Supporting Information

Rational Design, Synthesis and Testing of Novel Tricyclic Topoisomerase Inhibitors for the Treatment of Bacterial Infections Part 2

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Synthetic Procedures

General Information

All reactions were carried out using commercial materials and reagents without further purification unless otherwise noted. If the chiral building block was re-synthesised. It was done so following literature precedence, with matching spectra and therefore not reported. All reactions were monitored by thin layer chromatography (TLC). Visualization of the spots on TLC plates was achieved by UV light and by staining the TLC plates in potassium permanganate and charring with a heat gun, unless otherwise stated.

NMR spectral data was recorded on a LC Bruker AV400 using a 5 mm QNP probe or Bruker AVIII 400 Nanobay using a 5 mm BBFQ with z-gradients. Chemical shifts are expressed in parts per million values (ppm) and are designated as s (singlet); br s (broad singlet); d (doublet); t (triplet); q (quartet); quint (quintet) or m (multiplet). Where appropriate, COSY and NOE experiments were carried out to aid assignment.

Chromatography was performed on a an ISCO using silica (normal phase or C18 (reverse phase; or by flash-column chromatography using silica gel (Fluorochem silica gel 60A 40-63 μm).

LCMS methods

Method A: Instrument: **Waters Acquity UPLC H-Class system;** Column: Acquity BEH C18 1.7 μm 2.1 x 50 mm; eluent A: water, eluent B: acetonitrile, gradient: 0-0.3 min 5% B, 0.3-2.0 min 5-95% B with A, 2.0-2.6 min 95-5% B with A, 2.6-3 min 5% B; flow 0.6 ml/min; injection volume: 2 μL

Method B: Instrument: **Waters Acquity UPLC H-Class system;** Column: Column: Gemini NX C18. 5 prn, 50 x 2 mm; eluent A: water, eluent B: acetonitrile; gradient: 0-4.0 min 5-95% B with A, 4.0-4.45 min 95% B, 4.45-4.5 min 95-5% B with A, 4.5-5.0 min 5% B; flow 1.0 ml/min; injection volume: 10 μL

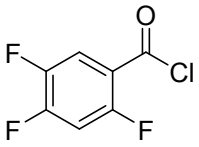
Method C: Instrument: **Waters Acquity UPLC H-Class system;** Column: Column: YMC Triart-C18 50 x 2 mm; eluent A: water, eluent B: acetonitrile, eluent C: 1 vol % formic acid in

50/50 water/acetonitrile; gradient: 0-2.0 min 95% A, 5% C, 2.0-12 min 0-95% B with A, 5% C throughout, 12-14 min 95% B with 5% C; flow 0.8 ml/min; injection volume: 5 µL

Method D: Instrument: **Waters Acquity UPLC H-Class system;** Column: Column: YMC Triart-C18 50 x 2 mm; eluent A: water, eluent B: acetonitrile, eluent C: 1 vol % ammonia (35%) in water /acetonitrile; gradient: 0-2.0 min 95% A, 5% C, 2.0-12 min 0-95% B with A, 5% C throughout, 12-14 min 95% B with 5% C; flow 0.8 ml/min; injection volume: 5 µL

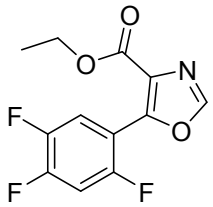
Intermediate 2 (step a)

1. 2,4,5-trifluorobenzoyl chloride



A suspension of 2,4,5-trifluorobenzoic acid (5 g, 28.4 mmol) in DCM (60 mL) was cooled to 0^{o}C. Oxalyl chloride (3.72 mL, 42.59 mmol) was added followed by 3 drops of DMF and the reaction allowed to warm to room temperature. Effervescence commenced on warming. The mixture was stirred at room temperature for 2 h then evaporated (co-evaporated from DCM x 3) and used without further purification

2. ethyl 5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylate



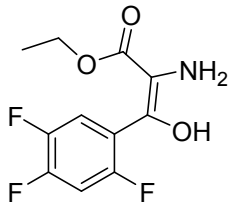
Ethyl isocyanoacetate (3.72 g, 31.23 mmol) in THF (30 mL) was cooled to 0^{o}C. Et_3N (11.81 mL, 85.19 mmol) was added drop wise followed by the addition of 2,4,5-trifluorobenzoyl chloride (5.52 g, 28.4 mmol) in THF (30 mL) over 5 min. The reaction was warmed to room temperature and stirred overnight. The mixture was diluted with DCM (100 mL) and washed with saturated aqueous $NaHCO_3$ (3 x 50 mL) and brine (50 mL). The organic phase was separated, dried over

Na_2SO_4. filtered and solvent removed *in vacuo* to give a brown solid. Purification by flash chromatography eluting with 0-80% EtOAc in Petroleum ether (40-60) gave ethyl 5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylate (3.7 g, 48% yield) as a cream solid.

LC-MS (Method A) 272.0 $[M+H]^+$; RT 1.97 min.

Intermediate 3 (step b)

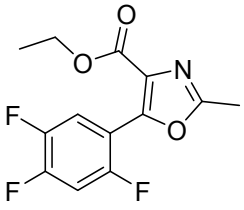
1. ethyl (*E/Z*)-2-amino-3-hydroxy-3-(2,4,5-trifluorophenyl)acrylate



A solution of ethyl 5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylate (2.70 g, 9.96 mmol) in 1,4-dioxane (50 mL) was treated with 1M aqueous HCl (50 mL). After stirring for 72 h at room temperature the solvent was removed *in vacuo* to give ethyl (Z)-2-amino-3-hydroxy-3-(2,4,5-trifluorophenyl)prop-2-enoate as a yellow oily solid (2.60 g) which was used without further purification.

LC-MS (Method A) 262.0 $[M+H]^+$; RT 0.72 min.

