

Supplementary Tables

Table S1. NMR spectral data of hygromycin A in DMSO-*d*₆. Related to Figure 1.

	δ_c	δ_{H^a} multi (J in Hz)
C-1	69.4	3.70, m
C-2	49.2	4.31 (ddd, 9.0, 6.0, 3.0)
C-3	69.4	3.57 (dd, 7.1, 3.8)
C-4	76.5	4.09 (dd, 6.4, 3.9)
C-5	77.1	4.02 (m)
C-6	69.9	4.02 (m)
-OCH ₂ O-	94.1	5.13 (s), 4.69 (s)
1-OH		5.02 (d, 5.7)
2-NH		7.25 (d, 9.1)
3-OH		5.11 (d, 4.3)
6-OH		5.06 (d, 5.8)
C-1'	169.0	
C-2'	131.2	
C-3'	132.1	7.14 (s)
C-4'	130.7	
C-5'	116.6	6.88 (d, 2.1)
C-6'	146.5	
C-7'	144.0	
C-8'	115.7	7.15 (d, 8.5)
C-9'	121.0	6.85 (dd, 8.5, 2.1)
2'-CH ₃	14.5	2.03 (d, 1.3)
C-1''	101.3	5.65 (d, 4.2)
C-2''	77.0	4.02 (m)
C-3''	76.0	4.19 (m)
C-4''	87.2	4.19 (m)
C-5''	207.6	
5''-CH ₃	25.9	2.02 (s)
3''-OH		5.86 (m)

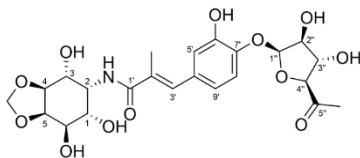


Table S2. Comparison of ¹³C NMR chemical shifts for hygromycin A (CD₃OD) isolated in this study with that in the literature (Yoshida et al., 1985). Related to Figure 1.

This study	Yoshida et al., 1985	Chemical shift difference
210.1	210.1	0
172.7	172.6	0.1
148.4	148.4	0
146.1	146.0	0.1
135.1	135.0	0.1
133.0	132.2	0.8
132.4	132.2	0.2
122.7	122.4	0.3
118.5	117.8	0.7
117.9	117.8	0.1
103.8	103.7	0.1
96.3	96.2	0.1
88.6	88.5	0.1
78.7	78.6	0.1
78.3	78.2	0.1
78.3	78.2	0.1
77.7	75.9	1.8
72.7	72.6	0.1
71.7	71.6	0.1
71.4	71.3	0.1
50.4	50.7	-0.3
26.3	26.2	0.1
14.7	14.6	0.1

Table S3. MIC of *E. coli* wild-type and mutant strains. Related to Table 1.

<i>E. coli</i> strain	Description	HygA (μg/ml)
WT	BW25113	512
ΔtolC	no efflux	16
WT-Pore	hyperporinated	512
ΔtolC-Pore	no efflux, hyperporinated	16

Table S4. Polymorphisms found in *B. turcica* KLEx1 as compared to its parental strain. All deletions and insertions are single-base. Related to Figure 3.

Locus Tag	Description	Polymorphism Type	Protein Effect
DB313_RS00990	RelA/SpoT Family CDS	SNP	Substitution
DB313_RS02155 (23S)	23S rRNA gene	SNP	N/A
DB313_RS03380	Alpha/beta fold hydrolase	Deletion	Frame Shift
DB313_RS04445	Hypothetical Protein	SNP	Substitution
DB313_RS04455	Hypothetical Protein	Substitution	Substitution
DB313_RS04455	Hypothetical Protein	SNP	Substitution
DB313_RS04455	Hypothetical Protein	SNP	Substitution
DB313_RS04480	DUF871 domain-containing protein	SNP	Substitution
DB313_RS04480	DUF871 domain-containing protein	SNP	Substitution

N/A = does not code for a protein (RNA)

Table S5. Polymorphisms found in *B. burgdorferi* KLEx2 as compared to its parental strain. All deletions and insertions are single-base. Related to Figure 3.

Locus Tag	Description	Polymorphism Type	Protein Effect
BB_0059	CBS domain-containing protein	SNP	Substitution
BB_0067	peptidase	SNP	Substitution
BB_0304	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D- alanine ligase	Insertion	Frame Shift
BB_0336	hypothetical protein	Deletion	Frame Shift
BB_0356	Conserved degenerate gene	Deletion	*
BB_0357	Conserved degenerate gene	Insertion	*
BB_0411	Conserved degenerate gene	Insertion	*
BB_0412	Conserved degenerate gene	Insertion	*
BB_0413	Conserved degenerate gene	Insertion	*
Intergenic	Between BB_0413 and 0414	Deletion	N/A
BB_r04	rrlB (23S rRNA)	SNP	N/A
BB_0511	Conserved degenerate gene	Deletion	*
BB_0511	Conserved degenerate gene	Deletion	*
BB_0511	Conserved degenerate gene	Deletion	*
BB_0511	Conserved degenerate gene	Deletion	*
Intergenic	Between BB_0542 and fusA	Deletion	N/A
BB_0634	recC	Deletion	Frame Shift
BB_0701	hypothetical protein	Deletion	Frame Shift
BB_t14	tRNA-Thr-2	Deletion	N/A
BB_t15	tRNA-Thr-3	Insertion	N/A
BB_t17	tRNA-Leu-4	Insertion	N/A

N/A = does not code for a protein (RNA or intergenic)

* = protein is not functional in neither the parental nor mutant strain

Table S6. Differentially expressed genes in *B. burgdorferi* hygromycin A resistant mutant KLEx2 in the presence of 4ug/ml hygromycin A, organized by P-value. Related to Figure 3.

ID	log2 Fold Change	P-value
16S	-1.80	0.003
bmpD	-1.67	0.005
BB_H41	-2.37	0.005
glpK	-1.62	0.006
BB_0183	-1.46	0.010
BB_J17	1.43	0.011
BB_0844	-1.41	0.013
BB_J45	1.22	0.019
BB_J31	1.22	0.020
BB_0758	1.32	0.020
BB_J27	1.18	0.023
BB_A07	-1.23	0.024
BB_U05	-1.14	0.025
BB_0839	-1.15	0.026
tRNA-Cys-1	-1.17	0.028
BB_0212	-1.15	0.030
BBC10	-8.07	0.032
BB_0206	1.07	0.033
BB_N03	1.15	0.034
BB_I18	1.16	0.034
BB_A34	1.06	0.034
BB_P03	1.06	0.036
BB_N04	1.06	0.036
BB_L03	1.06	0.036
BB_K42	-1.05	0.039
BB_J11	1.02	0.039
BBC11	-7.92	0.040
BB_O18	-1.04	0.040
BB_B13	1.12	0.041
BB_0731	-1.01	0.048

Table S7. Cytotoxicity and therapeutic index of hygromycin A. Each cell line was treated with hygromycin A, and 50% inhibitory concentration was calculated by resazurin (alamar blue/MABA) assay. Related to Figure 4.

Cell line	IC50 (µg/ml)	Therapeutic index
HepG2	>512	>2000
FaDu	>512	>2000
HEK293	>512	>2000