

Supporting Information

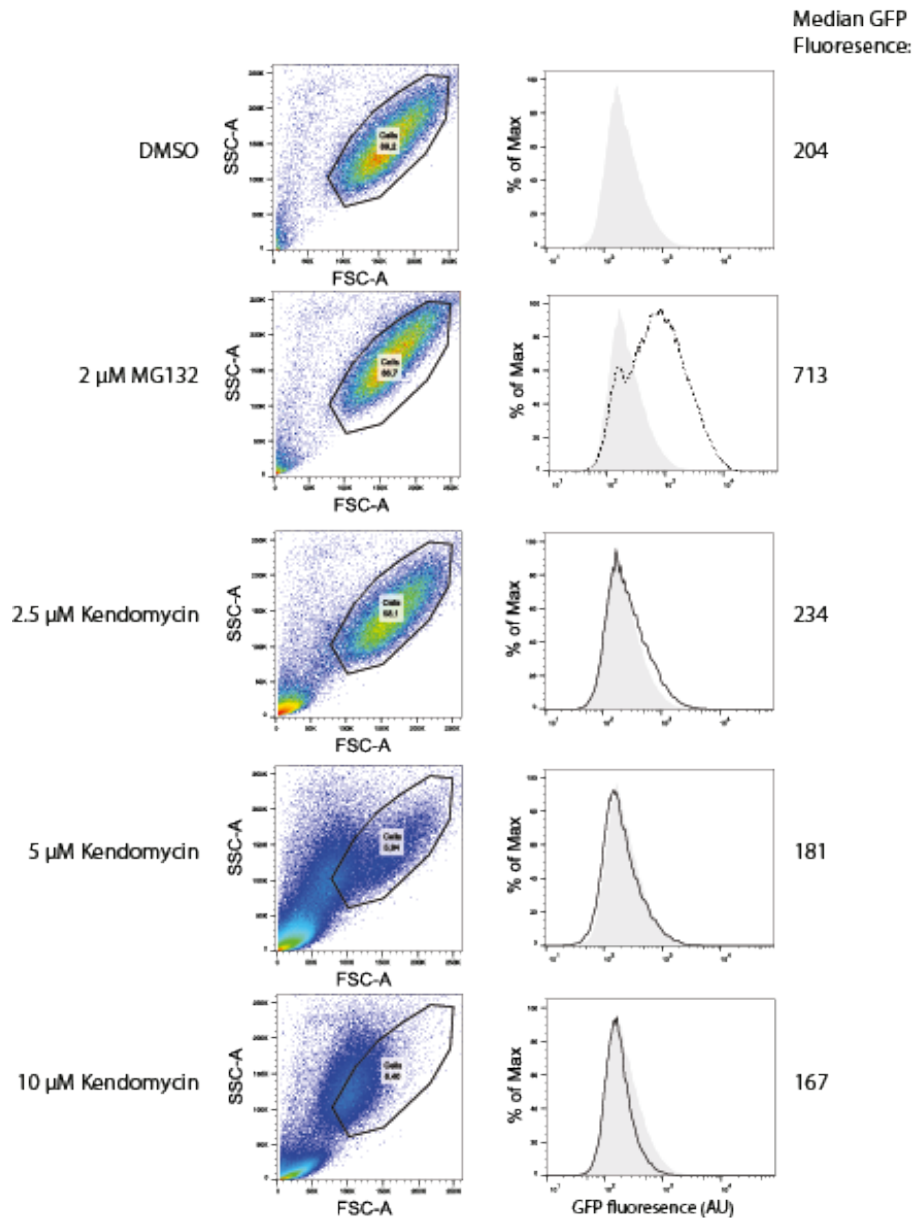
Kendomycin cytotoxicity against bacterial, fungal and mammalian cells is due to cation chelation.

