Accepted Manuscript

Aurone derivatives as promising antibacterial agents against resistant Gram-positive pathogens

Hamza Olleik, Samir Yahiaoui, Brayan Roulier, Elise Courvoisier-Dezord, Josette Perrier, Basile Pérès, Akram Hijazi, Elias Baydoun, Josette Raymond, Ahcène Boumendjel, Marc Maresca, Romain Haudecoeur

PII: S0223-5234(19)30032-7

DOI: https://doi.org/10.1016/j.ejmech.2019.01.022

Reference: EJMECH 11029

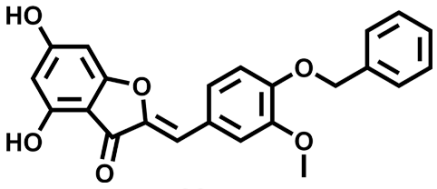
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 16 October 2018

Revised Date: 7 January 2019

Accepted Date: 10 January 2019

Please cite this article as: H. Olleik, S. Yahiaoui, B. Roulier, E. Courvoisier-Dezord, J. Perrier, B. Pérès, A. Hijazi, E. Baydoun, J. Raymond, Ahcè. Boumendjel, M. Maresca, R. Haudecoeur, Aurone derivatives as promising antibacterial agents against resistant Gram-positive pathogens, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.01.022.

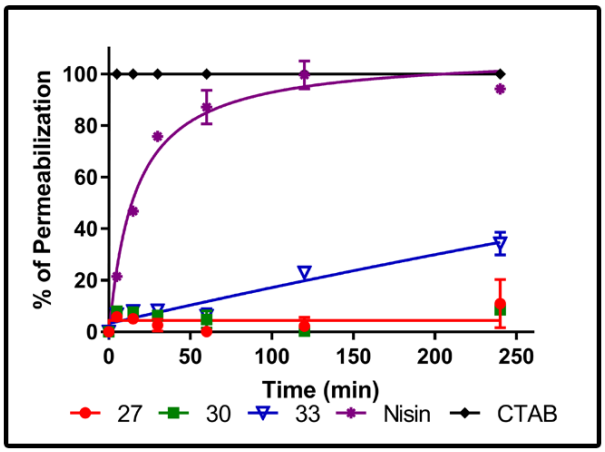


33

MIC = 3.9–250 μM

Gram+ *S. aureus, C. perfringens, B. subtilis, E. faecalis, L. lactis, etc.*

Gram– only *H. pylori* and *V. alginolyticus*



Aurone derivatives as promising antibacterial agents against resistant Gram-positive pathogens

Hamza Olleik,[a] Samir Yahiaoui,[1,b] Brayan Roulier,[b] Elise Courvoisier-Dezord,[a] Josette Perrier,[a] Basile Pérès,[b] Akram Hijazi,[c] Elias Baydoun,[d] Josette Raymond,[e] Ahcène Boumendjel,[b] Marc Maresca,[a,*] Romain Haudecoeur[b,*]

[a]Aix Marseille Univ., CNRS, Centrale Marseille, iSm2, Marseille, France.

E-mail: m.maresca@univ-amu.fr

[b]Univ. Grenoble Alpes, CNRS, DPM UMR 5063, F-38041, Grenoble, France.

E-mail: Romain.Haudecoeur@univ-grenoble-alpes.fr

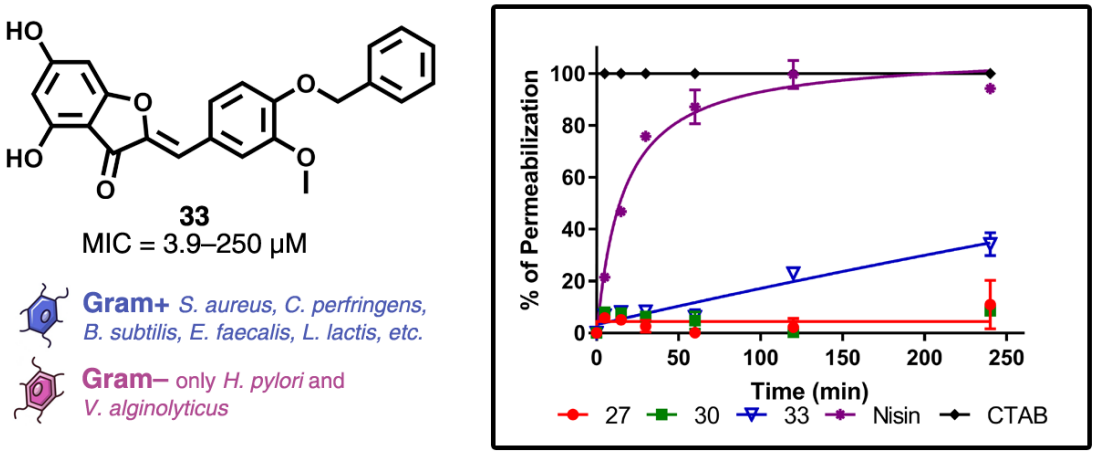
[c]Lebanese University, Doctoral School of Science and Technology, PRASE, Beirut, Lebanon.

[d]American University of Beirut, Department of Biology, Beirut 1107 2020, Lebanon.

[e]Université Paris 5, Hôpital Cochin, Service de bactériologie.

[1]Present address: Department of Drug Design and Optimization, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Campus E8.1, 66123 Saarbrücken, Germany.

Graphical abstract



Abstract

A set of variously substituted aurones was synthesized and evaluated against Methicillin-Resistant *S. aureus* (MRSA) and *P. aeruginosa*. Several analogues were found active against MRSA, but no effect was recorded against *P. aeruginosa*. Compounds **27**, **30** and **33** showed low cytotoxicity, and were tested against a full range of bacterial (Gram-positive and Gram-negative) and fungal species, including resistant strains. These aurones displayed a selective inhibition of Gram-positive bacteria with excellent Therapeutic Index values, while showing no significant action on several Gram-negative strains, *H. pylori* and *V. alginolyticus* being the only susceptible strains among the Gram-negative bacteria tested. A permeabilization assay showed that the antibacterial activity of at least some of the aurones could be linked to alterations of the bacterial membrane. Overall, this study endorses the use of the aurone scaffold for the development of new potent and selective antibacterial agents.

