

## Supplementary

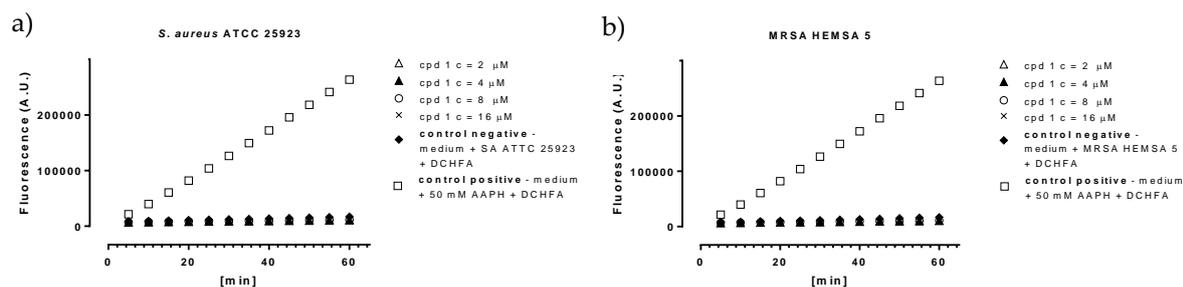
**Table S1.** Details of MIC values of the compounds 1-8 and ebselen against Gram-negative bacteria.

	Bacteria Strains	MIC* of the tested compounds (1-8) [ $\mu\text{g/ml}$ ]								Ebselen
		1	2	3	4	5	6	7	8	
<i>K. pneumoniae</i>	NRZ-00103	<u>1.24</u> <sup>1</sup>	13.76	<u>4-2.8</u>	2.88-5.76	46.08	46.08	<u>2.88</u>	5.76	$\geq$ 143.35
	KP 2151307	<u>0.62</u> <u>1.24</u>	6.88- 13.76	<u>1.4</u>	<u>2.88</u>	23.04- 46.08	11.52- 23.04	<u>2.88</u>	5.76	71.68- 143.36
	KP 1963584	<u>0.62</u>	6.88	<u>1.4</u>	<u>2.88</u>	11.52- 23.04	11.52- 23.04	<u>1.44</u> <u>2.88</u>	<u>2.88</u>	71.68
<i>Acinetobacter</i>	AC 2151300	<u>0.31-0.62</u>	<u>0.86</u>	<u>0.35-0.7</u>	<u>0.36-0.72</u>	<u>2.88</u>	<u>1.44</u>	<u>0.36</u> <u>0.72</u>	<u>1.44</u>	17.92
	AB 1995594	<u>0.62</u>	<u>1.72</u> <u>3.44</u>	<u>1.4</u>	<u>0.72</u>	2.88-5.76	<u>2.88</u>	<u>0.72</u>	<u>1.44</u> <u>2.88</u>	17.92- 35.84
	AB 4184/2/5	<u>0.31</u>	<u>0.86</u>	<u>0.35</u>	<u>0.36</u>	<u>2.88</u>	<u>0.72</u> <u>1.44</u>	<u>0.36</u>	<u>1.44</u>	17.92
<i>P. aeruginosa</i>	ATCC 27853	<u>2.48</u>	110.08	5.60-11	5.76-11.52	46.08- 92.16	92.16- 184.32	11.52- 23.04	23.04	71.68
	PA T18	<u>0.31</u>	<u>1.72</u>	<u>0.7</u>	<u>1.44</u>	11.52	11.52	<u>0.72</u>	1.44- 2.88	17.92
	PA54	<u>0.62-1.24</u>	13.76- 27.52	<u>1.4-2.8</u>	5.76	11.52	46.08- 92.16	5.76	5.76- 11.52	71.68- 143.36
	PA58	<u>0.62</u>	6.88- 13.76	<u>1.4</u>	<u>2.88</u>	11.52- 23.04	23.04	<u>2.88</u>	5.76	17.92- 35.84
<i>E. coli</i>	NCTC 13351	<u>1.24-2.48</u>	6.88- 13.76	<u>1.4</u>	<u>2.88</u>	11.52- 23.04	11.52	<u>2.88</u>	5.76	71.68- 143.36
	EC 2151612	<u>1.24-2.48</u>	6.88	<u>1.4</u>	<u>2.88</u>	11.52- 23.04	11.52	<u>1.4</u> <u>2.88</u>	5.76	71.68
	EC 1995591	<u>2.48</u>	6.88- 13.76	<u>1.4-2.8</u>	<u>2.88</u>	23.04	11.52	<u>2.88</u>	5.76	71.68
	EC 1227107	<u>1.24-2.48</u>	13.76	<u>2.8</u>	5.76	23.04	11.52- 23.04	<u>2.88</u>	5.76- 11.52	35.84- 71.68