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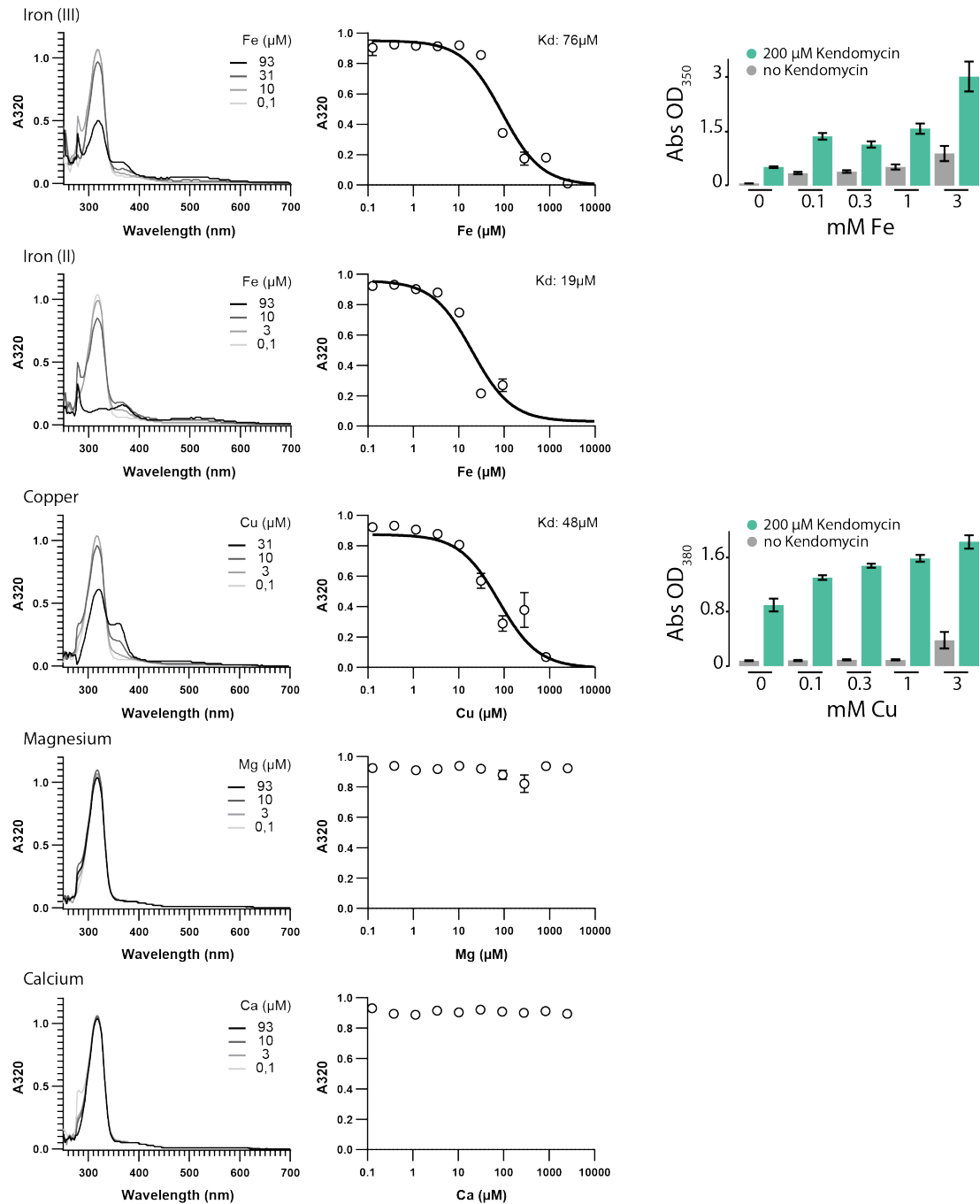
Figure S1



Effects of MG132 and Kendomycin on the cellular turnover of a destabilized Ub^{G76V}-GFP reporter. The gene for a destabilized Ub^{G76V}-GFP under an inducible TRE2 promoter was transduced into K562 cells with lentiviruses. 16h before the addition of the drugs, the expression of the Ub^{G76V}-GFP was induced with 1 μ g/ml doxycyclin. The induced cells were treated for 4h with 0.1% DMSO, MG132 or Kendomycin and then analysed with BD LSRFortessa flow-cytometer (BD Biosciences). The left-hand panels show the light-scattering of the analyzed cells, while the right-hand panels show the GFP-fluorescence of the gated cell populations. In the right-hand panels the fluorescence of the DMSO-treated reference sample is shown in grey, while the fluorescence of the drug-treated cells is shown with a black line.

Figure S2

UV-Vis analysis of kendomycin interaction with various cations



UV-Vis spectra of kendomycin in the presence of indicated concentrations of cations. A320 measurements (n=4) plotted against concentration and binding curve fitted to estimate K_{app} .

