**Supplementary Information**

**Conjugated Carbon Monoxide Releasing Molecules have broad-spectrum antimicrobial activity**

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**Tables and Figures**

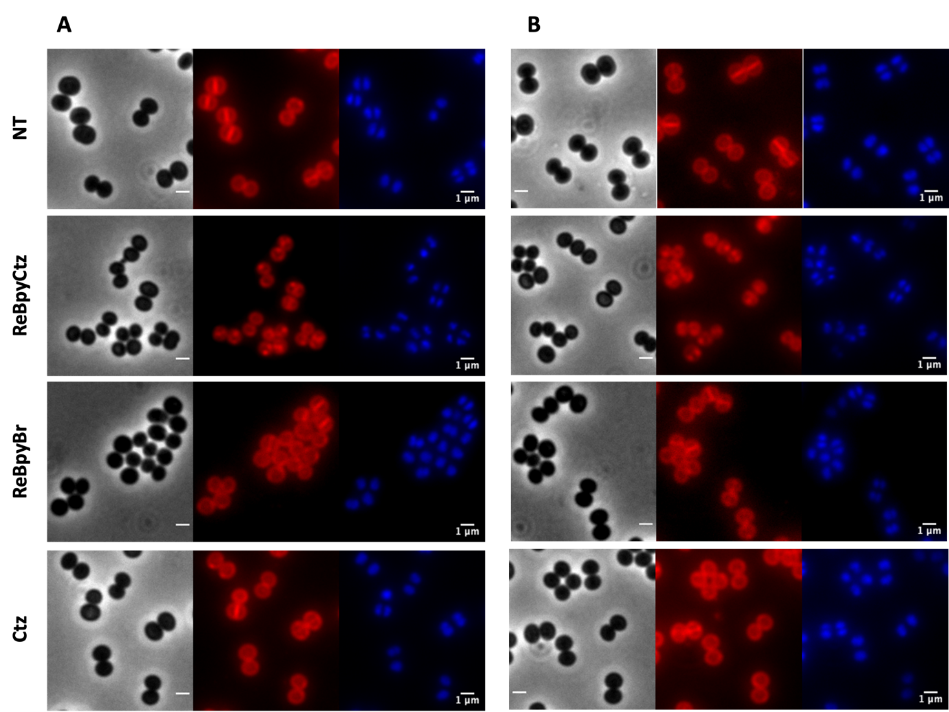
**Table S1.** **Toxicity of CORMs and clotrimazole to animal cells.**

Half-Maximum Inhibitory Concentration (IC50) is presented in µM. IC50 values of ReBpyBr, ReBpyCtz, and clotrimazole determined for mouse macrophages J774A.1, human hepatic (HepG2) and porcine kidney (LLC-PK1) cell lines.

|  |  |  |  |
| --- | --- | --- | --- |
| IC50 (µM) | | | |
|  | J774A.1 | HepG2 | LLC-PK1 |
| ReBpyCtz | 0.2 ± 0.2 | 1.4 ± 0.4 | 2.0 ± 0.8 |
| ReBpyBr | 16.9 ± 6.7 | 31.1 ± 11.0 | 16.4 ± 8.3 |
| Ctz | 1.5 ± 0.9 | 29.2 ± 21.9 | 11.6 ± 3.2 |

**Figure S1.** ***S. aureus* treated with ReBpyCtz.**

Images of *S. aureus* not treated (NT) andexposed to ReBpyCtz, ReBpyBr and Ctz (1 µM) for 4 min (A) and 60 min (B). Bacterial membrane (red) and DNA (blue) are marked by Nile Red and Hoechst dyes, respectively. Images are representative of three independent biological samples



**Videos S1 andS2. ReBpyCtz induces changes in the membrane morphology of *S. aureus.***

*S. aureus* untreated (*S. aureus* control) and exposed to ReBpyCtz 1 µM (*S. aureus* ReBpyCtz) were captured by super-resolution microscopy and images were collected each 3 min during 30 min. The time-lapse videos present 12 images in a total of 5 s, and are representative of two independent biological samples.

*S. aureus* ReBpyCtz

*S. aureus* control

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**Figure S2. Toxicity assays of ReBpyCtz.**

Viability of porcine kidney cells (LLC-PK1) following incubation with ReBpyCtz (A) and Ctz (B) for 24 h, 48 h and 72 h. Cellular viability was calculated by the Thiazol blue tetrazolium bromide (MTT) colorimetric assay with values normalized to untreated cells. Concentration range: 0-8 µg/ml (ReBpyCtz) and 0-32 µg/ml (Ctz) (DMSO of 1%).

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**Figure S3. Checkerboard assay of *S. aureus* exposed to CORM and clotrimazole.**

Growth ratio of *S. aureus* exposed to a combination of ReBpyBr (250 -2 μM) and clotrimazole (64 - 1 μM). Cells were grown in MHB medium at 37 °C, and after 24 h the ratio was determined by measuring OD600. Growth ratios are represented as 0-1.0 in a heatmap of the normalized growth relative to untreated cells, obtained in GraphPad Prism. Image is representative of two independent biological samples.



**Figure S4. Release of carbon monoxide in acidified media culture.**

*S. aureus* cells collected at the exponential phase were filtered and incubated with 10 µM of ReBpyCtz for 15 min in the presence of the COP-probe. Fluorescence intensity was measured at time zero (A) and after 30 min (B). Samples were excited at 419 nm and emission detection was measured between 500 to 700 nm. Data is representative of two independent biological samples.

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