Keywords

Aurones, antibacterial, resistance, MRSA, Gram-positive, bacterial membrane

1. Introduction

Flavonoids are a large and diverse class of natural products, including widespread flavones, chalcones, flavanones and derivatives, which provided promising antibacterial activities in the past decades [1]. Among flavonoids, aurones constitute a minor subclass occurring in a very limited number of advanced plant species, where they play a variety of roles, such as flower pigments, nectar guides or antioxidants [2], while being increasingly studied in medicinal chemistry contexts [3,4]. Among the hundred-plus acknowledged natural derivatives of this molecular subclass, three particular compounds, cephalocerone, hispidol and hispidol-4'-*O*-β-D-glucoside (Figure 1), have been identified as phytoalexins, produced by the taxa *Cephalocereus senilis* [5,6] and *Medicago truncatula* [7] in response to elicitation. In the first case, cephalocerone has been found to inhibit the growth of *Erwinia cacticida*, a Gram-negative plant pathogenic bacteria responsible for common cactus rot infections. In the second case, hispidol and its glucoside derivative showed significant inhibition of *Phoma medicaginis*, a fungal pathogen causing spring black stem and leaf spot disease in several Fabaceae species. Based on these natural antimicrobial properties, and since the related medicinal chemistry works reported so far mainly focused on heavy modifications of the aurone scaffold itself (*e.g.* prenylated aurones and corresponding cyclized analogues [8], 5-nitrofuran and 5-nitroimidazole aurones [9,10], ferrocene-based derivatives [11], 2,2-bisaminomethyl analogues [12], fused aurone–chromanones and aurone–quinolones [13,14],

see Figure 1), we thought that further exploring the diversity of aurone substituents could provide more active compounds against a broader spectrum of pathogens.

Indeed there is still a crucial need for novel antimicrobial agents, as the emergence of drug-resistant bacteria is progressively dismissing current standard antibiotics, leading to an elevating mortality rate of infectious diseases. Methicillin-resistant *Staphylococcus aureus* (MRSA) is of particular concern, as it was recently estimated that ~80 000 individuals are inflected in the United States annually, with ~11 000 deaths, making this strain the most deadly antibiotic-resistant bacteria [15]. Moreover, several other Gram positive and Gram negative pathogens have become resistant at a worrisome level, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* or *Enterococcus* species. In this context, nature's strategies for fighting microbial invasions could be a valuable source of inspiration for discovering new therapeutic options. We thus proposed to synthesize a series of aurones, variously substituted at positions 4, 6, 7, 2', 3', 4', 5' and/or 6' (Figure 1), and to engage them in antimicrobial and cytotoxicity assays.

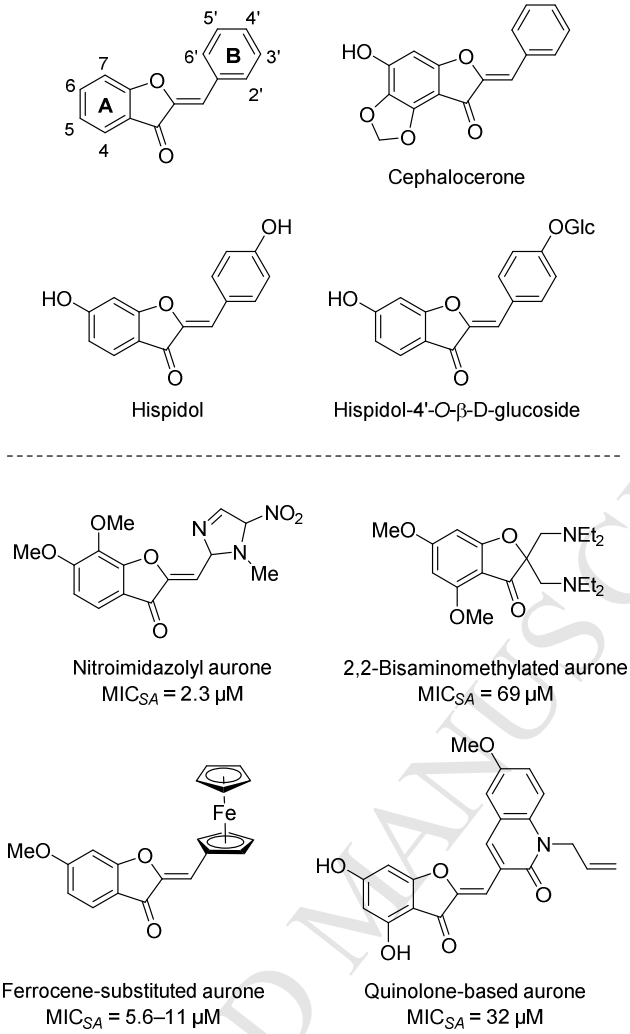
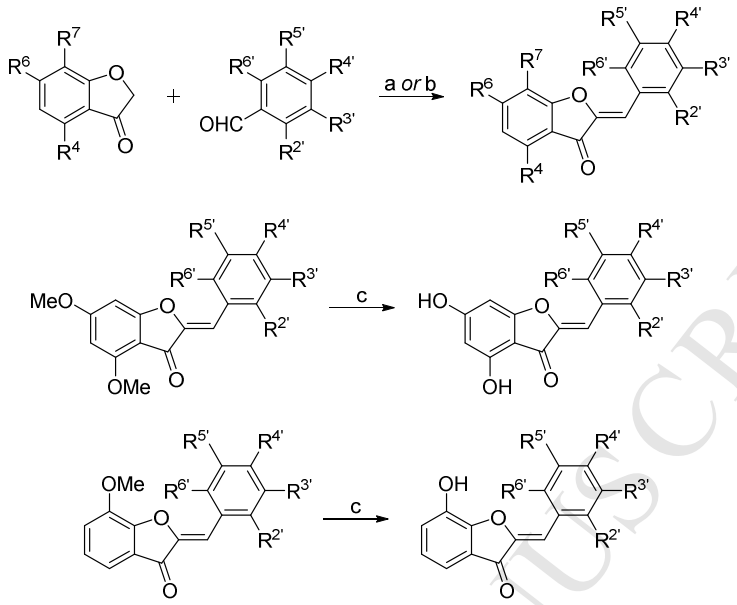


Figure 1. Structure of the aurone scaffold, natural antimicrobial aurones, and examples of synthetic, heavily modified analogues.