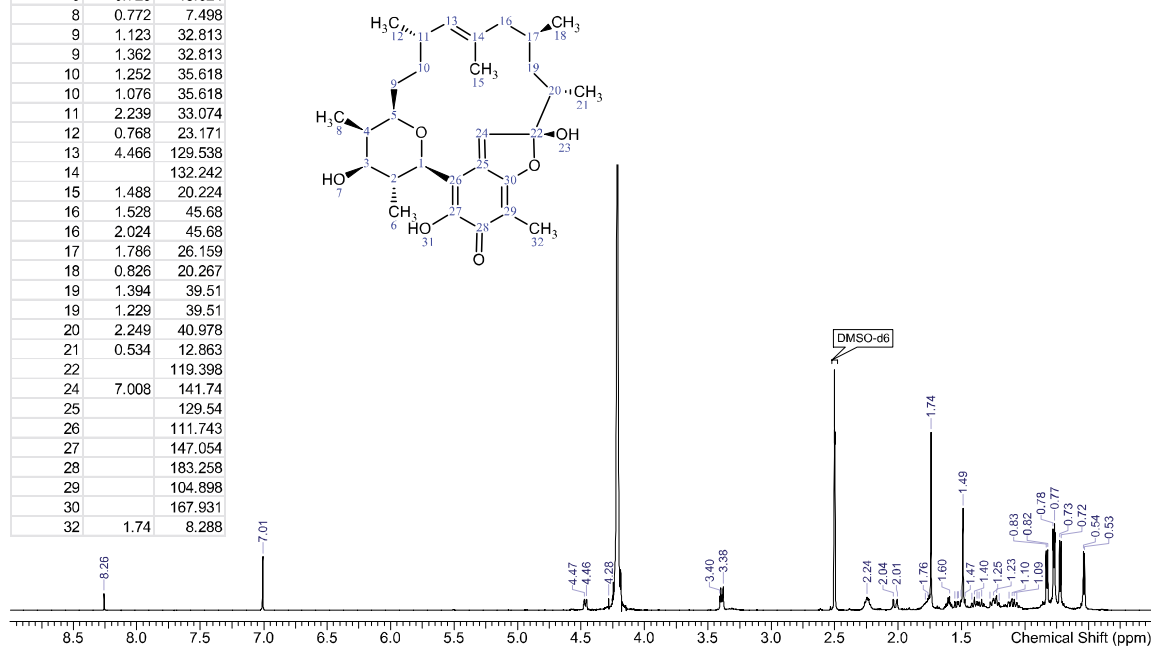
Figure S3

¹H-NMR analysis of kendomycin

Atom#	H Shift	C Shift
1	4.193	77.019
2	1.6	37.27
3	3.393	75.834
4	1.754	39.843
5	3.386	78.095
6	0.723	13.624
8	0.772	7.498
9	1.123	32.813
9	1.362	32.813
10	1.252	35.618
10	1.076	35.618
11	2.239	33.074
12	0.768	23.171
13	4.466	129.538
14		132.242
15	1.488	20.224
16	1.528	45.68
16	2.024	45.68
17	1.786	26.159
18	0.826	20.267
19	1.394	39.51
19	1.229	39.51
20	2.249	40.978
21	0.534	12.863
22		119.398
24	7.008	141.74
25		129.54
26		111.743
27		147.054
28		183.258
29		104.898
30		167.931
32	1.74	8.288