2. ethyl 2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylate

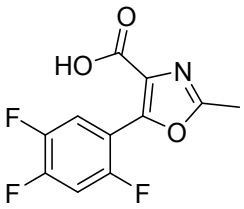


A mixture of ethyl (Z)-2-amino-3-hydroxy-3-(2,4,5-trifluorophenyl)prop-2-enoate (2.60 g, 9.95 mmol) and trimethyl orthoacetate (25.mL, 24.98 mmol) was heated under reflux at 110°C for 2 h. After consumption of starting material (monitored by LCMS) the mixture was concentrated *in vacuo* to give ethyl 2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylate (2.82 g) which was used without further purification.

LC-MS (Method A) 286.1 $[M+H]^+$; RT 1.70 min.

Intermediate 4 (step c)

1. 2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylic acid

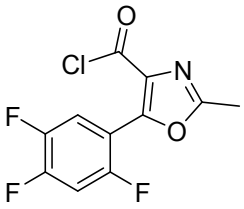


A solution of ethyl 2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylate (2.82 g, 9.9 mmol) in 1,4-dioxane (60 mL) was treated with 1M aq. LiOH (59.4 mL) and stirred at room temperature overnight. The mixture was evaporated to a minimum, partitioned with EtOAc (50 mL) and H_2O (80 mL) and the aq. washed with EtOAc (2 x 50 mL). The aq. was acidified with 1M aqueous HCl (80 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylic acid (2.50 g, 98 % yield) as a cream solid.

LC-MS (Method A) 258.0 $[M+H]^+$; RT 1.41 min.

Intermediate 5 (step d)

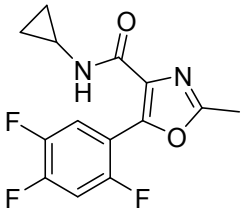
1. 2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carbonyl chloride



A suspension of 2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxylic acid (2.50 g, 9.72 mmol) in DCM (75 mL) was treated with oxalyl chloride (1.27 mL, 14.58 mmol) and cat. DMF

(1 drop) and stirred at room temperature for 1 h under N_2. The mixture was then evaporated and co-evaporated from DCM (3 x) to give a yellow powder, which was used immediately.

2. N-cyclopropyl-2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxamide

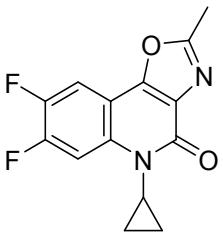


A solution of 2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carbonyl chloride (2.68 g, 9.72 mmol) in DCM (75 mL) was treated with cyclopropylamine (1.41 mL, 20.42 mmol) and stirred at room temperature overnight. The mixture was then diluted with DCM (50 mL) and washed with saturated aqueous $NaHCO_3$ (3 x 30 ml) and brine (30 ml). The organic phase was separated, dried over Na_2SO_4 filtered and solvent removed *in vacuo* to give N-cyclopropyl-2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxamide (2.30g 79 % yield) as a pale solid.

LC-MS (Method A) 297.1 $[M+H]^+$; RT 1.62 min

Intermediate 6 (step e)

5-cyclopropyl-7,8-difluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

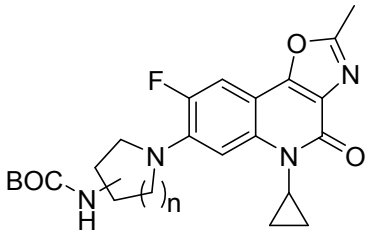


A solution of N-cyclopropyl-2-methyl-5-(2,4,5-trifluorophenyl)-oxazole-4-carboxamide (500 mg, 1.69 mmol) and 18-crown-6 (446 mg, 1.69 mmol) in DMSO (10 mL) was heated at 140°C for 50 min. On cooling the reaction was diluted with EtOAc (100 ml) and washed 5 x with H_2O followed by brine (30 ml). The organic phase was dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. The resulting residue was purified by flash chromatography eluting with 0-

100% EtOAc in heptane to give 5-cyclopropyl-7,8-difluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one (300 mg, 64 % yield) as a pale brown powder.

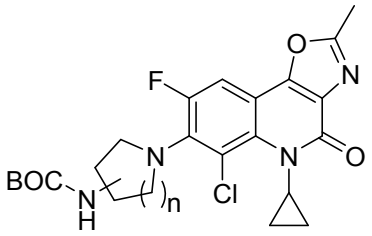
LC-MS (Method A) 277.1 $[M+H]^+$; RT 1.53 min

GP1 (step f)



A mixture of intermediate **5** (0.14 mmol), heterocyclic amine (0.19 mmol) and DIPEA (0.15 mL, 0.87 mmol) in DMSO (2 mL) were heated in the microwave at 140° C. for 80 min. On cooling the reaction mixture was diluted with EtOAc (50 mL) and washed with water (5x15 mL) , 0 . 5M aqueous HCl (2x30 mL) , saturated aqueous $NaHCO_3$, (50 mL) and brine (50 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent removed in vacuo to give intermediate **6** as a brown solid. Typical yields: 71-91%

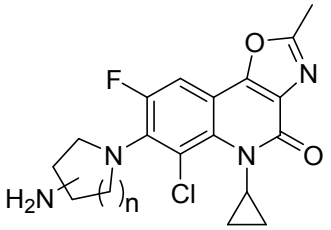
GP 2 (step g)



To a solution of intermediate **6** (0.16 mmol) in DCM (2 mL) was added 1,3-dichloro-5,5-dimethyl-imidazolidine-2,4-dione (46 mg, 0.24 mmol) in DCM (1.14 mL) and the reaction stirred at room temperature. After 1 h the reaction was quenched with aqueous sodium bisulfite (0.5 g in 5 mL) and diluted with DCM (5 mL). The layers were separated, and the organic layer washed with saturated aqueous $NaHCO_3$ and then H_2O, dried over $MgSO_4$,

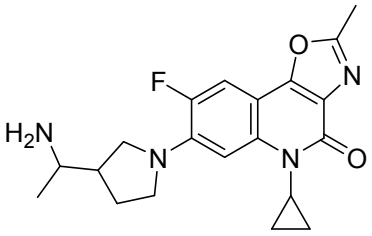
filtered and the solvent removed *in vacuo*. The resulting residue was purified by flash chromatography using 0-10% MeOH in DCM to give the desired intermediate **7.** 43-59%

GP3 (step h)