2. Chemistry

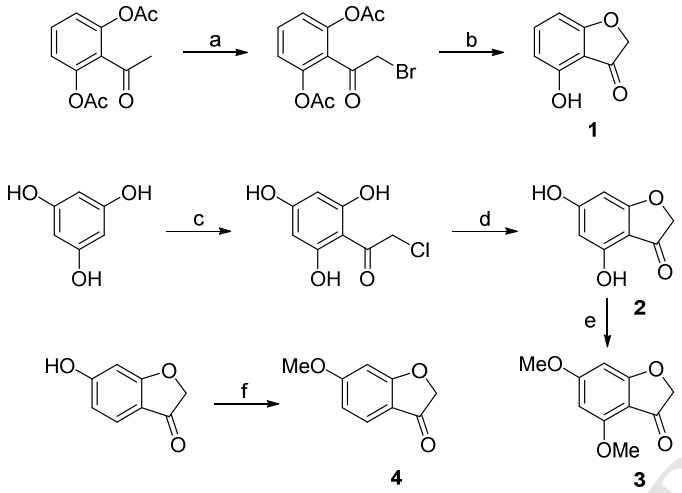
The aurone derivatives were prepared starting from substituted benzofuran-3(2*H*)-ones, through an aldol condensation with various benzaldehyde derivatives, either in basic or acidic conditions, depending of the substitution pattern of the compounds (Scheme 1). While methoxy- and polymethoxyaurones were obtained either by reaction with KOH/H_2O in MeOH at room temperature or by neutral Al_2O_3-mediated condensation, the hydroxy and

polyhydroxy counterparts needed stronger base- or acid-mediated conditions, or an additional deprotection step from the methoxy analogues with boron tribromide in dichloromethane [16].



Scheme 1. General pathways for the synthesis of aurone derivatives. Reagents and conditions: (a) KOH/H_2O, MeOH or EtOH, 25–80 °C, 5 min to 18 h; (b) Al_2O_3, CH_2Cl_2, 25 °C, 18 h; (c) BBr_3, CH_2Cl_2, rt, 24–72 h.

All these methods required the prior synthesis of various benzofuran-3(2*H*)-ones, as only benzofuran-3(2*H*)-one, 6-hydroxybenzofuran-3(2*H*)-one and 7-methoxybenzofuran-3(2*H*)-one were commercially available (Scheme 2). Compound **1** was obtained from the corresponding diacetoxyacetophenone, after a α-bromination / deprotection / cyclization sequence. The 4,6-dihydroxy analogue **2** was prepared from phloroglucinol, through a Houben-Hoesch reaction using chloroacetonitrile and zinc chloride in HCl / Et_2O, followed by one-pot hydrolysis and cyclization in refluxed H_2O. The methoxy derivatives **3** and **4** were synthesized from the corresponding hydroxy analogues, using Me_2SO_4 as the methyl donor agent.

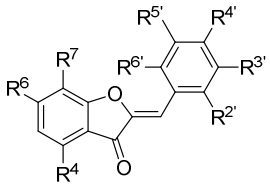


Scheme 2. Synthesis of benzofuran-3(2*H*)-one derivatives **1**–**4**. Reagents and conditions: (a) trimethylphenylammonium tribromide, THF, rt, 6 h; (b) KOH/H_2O, MeOH, 65 °C, 3 h; (c) $ClCH_2CN$, HCl, $ZnCl_2$, Et_2O, 0 °C, 3 h; (d) HCl, H_2O, 100 °C, 1 h; (e) Me_2SO_4, K_2CO_3, dimethoxyethane, 80 °C, 3 h; (f) Me_2SO_4, K_2CO_3, acetone, rt, 4 h.

3. Results & discussion

Antimicrobial activity of the aurone derivatives was first tested using MRSA (Methicillin-Resistant *S. aureus*, Gram positive) and *P. aeruginosa* (Gram negative) with the determination of MIC values as detailed in the Experimental section. Bacteria were exposed to increasing concentrations of compounds **5**–**38** (from 0 to 500 µM) and MICs were determined as the lowest concentration of the molecule able to prevent bacterial growth (Table 1). On the 34 derivatives tested, 15 were found active (*i.e.* compounds **8**–**10**, **15**, **16**, **23**–**25**, **27**–**33**) against MRSA (MIC_{MRSA} <250 µM) but not against *P. aeruginosa*, suggesting a specific activity against Gram positive bacteria. The determination of the Minimal Bactericidal Concentration (MBC) on MRSA showed that for all active derivatives, MBC and MIC were similar, suggesting a bactericidal mechanism of action as previously demonstrated for other aurones (data not shown) [11].

Table 1. Structure and antibacterial activity of aurone derivatives against MRSA (MIC_{MRSA}) and *P. aeruginosa* (MIC_{PA}). >500 µM indicates that MIC is higher than the highest dose tested (*i.e.* 500 µM).



Cd	R^4	R^6	R^7	$R^{2'}$	$R^{3'}$	$R^{4'}$	$R^{5'}$	$R^{6'}$	MIC_{MRSA} (µM)	MIC_{PA} (µM)
5	H	H	H	H	H	H	H	H	>500	>500
6	H	H	H	H	H	OH	H	H	>500	>500
7	OH	H	H	H	H	H	H	H	>500	>500
8	OH	H	H	H	H	OH	H	H	**125–250**	>500
9	OH	H	H	H	OH	OH	H	H	**125–250**	>500
10	OH	H	H	H	H	Et	H	H	**62.5–125**	>500
11	OH	H	H	H	H	*i*-Pr	H	H	>500	>500
12	OH	H	H	H	H	*n*-Bu	H	H	>500	>500
13	OH	H	H	H	H	*t*-Bu	H	H	>500	>500
14	OH	H	H	Me	H	Me	H	H	>500	>500
15	OH	H	H	OMe	H	H	H	OMe	**250**	>500
16	OH	H	H	OMe	H	OMe	H	OMe	**250**	>500
17	OH	H	H	H	H	*N*-MePpz	H	H	>500	>500
18	H	OH	H	H	H	OH	H	H	>500	>500
19	H	OH	H	H	OH	H	H	H	>500	>500
20	H	OH	H	OH	H	H	H	H	>500	>500
21	H	OH	H	H	H	*N*-MePpz	H	H	>500	>500
22	H	H	OH	H	H	H	H	H	>500	>500
23	H	H	OH	H	OH	OH	H	H	**125–250**	>500
24	H	H	OH	OH	OH	H	H	H	**250**	>500
25	H	H	OH	H	OH	OH	OH	H	**125–250**	>500
26	H	H	OH	H	H	CO_2H	H	H	>500	>500
27	OH	OH	H	H	H	H	H	H	**15.6–31.25**	>500
28	OH	OH	H	H	H	OH	H	H	**62.5–125**	>500
29	OH	OH	H	H	OH	H	H	H	**125–250**	>500
30	OH	OH	H	H	H	F	H	H	**7.8–15.6**	>500
31	OH	OH	H	H	H	*n*-Bu	H	H	**15.6–31.25**	>500
32	OH	OH	H	H	H	*c*-Hex	H	H	**7.8**	>500
33	OH	OH	H	H	OMe	OBn	H	H	**15.6**	>500
34	H	OMe	H	H	OMe	OMe	H	H	>500	>500
35	OMe	OMe	H	OH	H	H	H	H	>500	>500
36	OMe	OMe	H	Me	H	Me	H	H	>500	>500
37	OMe	OMe	H	H	H	*n*-Bu	H	H	>500	>500
38	OMe	OMe	H	OMe	H	OMe	H	H	>500	>500

