

Supplementary Information

3-*O*-Methyl-alkylgallates inhibit fatty acid desaturation in *Mycobacterium tuberculosis*

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Table S1 Minimal inhibitory concentration (MIC) of all compounds against *Staphylococcus aureus* (ATCC 25923), Methicillin-resistant *S. aureus* (MRSA, ATCC 700699), *Enterococcus faecalis* (ATCC 29212 and ATCC 51299), *Enterococcus faecium* (ATCC 35667) and Vancomycin-resistant *E. faecium* (VRE, ATCC 700221), and *Acinetobacter baumannii* (ATCC BAA-1605). Compounds were tested against *A. baumannii* in monotreatment and in combination with sublethal doses of colistin (COL, 0.31 μ M) or rifampicin (RIF, 2.5 μ M). MICs are shown in μ M.

	<i>S. aureus</i>		<i>E. faecalis</i>		<i>E. faecium</i>		<i>A. baumannii</i>		
	ATCC 25923	ATCC 700699	ATCC 29212	ATCC 51299	ATCC 35667	ATCC 700221	ATCC BAA-1605	ATCC BAA-1605, COL	ATCC BAA-1605, RIF
1	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
2	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
3	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
4	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
5	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
6	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
7	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
8	> 100	50	> 100	> 100	> 100	> 100	> 100	> 100	> 100
9	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
10	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
11	> 100	25	> 100	> 100	> 100	> 100	> 100	> 100	> 100
12	25	25	> 100	> 100	25	100	> 100	> 100	> 100
13	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
14	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
15	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
16	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
17	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
18	> 100	100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
19	100	> 100	> 100	> 100	> 100	100	> 100	> 100	> 100

Table S2 Cytotoxicity data of compounds 10, 11 and 12 against various human cancer cell lines. The data indicate the concentration (μM) reducing the cell growth by 50 % (IC_{50}) relative to the DMSO control. Cell lines and tissue origins are given. A concentration $>100 \mu\text{M}$ indicates no cytotoxic effects in this assay up to the highest tested concentration. For quantification, the resazurin dye reduction assay has been used.

Cell line	Origin	Compound 10	Compound 11	Compound 12
HepG2	Human liver carcinoma	100	> 100	> 100
CLS-54	Human lung carcinoma	> 100	> 100	> 100
HEK293	Human embryonic kidney	> 100	> 100	> 100
H4	Human brain neuroglioma	> 100	> 100	> 100
HUH7	Human liver carcinoma	> 100	> 100	100

Table S3 Checkerboard synergy assay. *M. tuberculosis* H37Rv cells were inoculated at a concentration of 10⁶ CFU/mL. Growth of bacteria was determined after 5 days via resazurin dye reduction assay. Table shows growth of bacteria as percent of controls. Growth below 10% relative to controls is marked in blue, growth greater than 10% is marked in purple. Wells relevant for FICI calculation are marked in red. RIF, rifampicin; INH, isoniazid; DELA, delamanid.

3-O-Methyl-butylgallate (µM)

RIF (µM)	50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0.098	0.049	0
1.250	-6.41	-2.97	-4.10	-3.10	-4.16	-4.50	-3.39	-2.59	-2.44	-2.38	-2.47	2.19
0.625	-2.96	-7.35	-8.81	-8.02	-7.48	-5.82	-7.39	-6.72	-7.81	-7.22	-7.24	-0.82
0.313	-1.21	-6.97	-6.92	-6.27	-4.77	-7.45	-4.65	-4.04	-4.29	-4.63	-3.75	-0.94
0.156	-0.70	-5.69	-5.37	-4.96	-4.48	-4.12	-2.71	-2.60	-2.56	-3.18	-3.05	27.69
0.078	0.31	-6.57	-5.69	-1.18	5.60	6.07	8.37	11.62	13.01	13.86	16.26	104.23
0.039	1.23	-5.17	-3.97	19.93	63.88	62.61	61.94	72.48	73.73	73.77	71.27	124.33
0.020	1.52	-3.89	-1.01	79.33	107.65	110.90	116.87	121.26	116.72	116.82	116.37	77.79
0	1.80	-2.47	10.83	128.04	163.76	169.69	163.33	172.47	176.96	161.40	149.05	100.00