To a solution of intermediate **7** (0.12 mmol) in DCM (3.5 mL) at room temperature was added TFA (0.11 mL, 1.4 mmol) and the mixture left stirring for 18 h . 0.5M aqueous HCl (20 mL) was added and the mixture washed with EtOAc (2x20 mL). The aqueous phase was basified with solid K_2CO_3 and extracted with DCM (3x30 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo* . The crude product was purified by flash chromatography eluting with 0-20 % MeOH in DCM to give compound **8** as an off - white solid. 85-99%

REDX05848 – 7-[3-(1-aminoethyl)pyrrolidin-1-yl]-5-cyclopropyl-8-fluoro-2-methyloxazolo[4,5-c]quinolin-4-one

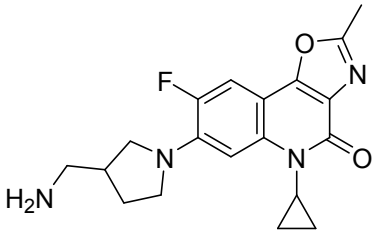


Prepared using tert-butyl N-[1-(pyrrolidin-3-yl)ethyl]carbamate as described in **GP1 and GP3**

^{1}H NMR (400MHz, CD_3OD): δ ppm 7.34 (d, *J* = 12.89 Hz, 1H), 7.03 (d, *J* = 7.93 Hz, 1H), 3.69-3.60 (m, 3H), 3.39-3.37 (m, 1H), 3.18-3.14 (m, 1H), 2.95-2.91 (m, 1H), 2.64 (s, 3H), 2.40-2.29 (m, 2H), 1.92-1.82 (m, 1H), 1.43-1.40 (m, 2H), 1.32 (d, *J* = 6.33 Hz, 3H), 0.88-0.85 (m, 2H);

LC-MS (Method A) 371.4 [M+H]+; RT 1.12 min.

REDX05762 – 7-[3-(aminomethyl)pyrrolidin-1-yl]-5-cyclopropyl-8-fluoro-2-methyloxazolo[4,5-c]quinolin-4-one

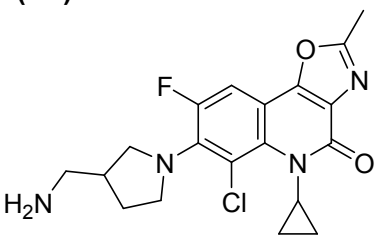


Prepared using tert-butyl N-(pyrrolidin-3-ylmethyl)carbamate as described in **GP1** and **GP3**. Product isolated as a HCl salt by stirring in 1M HCl in ether followed by removal of solvent.

^{1}H NMR (400MHz, DMSO-d6): δ ppm 7.99 (br s, 2H), 7.58 (d, *J* = 13.35 Hz, 1H), 7.07 (d, *J* = 7.97 Hz, 1H), 3.72-3.51 (m, 4H), 2.97 (m, 4H), 2.59 (s, 3H), 2.19 (m, 1H), 1.82 (m, 1H), 1.36 (m, 2H) 0.78 (m, 2H);

LC-MS (Method A) 357.3 [M+ H+]; RT 1.88 min.

REDX06598 - 7-(3-(aminomethyl)pyrrolidin-1-yl)-6-chloro-5-cyclopropyl-8-fluoro-2-methyloxazolo[4,5-c]quinolin-4(5H)-one

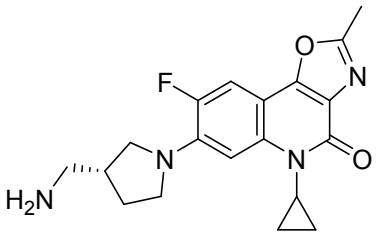


Prepared using tert-butyl N-(pyrrolidin-3-ylmethyl)carbamate as described in **GP1**, **GP2** and **GP3**

^{1}H NMR (400MHz, $CDCl_3$): δ ppm 7.57 (d, J = 11.6 Hz, 1H), 3.79-3.59 (m, 4H), 3.50- 3.43 (m, 1H), 3.18-3.08 (m, 2H), 2.66 (s, 4H), 2.34-2.24 (m, 1H), 1.91-1.80 (m, 1H), 1.30- 1.20 (m, 2H), 0.57-0 50 (m, 2H);

LC-MS (Method A) 391 4/393.4 [M+H]+; RT 1.65 min. Main isotope reported

REDX05840– 7-[(3R)-3-(aminomethyl)pyrrolidin-1-yl]-5-cyclopropyl-8-fluoro-2-methyloxazolo[4,5-c]quinolin-4-one

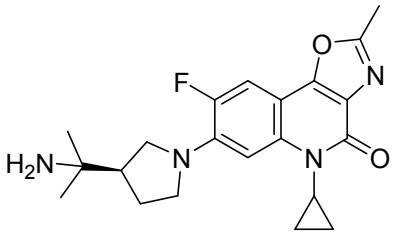


Prepared using (3S) tert-butyl N-(pyrrolidin-3-ylmethyl)carbamate as described in **GP1** and **GP3**

^{1}H NMR (400MHz, MeOD): δ ppm 7.36 (d, *J* = 13.5 Hz, 1H), 7.06 (d, *J* = 7.74 Hz, 1H), 3.59-3.56 (m, 1H), 3.46- 3.41 (m, 1H), 3.19-3.14 (m, 1H), 2.96-2.92 (m, 2H), 2.90-2.88 (m, 1H), 2.64 (s, 3H), 2.53-2.47 (m, 1H), 2.28-2.21 (m, 1H), 1.86-1.79 (m, 1H), 1.86-1.82 (m, 1H), 1.45-1.41 (m, 2H), 0.89- 0.85 (m, 2H);

LC-MS (Method A) 357.2 [M+H]+; RT 1.11 min.

