Supplementary Information

A New Antibiotic with Potent Activity Targets MscL

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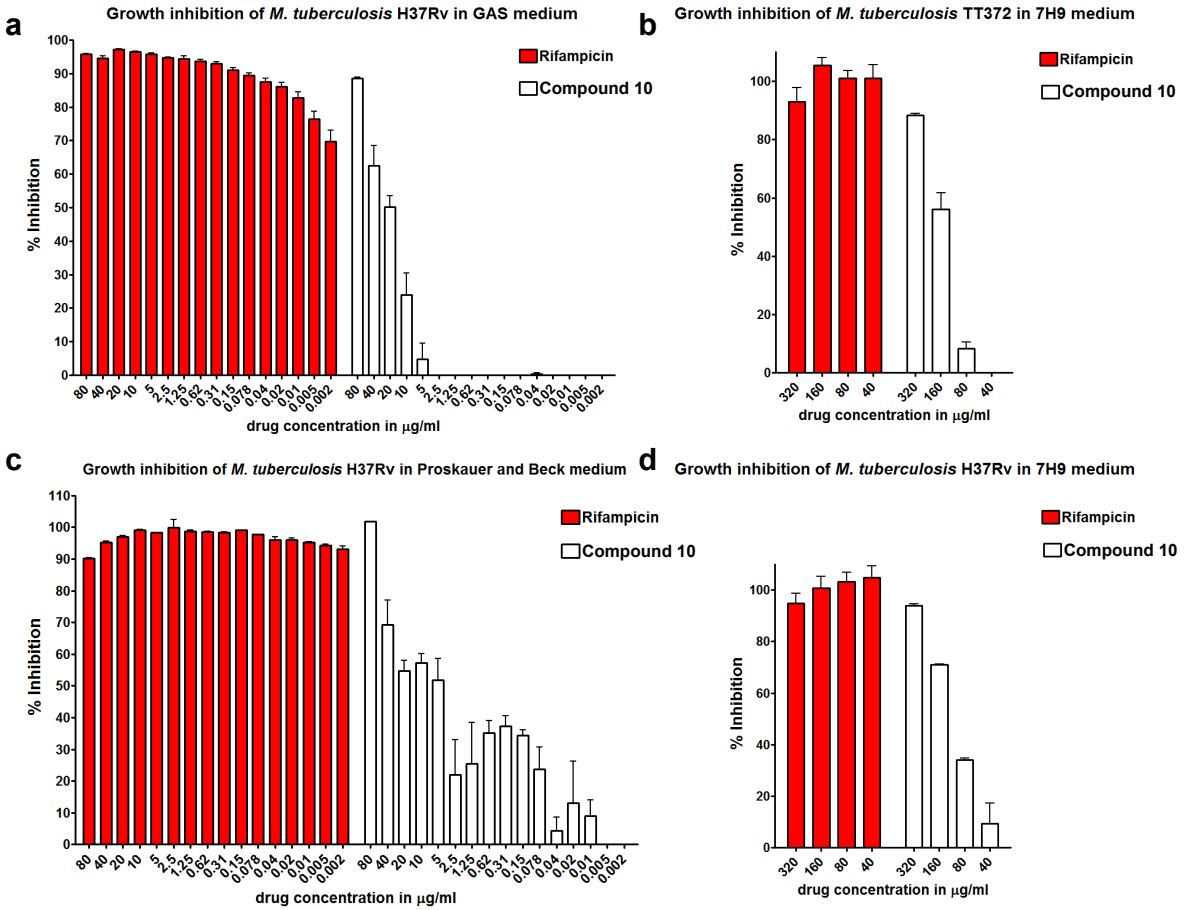

Fig. S1 | Comparison of efficacy of **10** and Rifampicin against multi-drug resistance *M. tuberculosis* H37RV (a, c and d) and TT372 strains (b). The 7H9 medium contains OADC (oleic acid, albumin, dextrose and catalase), which is needed for the growth of the bacteria. GAS medium has a final pH of 6.6 and the Proskauer and Beck medium has a final pH of 7.4.

