Functionalized Cyclopentenones and an Oxime Ether as Antimicrobial Agents

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Several naturally occurring cyclopentenones, such as palmenones and nigrosporiones, exhibit antimicrobial activity. Herein we describe the antimicrobial activity of cyclopentenones and derivatives that can be easily accessed from biomass derivatives furfural and 5-hydroxymethylfurfural. Upon screening a range of functionalized *trans*-diamino-cyclopentenones (DCPs) and δ-lactone-fused cyclopentenones (LCPs), an oxime ether derivative of DCP was identified that exhibited remarkable antimicrobial activity against Gram-positive bacteria, including resistant strains such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *E. faecalis* (VRE) strains.

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.[1] From that point on, MRSA infections have seen a dramatic increase.[2,3] Vancomycin is the gold standard for MRSA infections, however exhibits drawbacks such as the low oral bioavailability that limits its use to intravenous,[4] and vancomycin-associated nephron and ototoxicity that leads to the need of constant monitoring of serum vancomycin concentrations.[5] Moreover the appearance of vancomycin intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) is a cause of concern.[6] For this reason the search for new molecules with antibiotic properties, especially against MRSA[7–9] is urgently needed. Concerning antimicrobial naturally occurring products, examples such as Nigrosporione A–B[10] and Palmenone A–B[11] contains a cyclopentenone (CP) scaffold (Scheme 1 A).

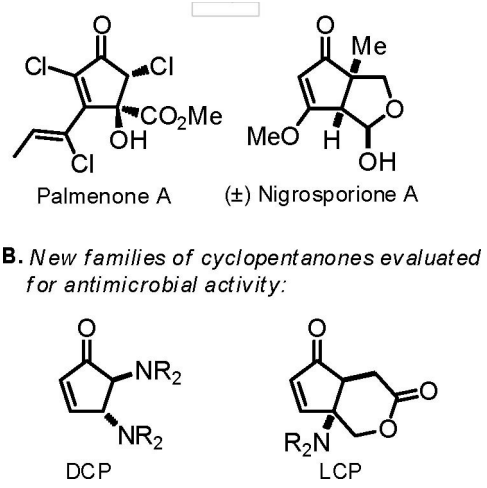
Inspired by these natural products, we envisioned that synthetic cyclopentenones easily prepared from biomass synthons[12] could provide access to a new sustainable scaffold with antimicrobial activity.

In particular, *trans*-diamino-cyclopentenones (DCPs) and δ-lactone-fused cyclopentenones (LCPs) can be prepared respectively from furfural and activated 5-hydroxymethylfurfural (HMF), both furan derivatives being derived from biomass (Scheme 1B).[13–24] 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] 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



Scheme 1. (A) Examples of relevant antimicrobial cyclopentenones and (B) new biobased cyclopentenones evaluated for antimicrobial activity.

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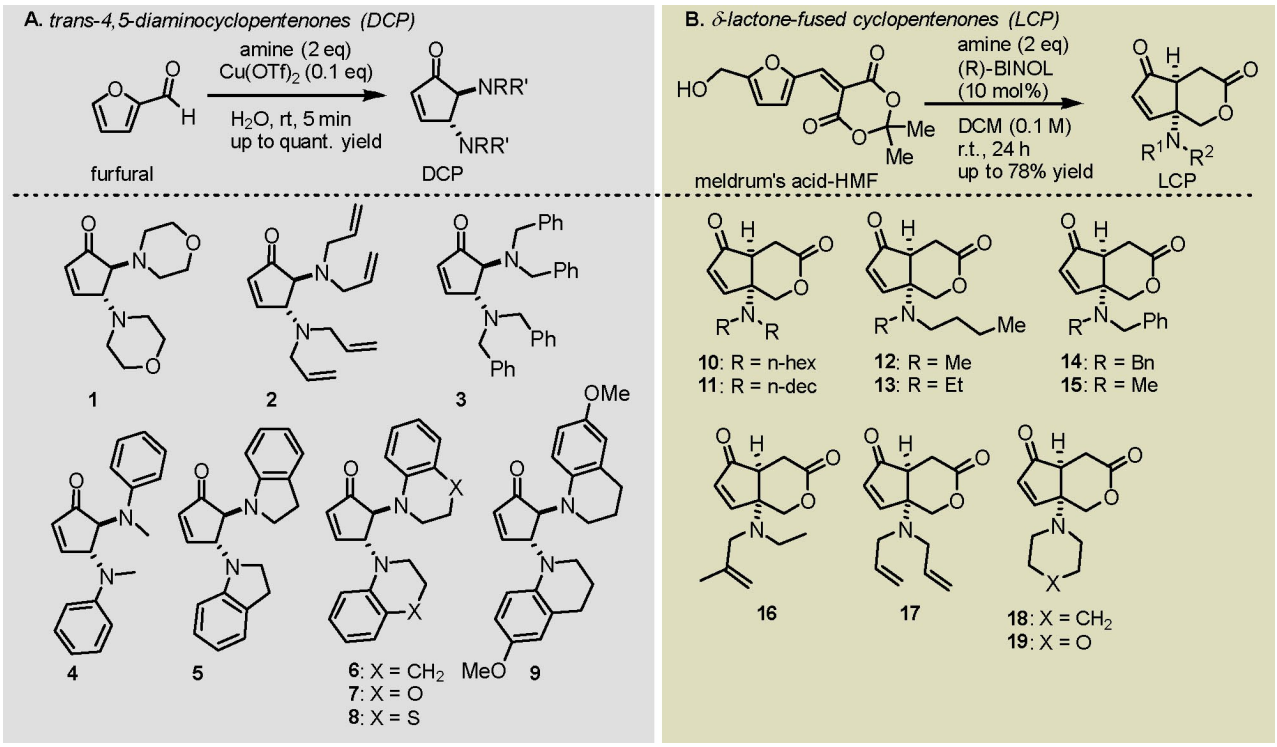