REDX05960 – 7-[(3R)-3-(2-aminopropan-2-yl)pyrrolidin-1-yl]-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

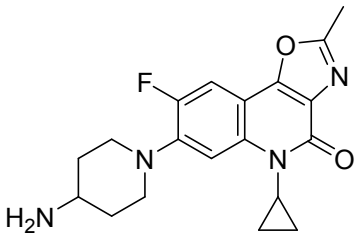


Prepared using tert-butyl N-(2-[(3R)-pyrrolidin-3-yl]propan-2-yl)carbamate as described in **GP1** and **GP3**

^{1}H NMR (400MHz, MeOD): δ ppm 7.39 (d, *J* = 13.04 Hz, 1H), 7.09 (d, *J* = 12.02 Hz, 1H), 3.73- 3.68 (m, 1H), 3.63-3.60 (m, 1H), 3.56-3.51 (m, 1H), 2.99-2.93 (m, 1H), 2.65 (s, 3H), 2.57-2.53 (m, 1H), 2.20-2.13 (m, 1H), 1.98-1.91 (m, 1H), 1.44-1.42 (m, 1H), 1.38 (s, 3H), 1.37 (s, 3H), 1.33-1.25 (m, 2H), 0.92-0.86 (m, 2H);

LC-MS (Method A) 385.2 [M+H]+; RT 1.14 min.

REDX05855- 7-(4-amino-1-piperidyl)-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

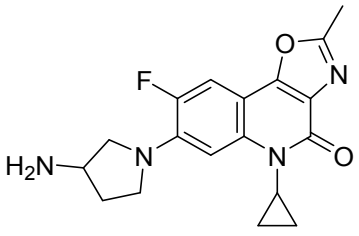


To a solution of tert-butyl N-[1-(5-cyclopropyl-8-fluoro-2-methyl-4-oxo-oxazolo[4,5-c]quinolin-7-yl)-4-piperidyl]carbamate (53.4 mg, 0.12 mmol) in DCM (3.5 mL) at room temperature was added TFA (0.11 mL, 1.4 mmol) and the mixture left stirring for 18 h. 0.5M aqueous HCl (20 mL) was added and the mixture washed with EtOAc (2 x 20 mL). The aqueous phase was basified with solid K_2CO_3 and extracted with DCM (3 x 30 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography eluting with 0-20 % MeOH in DCM to give 7-(4-amino-1-piperidyl)-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one (11 mg, 26 % yield) as an off-white solid.

^{1}H NMR (400MHz, MeOD): δ ppm 7.59 (d, *J* = 7.4 Hz, 1H), 7.56 (d, *J* = 7.83 Hz, 1H), 3.73-3.70 (m, 2H), 3.10-3.00 (m, 3H), 2.99- 2.94 (m, 2H), 2.67 (s, 3H), 2.11-2.09 (m, 2H), 1.27-1.19 (m, 2H), 0.94-0.91 (m, 2H);

LC-MS (Method A) 357.2 [M+H]+; RT 1.11 min.

REDX05761 – 7-(3-aminopyrrolidin-1-yl)-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

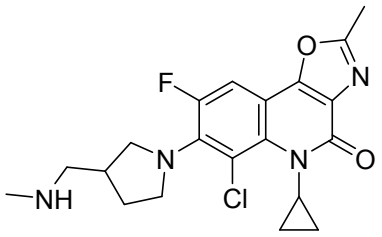


Prepared using tert-butyl N-(pyrrolidin-3-yl)carbamate as described in **GP1** and **GP3**. Product isolated as a formate salt

^{1}H NMR (400MHz, CD_3OD): δ ppm 8.20 (br s, 2H), 7.62 (d, *J* = 13.4 Hz, 1H), 7.09 (d, *J* =

8.03 Hz, 1H), 3.77-3.53 (m, 5H), 2.98 (m, 1H), 2.65 (s, 3H), 2.35-2.29 (m, 1H), 2.19-2.13 (m, 1H), 1.33-1.25 (m, 2H), 0.92-0.86 (m, 2H);
LC-MS (Method A) 343.2 [M+H]+; RT 1.80 min

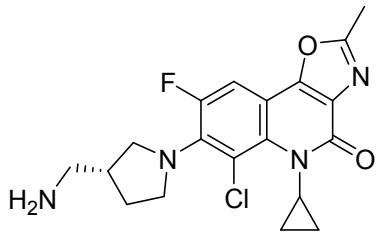
REDX07046- 6-chloro-5-cyclopropyl-8-fluoro-2-methyl-7-[3-(methylaminomethyl)pyrrolidin-1-yl]oxazolo[4,5-c]quinolin-4-one



Prepared using tert-butyl-(pyrrolidin-3-ylmethyl)carbamate as described **GP1, GP2** and **GP3** to give 6-chloro-5-cyclopropyl-8-fluoro-2-methyl-7-[3-(methylaminomethyl)pyrrolidin-1-yl]oxazolo[4,5-c]quinolin-4-one (44.92mg, 0.1054mmol, 32.451% yield) as a pale yellow solid.

^{1}H NMR ($CDCl_3$): δ ppm 7.4 (d, 1H), 3.7-3.8 (m, 1H), 3.5-3.7 (m, 3H), 3.4 (m, 1H), 2.7 (d, 2H), 2.6 (s, 3H), 2.5 (m, 4H), 2.1-2.2 (m, 1H), 1.7-1.8 (m, 1H), 1.2-1.3 (m, 2H), 0.5-0.6 (m, 2H);
LC-MS (method C) 405.5 $[M+H]^+$; RT 5.20 min. Main isotope reported.

REDX07815 - 7-[(3R)-3-(aminomethyl)pyrrolidin-1-yl]-6-chloro-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

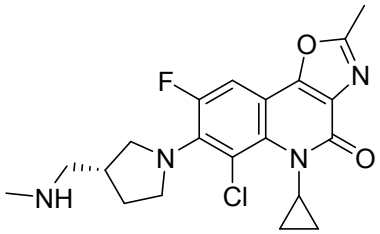


Prepared using tert-butyl (R)-(pyrrolidin-3-ylmethyl)carbamateas as described in **GP1**, **GP2** and **GP3**

^{1}H NMR (400MHz, $CDCl_3$): δ ppm 7.33-7.35 (d, 1H), 3.70-3.74 (m, 1H), 3.60-3.66 (m, 2H), 3.53-3.57 (m, 1H), 3.37-3.41 (m, 1H), 2.82-2.84 (d, 2H), 2.63 (s, 3H), 2.35-2.40 (m, 1H), 2.12-2.18 (m, 1H), 1.68-1.75 (m, 1H), 1.19-1.29 (m, 2H), 0.49-0.55 (m, 2H);

