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Supplemental Information

Mode of Action of Kanglemycin A, an Ansamycin Natural Product that Is Active against Rifampicin-Resistant *Mycobacterium tuberculosis*

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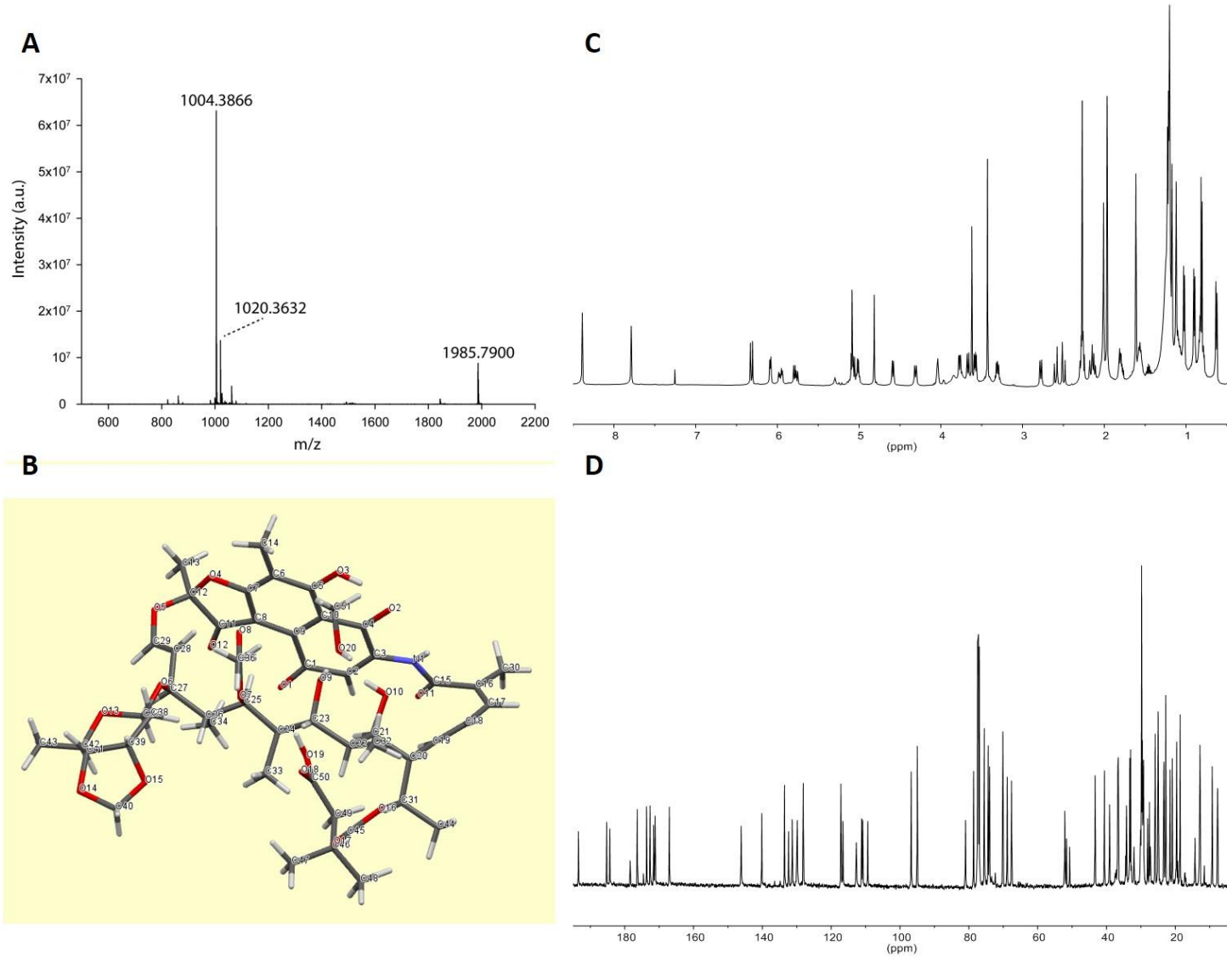
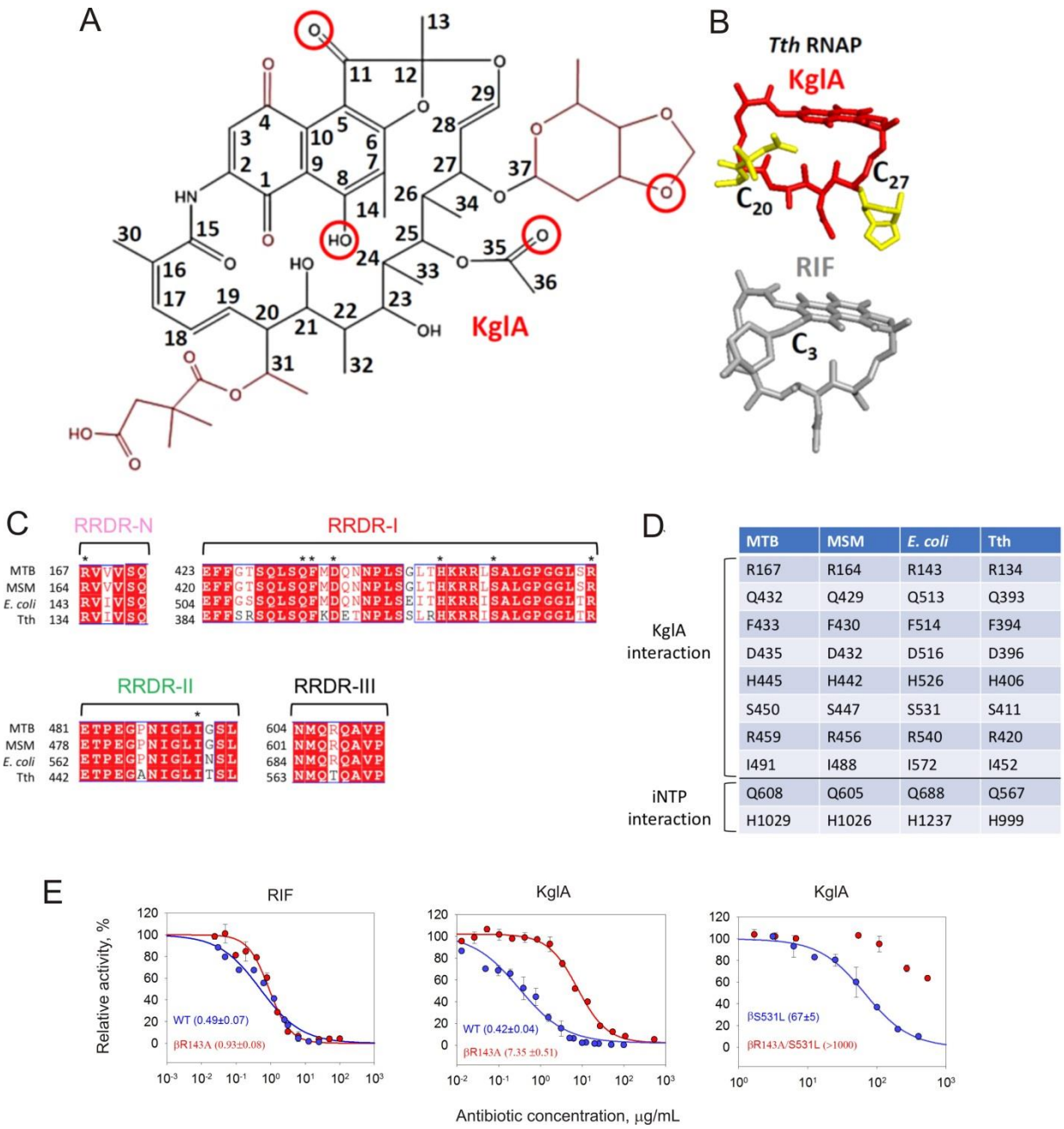