Scheme 2. Tested cyclopentenones prepared from biomass derived furfural and 5-hydroxymethylfurfural.

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	*Sa*		*MRSA*		*Ef*		*VRE*		*Ec*		*Sc*		*HEK 293T*
	MIC [μg·mL^{-1}]	MBC [μg·mL^{-1}]	MIC [μg·mL^{-1}]	MBC [μg·mL^{-1}]	MIC [μg·mL^{-1}]	MBC [μg·mL^{-1}]	MIC [μg·mL^{-1}]	MBC [μg·mL^{-1}]	MIC [μg·mL^{-1}]	MBC [μg·mL^{-1}]	MIC [μg·mL^{-1}]	MFC [μg·mL^{-1}]	[% viability at 20 μM]
6	7.81	>31.2	15.6	250	1.95	>15.6	7.81	>62.5	62.5	125	62.5	125	90
7	7.81	>62.5	3.91	>31.2	1.95	>15.6	3.91	>31.2	62.5	250	31.2	62.5	95
8	**3.91**	>31.2	3.91	>31.2	**0.49**	>3.91	**0.98**	15.6	62.5	250	62.5	125	65
9	7.81	250	62.5	250	15.6	500	nt	nt	nt	nt	31.2	125	59
20	7.81	>62.5	**0.976**	>7.81	**0.976**	>7.81	3.91	62.5	62.5	125	**15.6**	62.5	100
Vanco	1.95	500	3.91	nt	<0.49	>500	62.5	>500					
NOR									<0.49	500			
NYS											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. *Sa*: *Staphylococcus aureus*. MRSA: methicillin-resistant *Staphylococcus aureus*. *Ef*: *Enterococcus faecalis*. VRE: vancomycin-resistant *Enterococcus faecalis*. *Ec*: *Escherichia coli*. *Sc*: *Saccharomyces cerevisiae*. HEK: Human Embryonic Kidney.

concentrations (MICs) against a Gram-positive bacteria strain *Staphylococcus aureus* (*Sa*) and yeast *Saccharomyces cerevisiae* (*Sc*).

The initial screening revealed that DCPs **1**–**5** exhibited MIC > 62.5 μg·mL^{-1} against Gram-positive bacteria *Sa* and yeast *Sc*. However, amongst the DCP family, examples containing aryl amines (**4**–**9**) showed activity with MIC ranging from 3.91 to 7.81 μg·mL^{-1} in bacteria and moderate antifungal activity with MIC ranging from 31.2 to 62.5 μg·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 (*Staphylococcus aureus* ATCC 25923 (MSSA), *S. aureus* CIP 106760 (MRSA), *Enterococcus faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 (Low VRE)), Gram-negative (*Escherichia coli* ATCC 25922) bacteria and yeast (*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

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 $\mu g \cdot mL^{-1}$, CP **7** and **8**) and vancomycin-resistant *E. faecalis* (MIC of 0.98 $\mu g \cdot mL^{-1}$, 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 $\mu g \cdot mL^{-1}$ and 3.91 $\mu g \cdot mL^{-1}$, 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 $\mu g \cdot mL^{-1}$).

