Infectious diseases are a serious cause of morbidity and mortality. Amongst the several pathogens involved in infectious diseases, *Staphylococcus aureus* has developed resistance to most antibiotics. Barber described in 1961 methicillin-resistant *S. aureus* (MRSA) strains in clinical isolates derived from an hospital in England. From that point on, MRSA infections have seen a dramatic increase. Vancomycin is the gold standard scaffold exhibiting antimicrobial activity against Gram-positive bacteria, including resistant strains such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *E. faecalis* (VRE) strains.

MRSA\(^2\)–\(^9\) is urgently needed. Concerning antimicrobial naturally occurring products, examples such as Nigrosporionone A–B\(^10\) and Palmenone A–B\(^11\) contains a cyclopentenone (CP) scaffold (Scheme 1 A).

Inspired by these natural products, we envisioned that synthetic cyclopentenones easily prepared from biomass synthons\(^12\) could provide access to a new sustainable scaffold with antimicrobial activity.

In particular, trans-diamino-cyclopentenones (DCPs) and δ-lactone-fused cyclopentenones (LCPs) can be prepared respectively from furfural and activated δ-hydroxymethylfurfural (HMF), both furan derivatives being derived from biomass (Scheme 1B). In line with our interest regarding sustainable production of biologically active compounds, we have also developed choline-based ionic liquids with antibiotic activity.\(^{25}\) Herein we report a novel sustainable based cyclopentenone scaffold exhibiting antimicrobial activity against MRSA.

The aforementioned DCP family was previously prepared by us from the condensation of furfural and secondary amines in aqueous conditions in the presence of Cu(OTf)\(_2\)\(^{13\text{-}24}\) as depicted in Scheme 2A. Novel DCP 7, 8 and 9 were prepared using the same method in moderate to good yields.

The LCP family 10–19 was previously prepared by us from the condensation of activated HMF and secondary amines in dichloromethane promoted by (R)-BINOL.\(^{24}\)

The CPs antimicrobial activity was evaluated. An initial screening was performed by assessing the minimum inhibitory
concentrations (MICs) against a Gram-positive bacteria strain \textit{Staphylococcus aureus} (\textit{Sa}) and yeast \textit{Saccharomyces cerevisiae} (\textit{Sc}).

The initial screening revealed that DCPs 1–5 exhibited MIC $> 62.5$ $\mu$g · mL$^{-1}$ against Gram-positive bacteria \textit{Sa} and yeast \textit{Sc}. However, amongst the DCP family, examples containing aryl amines (4–9) showed activity with MIC ranging from 3.91 to 7.81 $\mu$g · mL$^{-1}$ in bacteria and moderate antifungal activity with MIC ranging from 31.2 to 62.5 $\mu$g · mL$^{-1}$ (Table 1, compounds 6–9).

LCP derivatives 10–19 despite the structural resemblance to naturally occurring Nigrosporiones (Scheme 1) exhibited no antimicrobial activity.

Next, we evaluated the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values against Gram-positive (\textit{Staphylococcus aureus} ATCC 25923 (MSSA), \textit{S. aureus} CIP 106760 (MRSA), \textit{Enterococcus faecalis} ATCC 29212 and \textit{E. faecalis} ATCC 51299 (Low VRE)), Gram-negative (\textit{Escherichia coli} ATCC 25922) bacteria and yeast (\textit{Saccharomyces cerevisiae} ATCC 2601) strains for the selected CP 6–9 (Table 1).

Due to the potential Michael acceptor character of the CP that can cause undesired ADMET properties (absorption, distribution, metabolism, elimination and toxicity), we prepared 20 as a model oxime derivative of DCP. Moreover, previous reports had shown that oxime and oxime ether lead to increased antimicrobial activity of the corresponding ketones.$^{26–30}$ The oxime ether