Figure S1. ***Related to Fig. 1B*****. A.** Mass spectrum of KglA showing peaks at 1004.3866, 1020.3632 and 1985.7900 Da, assigned as $[M+Na]^+$ $[M+K]^+$ and $[2M+Na]^+$ respectively. **B.** Crystallographically determined structure of KglA (from synchrotron data). Non-H atoms are labelled. **C**. 500 MHz ^{1}H NMR spectra of KglA in $CDCl_3$. **D**. 125 MHz $^{13}C\{^1H\}$ NMR spectra of KglA in $CDCl_3$.

Table S1. ***Related to Fig. 1B.*** Assignment of the ^{1}H and ^{13}C NMR data of KglA

Atom no.	δ_C	^{1}H					^{13}C-^{1}H (HMBC)
		δ_H	Protons	Multiplicity	*J* (Hz)	^{1}H-^{1}H (COSY)	
35	43.1	2.54	1	d	16.8	2.66 (**35**)	1.18 (**37**), 1.23 (**38**)
		2.66	1	d	16.8	2.54 (**35**)	
36	172.0	-	-	-	-	-	2.54 (**35**), 2.66 (**35**)
34	40.4	-	-	-	-	-	2.54 (**35**), 2.66 (**35**), 1.18 (**37**), 1.23 (**38**)
38	24.6	1.23	3	s	-	-	2.54 (**35**), 2.66 (**35**)
37	25.7	1.18	3	s	-	-	2.54 (**35**), 2.66 (**35**)
33	176.0	-	-	-	-	-	2.54 (**35**), 2.66 (**35**), 1.18 (**37**), 1.23 (**38**), 5.07 (**31**)
31	67.7	5.07	1	"q"	6.4	1.09 (**32**)	3.70 (**21**), 1.09 (**32**), 5.83 (**19**)
32	19.3	1.09	3	d	6.4	5.07 (**31**)	5.07 (**31**), 2.17 (**20**)
20	52.4	2.17	1	"t"	9.7	5.83 (**19**), 3.70 (**21**)	5.97 (**18**), 5.83 (**19**), 5.07 (**31**), 3.70 (**21**), 3.52 (OH), 1.87 – 1.81 (**22**)
19	133.6	5.83	1	dd	15.6, 9.7	5.97 (**18**), 2.17 (**20**)	6.17 (**17**), 5.97 (**18**), 5.07 (**31**), 3.70 (**21**), 2.17 (**20**), 2.06 (**30**)
18	127.9	5.97	1	dd	15.9, 6.2	6.17 (**17**), 5.83 (**19**)	5.83 (**19**), 2.17 (**20**), 2.06 (**30**)
17	129.6	6.17	1	"dt"	6.2, 1.3	5.97 (**18**), 2.06 (**30**)	5.97 (18), 5.83 (19), 2.06 (**30**)
16	132.5	-	-	-	-	-	5.97 (**18**), 5.83 (**19**), 2.06 (**30**)
30	20.5	2.06	3	s	-	6.17 (**17**)	6.17 (**17**)
15	171.0	-	-	-	-	-	8.35 (NH), 6.17 (**17**), 2.06 (**30**)
21	68.5	3.70	1	d	10.1	2.17 (**20**)	5.83 (**19**), 5.07 (**31**), 3.52 (OH), 2.86 (**23**), 2.17 (**20**), 1.87 – 1.81 (**22**), 0.95 (**39**)
22	33.5	1.87-1.81 (overlap **45**)	2[a]	m	-	0.95 (**39**)	2.86 (**23**), 2.17 (**20**), 0.95 (**39**)
39	12.4	0.95	3	d	7.0	1.87 – 1.81 (**22**)	3.70 (**21**), 2.86 (**23**), 1.87 – 1.81 (**22**)
23	78.5	2.86	1	d	10.3	1.60 (**24**)	4.39 (**25**), 3.70 (**21**), 1.87 – 1.81 (**22**), 1.60 (**24**), 0.95 (**39**), 0.70 (**40**)