LC-MS (Method C) 391.0 [M]; RT 5.08 min. Main isotope reported

REDX07948 - 6-chloro-5-cyclopropyl-8-fluoro-2-methyl-7-[(3R)-3-(methylaminomethyl)pyrrolidin-1-yl]oxazolo[4,5-c]quinolin-4-one

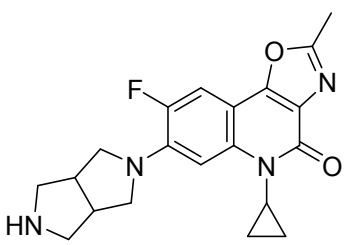


Prepared using tert-butyl (S)-(pyrrolidin-3-ylmethyl)carbamate as described in **GP1**, **GP2** and **GP3**

^{1}H NMR ($CDCl_3$): δ ppm 7.3-7.4 (d, 1H), 3.7-3.8 (m, 1H), 3.6-3.7 (m, 2H), 3.5-3.6 (m, 1H), 3.4 (m, 1H), 2.7 (m, 2H), 2.6 (s, 3H), 2.5 (s, 4H), 2.2 (m, 1H), 1.7-1.8 (m, 1H), 1.2-1.3 (m, 2H), 0.5-0.6 (m, 2H);

LC-MS (short acidic) 405.1 [M]; RT 1.56 min. Main isotope reported

REDX06921- 7-(2,3,3a,4,6,6a-hexahydro-1H-pyrrolo[3,4-c]pyrrol-5-yl)-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

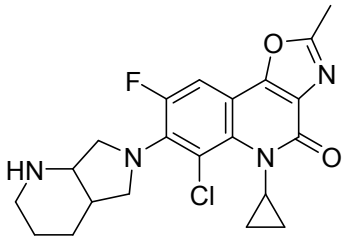


Prepared with 1,2,3,3a,4,5,6,6a-octahydropyrrolo[3,4-c]pyrrole as described in **GP1**

^{1}H NMR ($CDCl_3$): δ ppm 7.43 (d, 1H), 7.27 (s, 1H), 7.15 (d, 1H), 3.6-3.55 (m, 2H), 3.39 (d, 2H), 3.21-3.12 (m, 2H), 3.00- 2.85 (m, 5H), 2.65 (s, 3H), 1.45-1.35 (m, 2H), 0.95-0.5 (m, 2H);

LC-MS (Method B) 369.5 $[M+H]^+$; RT 1.65 min.

REDX06876- 7-(1,2,3,4,4a,5,7,7a-octahydropyrrolo[3,4-b]pyridin-6-yl)-6-chloro-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

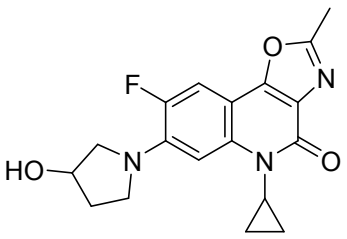


Prepared using tert-butyl octahydro-1H-pyrrolo[3,4-b]pyridine-1-carboxylate as described in **GP1**, **GP2** and **GP3**

^{1}H NMR ($CDCl_3$): δ ppm 7.38 (s, 1H), 7.31 (s, 1H), 3.97-3.86 (m, 2H), 3.75-3.70 (m, 1H), 3.53-3.35 (m, 2H), 3.31 (d, 1H), 3.05 (d, 1H), 2.75-2.6 (m, 1H), 2.63 (s, 3H), 2.45-2.34 (m, 1H), 1.9-1.74 (m, 2H), 1.75-1.6 (m, 1H), 1.54-1.48 (m, 1H), 1.38-1.15 (m, 2H), 0.6-0.45 (m, 2H);

LC-MS (Method D) 417.4 $[M+H]^+$; RT 7.06 min. Main isotope reported.

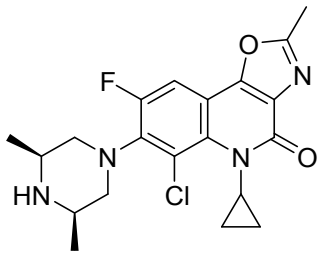
REDX05944 – 5-cyclopropyl-8-fluoro-7-(3-hydroxypyrrolidin-1-yl)-2-methyl-oxazolo[4,5-c] quinolin-4-one



Prepared using pyrrolidin-3-ol as described in **GP1**

^{1}H NMR (400MHz, $CDCl_3$): δ ppm 7.18 (d, *J* = 13.0 Hz, 1H), 6.91 (d, *J* = 7.99 Hz, 1H),4.66 (m, 1H), 3.85-3.77 (m, 2H), 3.73-3.69 (m, 1H), 3.58-3.54 (m, 1H), 2.96- 2.92 (m, 1H), 2.21-2.16 (m, 2H), 1.37-1.33 (m, 2H), 0.87-0.84 (m, 2H);
LC-MS (Method A) 344.0 [M+H]+; RT 1.39 min.

REDX06803- 6-chloro-5-cyclopropyl-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

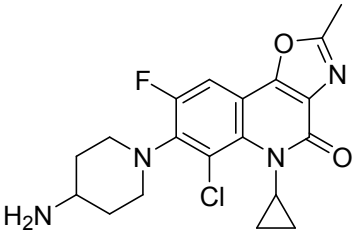


Prepared using (2R,6S)-2,6-dimethylpiperazine as described in **GP1** and **GP3**

^{1}H NMR ($CDCl_3$): δ ppm 7.38 (d, 1H), 7.28 (s, 1H), 3.8-3.7 (m, 1H), 3.20-3.10 (m, 4H), 2.95-2.85 (m, 2H), 2.65 (s, 3H), 1.30-1.25 (m, 3H), 0.9 (d, 6H), 0.55-0.50 (m, 2H);

LC-MS (Method C) 405.4 $[M+H]^+$; RT 4.81 min. Main isotope reported.