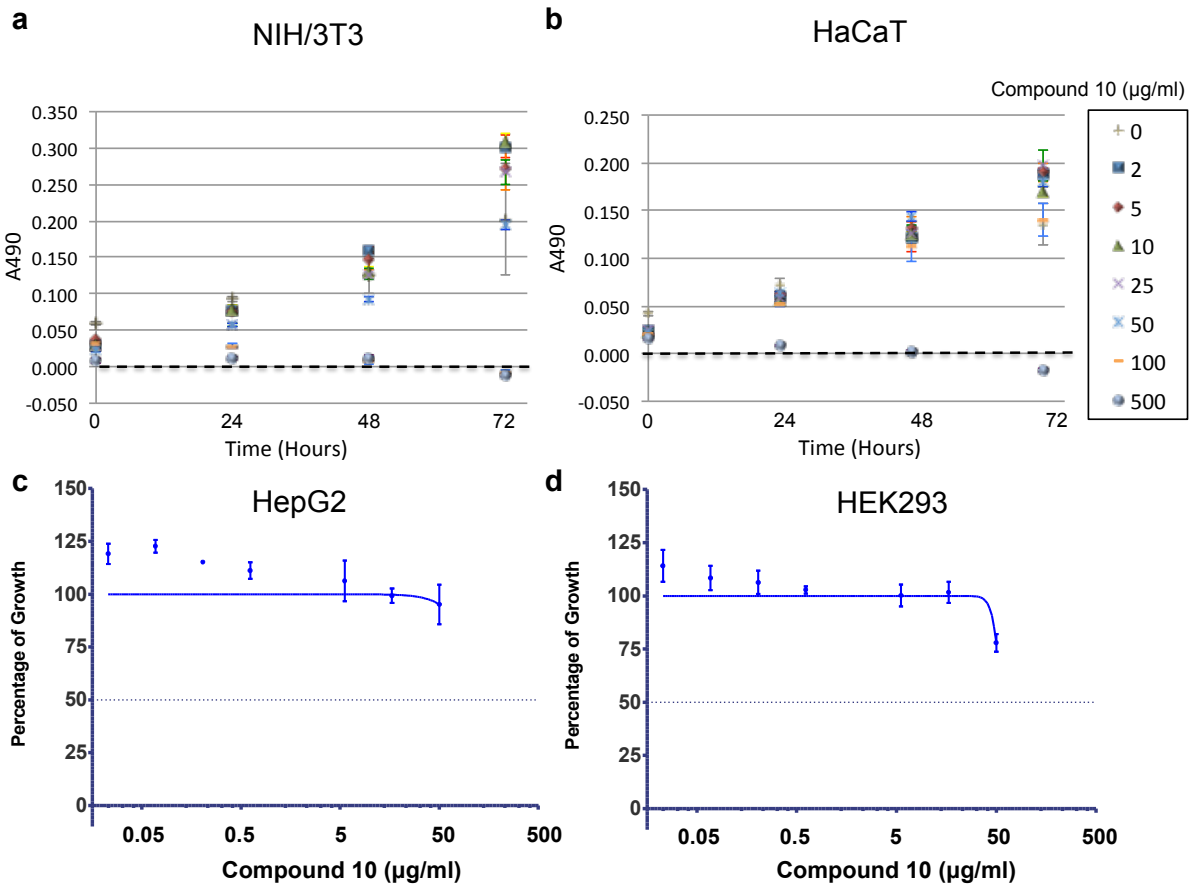

Fig. S2 | Cytotoxicity studies of Ramizol®. (a) Cytotoxicity in NIH/3T3 fibroblasts and (b) immortalised keratinocytes (HaCaT). (c) Cytotoxicity in hepatocellular carcinoma (HepG2 ATCC HB-8065) and (d) human embryonic kidney cells (HEK293 ATCC CRL-1573). Statistical differences in cell viability were observed between NIH/3T3 fibroblasts in the presence of **10** at 2 µg/mL and 500 µg/mL after 72 hrs compared to the control ($p=0.0256$, $n=3$ Student *t*-test). Statistical differences were observed between HaCaT cells in the presence of **10** at 500 µg/mL after 72 hrs compared to the control ($p=0.0256$, $n=3$ Student *t*-test unpaired).