24	36.6	1.60	1	m	-	2.86 (**23**), 0.70 (**40**), 4.39 (**25**)	2.86 (**23**), 0.70 (**40**), 1.87 – 1.81 (**22**)
40	8.9	0.70	3	d	6.7	1.60 (**24**)	4.39 (**25**), 2.86 (**23**), 1.60 (**24**)
25	73.7	4.39	1	d	9.9	2.13-2.08 (**26**), 1.60 (**24**)	3.86 (**27**), 2.86 (**23**), 2.06 (**30**), 0.70 (**40**), 0.39 (**43**)
41	173.6	-	-	-	-	-	4.39 (**25**), 2.03 (**42**)
42	21.3	2.03	3	s	-	-	-
26	36.7	2.13-2.08	1	m	-	0.39 (**43**), 4.39 (**25**), 3.86 (**27**)	5.14 (**28**), 4.39 (**25**), 3.86 (**27**), 0.39 (**43**)
43	12.9	0.39	3	d	7.2	2.13-2.08 (**26**)	4.39 (**25**), 3.86 (**27**), 2.13-2.08 (**26**)
27	80.9	3.86	1	dd	9.1, 3.0	5.14 (**28**), 2.13-2.08 (**26**)	6.37 (**29**), 4. 66 (**44**), 4.39 (**25**), 2.13-2.08 (**26**), 0.39 (**43**)
28	112.8	5.14 (overlap **47**)	2	dd	12.8, 9.1	3.86 (**27**), 6.37 (**29**)	6.37 (**29**), 3.86 (**27**),), 2.13-2.08 (**26**)
29	145.8	6.37	1	d	12.8	5.14 (**28**)	5.14 (**28**), 3.86 (**27**), 1.68 (**13**), 0.39 (**43**)
44	96.8	4.66	1	dd	8.9, 3.3	1.87 – 1.81 (**45**), 2.22 (**45**)	4.11 (**46**), 3.86 (**27**), 3.37 (**49**), 2.22 (**45**), 1.87 – 1.81 (**45**)
49	70.0	3.37	1	"dq"	9.0, 6.2	1.28 (**50**), 3.63 (**48**)	4.87 (**47**), 4.66 (**44**), 4.11 (**46**), 1.28 (**50**)
48	75.5	3.63	1	dd	9.0, 5.2	3.37 (**49**), 4.11 (**46**)	5.13 (**47**), 4.87 (**47**), 3.37 (**49**), 2.22 (**45**), 1.28 (**50**)
47	94.9	5.13 (overlap **28**)	2	"s"	-	-	3.63 (**48**)
		4.87	1	"s"	-	-	
46	74.3	4.11	1	"td"	4.8, 2.8	1.87 – 1.81 (**45**), 2.22 (**45**), 3.63 (**48**)	5.13 (**47**), 4.87 (**47**), 4.66 (**44**), 3.63 (**48**), 2.22 (**45**)
45	33.0	2.22	1	"dt"	14.5, 2.4	1.87 – 1.81 (**45**), 4.11 (**46**), 4.66 (**44**)	4.66 (**44**)
		1.87-1.81 (overlap **22**)	2[a]	m	-	2.22 (**45**), 4.11 (**46**), 4.66 (**44**)	
50	18.3	1.28	3	d	6.2	3.37 (**49**)	3.63 (**48**), 3.37 (**49**)
13	23.1	1.68	3	s	-	-	-
12	109.3	-	-	-	-	-	6.37 (**29**), 1.68 (**13**)
11	193.5	-	-	-	-	-	1.68 (**13**)
6	171.4	-	-	-	-	-	2.34 (**14**)
14	7.3	2.34	3	s	-	-	-
7	116.4	-	-	-	-	-	2.34 (**14**)
5	111.3	-	-	-	-	-	2.34 (**14**)
8	166.9	-	-	-	-	-	2.34 (**14**)
9	110.9	-	-	-	-	-	2.34 (**14**)
10	131.5	-	-	-	-	-	7.80 (**3**), 2.34 (**14**)
1/4	185.3	-	-	-	-	-	8.35 (NH), 7.80 (**3**)
1/4	184.3	-	-	-	-	-	8.34 (NH)
2	140.4	-	-	-	-	-	7.80 (**3**)
3	116.7	7.80	1	s	-	-	8.35 (NH)
NH	-	8.35	1	-	-	-	-
Ar-OH	-	12.60	1	-	-	-	-
OH (**21**)	-	3.52	1	-	-	-	-