13C Shifts extracted from HSQC and HMBC

¹H-NMR of Kendomycin in DMSO-d₆ with 15% D₂O



HSQC of kendomycin

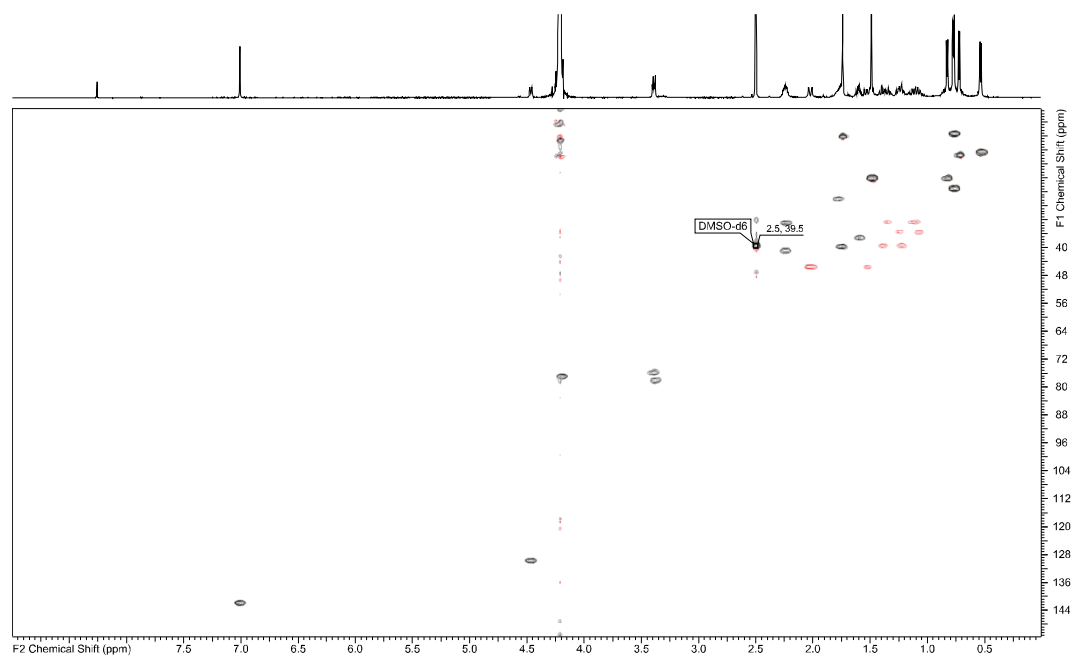
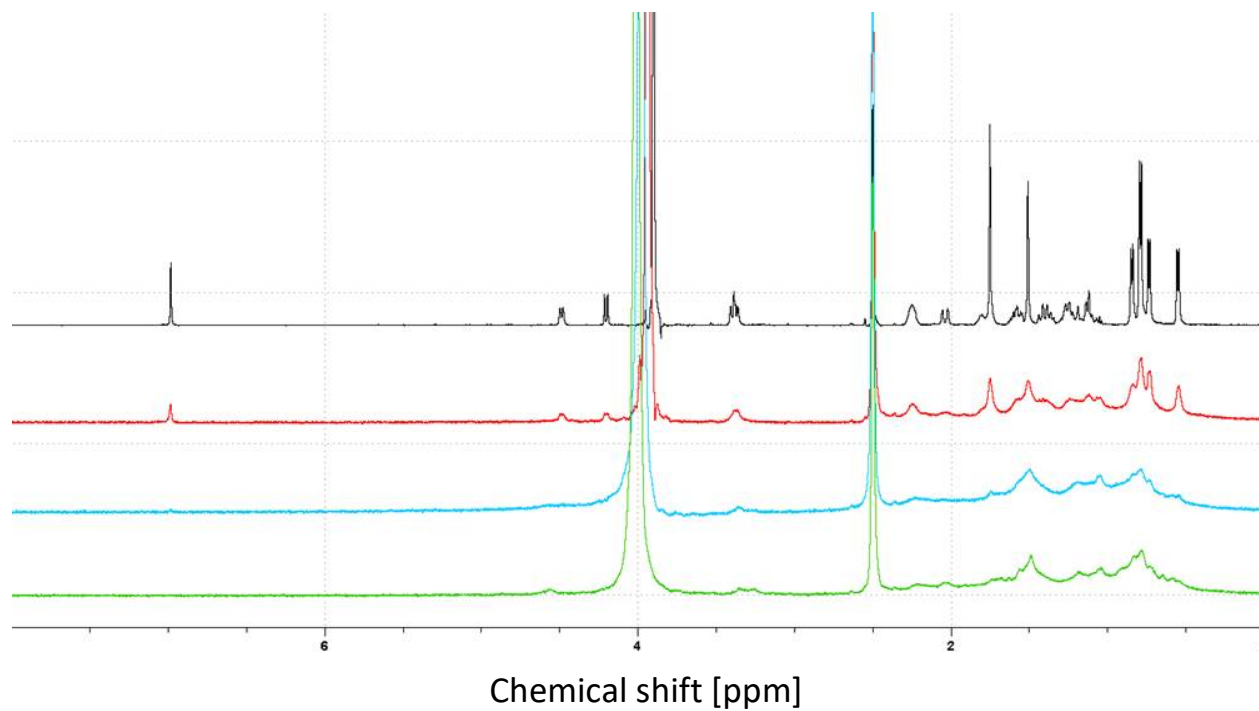


