The reaction mixture was stirred under argon at 40 °C for 2.5 h. The reaction was allowed to cool and then concentrated *in vacuo*. The mixture was then separated with EtOAc and water, extracted 3 x 10 mL EtOAc, washed 2 x 10 mL of brine, organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The crude product was purified using column chromatography (20:80 to 50:50 EtOAc/Hexane) to afford 0.0359 g of product (68%): $^1$H

NMR (600 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.1, 2H), 7.47 (d, *J* = 8.2, 2H), 7.11 (d, *J* = 7.8, 2H), 7.06 (d, *J* = 7.9, 2H), 5.07 (s, 1H), 4.85 (s, 1H), 4.17 (s, 2H), 3.84 (s, 1H), 3.48 (dd, *J* = 12.8, 35.2, 2H), 2.75 – 2.68 (m, 1H), 2.46 (d, *J* = 7.2, 2H), 2.38 (d, *J* = 4.5, 2H), 2.20 (dd, *J* = 9.0, 16.0, 1H), 2.11 – 2.03 (m, 1H), 1.89 – 1.80 (m, 1H), 1.47 (s, 9H), 1.25 (s, 1H), 0.90 (d, J = 6.5, 6H); $^{13}C$ NMR (600 MHz, $CDCl_3$) δ 155.6, 141.0, 140.7, 135.7, 132.6, 129.4, 128.8, 127.6, 127.3, 89.4, 81.9, 60.6, 59.8, 53.1, 52.3, 45.4, 32.9, 31.4, 30.6, 30.0, 28.7, 22.7; IR (thin film) 3291, 2956, 2925, 2252, 1692 $cm^{-1}$; $[\alpha]_D$ = 24.6 (c 0.57, MeOH) (t = 20.2 °C); HRMS *m/z* $(M+H)^+$ 526.2661, found 526.2708.



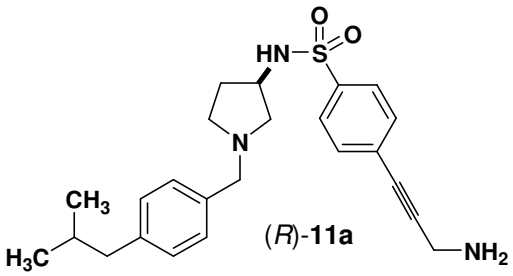
(*S*)-**11**

**((*S*)-11)**. To a solution of ***(S)*-6** (0.050 g, 0.100 mmol) in 2 mL DMF was added Boc protected-propargyl amine (0.023 g, 0.15 mmol), $Pd(PPh_3)_4$ (0.006 g, 0.005 mmol), copper iodide (0.002 g, 0.01 mmol) and triethylamine (0.0418 mL, 0.300 mmol). The reaction mixture was stirred under argon at 40 °C for 2.5 h. The reaction was allowed to cool and then concentrated *in vacuo*. The mixture was then separated with EtOAc and water, extracted 3 x 10 mL EtOAc, washed 2 x 10 mL of brine, organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The crude product was purified using column chromatography (20:80 to 50:50 EtOAc/Hexane) to afford 0.046 g of product (88%): $^1H$ NMR (600 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.4, 2H), 7.47 (d, *J* = 8.4, 2H), 7.12 (d, *J* = 7.9, 2H), 7.07 (d, *J* = 7.9, 2H), 5.15 (s, 1H), 4.84 (s, 1H), 4.17 (s, 2H), 3.85 (s, 1H), 3.49 (dd, *J* = 12.8, 34.7, 2H), 2.76 – 2.70 (m, 1H), 2.46 (d, *J* = 7.2, 2H), 2.39 (d, *J* = 5.2, 2H), 2.21 (dd, *J* = 8.9, 16.1, 1H), 2.11 – 2.04 (m, *J* = 8.8, 1H), 1.89 – 1.80 (m, 1H), 1.47 (s, 9H), 1.25 (s, 1H), 0.90 (d, J = 6.6, 6H); $^{13}C$ NMR (600 MHz, $CDCl_3$) δ 155.6, 141.0, 140.6, 135.5, 132.5, 129.4, 128.9, 127.6, 127.2, 89.4, 81.9, 60.5, 59.7, 53.1, 52.3, 45.4, 32.9, 31.4, 30.6, 30.0, 28.7, 22.7; IR (thin film) 3292, 2956, 2926, 2251, 1692 $cm^{-1}$; $[\alpha]_D$ = -25.9 (c 0.70, MeOH) (t = 20.6 °C); HRMS *m/z* $(M+H)^+$ 525.2661, found 526.2728.