[a]The integration of the signals in the 1.87 – 1.81 ppm region corresponded to 3 protons. 2 of these protons are assigned as C^{45}-H and C^{22}-H. It is possible that the remaining signal corresponds to an OH.



	MTB	MSM	*E. coli*	Tth
KglA interaction	R167	R164	R143	R134
	Q432	Q429	Q513	Q393
	F433	F430	F514	F394
	D435	D432	D516	D396
	H445	H442	H526	H406
	S450	S447	S531	S411
	R459	R456	R540	R420
	I491	I488	I572	I452
iNTP interaction	Q608	Q605	Q688	Q567
	H1029	H1026	H1237	H999

Figure S2. ***Related to Fig. 2.*** **A**. The chemical structure of Kanglemycin A (KglA). The four oxygen atoms forming hydrogen bonds with the RIF-binding pocket of RNAP are marked by red circles. **B.** Conformations of KglA and RIF in the RIF-binding pocket of *T. thermophilus* RNAP. KglA (red) and RIF (grey) are shown as stick models; the KglA's *ansa*-bridge C20 and C27 side chains are shown in yellow and labeled; the C3 side chain of RIF is labeled. **C.** Sequence alignment spanning the RIF resistance-determining regions (RRDRs) in the β subunit (RpoB) of RNAPs from *M. tuberculosis* H37RV (MTB), *M. smegmatis* (MSM), *E. coli* and *T. thermophilus* (Tth). Amino acid residues involved in the KglA interaction (Fig. 2) and also having mutations to generate the RIF-resistant RNAPs (Figs. 1 and 3) investigated in this study are indicated by asterisks.