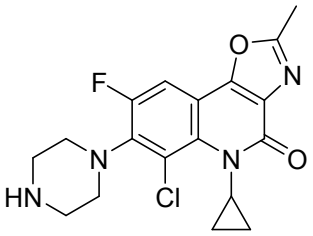
REDX07181- 7-(4-amino-1-piperidyl)-6-chloro-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one



Prepared using tert-butyl piperidin-4-ylcarbamate as described in **GP1**, **GP2** and **GP3** as a pale-yellow solid.

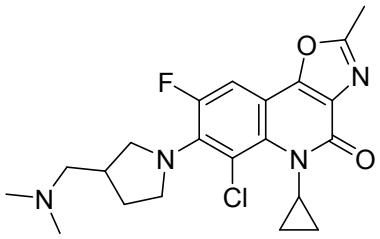
^{1}H NMR ($CDCl_3$): δ ppm 7.54 (d, 1H), 3.73 (m, 1H), 3.37 (m, 4H), 2.84 (m, 1H), 2.65 (s, 3H), 1.93 (m, 2H), 1.63 (m, 2H), 1.23 (m, 3 H). 0.90 (bs, 1H), 0.51 (m, 2H);
LC-MS (Method C) 391.5 $[M+H]^+$; RT 6.34 min. Main isotope reported

REDX06937- 6-chloro-5-cyclopropyl-8-fluoro-2-methyl-7-piperazin-1-yl-oxazolo[4,5-c]quinolin-4-one



Prepared using tert-butyl piperazine-1-carboxylate as described in **GP1**, **GP2** and **GP3** as a reddish brown solid.
^{1}H NMR ($CDCl_3$): δ ppm 7.4 (d, 1H), 3.75 (m, 1H), 3.4 (m, 4H), 3.1 (m, 4H), 2.6 (s, 3H), 1.75 (m, 2H), 0.5 (m, 2H);
LC-MS (Method C) 377.1 $[M+H]^+$; RT 6.00 min. Main isotope reported.

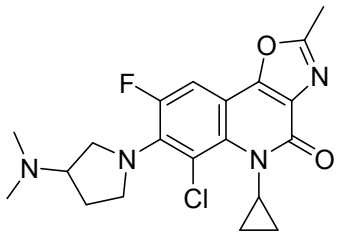
REDX07156 - 6-chloro-5-cyclopropyl-7-[3-[(dimethylamino)methyl]pyrrolidin-1-yl]-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one



Prepared using N,N-dimethyl-1-(pyrrolidin-3-yl)methanamine as described in **GP2** and **GP3** as a pale yellow solid

^{1}H NMR ($CDCl_3$): δ ppm 7.35 (d, *J* = 7.6 Hz, 1H), 3.72 (m, 2H), 3.65 (q, *J* = 8.0 Hz, 2H), 3.50 (m, 1H), 3.40 (t, *J* = 8.0 Hz, 1H), 2.65 (s, 3H), 2.50 (quin, *J* = 8.0 Hz, 1H), 2.40 (m, 2H), 2.30 (s, 6H), 2.15 (m, 1H), 1.72 (m, 1H), 1.26 (m, 3H), 0.55 (m, 2H);
LC-MS (Method D) 419.5/421.5 [M^+ H^+], RT 7.73 min. Main isotope reported

REDX06885 - 6-chloro-5-cyclopropyl-7-[3-(dimethylamino)pyrrolidin-1-yl]-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one

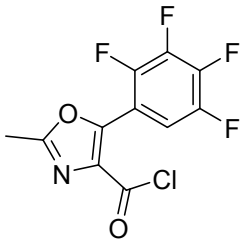


Prepared using N,N-dimethylpyrrolidin-3-amine as described in **GP2** and **GP3** as a pale yellow solid

^{1}H NMR ($CDCl_3$): δ ppm 7.35 (d, *J* = 7.4 Hz, 1H), 3.75 (m, 2H), 3.62 (m, 1H), 3.50 (m, 2H), 2.95 (quin, *J* = 8.0 Hz, 1H), 2.65 (s, 3H), 2.32 (s, 6H), 2.20 (m, 1H), 1.95 (m, 1H), 1.25 (m, 2H), 0.50 (m, 2H);
LC-MS (Method C) 405.4 [M^+ H^+], RT 4.89 min. Main isotope reported

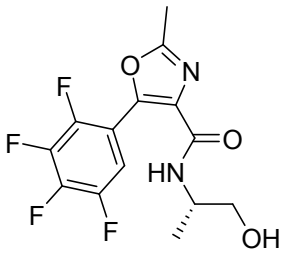
REDX06215 – (2S)-6-[3-(aminomethyl)pyrrolidin-1-yl]-7-fluoro-2,12-dimethyl-4,11-dioxa-1,13-diazatetracyclo[7.6.1.0^{5},^{16}.0^{10},^{14}]hexadeca-5(16),6,8,10(14),12-pentaen-15-one

1. 2-methyl-5-(2,3,4,5-tetrafluorophenyl)oxazole-4-carbonyl chloride



Prepared using 2,3,4,5-tetrafluorobenzoic acid (compound 10) as described in intermediate 2 (step a) and used directly in the following step.

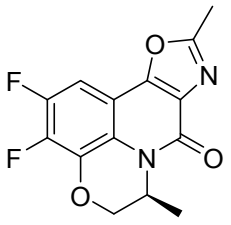
2. (S)-N-(1-hydroxypropan-2-yl)-2-methyl-5-(2,3,4,5-tetrafluorophenyl)oxazole-4-carboxamide



2-Methyl-5-(2,3,4,5-tetrafluorophenyl)oxazole-4-carbonyl chloride (1.54 g, 5.23 mmol) in DCM (75 mL) was treated with (2S)-(+)-2-aminopropan-1-ol (0.85 mL, 10.98 mmol) and stirred at room temperature overnight. The mixture was then diluted with DCM (50 mL) and washed with 0.5N aqueous HCl (2 x 50 ml) then saturated aqueous.$NaHCO_3$. (3 x 30 mL) and brine (30 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to *in vacuo* to give N-[(1S)-1-hydroxpropan-2-yl]-2-methyl-5-(2,3,4,5-tetrafluorophenyl)oxazole-4-carboxamide (1.34 g, 77 % yield) as a yellow powder.