The five most active derivatives (*i.e.* compounds **27** and **30**–**33** with MIC_{SA} = 7.8–31.25 µM) were selected for further evaluation. Their innocuity was evaluated using normal human cells as explained in the Experimental section. The five derivatives displayed a dose-dependent reduction of human cell viability after 48 h of incubation (Supporting Information). Results showed that IC_{50} (the inhibitory concentrations reducing 50% of the cell viability) values for BEAS-2B and IMR-90 cells were similar for each derivative (Table 2). Compounds **27**, **30** and **33** were found as the least toxic ones (IC_{50} = 191–623 µM) whereas compounds **31** and **32**, bearing alkyl groups at position 4', showed more toxicity (IC_{50} = 31–87 µM).

Table 2. Evaluation of the toxicity of selected aurone derivatives against normal human cells (IC_{50} values). Human normal lung epithelial (BEAS-2B) and fibroblast (IMR-90) were tested.

Compounds	**27** (µM)	**30** (µM)	**31** (µM)	**32** (µM)	**33** (µM)
BEAS-2B	280 ± 50	590 ± 90	90 ± 20	33 ± 8	190 ± 30
IMR-90	250 ± 50	620 ± 90	80 ± 20	31 ± 6	340 ± 70

The spectrum of antimicrobial activity of the three most active and least toxic derivatives (*i.e.* compounds **27**, **30**, **33**) was further tested using a larger panel of microorganisms (Table 3). Results showed that compounds **27**, **30** and **33** were active against all tested Gram positive bacteria, however with differences in terms of sensitivity. Thus, compound **27** was more active against *C. perfringens* whereas compound **33** was found more active on *B. subtilis*. On the other hand, *E. faecalis* was more sensitive to compounds **27** and **33** than compound **30**, whereas Methicillin-resistant *S. aureus*, *P. acnes*, *L. lactis* or *A. gandavensis* gave similar sensitivity for the three compounds. Compounds **27**, **30** and **33** were found inactive against most of the Gram negative strains tested, except *H. pylori* that was found sensitive to compounds **27** and **33** and *V. alginolyticus* that was found sensitive to compounds **27**, **30** and **33**. No activity was detected against *M. smegmatis*. In order to extend our investigation to other infectious pathogens, we evaluated their antifungal activity on various unicellular and

filamentous fungi causing infection in animals, humans or plants. Compound **27** was found active against *A. niger* and *F. graminearum*. Compound **33** was only found active against *C. albicans* whereas compound **30** was found inactive in all anti-fungal assays. The determination of MBC values demonstrated that the antimicrobial effect of compounds **27**, **30** and **33** was due to a bactericidal action since their MBC and MIC values were identical (data not shown).

Table 3. Spectrum of activity of the most active aurone derivatives **27**, **30** and **33** on various selected microorganisms (MIC values). >500 µM indicates that MIC is higher than the highest dose tested (*i.e.* 500 µM).

Compounds		**27** (µM)	**30** (µM)	**33** (µM)
Gram positive bacteria	*A. gandavensis*	**31.25**	**15.6**	**31.25**
	B. subtilis	**62.5–125**	**62.5**	**7.8**
	B. subtilis (Nisin-resistant)	**31.25**	**31.25**	**3.9**
	C. botulinum	**500**	**500**	**62.5**
	C. coccoides	**500**	>500	**250**
	C. difficile	**62.5**	**62.5**	**31.25**
	C. perfringens	**7.8**	**31.25–62.5**	**31.25–62.5**
	E. faecalis	**15.6**	**125–250**	**15.6–31.25**
	L. lactis	**15.6**	**15.6**	**31.25**
	P. acnes	**31.25**	**31.25**	**31.25**
	S. aureus MRSA	**15.6–31.25**	**7.8–15.6**	**15.6**
	S. thermophilus	**31.25**	**31.25**	**125**
Gram negative bacteria	*A. baumannii*	>500	>500	>500
	A. baumannii MRS	>500	>500	>500
	B. thetaioataomicron	>500	>500	>500
	C. farmer	>500	>500	>500
	C. rodentium	>500	>500	>500
	E. coli	>500	>500	>500
	E. coli MRS	>500	>500	>500
	E. coli EHEC K88	>500	>500	>500
	H. pylori	**15.6**	>500	**15.6**
	K. pneumoniae	>500	>500	>500
	K. variicola	>500	>500	>500
	S. enterica	>500	>500	>500

	S. flexneri	>500	>500	>500
	V. alginolyticus	**31.25**	**125**	**3.9**
	V. diabolicus	>500	>500	>500
Mycobacterium	*M. smegmatis*	>500	>500	>500
Fungi	*A. flavus*	>500	>500	>500
	A. ochraceus	>500	>500	>500
	A. niger	**62.5**	>500	>500
	C. albicans	>500	>500	**125**
	F. graminearum	**31.25**	>500	500
	F. oxysporum	>500	>500	>500
	F. verticillioides	>500	>500	>500
	P. verrucosum	>500	>500	>500
	S. chartarum	>500	>500	>500

Finally, the Therapeutic Index (T.I) of compounds **27**, **30** and **33** was determined by comparing their antimicrobial and cytotoxic dose-effects. The T.I was determined by dividing the IC_{50} value found with one compound on the more sensitive normal human cells by the MIC value found with the same compound on the more sensitive microorganism. Using this equation, T.Is of compounds **27**, **30** and **33** were found equal to 32, 76 and 49, respectively (Figure 2).

