In-silico identified new natural sortase A inhibitors disrupt *S. aureus* biofilm formation

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Supplementary Materials:

**Figure S1:** Dose response of known inhibitors, curcumin (A) and chlorogenic acid (B), on sortase A. Inhibition of SrtA activity was measured at 24h using two SrtA substrates. The results are mean values and SE of three replicates.
Figure S2: Effect of hit compounds and known inhibitors on the growth of *S. aureus* ATCC 25923: N1287 (A), N2576 (B), curcumin (C), and chlorogenic acid (D). The results are mean values and SE of three replicates.
**Figure S3**: Effect of hit compounds and known inhibitors on the growth of *S. aureus* ATCC 33591 (MRSA): N1287 (A), N2576 (B), curcumin (C), and p-HMB (D). The results are mean values and SE of three replicates.
Figure S4. Effect of the known inhibitors of SrtA and selected hits on S. aureus ATCC 33591 (MRSA) biofilm formation and pre-formed biofilms. Inhibition of S. aureus biofilm formation was determined using CV assay. N1287 (A), N2576 (B), curcumin (C) and p-HMB (D). The results data are presented as the mean percent inhibition ± S.E of three replicates (two independent experiments) relative to the untreated control (wild type). *p<0.05, **p<0.01, ***p<0.001 and, ****p<0.0001.
**Figure S5:** Crystal violet assay to assess the antibiofilm activity of selected hits and known inhibitors of SrtA on *S. aureus* ATCC 33591 (MRSA) biofilm formation (A) and pre-formed biofilms (B).
Figure S6: Dose-response of the known inhibitors of SrtA and selected hits on *S. aureus* ATCC 33591 (MRSA) biofilm formation and pre-formed biofilms. Inhibition of *S. aureus* biofilm was determined using PrestoBlue cell viability reagent. N1287 (A), N2576 (B), curcumin (C), and p-HMB (D). The results data
are presented as the mean percent inhibition ± S.E of three replicates (two independent experiments) relative to the untreated control (wild type). *p<0.05, **p<0.01, ***p<0.001 and, ****p<0.0001.
Figure S7: Effect of the known inhibitors of SrtA and selected hits on *E.coli* ATCC 25922 biofilm formation. Inhibition of *E.coli* ATCC 25922 biofilm formation was determined using CV assay. N1287 (A), N2576 (B), curcumin (C) and p-HMB (D). The results data are presented as the mean percent biofilm formation ± S.E of three replicates (two independent experiments) relative to the untreated control (wild type).
| Compound | \( S. \text{aureus} \) MIC (µM) | \( S. \text{aureus} \) ATCC 25923 Newman | \( S. \text{aureus} \) ATCC 3591 (MRSA) | Mammalian cell lines IC\(_{50}\) (µM) | Biofilm Inhibition IC\(_{50}\) (µM) | Fibrinogen adherence inhibition IC\(_{50}\) (µM) |
|----------|-------------------------------|-----------------------------------------|-----------------------------------|---------------------------------|-----------------------------|-----------------------------|
|          | ATCC 25923 Newman             | ATCC 3591 (MRSA)                        | HepG2                             | A549                            | Biofilm formation Pre-formed | Biofilm formation Pre-formed |
| N1287    | 7.2 ± 0.1                    | 7.3±0.8                                 | 80.1±6.3                          | 63.9±5.1                        | 12.2±4.1                   | NA                          | 41.9                      | NA                           | 9.5±0.5         |
| N2576    | 6.3 ± 0.1                    | 5.8±1.3                                 | 25.2±5.5                          | 65±7.4                          | 2.9±0.4                    | 10±1.2                     | 24.8                      | >200                         | 5.7±0.8         |
| Curcumin | > 200                        | >200                                    | >200                              | 39.6±5.3                        | 51.2±4.4                   | NA                          | >150                      | NA                           | 46.5±6.2         |
| Chlorogenic acid | > 200       | >200                                    | NT                                | >200                            | NT                          | NT                          | NT                        | NT                           | NT               |
| p-hydroxymercuribenzoic acid (p-HMB) | NT | NT | 6.7±1.5 | NT | NT | 11.9±2.8 | 43±3.6 | >150 | NA | 37.38±5.3 |

NA – no activity, NT - not tested