LC-MS (Method A) 333.0 [M^+ H^+], RT 3.42 min.

3. Intermediate 11 - (S)-2,3-difluoro-6,10-dimethyl-5,6-dihydro-8H-[1,4]oxazino[2,3,4-ij]oxazolo[4,5-c]quinolin-8-one

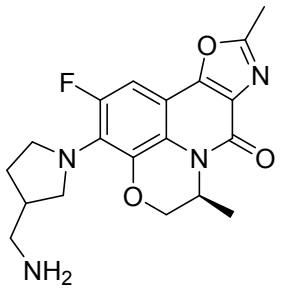


A mixture of N-[(1S)-1-hydroxypropan-2-yl]--2-methyl-5-(2,3,4,5-tetrafluorophenyl)oxazole-4-carboxamide (1.51 g, 4.54 mmol), 18-crown-6 (1.35 g, 5.09 mmol) and K_2CO_3 (3.14 g, 22.72 mmol) in DMSO (30 mL) was heated at 140^{o}C for 40 min The mixture was diluted with EtOAc

(200 mL) and washed with H_2O (5 x 50 ml) then brine (100 ml). The organic extract was dried over Na_2SO_4 and solvent removed *in vacuo* to give a brown oil which was purified by flash chromatography eluting with 0-100% EtOAc in heptane to give (2S)-6,7-difluoro-2,12-dimethyl-4,11-dioxa-1,13-diazatetracyclo [7.6.1.0^{5},^{16}.0^{10},^{14}] hexadeca-5(16),6,8,10(14),12-pentaen-15-one (490 mg, 37 % yield) as a yellow foam.

LC-MS (Method A) 293.1 [M^+ H^+], RT 3.43 min.

4. (6S)-3-(3-(aminomethyl)pyrrolidin-1-yl)-2-fluoro-6,10-dimethyl-5,6-dihydro-8H-[1,4]oxazino[2,3,4-ij]oxazolo[4,5-c]quinolin-8-one

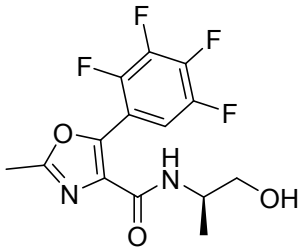


Prepared using tert-butyl N-(pyrrolidin-3-ylmethyl)carbamate and (2S)-6,7-difluoro-2,12-dimethyl-4,11-dioxa-1,13-diazatetracyclo [7.6.1.0^{5},^{16}.0^{10},^{14}] hexadeca-5(16),6,8,10(14),12-pentaen-15-one as described in **GP1** and **GP3**

^{1}H NMR (400MHz, CD_3OD): δ ppm 8.35 (br s, 1H), 7.23 (m, 1H), 5.15 (m, 1H), 4.56 (d, *J* = 11.14Hz, 1H), 4.13 (m, 1H), 3.76-3.48 (m, 4H), 3.25-3.05 (m, 2H), 2.67 (s, 3H), 2.61 (m, 1H), 2.28 (m, 1H), 1.80 (m, 1H), 1.38 (m, 3H);
LC-MS (Method A) 373.1 [M^+ H^+], RT 2.27 min.

REDX06415 - (6S)-3-(3-(aminomethyl)pyrrolidin-1-yl)-2-fluoro-6,10-dimethyl-5,6-dihydro-8H-[1,4]oxazino[2,3,4-ij]oxazolo[4,5-c]quinolin-8-one

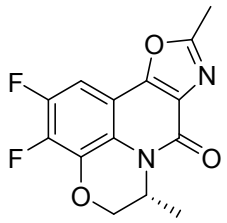
1. N-[(1R)-2-hydroxy-1-methyl-ethyl]-2-methyl-5-(2,3,4,5-tetrafluorophenyl)oxazole-4-carboxamide



Prepared as described for REDX06215, step 2, using (R)-(-)-2-amino-1-propanol as a yellow powder.

LC-MS (Method A) 293 .1 $[M+H]^+$, RT 3 .43 min.

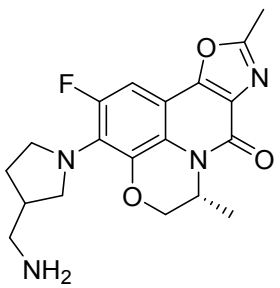
2. (R)-2,3-difluoro-6,10-dimethyl-5,6-dihydro-8H-[1,4]oxazino[2,3,4-ij]oxazolo[4,5-c]quinolin-8-one



Prepared as described for REDX06215, step 3.

^{1}H NMR (400MHz, $CDCl_3$): δ ppm 8.14 (s, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.32–7.23 (m, 1H), 7.08 (dd, *J* = 11.2, 6.4 Hz, 1H), 6.59 (dd, *J* = 10.7, 7.5 Hz, 1H), 5.36–5.25 (m, 1H), 4.42 (dd, *J* = 11.4, 1.3 Hz, 1H), 4.15 (dd, *J* = 11.4, 2.4 Hz, 1H), 3.95 (s, 2H), 1.45 (d, *J* = 6.6 Hz, 3H);
LC-MS (Method B) 370.4 $[M+H]^+$; RT 2.54 min.

3. (6R)-3-(3-(aminomethyl)pyrrolidin-1-yl)-2-fluoro-6,10-dimethyl-5,6-dihydro-8H-[1,4]oxazino[2,3,4-ij]oxazolo[4,5-c]quinolin-8-one

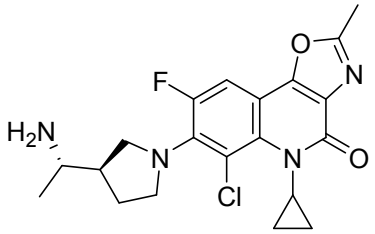


Prepared using 2S)-6-(4-amino-2,5-difluorophenyl)-2-methyl-4,11-dioxa-1,13-diazatetracyclo[7.6.1.0^{5},^{16}.0^{10},^{14}]hexadeca-5(16),6,8,10(14),12-pentaen-15-one and tert-butyl (pyrrolidin-3-ylmethyl)carbamate as described in **GP1** and **GP3** to give a white solid

^{1}H NMR ($CDCl_3$): δ ppm 7.13 (d, J= 16.6 Hz, 1H), 5.25 (m, , 1H), 4.45 (m, 1H), 4.16 (m, 1H), 3.82-3.55 (m, 4H, diastereomers), 2.82 (m, 2H), 2.66 (s, 3H), 2.34 (m, 1H), 2.10 (m, 1H), 1.68 (m, 1H), 1.40 (m, 3H);

LC-MS (Method C) 373 $[M+H]^+$; RT 5.01 min.

