

Supporting Information

The Structural Features of Novel Bacterial Topoisomerase Inhibitors that Define Their Activity on Topoisomerase IV

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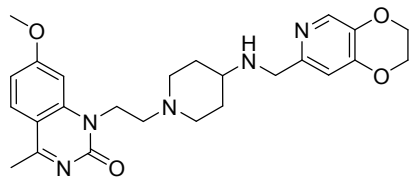
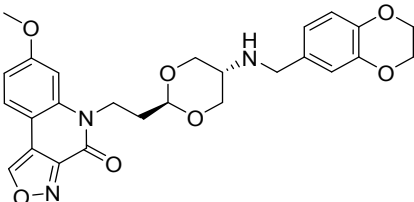
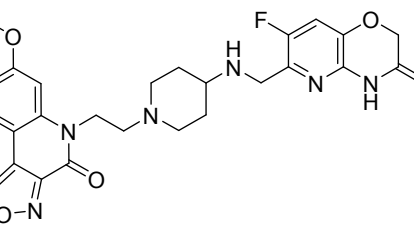
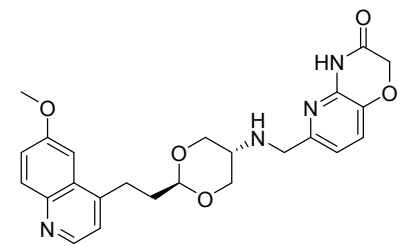
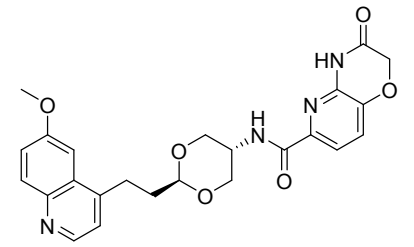
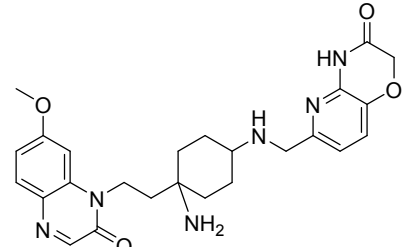
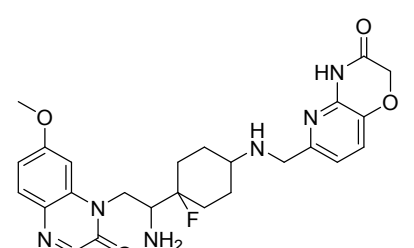
Representatives of NBTI antibacterials targeting topoIV enzymes

Table S1. Comparison of topoIV inhibition data for the most representative Gram-positive (e.g., *S. aureus*) and Gram-negative (e.g., *E. coli*) bacterial pathogens for a panel of selected NBTIs.

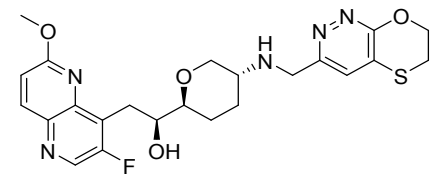
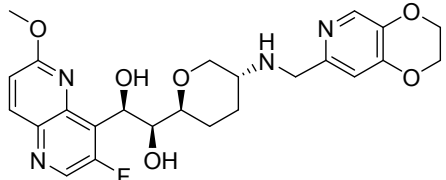
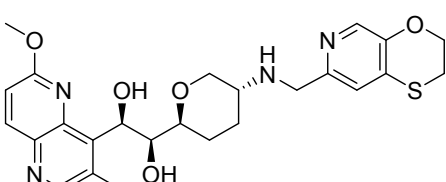
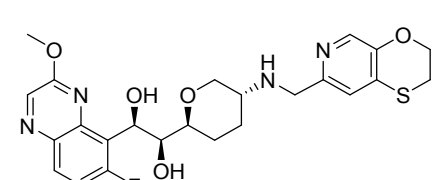
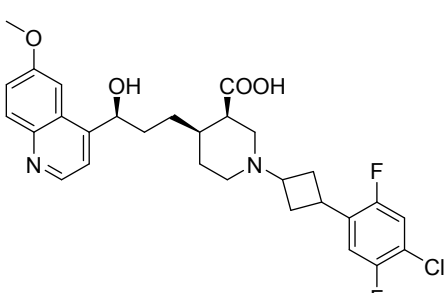
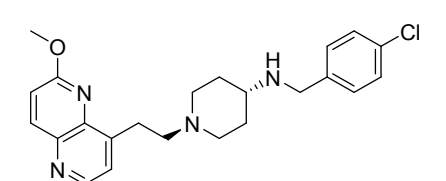
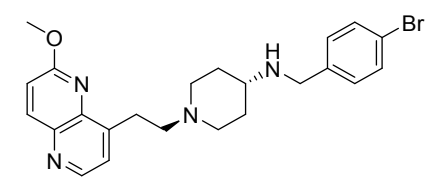
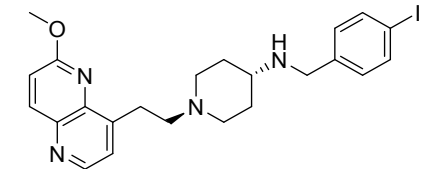
ID	Structure	topoIV IC ₅₀ [μM] ^{a,b,c,d}		Ref. (No. of ref. in the paper)
		<i>S. aureus</i>	<i>E. coli</i>	
1		ND	4.680	1 (53)
2		ND	0.036	1 (53)
3		15	ND	2 (42)
4		6.25	ND	3 (36)
5		ND	0.0013	4 (50)
6		ND	0.002	4 (50)

