Supporting Information

Mechanistic and Structural Basis for the Actions of the Antibacterial Gepotidacin against *Staphylococcus aureus* Gyrase

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Table S1

Data collection		
Beamline	ESRF ID23-2	ESRF
Space group	$P6_1$	$P2_1$
Cell dimensions a,b,c (Å); α, β, γ (°)	92.73, 92.73, 408.78; 90.0, 90.0, 120.0	86.34, 123.65, 94.10; 90.0, 117.1, 90.0
Wavelength (Å)	0.8726	0.97242
Resolution range (Å)	40-2.31 (2.35-2.31)	50.0-2.37 (2.46-2.37)
No. of unique reflections	85905 (4236)	70324 (6522)
Multiplicity	5.5 (5.6)	3.4 (3.4)
Completeness (%)	99.7 (99.9)	99.0 (93.9)
R_{merge} (%)	9.4 (48.0)	9.9 (112.4)
I/σI	19.2 (3.7)	7.3 (0.9)
Refinement		
Resolution (Å)	2.31 (2.37-2.31)	2.37 (2.43-2.37)
No. reflections (work/free)	82431 /3426	66745 / 3535
R_{work}/ R_{free} (%)	16.7/20.5 (22.3/28.3)	18.5/22.5 (29.7/36.2)
No. Atoms	12711	12243
Protein	10926	10675
DNA	850	872
Ligand/ion	130	86
Water	805	610
B-factors		
Protein	35.6	53.9
DNA	36.8	49.2
Ligand/ion	44.9*	43.7
Water	40.3	50.2
R.m.s deviations		
Bond lengths (Å)	0.007	0.002
Bond angles (°)	1.46	0.778

Table S1. X-ray data collection and refinement statistics. Crystallographic parameters for the structures of the two gepotidacin complexes with *S. aureus* gyrase and DNA are given in the table. The 2.31 Å structure containing the *S. aureus* gyrase core fusion truncate that carries a $GyrA^{Y123F}$ mutation and nicked DNA is shown in the middle column and the 2.37 Å structure containing the wild-type gyrase truncate and intact DNA is shown in the right column. *Includes 9 glycerols; average B-factor for gepotidacin is 37.3.

Table S2

PDB codes (and resolution in Å) for structures with this DNA	DNA DUPLEX NAME		DNA SEQUENCE Note all DNAs, when annealed, form 20 base-pair duplexes.
	Position* 5'-3' 3'-5'		-8 -5 -4 **-1** **1** 2 3 4 5 8 9 12 Ile Ile 12 9 8 5 4 3 2 **1** **-1** -4 -5 -8
x944 (2.31)	20-12p-8 20-12p-8	5'-3' 3'-5'	AGCC GTA**G** **^{P}G** **T** **A** C CTAC GGCT TCGG CAT C **A** **T** **G^{P}** **G**ATG CCGA
x944 (2.37)	20-444T 20-444T	5'-3' 3'-5'	GAGC GTAC A **G** **C** T GTAC GCTT TTCG CATG T **C** **G** A CATG CGAG
2xcs(2.1), 2xcr (3.5), *5bs3(2.65), 4plb(2.69)*	20-20 20-20	5'-3' 3'-5'	AGCC GTAG G **G** **C** C CTAC GGCT TCGG CATC C **C** **G** G GATG CCGA
5iwm (2.5)	20-21 20-21cmp.	5'-3' 3'-5'	TGTG CGGT G **A** **A** C CTAC GGCT ACAC GCCA C **T** **T** G GATG CCGA
4bul(2.6)	20-23 20-23cmp.	5'-3' 3'-5'	TGTG CGG**T** **G** **T** **A** C CTAC GGCT ACAC GCCA C **A** **T** **G** **G**ATG CCGA
5iwi (1.98)	20-12-8^{23} 20-23cmp.	5'-3' 3'-5'	TGTG CGG**T** **G** **T** **A** C CTAC GGCT ACAC GCCA C **A** **T** G GATG CCGA

Table S2. Comparison of DNA sequences used in *S. aureus* gyrase core fusion truncate crystal structures with twofold-axis pockets that bind NBTIs. DNAs are self-complementary and form 20 base-pair duplexes; the left-hand side of gepotidacin sits in the middle of the DNA between bases, 2 and 3 (and 3 and 2). The 20-12p-8 DNA duplex has a nick in the DNA at each cleavage site, and the 5' nucleotide of the 12mer includes a 5' phosphate (indicated by a ^{P}). The nucleotides on either side of the DNA-cleavage site are in red letters. If the two DNA nucleotides are covalently linked in the crystal structure, this is indicated by a red line. The bottom three DNA sequences are heteroduplexes (20-21/20-21cmp, 20-23/20-23cmp., and 20-12-8^{23}/20-23cmp) and crystal structures with these DNAs had static disorder and with the DNA observed in two orientations related by the twofold axis of the complex. By convention, for DNA sites cleaved by type IIA topoisomerases, the DNA sequences are numbered relative to the cleavage position between -1 and 1 nucleotides. In the crystal structures in the table, there is no ambiguity as to the register of oligos (because there is clear electron density for twenty nucleotides in at least one strand).