REDX07965 - 7-[(3R)-3-[(1S)-1-Aminoethyl]pyrrolidin-1-yl]-6-chloro-5-cyclopropyl-8-fluoro-2-methyl-oxazolo[4,5-c]quinolin-4-one



Prepared using intermediate **6** and tert-butyl ((S)-1-((R)-pyrrolidin-3-yl)ethyl)carbamate as described in **GP1, GP2** and **GP3** to give a white solid

^{1}H NMR (400MHz, DMSO-d_6) δ 7.60 (d, *J* = 12.13 Hz, 1H), 3.66-3.72 m, 1H), 3.54 – 3.59 (m, 1H), 3.48-3.52 (m, 2H), 3.39-3.45 (m, 2H), 2.70-2.77 (m, 1H), 2.60 (s, 3H), 1.95-2.09 (m, 3H), 1.58-1.67 (m, 1H), 1.08-1.21 (m, 2H), 1.04-1.08 (d, *J* = 6.27, 3H), 0.39-0.49 (m, 2H);

LC-MS (Method C) 405.1 / 407.0 $[M+H]^+$; RT 5.20 min.

Antibacterial susceptibility testing

Culturing and antibacterial susceptibility testing were performed in line with CLSI guidelines[1]. MICs were determined by the broth microdilution method for routine testing and by the agar dilution method for *N*. *gonorrhoeae*. Clinical isolate testing of *Staphylococcus* spp. and *Streptococcus* spp. was performed by IHMA Europe Sàrl (Epalinges, Switzerland) according to CLSI guidelines. Susceptibility testing of clinical isolates included a panel consisting of *Staphylococcus aureus* from 29 different countries.

Table S1 MIC_{90} for REDX07965 against panels of *S. aureus* strains:

Resistance Phenotype	No. Strains (Grew)	Levofloxacin	Vancomycin	Linezolid	REDX07965
All	246 (245)	8	1	2	0.5
MRSA, all	75 (75)	>16	2	2	1
MRSA, LFX-S	35 (35)	0.25	1	2	≤0.12
MRSA, LFX-R	26 (26)	>16	1	2	1
MSSA, all	65 (65)	16	1	2	0.5
MSSA, LFX-S	39 (39)	0.25	1	2	≤0.12
MSSA, LFX-R	21 (21)	>16	1	2	0.5

Enzyme assays

DNA supercoiling, decatenation and cleavage complex assays were all performed by Inspiralis (Norwich, UK). Briefly, one unit of *S. aureus* DNA gyrase was incubated with 0.5 mg of relaxed pBR322 and one unit of topoisomerase IV or human topoisomerase II was incubated with 200 ng of kDNA, all in a reaction volume of 30 mL at 37°C for 30 min in the presence of a series of

concentrations of the test compound. Human topoisomerase II activity was assessed in the presence of 100 mM test compound. Supercoiling reactions were conducted under the following conditions: 40 mM HEPES.KOH (pH 7.6), 10 mM magnesium acetate, 10 mM DTT, 2 mM ATP, 500 mM potassium glutamate and 0.05 mg/mL BSA. *S*. *aureus* topoisomerase IV decatenation reactions were conducted under the following conditions: 50 mM Tris-HCl (pH 7.5), 5 mM MgCl2, 5 mM DTT, 1.5 mM ATP, 350 mM potassium glutamate and 0.05 mg/mL BSA. Human topoisomerase II reactions were conducted under the following conditions: 50 mM Tris-HCl (pH 7.5),125 mM NaCl, 10 mMMgCl2, 5 mM DTT, 0.5 mM EDTA, 0.1 mg/mL BSA and 1 mM ATP. Reactions were stopped using 30 mL of chloroform/isoamylalcohol (26: 1) and 20 mL of Stop Dye [40% sucrose, 100 mM Tris-HCl(pH 7.5), 1 mM EDTA and 0.5 mg/mL bromophenol blue]. Topoisomers were visualized by ethidium bromide staining, resolved and quantified by gel electrophoresis and the band intensities analysed by gel documentation equipment (Syngene, Cambridge, UK) and quantified using Syngene Gene Tools software. Raw data were converted to a percentage of the inhibitor-free control and analysed using SigmaPlot Version 12.5. Non-linear regression was used to calculate the IC_{50}. The human topoisomerase II inhibitor etoposide was used as a positive control for inhibition for this assay. For cleavage complex assays, *S. aureus* DNA gyrase (one unit) was incubated with 0.5 mg of supercoiled pBR322 DNA at 37 ^{o}C for 60 min. Reactions were performed in a volume of 30 mL using the following conditions:40 mM HEPES.KOH (pH 7.6), 10 mM magnesium acetate, 10 mMDTT, 500 mM potassium glutamate and 0.05 mg/mL BSA. Following this, reactions were incubated for 30 min with 0.2% SDS and 0.5 mg/mL proteinase K. Reactions were stopped in the same manner as for the supercoiling and decatenation assays. Topoisomers and cleavage products were visualized by gel electrophoresis. Cleavage products were

expressed as a percentage of the fully supercoiled inhibitor-free control as described for the supercoiling and decatenation assays.

Table S2: Enzyme data of selected compounds

Compound ID	*S. aureus* DNA gyrase Supercoiling IC_{50} (μM)	*S. aureus* Topoisomerase IV Decatenation IC_{50} (μM)	Human Topoisomerase II Decatenation IC_{50} (μM)
Ciprofloxacin	21.5	4.04	>100
REDX07965	1.82	6.10	42.2

References

1. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard–Ninth Edition M07-A9*. CLSI, Wayne, PA, USA, 2012.