INH (µM)	50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0.098	0.049	0
5.000	35.14	30.26	22.49	22.39	21.33	22.69	20.26	21.56	15.79	21.41	15.80	20.07
2.500	29.95	13.39	14.12	15.57	19.31	17.27	16.63	16.81	14.31	14.52	14.28	18.49
1.250	26.68	9.49	11.72	13.95	13.80	17.84	17.86	17.63	17.00	13.78	15.79	21.10
0.625	17.61	8.03	10.64	13.08	15.09	15.89	14.99	16.19	12.95	13.01	12.49	17.33
0.313	11.49	4.90	8.31	11.18	14.14	14.07	15.39	18.66	15.13	13.22	12.52	73.44
0.156	1.56	2.35	9.33	14.42	17.06	19.25	19.36	19.48	21.77	19.32	20.83	108.83
0.078	-1.10	-1.49	11.79	63.87	86.98	91.45	101.14	68.20	94.93	78.84	103.20	97.93
0	-0.85	-0.46	7.66	80.32	91.13	96.20	98.19	93.29	98.04	94.67	97.08	100.00

DELA (µM)	50	25	12.5	6.25	3.125	1.563	0.781	0.391	0.195	0.098	0.049	0
2.340	-0.43	-4.86	-2.42	-2.34	-2.22	-2.03	-1.42	-3.77	-1.15	-0.21	0.94	5.04
1.170	2.39	0.5	1.00	0.79	0.28	1.68	1.24	0.92	3.25	2.40	2.15	6.40
0.585	1.53	-0.35	3.10	-0.10	1.24	0.77	1.01	0.80	1.37	1.40	2.57	8.91
0.293	-0.42	-2.35	-1.78	-0.57	0.81	-1.53	1.60	2.55	3.72	3.31	4.08	25.12
0.146	-0.61	-2.60	-1.87	2.70	8.45	11.56	10.27	14.65	18.82	17.12	13.89	55.16
0.073	-0.57	-2.40	0.76	14.66	48.45	48.71	53.63	52.31	47.36	50.49	48.05	66.45
0.037	0.06	0.22	0.94	61.34	71.63	89.27	69.22	70.47	59.91	65.13	69.98	79.24
0	-1.96	-2.23	-0.65	72.89	87.48	83.74	90.04	87.87	85.14	85.98	83.55	100.00