Figure S1

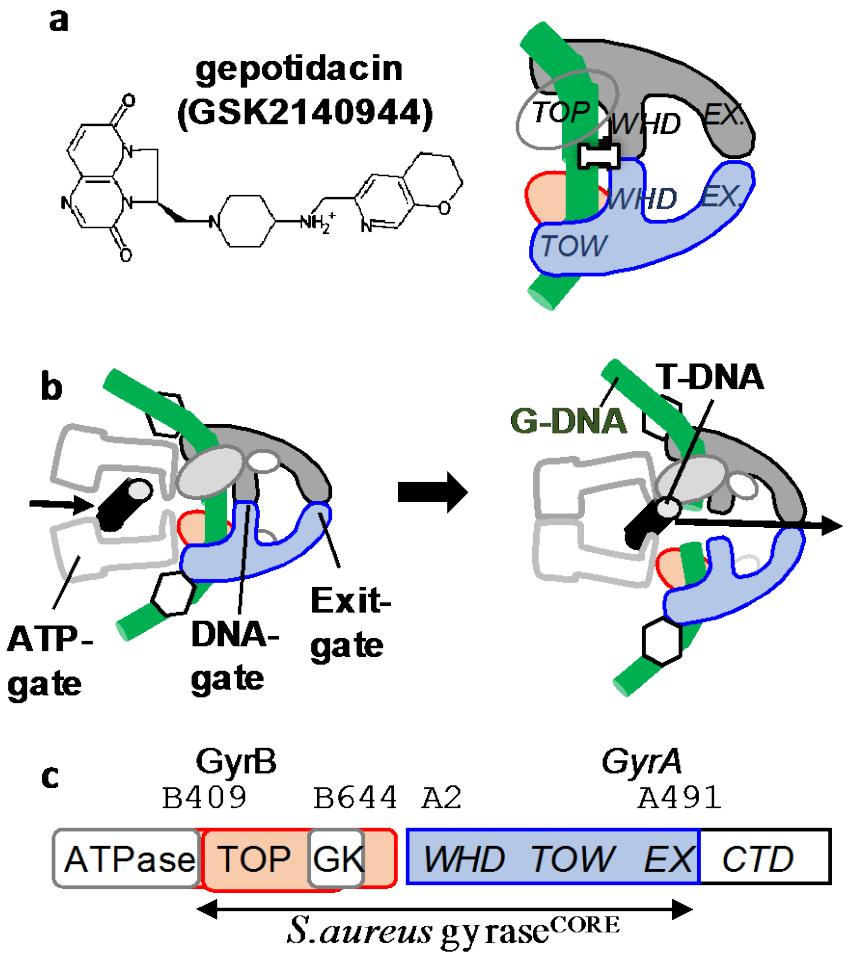

Figure S1. Schematic of gepotidacin interactions with *S. aureus*, DNA gyrase, and DNA. **(a)** A simplified schematic of how the inhibitor gepotidacin (represented by the letter **I** in the schematic) binds to the *S. aureus* gyrase core dimer (one covalently fused GyrBA subunit shown in red and blue the other in grey) and a 20 base-pair G-DNA duplex (green cylinder). Note the compound is in similar orientations in both parts of the panel. **(b)** Simplified schematic of reaction carried out by type IIA topoisomerases. The gate or G-DNA (green cylinder) is cleaved and another DNA duplex, the T (or transport segment - black) is passed through the cleaved DNA before religation. In this view the T-segment is passed from left to right. **(c)** DNA gyrase consists of two subunits, GyrB and GyrA. The *S. aureus* gyrase core fusion truncate construct used to determine crystal structures discussed in this paper is a fusion of the C-terminal TOPRIM (TOP) domain of GyrB with the N-terminal winged helical domain (WHD), tower (TOW) and exit-gate (EX) domains from GyrA. The small Greek key (GK) domain has been deleted.