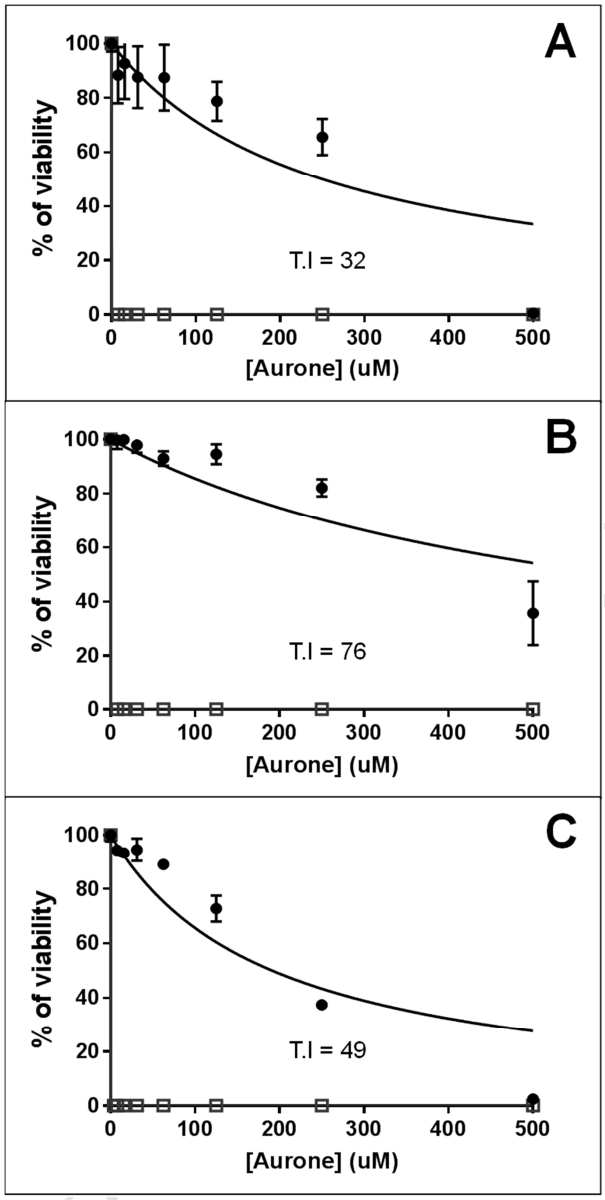


Figure 2. Evaluation of the Therapeutic Index (T.I) of compounds **27** (A), **30** (B) and **33** (C). T.I was calculated by dividing IC_{50} values obtained from cytotoxicity curves (closed circles) by MIC values obtained from antimicrobial curves (open squares). Data used were: (a) for compound **27** (A): MIC on *C. perfringens* and IC_{50} on IMR90 cells, (b) for compound **30** (B): MIC on MRSA and IC_{50} on BEAS-2B cells and (c) for compound **33** (C): MIC on *B. subtilis* and IC_{50} on BEAS-2B cells.

Overall, if some 4-hydroxy (**8**–**10**, **15**, **16**) and 7-hydroxyaurones (**23**–**25**) showed residual activity, the five most active compounds against MRSA were all 4,6-dihydroxyaurones (**27**, **30**–**33**), while strongly varying in terms of B-ring substitution. Such a substitution pattern at

the A-ring appeared as a crucial feature, since even closely related analogues were completely inactive. As an illustrative example, compound **31** showed a MIC_{MRSA} value of 15.6–31.25 µM, *versus* the 6-deoxy analogue **12** and the dimethoxy counterpart **37** having MIC_{MRSA} >500 µM. The same trend was recorded when comparing compound **27** with analogues **5** and **7** (MIC_{MRSA} = 15.6–31.25 µM versus >500 µM, respectively). This is reminiscent of antibacterial activities measured from other natural and synthetic flavonoids, which often share the same resorcinol-type hydroxylation pattern at the A-ring [1]. However in some cases the aurone scaffold afforded better results. For example, compound **27** yielded a MIC_{MRSA} value of 15.6–31.25 µM, while the analogous chalcone, flavone and flavanone (respectively 2',4'-dihydroxychalcone, chrysin and pinocembrin) were reported earlier as far weaker inhibitors (MIC_{MRSA} = 131, 407 and 488 µM, respectively) [17]. In addition, as direct B-hydroxylated analogues **28** and **29** did not share the potency of **27** and **30**–**33**, a hydrophobic environment could be mandatory at the B-ring for reaching decent antibacterial activity, an interesting observation that runs contrary to previous global structure-relationship studies regarding flavonoids, which highlighted a beneficial effect of OH groups at B-rings of chalcones, flavonols or flavanols [1]. In terms of global Gram positive antibacterial potency, aurones **27** and **33** share similar MIC values with isolupalbigenin or licochalcone A, which were ranked among the most potent antibacterial flavonoids [18]. Finally, none of the evaluated molecules were inhibitors of Gram negative strains growth (with the notable exceptions of *H. pylori* and *V. alginolyticus*), indicating a good selectivity towards Gram positive strains. Again, this finding seems surprising, as overall reported flavonoid derivatives often showed similar activities against Gram positive and Gram negative strains (*e.g.* the flavonol and flavanols analogues of **27**, *i.e.* galangin or pinocembrin) [19].

To date, the mechanism of action of aurones and derivatives described as antimicrobial is uncharacterized. Classically, antibiotics act either through an inhibition of macromolecules

biosynthesis and/or through pore-forming activity. More specifically, flavonoids were often associated with the targeting of cytoplasmic membrane, nucleic acid synthesis, and energy metabolism of bacteria [18]. Thus, both three hypotheses constitute plausible mechanisms of action for aurones. The fact that MIC and MBC values of compounds **27**, **30** and **33** were similar suggests that they possess bacteriolytic activity, potentially through pore-forming effect. Thus, the influence of compounds **27**, **30** and **33** on membrane permeability was evaluated on *C. perfringens*, since it was found as one of the most sensitive bacteria in the antimicrobial assay (Table 3). Membrane integrity was measured using propidium iodide assay, as documented in the Experimental section (Figure 3). Results showed that only compound **33** caused a partial permeabilization of the bacterial membrane (around 30% after 240 min), whereas compounds **27** and **30** did not affect membrane integrity, suggesting these two compounds have target(s) distinct from the bacterial membrane.

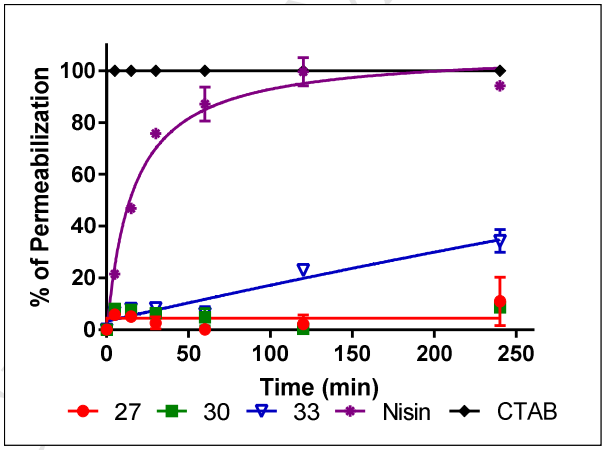


Figure 3. Evaluation of the impact of compounds **27**, **30** or **33** on the membrane integrity of *C. perfringens*. *C. perfringens* were exposed to compounds **27**, **30** or **33** or positive control of membrane permeabilization (CTAB or Nisin) in the presence of the cell-impermeable DNA probe propidium iodide. Fluorescent signal (excitation at 530 nm and emission at 590 nm) corresponding to membrane permeabilization and propidium iodide cell entry was measured over 240 min at 37 °C in anaerobic condition. Results corresponding to means ± SD (n = 3) were fitted using Graph Pad Prism software.