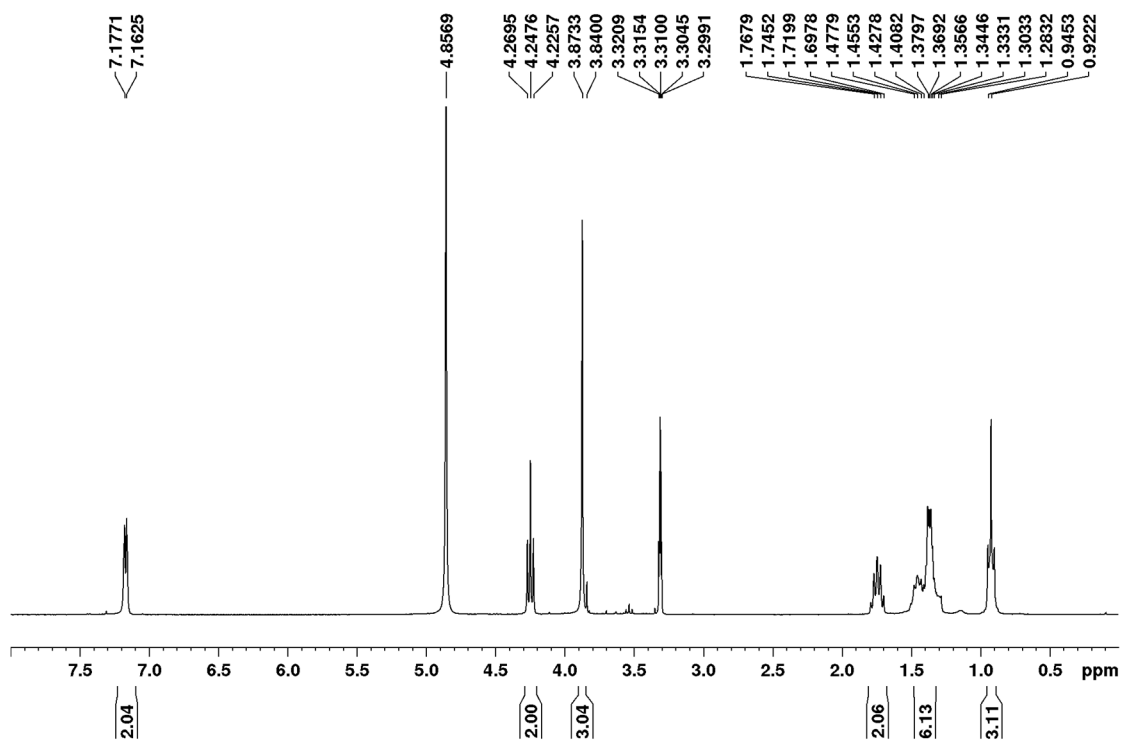


Figure S1 ^1H NMR spectrum (CD $_3$ OD, 300 MHz) of compound 11.

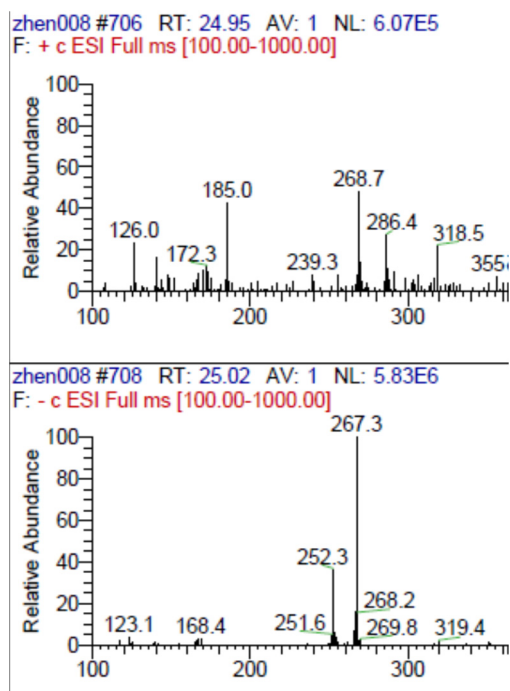


Figure S2 MS spectra of compound 11.