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**Table 1.** Selected observed of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) values for the synthesized cyclopentenones.$^{[a]}$

| CP | \textit{Sa} | MRSA | \textit{Ef} | VRE | \textit{Ec} | \textit{Sc} | HEK 293T |
|----|------------|------|-----------|------|---------|---------|-----------|
|    | [\(\mu\text{g} \cdot \text{mL}^{-1}\)] | [\(\mu\text{g} \cdot \text{mL}^{-1}\)] | [\(\mu\text{g} \cdot \text{mL}^{-1}\)] | [\(\mu\text{g} \cdot \text{mL}^{-1}\)] | [\(\mu\text{g} \cdot \text{mL}^{-1}\)] | [\(\mu\text{g} \cdot \text{mL}^{-1}\)] | [% viability at 20 $\mu$M] |
| 6  | 7.81       | >31.2 | 15.6      | 250  | 1.95    | >15.6   | >62.5     | 62.5      | 125       | 125     | 90      |
| 7  | 7.81       | >62.5 | 3.91      | >31.2 | 1.95    | >15.6   | 7.81      | >62.5     | 62.5      | 125     | 125     | 95      |
| 8  | 3.91       | >31.2 | 3.91      | >31.2 | >0.49   | >3.91   | 7.81      | >15.6     | 62.5      | 125     | 125     | 65      |
| 9  | 7.81       | 250   | 62.5      | 250  | 15.6    | 500     | nt        | nt        | nt        | 31.2    | 125     | 59      |
| 20 | 7.81       | >62.5 | 0.976     | >7.81 | >0.49   | >7.81   | >0.976    | >7.81     | >0.49    | >7.81   | >0.49   | >7.81   |
| Vanco | 1.95 | 500  | 3.91      | nt   | <0.49   | >500    | <0.49    | <0.49    | 500      | 15.6    | 500     |
| NOR | 1.95      | 500   | 3.91      | nt   | <0.49   | >500    | <0.49    | <0.49    | 500      | 15.6    | 500     |
| NYS | 1.95        | 500   | 3.91      | nt   | <0.49   | >500    | <0.49    | <0.49    | 500      | 15.6    | 500     |

$^{[a]}$ The MIC corresponding to the lowest concentration at which no visible growth was observed, was assessed by the microdilution method. For MBC evaluation, the bacterial suspension on the wells was homogenized, serial-diluted, triplicate spread on appropriate medium and incubated at 37°C for 24 h. Data represent the median values of at least three replicates. Vanco: vancomycin. NOR: norfloxacin. NYS: nystatin. \textit{Sa}: \textit{Staphylococcus aureus}. MRSA: methicillin-resistant \textit{Staphylococcus aureus}. \textit{Ef}: \textit{Enterococcus faecalis}. VRE: vancomycin-resistant \textit{Enterococcus faecalis}. EC: \textit{Escherichia coli}. \textit{Sc}: \textit{Saccharomyces cerevisiae}. HEK: Human Embryonic Kidney.
of DCP 6 was prepared by condensation with O-benzylhydroxylamine hydrochloride under basic conditions in good yield (Scheme 3). The formation of an oxime using the corresponding free hydroxylamine was not possible, DCP 6 underwent decomposition and no oxime was observed despite full conversion of the starting material.

Overall selected DCP 6–9 were active in Gram-positive strains, including methicillin-resistant S. aureus (MIC of 3.91 µg·mL⁻¹, CP 7 and 8) and vancomycin-resistant E. faecalis (MIC of 0.98 µg·mL⁻¹, CP 8). No activity was observed against Gram-negative strain E. coli nor in yeast strain S. cerevisiae. Despite the relevant antimicrobial activity, CP 6–9 also exhibited toxicity in healthy cell line HEK 293T, in particular CP 8 and 9 decreased the cell viability at 20 µM in 65 and 59%, respectively.

On the other hand, oxime derivative 20 remained active in MRSA and VRE (MIC of 0.976 µg·mL⁻¹ and 3.91 µg·mL⁻¹, respectively) yet did not induce cell death in HEK 293T. Moreover 20 was active against yeast strain S. cerevisiae with MIC value similar to positive control nystatin (MIC of 15.6 µg·mL⁻¹).

Focusing on the lead compound 20, the analysis of its effect on bacterial growth over time was performed against S. aureus MRSA. In order to address the bacteriostatic and bactericidal properties of 20, viable cells (CFU/mL) were determined in the presence of different concentrations of 20 (Figure 1).