Figure S4

NMR spectra of Kendomycin with increasing concentrations of FeSO_4



^1H NMR spectra of 4 mM kendomycin with increasing concentrations of FeSO_4 ; 0.00 eq (black), 0.33 eq (red), 0.66 eq (blue) and 1.00 equivalent (green). Disappearance of the H24 signal between 0.33 and 0.66 equivalents indicates the formation of a 1:2 Fe(II) : kendomycin complex.

Figure S4

BS17004214795QE #592-601 RT: 6.00-6.07 AV: 5 NL: 2.79E7
T: FTMS + p ESI Full lock ms [133.4000-2000.0000]

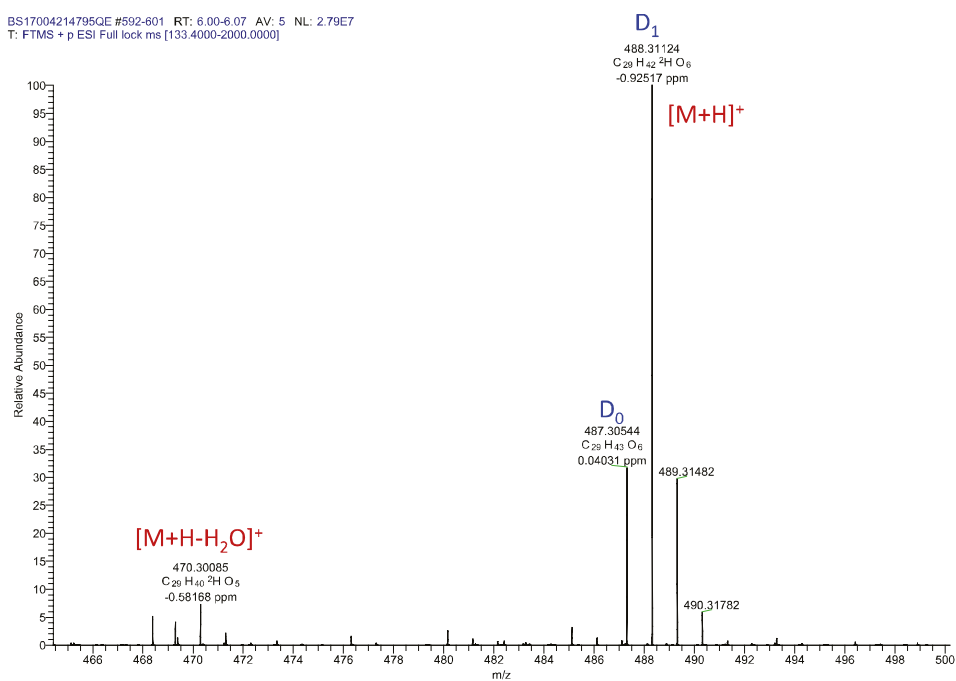


Table S1

Summary of SNP analysis of 6 kandomycin resistant HCT116 mutant cell lines

Gene name	Gene description
ALPK3	alpha kinase 3
CCM2	CCM2 scaffolding protein
CROCC	rootletin, ciliary rootlet coiled-coil
DCLRE1C	DNA cross-link repair 1C
GTF2IRD1	GTF2I repeat domain containing 1 protein
HSP90AA1	heat shock protein 90 alpha family class A member 1
IRF3	interferon regulatory factor 3
KANK3	KN motif and ankyrin repeat
MAP4K4	mitogen-activated protein kinase kinase kinase 4
NUMBL	NUMB like, endocytic adaptor protein
POLG	DNA polymerase gamma, catalytic subunit
RBMX	RNA binding motif protein, X-linked
TFAP4	transcription factor AP-4
TM7SF2	transmembrane 7 superfamily member 2
TXNRD3	thioredoxin reductase 3
UQCRC1	Rieske Fe-S protein
WSCR27	Williams-Beuren syndrome chromosomal region 27 protein
ZBBX	Zinc finger B-box domain-containing protein 1