7		ND	0.005	4 (50)
8		8	0.125	5 (48)
9		ND	0.125	6 (49)
10		0.98	ND	7 (43)
11		>100	ND	7 (43)
12		0.5	0.125	8 (37)
13		0.5	0.125	8 (37)
14		8	0.125	8 (37)
15		8	0.125	5 (48)

16		8	0.5	5 (48)
17		8	0.125	8 (37)
18		2	0.125	8 (37)
19		0.5	0.03	8 (37)
20		ND	0.028	4 (50)
21		ND	0.022	4 (50)
22		12.8	0.22	9 (56)
23		ND	0.005	4 (50)
24		ND	0.78	4 (50)

25		ND	0.082	4 (50)
26		>200	ND	7 (43)
27		ND	0.10	10 (47)
28		>100	ND	11 (45)
29		0.19	ND	12 (46)
30		ND	0.002	13 (52)
31		ND	0.005	13 (52)

32		>8	0.125	8 (37)
33		>8	0.125	8 (37)
34		8	0.125	8 (37)
35		>8	0.125	8 (37)
36		ND	0.03	6 (49)
37		ND	0.125	6 (49)
38		18 ^b 0.3 ^c	2.2 ^b 0.2 ^c	14 (19)
39		32	2	5 (48)
40		18	ND	7 (43)

41		8	0.125	5 (48)
42		8	0.125	8 (37)
43		8	0.125	8 (37)
44		8	0.125	8 (37)
45		120	ND	2 (42)
46		16.37	0.031	15 (38)
47		7.22	0.042	15 (38)
48		2.13	0.021	15 (38)

49		0.24	0.105	16 (41)
50		0.05	0.033	16 (41)

^aATPase activity assay: references 1,4,13; ^btopoIV Relaxation Assay: references 5,6,8,14; ^ctopoIV Relaxation High Throughput Plate Assay: references 15,16; ^dtopoIV Decatenation Assay: references 2,3,7,9,11,12,14; ND = not determined.