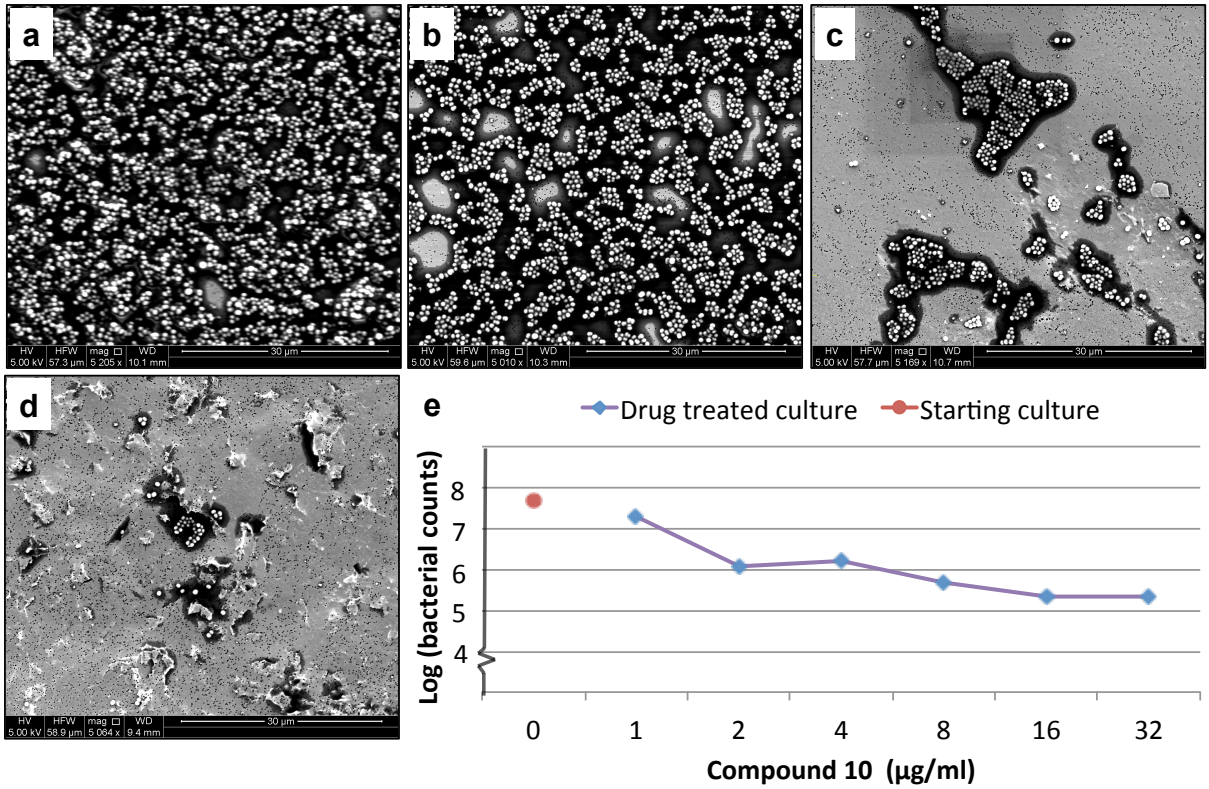

Fig. S3 | SEM images of *S. aureus* (ATCC 29213) treated with **10** at (a) 0.5xMIC (0.5 µg/mL), (b) 1xMIC (1.0 µg/mL), (c) 2xMIC (2.0 µg/mL) and (d) 1xMBC (32.0 µg/mL). Images were taken at approximately x5000 magnification. Scale bar is 30 µm. (e) *In-vitro* efficacy of **10** against *S. aureus* showing a 2-fold decrease at 16-32 µg/mL compared to the starting culture. Untreated *S. aureus* and cultures treated with 0.5 µg/mL showed log (CFU/mL) > 9.

Table S1 | Autodock parameters used

Description	Value
Number of runs	256
Number of points	56 50 50
Grid Centre	4.0, -0.5353, 0.2711
Energy profile calculation	
Number of runs	30
Number of points	56 40 40
Common parameters for all dockings	
tran0 (initial coordinates)	random
quat0 (initial quaternion)	random
ndihe (number of initial tortions)	depends on ligand
dihe0 (initial torsions)	random
non-hydrogen torsional coeff	0.3113
tstep (translation step/A)	2.0
qstep (quaternion step/deg)	50.0
dstep (torsion step/deg)	50.0
trnrf (trans reduction factor/cycle)	1
quarf (quat reduction factor/cycle)	1
dihrf (tors reduction factor/cycle)	1
e0max (max. allowable initial energy, max. number of retries)	10000
Lamarckian Genetic Algorithm (LGA) parameters	
ga_pop_size (number of individuals in population)	50
ga_num_evals (maximum number of energy evaluations)	250000
ga_num_generations (maximum number of generations)	27000
ga_elitism (num. of top individuals that automatically survive)	1
ga_mutation_rate (rate of gene mutation)	0.02
ga_crossover_rate (rate of crossover)	0.80
ga_window_size (num. of generations for picking worst individual)	10
ga_cauchy_alpha (~mean of Cauchy distribution for gene mutation)	0
ga_cauchy_beta (~varience of Cauchy distribution for gene mutation)	1

Table S2 | Spatial dimensions of Eco-MscL versus spatial dimensions between α, β and γ hydrogen atoms in the ligands. The distance between the H atoms are as follows α-γ > β-γ > α-β.

	α-β	β-γ	α-γ	α β^γ	βα^γ	αγ^β	Lowest Docking Energy (kcal/mol)
	(Å)			(Degrees)			
Spatial description of Eco-MscL	6.32	9.60	14.47	129.57	20.37	30.06	
Ligand							
1	7.11	7.78	12.91	120.20	31.39	28.42	-9.89
2	6.23	6.41	6.76	64.67	59.00	56.34	-10.43
3	9.73	10.33	10.83	65.29	60.02	54.69	-5.96
4	7.23	9.95	12.71	94.13	51.31	34.57	-8.40
5	9.52	10.30	11.14	68.22	59.22	52.56	-8.21
6	5.22	8.40	9.67	87.21	60.16	32.63	-7.56
7	2.43	10.13	10.26	96.23	80.13	13.64	-7.62