D. Amino acid residues of the β subunit of RNAPs involved in the KglA and initiating NTP (iNTP) interactions. **E.** Involvement of KglA-specific interaction with βR143 in KglA activity. Error bars are ±SD. The brackets contain calculated IC50±SE. IC_{50} of KglA for double mutant was beyond our measurement limit.

Table S2. Oligonucleotides used in the study.

Oligonucleotides		
D516V (GAC =>GTC) rpoB_D516V_d: GCT GTC TCA GTT TAT GGT CCA GAA CAA CCC GCT G rpoB_D516V_r: CAG CGG GTT GTT CTG GAC CAT AAA CTG AGA CAG C	This paper	N/A
S531L (TCC =>TTG) rpoB_S531_d: AAA CGT CGT ATC TTG GCA CTC GGC CC rpoB_S531_r: GGG CCG AGT GCC AAG ATA CGA CGT TT	This paper	N/A
H526P (CAC =>CCG) rpoB_H526Y_d: CTG AGA TTA CGC CGA AAC GTC GTA T rpoB_H526Y_r: ATA CGA CGT TTC GGC GTA ATC TCA G	This paper	N/A
H526Y (CAC =>TAC) rpoB_H526Y_d: CTG AGA TTA CGT ACA AAC GTC GTA T rpoB_H526Y_r: ATA CGA CGT TTG TAC GTA ATC TCA G	This paper	
I572F (ATC =>TTC) rpoB_I572_d: CAT CGG TCT GTT CAA CTC TCT GTC CG rpoB_I572_r: CGG ACA GAG AGT TGA ACA GAC CGA TG	This paper	N/A
Q513L (CAG =>CTG) rpoB_Q513L_d: CCA GCC AGC TGT CTC TGT TTA TGG ACC rpoB_Q513L_r: GGT CCA TAA ACA GAG ACA GCT GGC TGG	This paper	N/A
D516Y (GAC =>TAC) rpoB_D516Y_d: GCT GTC TCA GTT TAT GTA TCA GAA CAA CCC GCT G rpoB_D516Y_r: CAG CGG GTT GTT CTG ATA CAT AAA CTG AGA CAG C	This paper	N/A
H526R (CAC =>CGC) rpoB_H526R_d: CTG AGA TTA CGC GCA AAC GTC GTC G rpoB_H526R_r: CGA CGA CGT TTG CGC GTA ATC TCA G	This paper	N/A
D516V (GAC =>GTC) rpoB_D516V_d: GCT GTC TCA GTT TAT GGT CCA GAA CAA CCC GCT G rpoB_D516V_r: CAG CGG GTT GTT CTG GAC CAT AAA CTG AGA CAG C	This paper	N/A
S531L (TCC =>TTG) rpoB_S531_d: AAA CGT CGT ATC TTG GCA CTC GGC CC rpoB_S531_r: GGG CCG AGT GCC AAG ATA CGA CGT TT	This paper	N/A
H526P (CAC =>CCG) rpoB_H526Y_d: CTG AGA TTA CGC CGA AAC GTC GTA T rpoB_H526Y_r: ATA CGA CGT TTC GGC GTA ATC TCA G	This paper	N/A
H526Y (CAC =>TAC) rpoB_H526Y_d: CTG AGA TTA CGT ACA AAC GTC GTA T rpoB_H526Y_r: ATA CGA CGT TTG TAC GTA ATC TCA G	This paper	N/A
I572F (ATC =>TTC) rpoB_I572F_d: CAT CGG TCT GTT CAA CTC TCT GTC CG rpoB_I572F_r: CGG ACA GAG AGT TGA ACA GAC CGA TG	This paper	N/A
R143A (CGT=>GCT) rpoB_R143A_d: TGT TAT CAA CGG TAC TGA GGC TGT TAT CGT TTC CCA GCT G rpoB_R143A_r: CAG CTG GGA AAC GAT AAC AGC CTC AGT ACC GTT GAT AAC A	This paper	N/A
pyrG non-template: TAT AAT GGG AGC TGG CTC TGA TGC AGG *pyrG* template: CCT GCA TCA GAG CCC AAA ATA C	Murakami et al., 2017	N/A