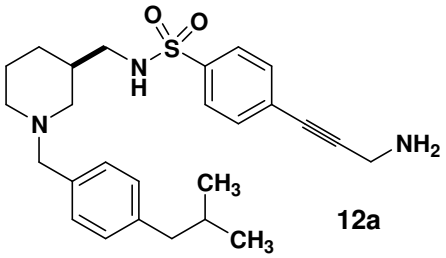



12

**(12)**. To a solution of **10** (0.050 g, 0.095 mmol) in 2 mL DMF was added Boc protected-propargyl amine (0.022 g, 0.14 mmol), $Pd(PPh_3)_4$ (0.0055 g, 0.0047 mmol), copper iodide (0.0018 g, 0.009 mmol) and triethylamine (0.037 mL, 0.285 mmol). The reaction mixture was stirred under argon at 40 °C for 2.5 h. The reaction was allowed to cool and then concentrated *in vacuo*. The mixture was then separated with EtOAc and water, extracted 3 x 10 mL EtOAc, washed 2 x 10 mL of brine, organic layers were dried with $MgSO_4$, and concentrated *in vacuo*. The crude product was purified using column chromatography (33:66 to 75:25 EtOAc/Hexane) to afford 0.020 g of product (39%): $^1$H NMR (600 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.4, 2H), 7.50 (d, *J* = 8.2, 2H), 7.16 (d, *J* = 7.9, 2H), 7.09 (d, *J* = 7.8, 2H), 4.81 (s, 1H), 4.19 (s, 2H), 3.40 (dd, *J* = 12.9, 27.9, 2H), 2.97 – 2.82 (m, 2H), 2.62 (s, 1H), 2.54 (s, 1H), 2.46 (d, *J* = 7.2, 2H), 2.16 (s, 1H), 1.92 – 1.81 (m, 2H), 1.78 – 1.72 (m, 1H), 1.72 – 1.67 (m, 1H), 1.65 – 1.60 (m, 1H), 1.47 (s, 9H), 1.25 (s, 2H), 1.08 (s, 1H), 0.90 (d, J = 6.5, 6H); $^{13}$C NMR (600 MHz, $CDCl_3$) δ 155.5, 140.8, 139.6, 132.4, 130.1, 129.2, 128.7, 128.7, 127.4, 127.1, 106.4, 89.3, 81.8, 63.3, 57.3, 55.5, 54.1, 47.4, 45.3, 30.4, 28.6, 28.4, 22.6; IR (thin film) 3280, 2926, 2867, 2250, 1694 cm$^{-1}$; $[\alpha]_D$ = -9.72 (c 0.51, MeOH) (t = 21.1 °C); HRMS *m/z* $(M+H)^+$ 554.2974, found 554.3053.




(*R*)-**11a**




**12a**

(*R*)-**11a,** (*S*)-**11a, 12a**: A solution of 10% TFA in DMF was added to **(*R*)-11, (*S*)-11, 12** until compounds dissolved, then the solution was concentrated *in vacuo*.

**Preparation of Cell Lysate.** Inoculate two tubes of 5 mL of LB with *E. coli* strain DRC39 and allow to grow overnight at 37 °C. Using the 5 mL overnight cultures, inoculate two 1 L bottles of LB and allow to grow at 37 °C. After 4 hours of growth, test OD600 to confirm sufficient growth, harvest cells by centrifuging cells for 15 min at 3500rpm and washing with 0.9% saline. Resuspend washed cell pellet in HNB (50mM Hepes pH 7.0, 150 mM NaCl) and add protease inhibitor cocktail and PMSF. Lyse cells using microfluidizer and centrifuge lysate for 15 mins at 15,000xg to clear lysate. Decant supernatant and save as lysate.

**Pull-down Experiment.** Equilibrate liganded resin prepared above by centrifuging and washing three times with 500 µL of HNB. After final wash add lysate to resin, add 500 µL of lysate per 50 µL of liganded resin and any necessary competitor compounds. Incubate at 4 °C for 2 hours with gentle shaking. Pellet the resin and remove supernatant. Washing resin three times with 500 µL of HNB and resuspend resin in 50 µL of 1x SB and boil. Run 5-20 µL of each sample on a 4-12% tris-glycine gel and stained with Coomassie Blue.


**Figure S4.** Typical results from a pull-down experiment with compounds **13-15** using the protocol above.

**References**

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