Figure S2

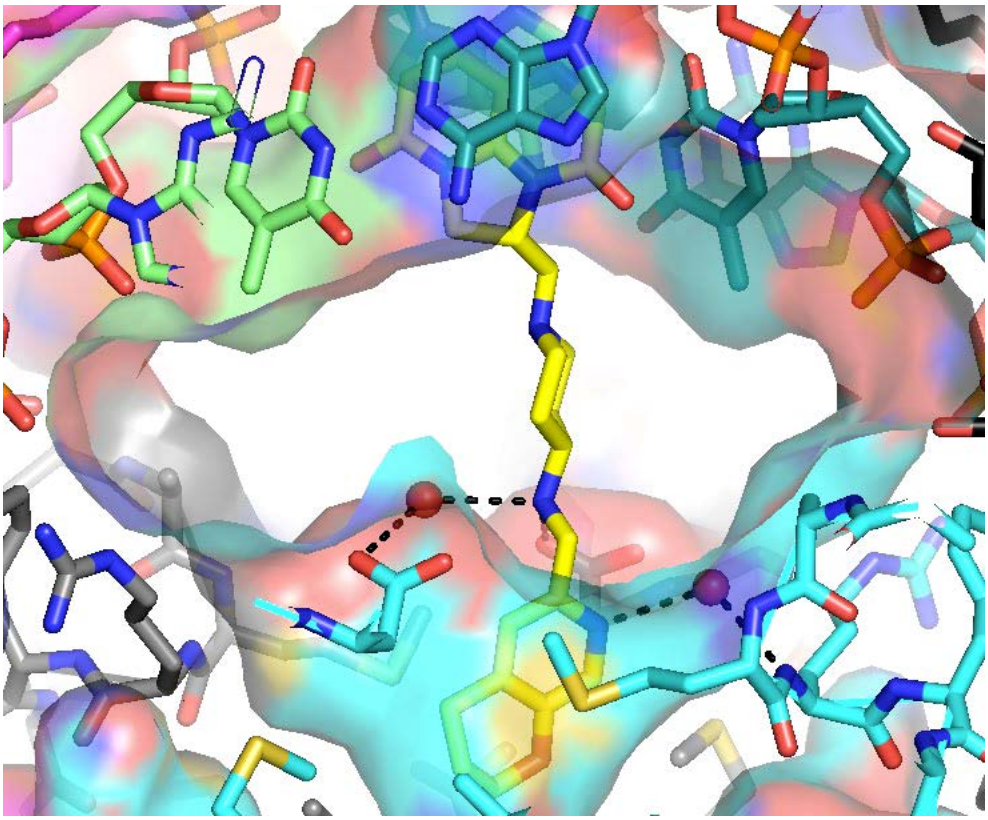

Figure S2. Animation of the interaction between gepotidacin and the *S. aureus* gyrase-DNA complex based on the 2.31Å crystal structure formed with doubly nicked duplex DNA. Gepotidacin is shown in yellow (carbon atoms) and blue (nitrogen atoms) and water molecules are shown in red spheres. For other assignments see Figure 11.

Figure S3

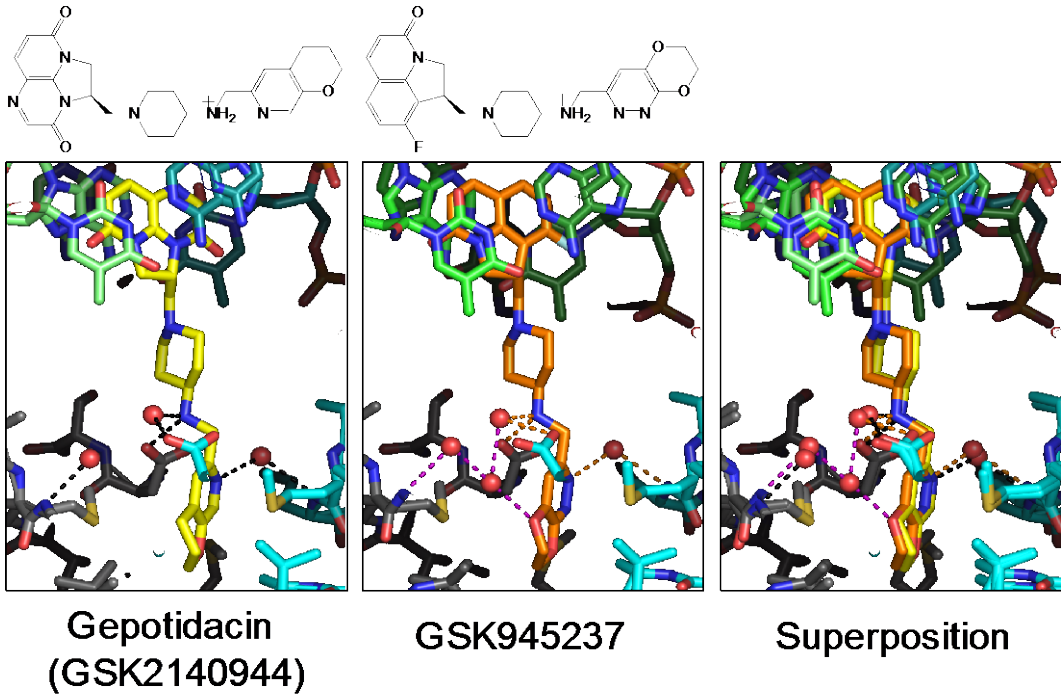

Figure S3: Comparison of complexes formed with gepotidacin or GSK945237 and *S. aureus* gyrase and DNA. The left panel shows the 2.31Å resolution complex with gepotidacin (yellow carbons) and the middle panel shows a 1.98Å resolution complex with GSK945237 (orange carbons) (PDB code: 5iwi). The chemical structures of the compounds are shown above the corresponding panels. The right panel shows the superposition of both complexes. Colors are as shown in Figure 11, except that the carbon atoms in GSK945237 (middle and left panels) are shown in orange.