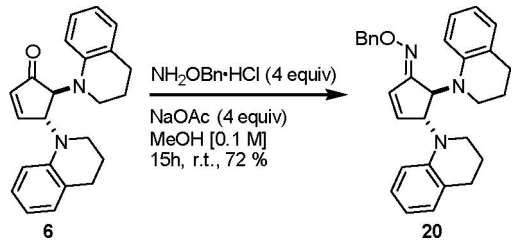
Focusing on the lead compound **20**, the analysis of its effect on bacterial growth over time was performed against *S. aureus* MRSA. In order to address the bacteriostatic and bactericidal properties of **20**, viable cells (CFU/mL) were determined in the presence of different concentrations of **20**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

Compound **20** displayed delay and decrease of the growth rate of MRSA at all tested concentrations. This decreased is more noticeable for the concentration of 0.488 $\mu g \cdot mL^{-1}$. 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 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 $NaBH_4$ (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



Scheme 3. Preparation of oxime ether **20** from *trans*-diamino-cyclopent-2-enone **6.**

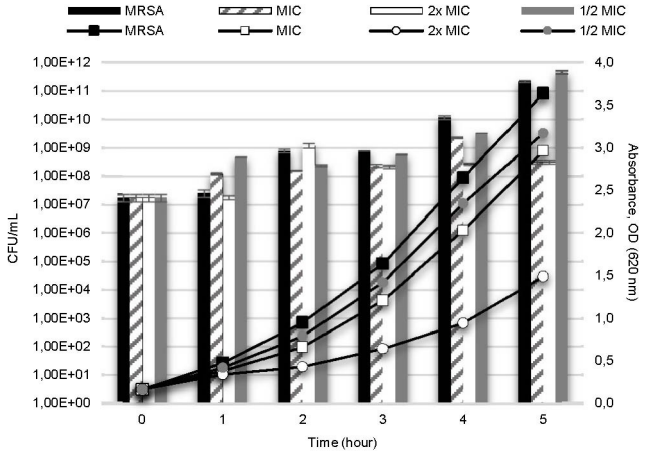
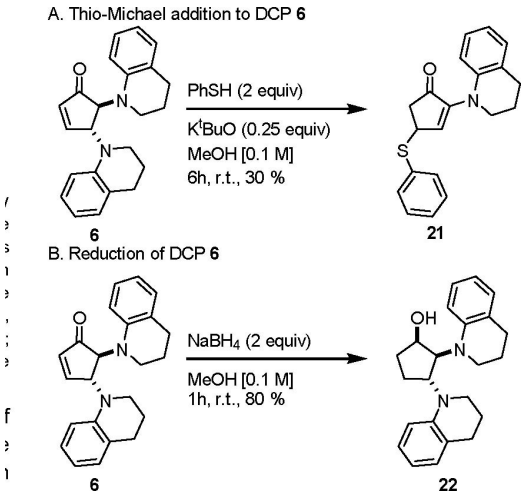


Figure 1. Monitoring of bacterial *S. aureus* MRSA strain growth curve by following the optical density (OD 620 nm), represented by a line chart on the right-side axis, and by determining the number of colony forming units (CFU/mL), represented by a column chart on the left side axis. The growth curves were determined in the absence (black bars and squares) or in the presence of compound **20** at MIC (0.976 $\mu g \cdot mL^{-1}$; striped bar and white square), at 2×MIC (1.952 $\mu g \cdot mL^{-1}$; withe bar and circle) and at $^1/_2$ MIC (0.488 $\mu g \cdot mL^{-1}$; grey bar and circle). Mean standard deviation (SD) values calculated for three independent bioassays, SD = ±0.48.



Scheme 4. Preparation of cyclopentenone derivatives of **6** by A) Michael addition and B) reduction with $NaBH_4$.

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

CP	*Staphylococcus aureus, Sa* MIC [$\mu g \cdot mL^{-1}$]	MBC [$\mu g \cdot mL^{-1}$]
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
6	4.31	344.4	3	0	23.55
7	3.03	348.4	5	0	42.02
8	3.79	380.5	3	0	23.55
20	6.63	449.6	4	0	28.07

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 tetrahydroquinoline 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 $\mu g \cdot mL^{-1}$ and 3.91 $\mu g \cdot mL^{-1}$ 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.

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.

Keywords: antimicrobial · biomass · cyclopentenones · sustainability · furfurals

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