Compound 20 displayed delay and decrease of the growth rate of MRSA at all tested concentrations. This decrease is more noticeable for the concentration of 0.488 µg·mL⁻¹. The comparison between the growth and the viability profiles showed that the cells remain viable, indicating a bacteriostatic effect for this compound.

Previous reports show the thio-Michael addition to DCP lead to the release of the amine in position 4 to reform the enone system. To evaluate the possibility of such event (e.g. CP 20 undergoing non-specific Michael addition in the bacteria cells releasing the corresponding thio-amino CP compound and tetrahydroquinoline (THQ)) both the thiol adduct and the THQ were tested as antimicrobial inhibitors. Derivative 21 was prepared by addition of thiophenol under basic conditions (Scheme 4A). Also reduced derivative 22 was prepared by reduction with NaBH₄ (Scheme 4B) in order to evaluate the importance on the activity of the enone functionality.

Upon antimicrobial assays, was observed low activity when the bacteria was incubated either with 21 or free amine THQ (Table 2). In addition, similar behavior was observed upon reduction of the enone (compound 22, Table 2). The combined results highlight the importance of the enone system.

Finally, the drug-like properties of the enones were accessed and are depicted in Table 3. CPs 6, 7 and 8 exhibits good drug-like properties, with low molecular weights, cLogP between 3.03 to 4.31. No hydrogen bond donors (HBD) and only 3–5 hydrogen bond acceptors (HBA). TPSA between 23 and 42. The calculated properties fits the Lipinski’s rule of 5 and also the rules described by Veber et al. [31] < 140 PSA and < 12 rotatable bonds. Oxime derivative 20 exhibits similar properties with the exception of cLogP. The value is 6.63, higher than the...
Experimental Section

Details for chemical synthesis, analytical and biological methods together with characterization data are described in the Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 2. Observed Minimum Inhibitory Concentration (MIC) values for the synthesized CPs[a]

| CP  | Staphylococcus aureus, Sa (µg · mL⁻¹) | MBC (µg · mL⁻¹) |
|-----|--------------------------------------|-----------------|
| 6   | 7.81                                 | > 31.2          |
| 21  | 125                                  | 500             |
| 22  | 62.5                                 | > 500           |
| THQ | 125                                  | > 500           |
| Vanco | 1.95                           | 500             |

[a] The MIC corresponding to the lowest concentration at which no visible growth was observed, was assessed by the microdilution method. For MBC evaluation, the bacterial suspension on the wells was homogenized, serial-diluted, triplicate spread on appropriate medium, and incubated at 37°C. Data represent the median values of at least three replicates. Vanco: vancomycin. Sa: Staphylococcus aureus.

Table 3. Calculated properties of relevant cyclopentenones.

| CP  | cLogP | MW  | HBA | HBD | TPSA | IS | RDK | TRD |
|-----|-------|-----|-----|-----|------|----|-----|-----|
| 6   | 4.31  | 344.4 | 3   | 0   | 23.55 | 9  | 25  | 10  |
| 7   | 3.03  | 348.4 | 5   | 0   | 42.02 | 11 | 14  | 16  |
| 8   | 3.79  | 380.5 | 3   | 0   | 23.55 | 11 | 14  | 16  |
| 20  | 6.63  | 449.6 | 4   | 0   | 28.07 | 10 | 14  | 20  |

Recommended value of < 5. However the remaining parameters point towards acceptable drug-like properties.

In summary, we observed that amongst the tested cyclopentenones the trans-4,5-diamino-cyclopent-2-enones are the most promising antibacterial agents. In particular tetrahydroquinalnine analogs show activity against Gram-positive strains, including MRSA and VRE. Although enones are Michael acceptors and possible PAINS, the corresponding oxime ether 20 exhibit enhanced activity in MRSA and VRE (MIC of 0.976 µg · mL⁻¹ and 3.91 µg · mL⁻¹ respectively) and better toxicity profile in HEK 293T cell lines. Further studies on the identification of the target for this scaffold and optimization of the activity are ongoing.

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