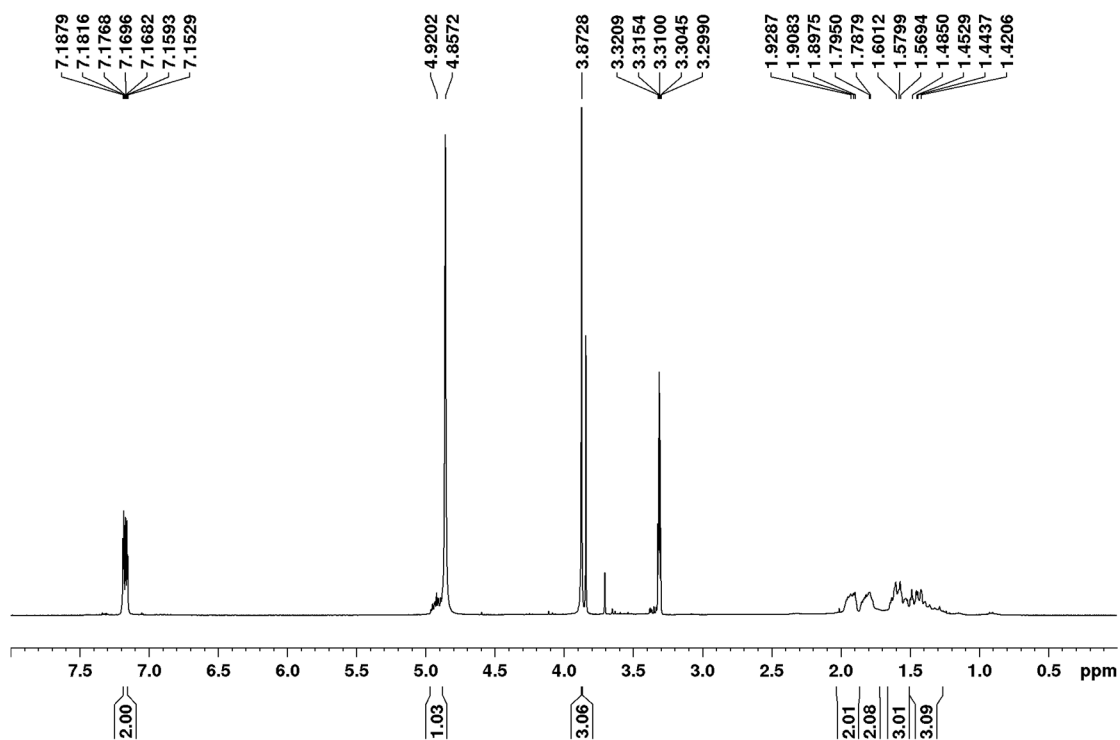


Figure S3 ^1H NMR spectrum (CD $_3$ OD, 300 MHz) of compound 13.

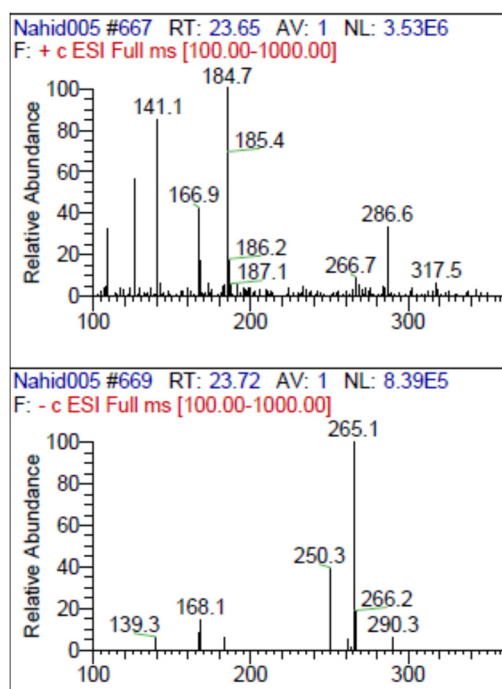


Figure S4 MS spectra of compound 13.

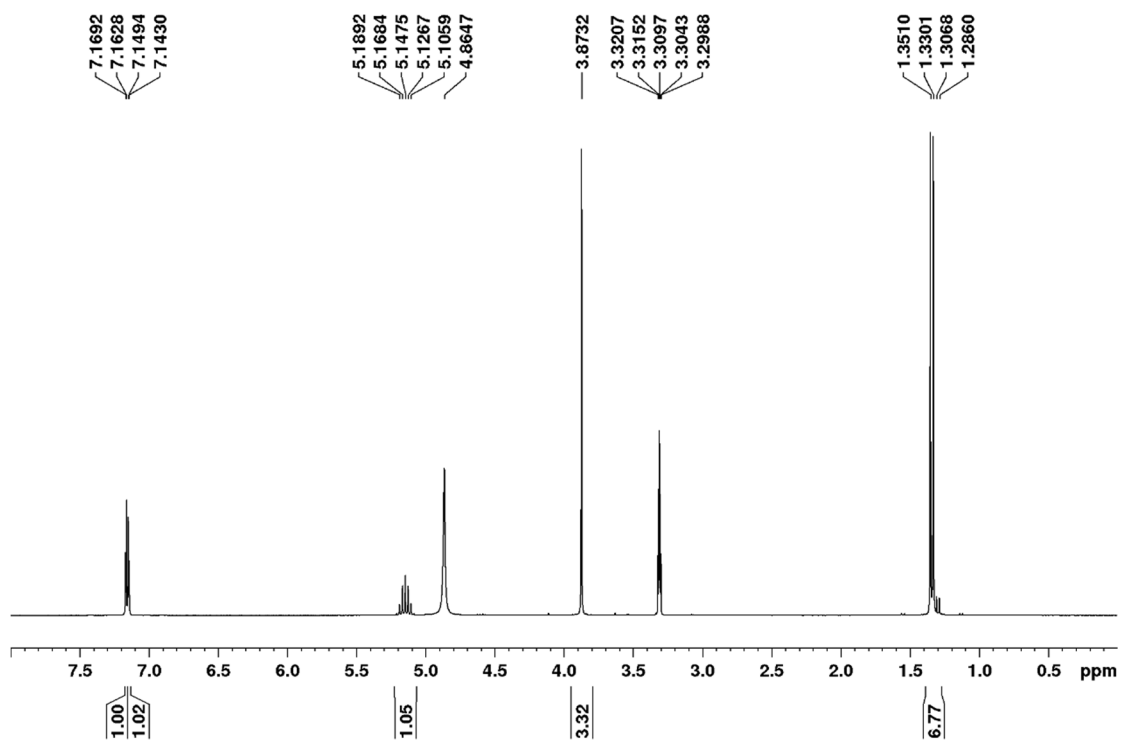


Figure S5 ^1H NMR spectrum (CD₃OD, 300 MHz) of compound 15.