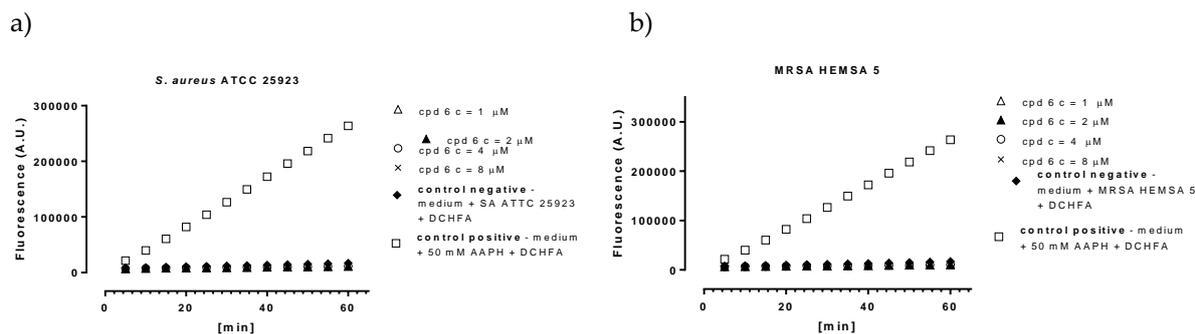
\* Particularly potent antibacterial activities (MIC < 5  $\mu\text{g/ml}$ ) are underlined.

### 3.3. Evaluation of ROS formation

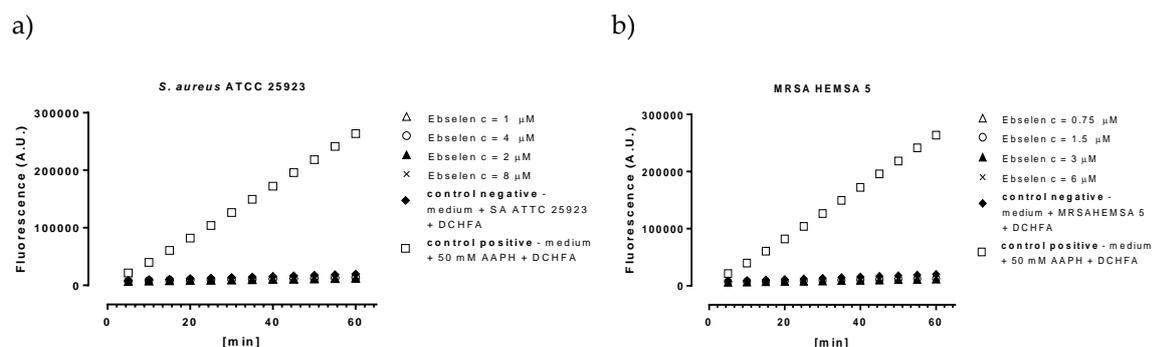
To analyze the effect of the selenazolinium salts tested on intracellular oxidative stress production in *S. aureus* DCHFA assay was performed. For this purpose, the impact of the most active compounds which were identified in the previous studies (the compounds 1 and 6) and ebselen on ROS release was determined in the reference *S. aureus* ATCC 25923 strain and the clinical isolate MRSA HEMSA 5 (Figure S1-S3)



**Figure S1.** Generation of intracellular ROS in the reference *S. aureus* ATCC 25923 strain (a) and the clinical MRSA HEMSA 5 isolate (b) upon exposure to the different concentrations of the compound 1. 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH) was included as a positive control in the assay. The level of oxidative stress was detected by the use of fluorogenic dye 2', 7'-dichlorodihydrofluorescein diacetate (DCHFA) which in the presence of cellular esterases and ROS is converted to highly fluorescent 2', 7'-dichlorofluorescein (DCFA). Values represent means with standard deviation (SD) bars from at least four repeats. Statistical significances were calculated using a one-way ANOVA followed by Bonferroni's multiple comparison test (**Figure S1 a, b**:  $p > 0.05$ ).



**Figure S2.** Generation of intracellular ROS in the reference *S. aureus* ATCC 25923 strain (a) and the clinical MRSA HEMSA 5 isolate (b) upon exposure to the different concentrations of compound 6. For further details refer to the **Figure S1**.



**Figure S3.** Generation of intracellular ROS in the reference *S. aureus* ATCC 25923 strain (a) and the clinical MRSA HEMSA 5 isolate (b) upon exposure to the different concentrations of ebselen. For further details, refer to the **Figure S1**.