4. Conclusion

In conclusion, we synthesized 34 compounds through substitution of the naturally occurring aurone scaffold. Among the derivatives possessing preliminary antimicrobial activity, mainly directed against Gram-positive bacteria, three compounds were selected based on innocuousness assays. These molecules shared structural similarity, such as a 4,6-dihydroxy substitution pattern. Interestingly, although they were active against a large number of Gram-positive bacteria, possibly through alterations of the bacterial membrane (as suggested by preliminary membrane permeabilization assays), two of them (**27** and **33**) showed an unexpected potency against Gram-negative human pathogens *H. pylori* and *V. alginolyticus*. It is worth noting that the aurone scaffold itself bears the activity herein, unlike some reported derivatives incorporating already known antibacterial moieties embedded on aurones (*e.g.* ferrocene, nitroimidazole, nitrofuran) [9–11]. In addition, far better T.Is were obtained (T.I = 32–76 for **27**, **30** and **33**), as those from the literature were crippled by collateral high cytotoxicity values (*e.g.* T.I ≤ 0.72 for ferrocene-substituted aurones) [11,12]. Taken together, our results demonstrate that the aurone scaffold itself is a well-suited platform for antibacterial agents design, and that diversifying the substitution pattern could be an efficient way to produce new antibiotics active against pathogenic bacteria and safe for human cells.

5. Experimental

5.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on an AC-400 instrument (Bruker, 400 MHz for ^{1}H, 100 MHz for ^{13}C). Chemical shifts are reported in ppm relative to the solvent in which the

spectrum was recorded [^{1}H: δ (d_6-DMSO) = 2.50 ppm, δ ($CDCl_3$) = 7.27 ppm; ^{13}C: δ (d_6-DMSO) = 39.52 ppm, δ ($CDCl_3$) = 77.16 ppm]. Electrospray ionization (ESI) high resolution mass spectra were acquired at the ICMG PSM Platform on a LTQ Orbitrap XL Thermo Scientific instrument. Combustion analyses were performed at the analysis facilities of DCM; all compounds had purity higher than 95%. IR spectra were recorded with a Perkin Elmer Spectrum Two FT-IR spectrometer using an ATR unit Silica gel F-254 plates (0.25 mm; Merck) were used for thin-layer chromatography (TLC), and silica gel 60 (200–400 mesh; Merck) was used for flash chromatography. Unless otherwise stated, reagents were obtained from commercial sources and were used without further purification. The synthesis and characterization of compounds **1**–**3**, **6**–**20**, **22**, **24**–**32** and **35**–**37** have already been reported elsewhere [16,20–23].

*5.1.1. 6-Methoxybenzofuran-3(2*H*)-one (**4**)*

To a suspension of 6-hydroxybenzofuran-3(2*H*)-one (1.00 g, 6.66 mmol) in acetone (5 mL) were added K_2CO_3 (1.38 g, 9.99 mmol) and Me_2SO_4 (690 µL, 7.28 mmol), and the mixture was stirred at room temperature for 4 h. Solvents were removed under reduced pressure, H_2O was added, and the mixture was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. The residue was washed with cold MeOH, filtered and dried to afford **4** (735 mg, 68%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.47 (d, 1H, J = 8.5 Hz), 6.57 (d, J = 8.5 Hz), 6.47 (s, 1H), 4.55 (s, 2H), 3.81 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 197.5, 176.5, 168.2, 125.0, 114.3, 111.7, 96.3, 75.5, 55.9.

*5.1.2. (*Z*)-2-Benzylidenebenzofuran-3(2*H*)-one (**5**)*

To a solution of benzofuran-3(2*H*)-one (300 mg, 2.24 mmol) in anhydrous CH_2Cl_2 (25 mL) was added benzaldehyde (0.272 mL, 2.69 mmol) and Al_2O_3 (8.0 g), and the suspension was stirred at room temperature for 18 hours. The solid was removed by filtration, and the filtrate

was concentrated under reduced pressure. The residue was washed with MeOH, filtered and dried to afford 5 (231 mg, 47%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.94 (m, 2H), 7.83 (m, 1H), 7.67 (ddd, 1H, J_1 = 8.6 Hz, J_2 = 7.3 Hz, J_3 = 1.4 Hz), 7.45 (m, 3H), 7.34 (d, 1H, J = 8.3 Hz), 7.23 (m, 1H), 6.91 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 185.0, 166.3, 147.0, 137.1, 132.4, 131.7, 130.0, 129.0, 124.8, 123.6, 121.8, 113.2, 113.1. IR (ATR) υ 1712, 1596, 1475, 1459, 1299, 1091, 1024, 857, 754, 697, 554 cm^{-1}. HRMS (ESI) calc. for $C_{15}H_{11}O_2$ $[M + H]^+$ 223.0754, found 223.0748.

*5.1.3. (Z)-2-(4-(4-Methylpiperazin-1-yl)benzylidene)-6-hydroxybenzofuran-3(2*H*)-one (**21**)*

To a solution of 6-hydroxybenzofuran-3(2*H*)-one (73 mg, 0.49 mmol) in MeOH (10 mL) were added an aqueous solution of KOH (50%, 0.75 mL) and 4-(4-methylpiperazin-1-yl)benzaldehyde (100 mg, 0.49 mmol), and the mixture was refluxed for 3 hours. After cooling, solvents were removed under reduced pressure, then the residue was diluted with H_2O and an aqueous solution of HCl (1 N) was added to adjust the pH to 6–7. The resulting precipitate was filtered, washed with Et_2O and dried to afford the hydrochloride salt of **21** (182 mg, 100%) as an orange solid. ^{1}H NMR (d_6-DMSO) δ 11.23 (s, 1H), 10.95 (br s, 1H, NH^+), 7.86 (d, 2H, J = 8.4 Hz), 7.60 (d, 1H, J = 8.4 Hz), 7.11 (d, 2H, J = 8.4 Hz), 6.77 (m, 3H), 4.02 (m, 2H), 3.46 (m, 2H), 3.25 (m, 4H), 2.81 (s, 3H). ^{13}C NMR (d_6-DMSO) δ 181.1, 167.4, 166.3, 150.1, 146.0, 132.7, 125.7, 122.8, 115.2, 113.2, 112.9, 111.2, 98.6, 51.8, 44.3, 41.9. IR (ATR) υ 3368 (br), 1603, 1568, 1517, 1456, 1393, 1341, 1293, 1252, 1144, 1123, 922, 818, 804, 770, 701, 665, 612, 550, 505 cm^{-1}. HRMS (ESI) calc. for $C_{20}H_{21}O_3N_2$ $[M + H]^+$ 337.1547, found 337.1540.