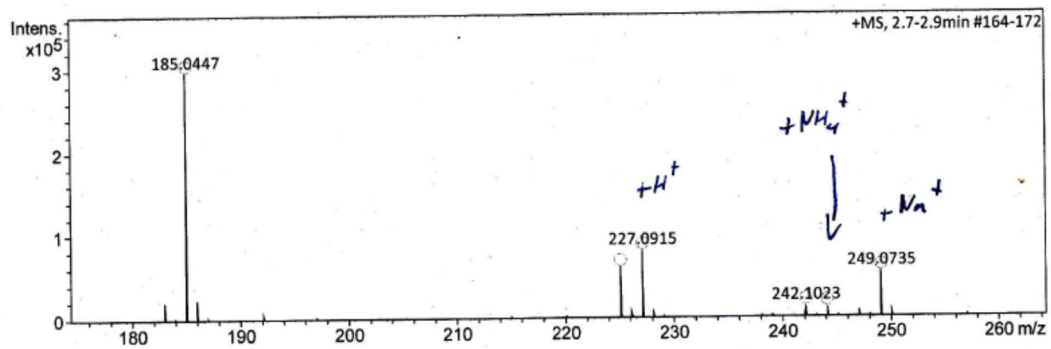


Figure S6 MS spectrum of compound 15.

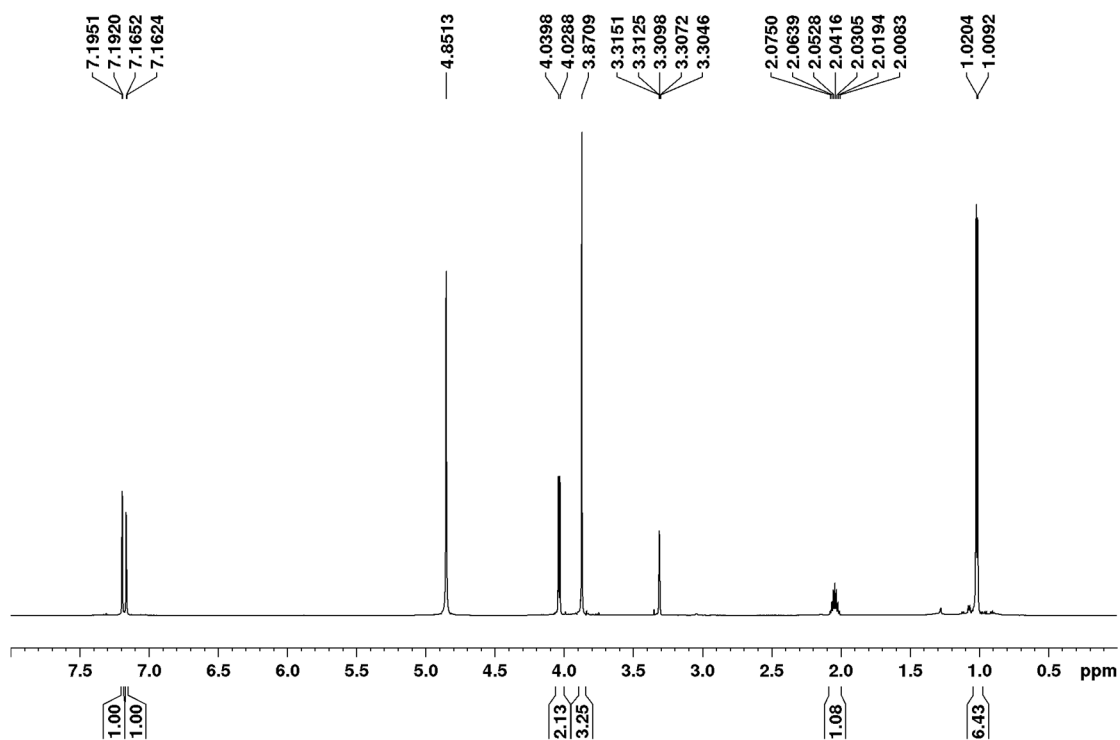


Figure S7 ^1H NMR spectrum (CD $_3$ OD, 300 MHz) of compound 16.