Superposition of co-crystal ligands from DNA gyrase/DNA/NBTI complexes

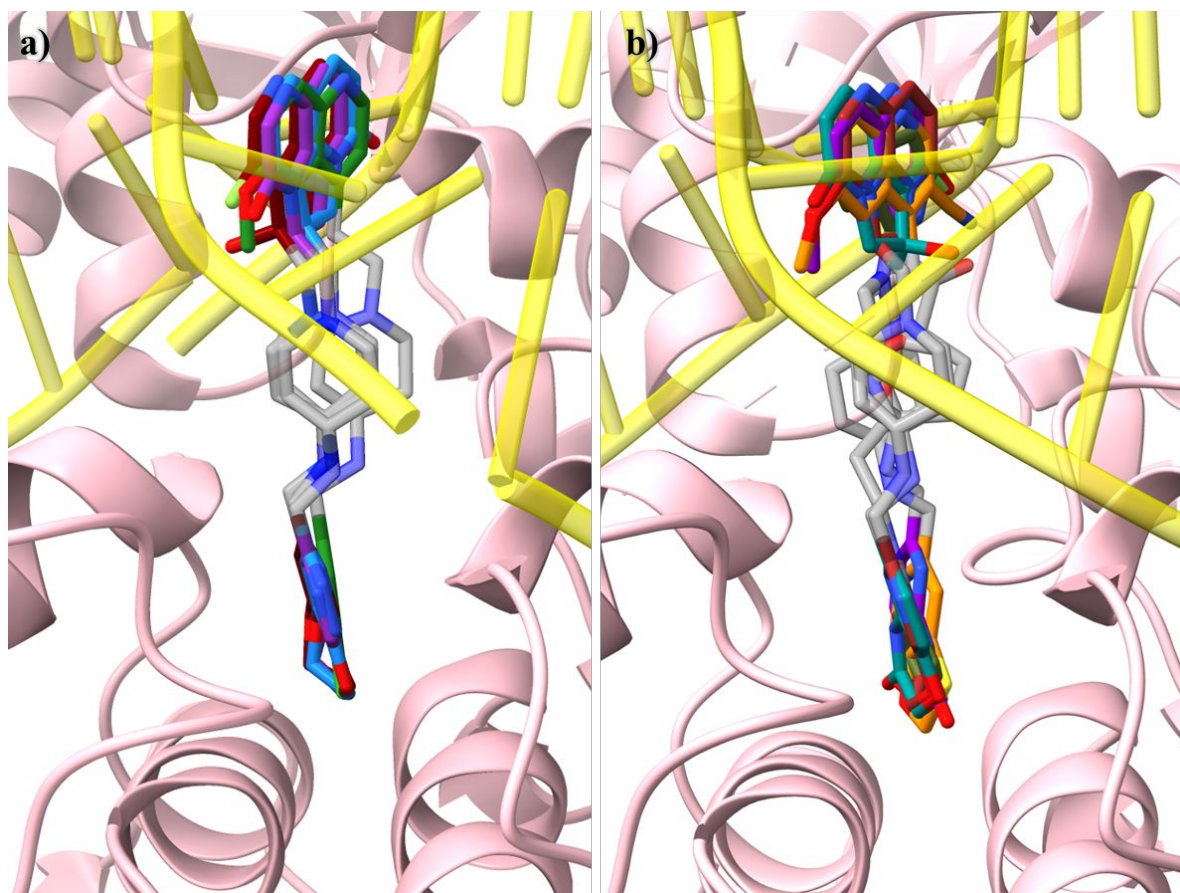


Figure S1. Structural superposition of the co-crystal ligands from available *S. aureus* DNA gyrase-DNA-NBTI complexes. a) Central base pairs are thymine (T) and adenine (A). The ligand from complex with PDB ID 6Z1A¹⁷ is in forest green, 4BUL¹⁸ in dark red, 5IWI¹⁹ in dodger blue, and 6QTK²⁰ in light violet. b) The central DNA base pairs are guanine (G) and cytosine (C). The ligand from complex with PDB ID 2XCS²¹ is in dark orange, 4PLB²² in dark violet, 6FM4²³ in dark olive green, 6QTP²⁰ in brown, and 5BS3 in dark cyan. LHS and RHS fragments are colored with the mentioned colors, while linker moieties are in transparent gray. DNA gyrase and DNA are in ribbon presentation and colored in pink and yellow, respectively.

Comparison of GyrA and ParC amino acid sequences of various Gram-positive and Gram-negative bacteria

<i>S. aureus</i> GyrA	61	DKSYKKSARIVGDVVGKYHPHGDL	LSIYEAMVRMAQDFSYRY	101	
<i>S. aureus</i> ParC	57	DKNFRKSAKTVGDVIGQYHPHGDS	SSVYEAMVRLSQDWKLRH	97	
<i>S. agalactiae</i> GyrA	58	DKPHKKSARITGDVVGKYHPHGDS	SSIYEAMVRMAQWWSYRH	98	
<i>S. agalactiae</i> ParC	56	EKGFRKSAKSVGNVMGNFHPHGDS	SIYDAMVRMSQDWKNRE	96	
<i>E. faecalis</i> GyrA	61	DKPHKKSARIVGDVVGKYHPHGDS	SAIYESMVRMAQPFYSYA	101	
<i>E. faecalis</i> ParC	59	DKGFRKSAKSVGNI	MGNYPHGHGDS	SIYEAMVRLSQDWKLRE	99
<i>E. coli</i> GyrA	60	NKAYKKSARVVG	DVIGKYHPHGDS	SAVYDTIVRMAQPFSLRY	100
<i>E. coli</i> ParC	57	SAKFKKSARTVGD	VLGKYHPHGDS	SACYEAMVLMAQPFYSRY	97
<i>K. pneumoniae</i> GyrA	60	NKAYKKSARVVG	DVIGKYHPHGDI	AVYDTIVRMAQPFSLRY	100
<i>K. pneumoniae</i> ParC	57	SAKFKKSARTVGD	VLGKYHPHGDI	ACYEAMVLMAQPFYSRY	97
<i>S. alachua</i> GyrA	60	NKAYKKSARVVG	DVIGKYHPHGDS	SAVYDTIVRMAQPFSLRY	100
<i>S. alachua</i> ParC	57	SAKFKKSARTVGD	VLGKYHPHGDS	SACYEAMVLMAQPFYSRY	97
<i>P. aeruginosa</i> GyrA	60	NKPYKKSARVVG	DVIGKYHPHGDT	AVYDTIVRMAQPFSLRY	100
<i>P. aeruginosa</i> ParC	64	DSKHKKSARTVGD	VLGKFHPHGDS	SACYEAMVLMAQPFYSRY	104
<i>N. gonorrhoeae</i> GyrA	68	NAAKKSARIVGD	VIGKYHPHGDS	SAVYDTIVRMAQNFMRY	108
<i>N. gonorrhoeae</i> ParC	64	GAKPVKSARVVG	GEILGKYHPHGDS	SSAYEAMVRMAQDFTLRY	104

Figure S2. Comparison of amino acid sequences of DNA gyrase GyrA and topoIV ParC subunits from various Gram-positive (*S. aureus*, *S. agalactiae*, *E. faecalis*, in yellow) and Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *S. alachua*, *P. aeruginosa*, *N. gonorrhoeae*, in green). The key amino acid residues for binding of NBTIs are highlighted in blue and red.

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