*5.1.4. (Z)-2-(3,4-Dihydroxybenzylidene)-7-hydroxybenzofuran-3(2*H*)-one (**23**)*

To a solution of 7-methoxybenzofuran-3(2*H*)-one (150 mg, 0.914 mmol) in anhydrous CH_2Cl_2 (25 mL) was added 3,4-dimethoxybenzaldehyde (183 mg, 1.10 mmol) and Al_2O_3 (3.4

g), and the suspension was stirred at room temperature for 2 hours. The solid was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was washed with MeOH, filtered and dried to afford (*Z*)-2-(3,4-dimethoxybenzylidene)-7-methoxybenzofuran-3(2*H*)-one (113 mg, 40%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.64 (d, 1H, J = 2.0 Hz), 7.52 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.40 (dd, 1H, J_1 = 6.8 Hz, J_2 = 2.0 Hz), 7.16 (m, 2H), 6.97 (d, 1H, J = 8.4 Hz), 6.91 (s, 1H), 4.03 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 184.7, 155.7, 151.1, 149.3, 146.2, 146.1, 126.3, 125.5, 123.9, 123.5, 118.7, 116.1, 114.3, 114.0, 111.4, 56.7, 56.1, 56.0. To a solution of (*Z*)-2-(3,4-dimethoxybenzylidene)-7-methoxybenzofuran-3(2*H*)-one (100 mg, 0.320 mmol) in anhydrous CH_2Cl_2 (20 mL) was added BBr_3 (0.618 mL, 6.40 mmol) at 0 °C, and the mixture was stirred at room temperature for 3.5 hours. Cold H_2O was then added, and the resulting mixture was extracted with AcOEt. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (eluent AcOEt/cyclohexane) to afford **23** (60 mg, 69%) as a red solid. ^{1}H NMR (d_6-DMSO) δ 10.51 (br s, 1H), 9.81 (br s, 1H), 9.33 (br s, 1H), 7.48 (d, 1H, J = 2.1 Hz), 7.39 (dd, 1H, J_1 = 8.3 Hz, J_2 = 2.1 Hz), 7.22 (dd, 1H, J_1 = 7.7 Hz, J_2 = 1.2 Hz), 7.18 (dd, 1H, J_1 = 7.7 Hz, J_2 = 1.2 Hz), 7.09 (t, 1H, J = 7.7 Hz), 6.87 (d, 1H, J = 8.3 Hz), 6.78 (s, 1H). ^{13}C NMR (d_6-DMSO) δ 183.6, 153.9, 148.6, 145.6, 144.8, 143.6, 125.2, 124.2, 123.3, 122.9, 122.8, 118.6, 116.1, 114.0, 113.8. IR (ATR) υ 3382 (br), 3167 (br), 1644, 1579, 1506, 1289, 1209, 1158, 1137, 1057, 1024, 909, 862, 791, 746, 732, 691, 620, 573, 557, 524 cm^{-1}. HRMS (ESI) calc. for $C_{15}H_{11}O_5$ $[M + H]^+$ 271.0601, found 271.0601.

*5.1.5. (Z)-2-(4-Benzyloxy-3-methoxybenzylidene)-4,6-dihydroxybenzofuran-3(2H)-one (**33**)*

To a solution of 4,6-dihydroxybenzofuran-3(2*H*)-one (100 mg, 0.602 mmol) in EtOH (2 mL) were added an aqueous solution of KOH (50%, 3 mL) and 4-benzyloxy-3-methoxybenzaldehyde (3 mL), and the mixture was refluxed for 2 hours. After cooling, solvents were removed under reduced pressure, then the residue was diluted with H_2O and an aqueous solution of HCl (1 N) was added to adjust the pH to 2–3. The resulting mixture was extracted three times with AcOEt. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from MeOH to afford **33** (150 mg, 64%) as a yellow solid. ^{1}H NMR (d_6-DMSO) δ 10.92 (s, 1H), 10.85 (s, 1H), 7.32–7.54 (m, 7H), 7.14 (d, 1H, J = 8.5 Hz), 6.59 (s, 1H), 6.23 (d, 1H, J = 1.7 Hz), 6.08 (d, 1H, J = 1.7 Hz), 5.15 (s, 2H), 3.83 (s, 3H). ^{13}C NMR (d_6-DMSO) δ 179.0, 167.6, 167.1, 158.2, 149.0, 148.8, 146.6, 136.7, 128.4, 128.0, 127.9, 125.3, 124.3, 114.2, 113.5, 108.8, 102.7, 97.7, 90.6, 69.8, 55.5. IR (ATR) υ 3541 (br), 3059 (br), 1603, 1586, 1509, 1456, 1272, 1243, 1231, 1145, 1075, 1003, 885, 818, 741, 697, 679, 635, 626, 562, 541, 519 cm^{-1}. HRMS (ESI) calc. for $C_{23}H_{19}O_6$ $[M + H]^+$ 391.1176, found 391.1172.

*5.1.6. (Z)-2-(3,4-Dimethoxybenzylidene)-6-methoxybenzofuran-3(2H)-one (**34**)*

To a solution of 6-methoxybenzofuran-3(2*H*)-one (58 mg, 0.354 mmol) in MeOH (5 mL) were added an aqueous solution of KOH (50%, 0.30 mL) and 3,4-dimethoxybenzaldehyde (59 mg, 0.354 mmol), and the mixture was stirred at room temperature for 2 hours. The solid was then filtered, washed with cold MeOH and dried. The crude product was recrystallized from MeOH to afford **34** (25 mg, 23%) as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 1H, J = 8.3 Hz), 7.51 (d, 1H, J = 1.9 Hz), 7.48 (dd, 1H, J_1 = 8.3 Hz, J_2 = 1.9 Hz), 6.94 (d, 1H, J = 8.3 Hz), 6.80 (s, 1H), 6.77 (dd, 1H, J_1 = 8.3 Hz, J_2 = 2.1 Hz), 6.76 (s, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 3.94 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 183.0, 168.4, 167.4, 150.8, 149.2, 147.0, 126.0, 125.9, 125.6, 115.3, 113.9, 112.6, 112.2, 111.4, 96.8, 56.3, 56.2, 56.2. IR (ATR) υ 1596, 1516, 1442,

1424, 1292, 1283, 1261, 1169, 1145, 1122, 1091, 1043, 1015, 949, 896, 862, 835, 816, 771, 700 cm^{-1}. HRMS (ESI) calc. for $C_{18}H_{17}O_5$ $[M + H]^+$ 313.1071, found 313.1065.