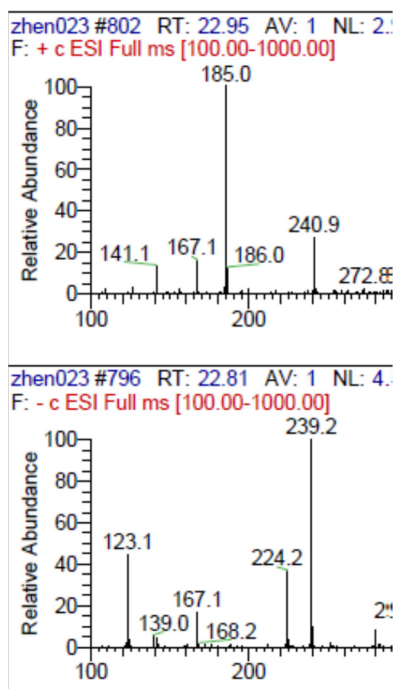


Figure S8 MS spectra of compound 16.

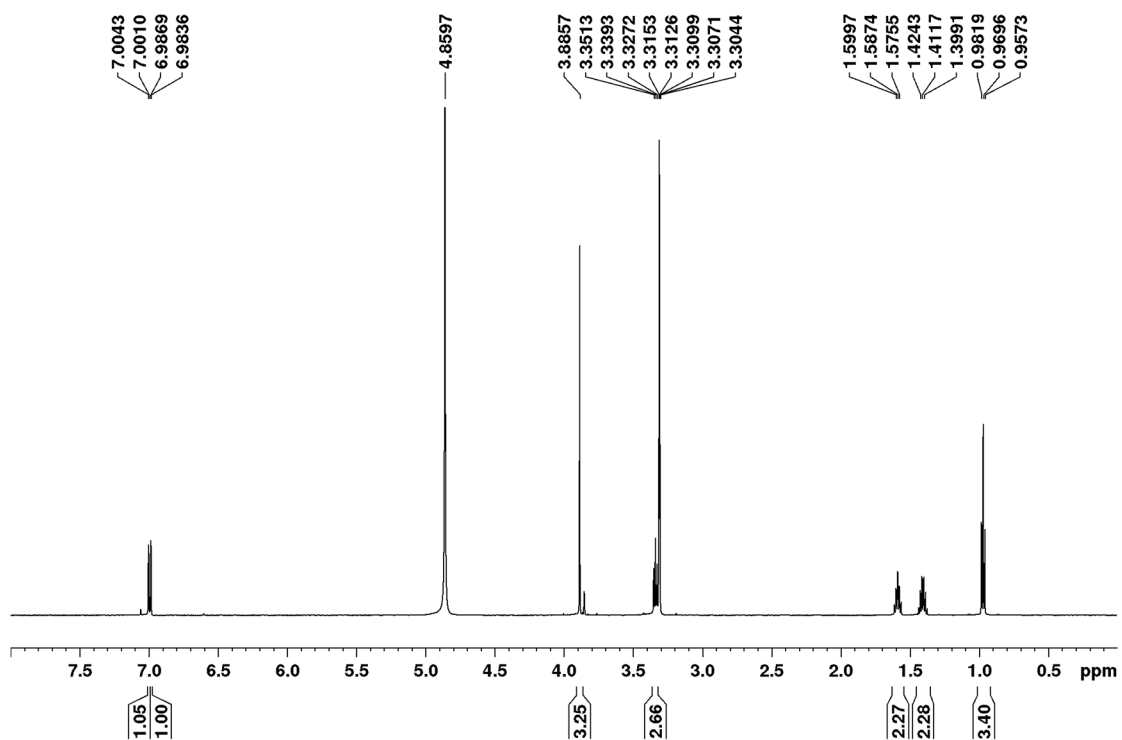


Figure S9 ^1H NMR spectrum (CD_3OD , 300 MHz) of compound 17.

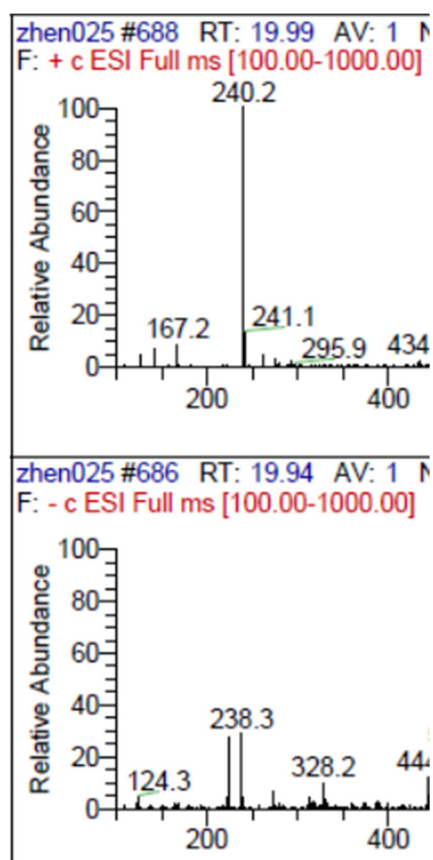


Figure S10 MS spectra of compound 17.

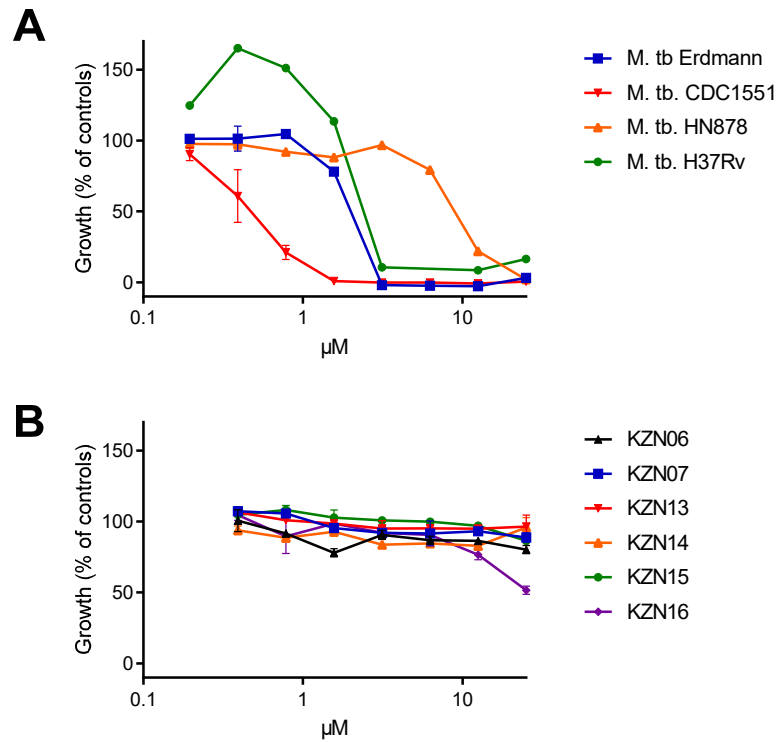


Figure S11 Sensitivity towards 3-*O*-methyl-butylgallate of drug-sensitive (A) and extensively drug-resistant (XDR) (B) clinical isolates of *M. tuberculosis*. Growth was quantified using the resazurin dye reduction assay. Data represent single measurements.

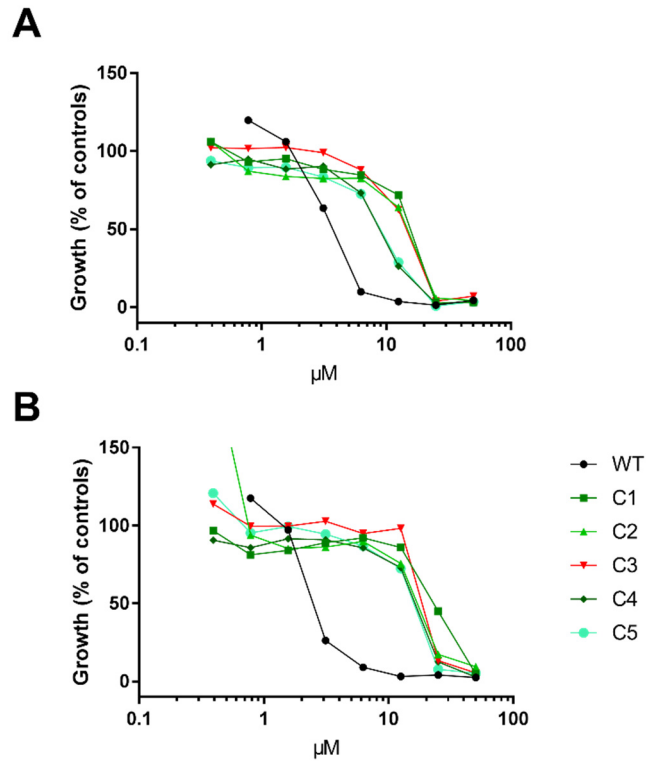


Figure S12 Cross resistance of spontaneous resistant *M. tuberculosis* mutants (C1-C5) generated with 3-*O*-methyl-butylgallate against 3-*O*-methyl-hexylgallate (A) and 3-*O*-methyl-octylgallate (B) in comparison to *M. tuberculosis* H37Rv WT. Growth was quantified using the resazurin dye reduction assay. Data represent single measurements.

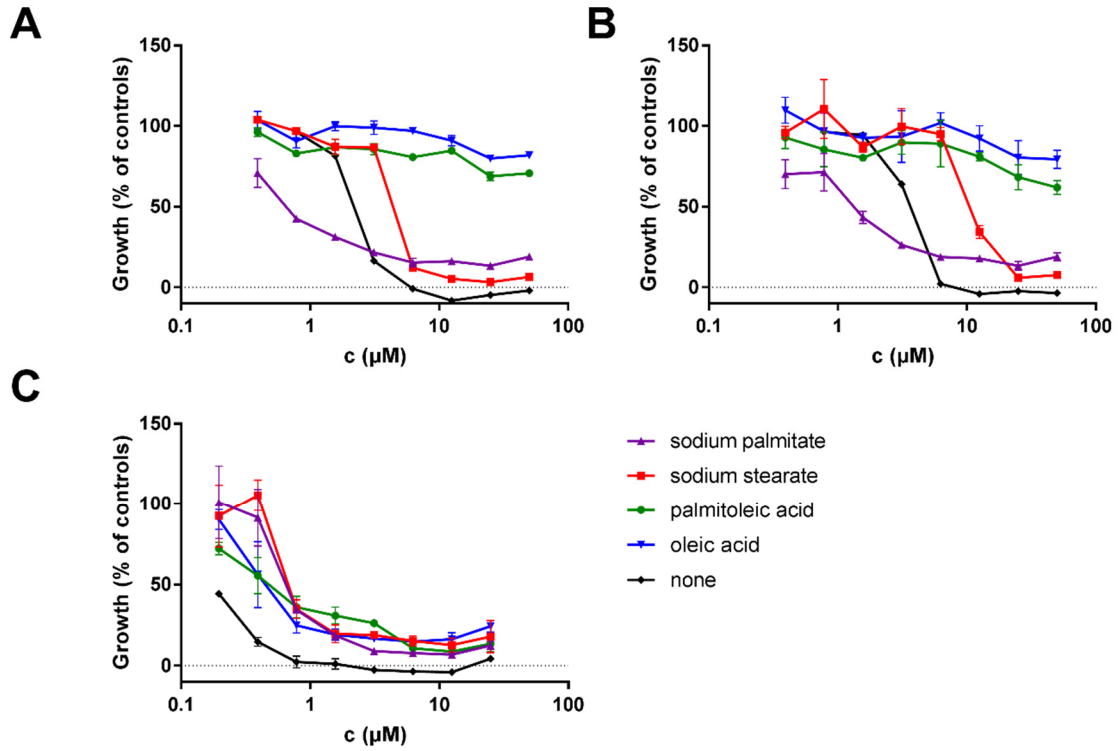


Figure S13 Dose-response curves of 3-*O*-methyl-hexylgallate (A), 3-*O*-methyl-octylgallate (B) and isoxyl (C) against *M. tuberculosis* H37Rv during supplementation with sodium palmitate (triangle), sodium stearate (square), palmitoleic acid (circle), oleic acid (reversed triangle) and none supplements (rhombus). Growth was quantified using the resazurin dye reduction assay. Data are means of triplicates \pm SD.