*5.1.7. (Z)-2-(2,4-Dimethoxybenzylidene)-4,6-dimethoxybenzofuran-3(2*H*)-one (**38**)*

To a solution of 4,6-dimethoxybenzofuran-3(2*H*)-one (245 mg, 2.06 mmol) in MeOH (30 mL) were added an aqueous solution of KOH (50%, 2 mL) and 2,4-dimethoxybenzaldehyde (315 mg, 3.09 mmol), and the mixture was stirred at room temperature for 2 hours. The solid was then filtered, washed with cold MeOH and dried. The crude product was recrystallized from MeOH to afford **38** (394 mg, 91%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 8.19 (d, 1H, J = 8.7 Hz), 7.27 (s, 1H), 6.57 (dd, 1H, J = 8.7 Hz, J = 2.3 Hz), 6.45 (d, 1H, J = 2.3 Hz), 6.36 (d, 1H, J = 1.7 Hz), 6.11 (d, 1H, J = 1.7 Hz), 3.94 (s, 3H), 3.90 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 180.6, 168.5, 168.4, 162.2, 160.1, 159.2, 146.8, 132.8, 114.6, 105.6, 105.5, 105.3, 97.9, 93.7, 89.0, 56.1, 56.0, 55.5, 55.4. IR (ATR) υ 1591, 1566, 1426, 1318, 1240, 1208, 1157, 1088, 1042, 1030, 945, 916, 883, 845, 806, 783, 696, 675, 550, 507 cm^{-1}. HRMS (ESI) calc. for $C_{19}H_{19}O_6$ $[M + H]^+$ 343.1176, found 343.1170.

5.2. Biology

5.2.1. Microorganism strains and growth conditions

Bacterial strains used in this study were reference strains and were obtained from either the American Type Culture Collection (ATCC), the German Leibniz Institute **(**DSMZ) or the French Pasteur Institute (CIP). Gram negative bacterial strains used were: *Acinetobacter baumannii* (CIP 110431), *Acinetobacter baumannii* multi resistant strain (MRS) (CIP 110431), *Bacteroides thetaioataomicron* (DSM 2255), *Citrobacter farmeri* (ATCC 51631), *Citrobacter rodentium* (ATCC 51116), *Escherichia coli* (ATCC 8739), *Escherichia coli* multi resistant strain (MRS) (DSM 22314), Enterohemorrhagic *E. coli* -EHEC (K88), *Helicobacter pylori* (ATCC 43504), *Klebsiella pneumoniae* (DSM 26371), *Klebsiella variicola* (DSM 15968), *Pseudomonas aeruginosa* (CIP 107398), *Salmonella enterica* (CIP 80.39), *Shigella*

flexneri (ATCC 12022), *Vibrio alginolyticus* (DSM 2171), *Vibrio diabolicus* (generous gift from Dr Aurélie Tasiemski from EEP UMR 8198). Gram positive bacterial strains used were: *Arthrobacter gandavensis* (DSM 2446), *Bacillus subtilis* (ATCC 6633), Nisin-resistant *B. subtilis* (DSM 347), *Clostridium botulinum* (DSM 1985), *Clostridium coccoides* (DSMZ 935), *Clostridium difficile* (DSMZ 1296), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecalis* (DSM 13591), *Lactococcus lactis* (MG1363), *Propionibacterium acnes* (ATCC 6919), *Streptococcus thermophilus* (LMD-9), and Methicillin Resistant *S. aureus* strain MRSA USA300 (ATCC BAA-1717 USA 300 CA-MRSA). In addition to Gram negative and Gram positive strains, *Mycobacterium smegmatis* (mc2155, ATCC 700084) was also used as model of Mycobacterium species. Bacteria were cultured as previously described [24–26]. Briefly, all bacterial strains (except *B. thetaioataomicron*, *C. botulinum*, *C. coccoides*, *C. difficile*, *C. perfringens*, *E. faecalis*, *P. acnes*, *M. smegmatis* and *S. thermophilus*) were grown on Luria Bertani (LB) agar plates and in LB broth at 37 °C in aerobic condition. *M. smegmatis* was cultured in Middlebrook 7H10 agar plate and Middlebrook 7H10 broth at 37 °C in aerobic condition. *B. thetaioataomicron*, *C. botulinum*, *C. coccoides*, *C. difficile*, *C. perfringens*, *E. faecalis*, *P. acnes*, *M. smegmatis and S. thermophilus* were cultured in Brain Heart Infusion (BHI) agar plates and BHI broth supplemented or not with fetal calf serum (10%) at 37 °C in an anaerobic cabinet (Coy Laboratory Products, Grass Lake, MI). Fungi used in this study were *Aspergillus flavus* (DSMZ 1959), *A. niger* (ATCC 9142), *A. ochraceus* (DSMZ 824), *Candida albicans* (DSM 10697), *Fusarium graminearum* (DSMZ 1095), *F. oxysporum* (DSMZ 62316), *F. verticillioides* (DSMZ 62264), *Penicillium verrucosum* (DSMZ 12639), *Stachybotrys chartarum* (DSMZ 2144). *C. albicans* was grown on LB agar plates and in liquid RMPI medium (buffered with MOPS of final concentration 0.165 M for pH 7.0 from Thermofisher).

All other fungal strains were grown on Potatoes Dextrose agar and RMPI medium at room temperature for 2 to 5 days.

5.2.2. Antimicrobial activity assay

Antimicrobial activity of aurone derivatives was evaluated using determination of the minimal inhibitory concentration (MIC) using two-fold serial dilutions of antimicrobial peptides in bacterial liquid media following the National Committee of Clinical Laboratory Standards (NCCLS, 1997) as previously described [24–26]. Briefly, single colonies of the different bacterial strains cultured on specific agar plates were used to inoculate 3 mL of Mueller-Hinton (MH) broth for all species except for *B. thetaioataomicron, C. botulinum, C. coccoides, C. difficile, C. perfringens, E. faecalis, P. acnes and S. thermophilus* that were cultured in 3 mL of BHI or *M. smegmatis* that was cultured in 3 mL of Middlebrook 7H10 broth. Tubes were then incubated overnight (for approximately 16 h) at 37 °C under stirring (200 rpm). The next day, optical density (OD) of the bacterial suspensions were read at 600 nm, adjusted to 1 with medium before bacteria were diluted 1/100 in 3 mL of fresh medium and incubated at 37°C, 200 rpm until bacteria reached log phase growth (OD_{600nm} around 0.6). Stock solutions of aurone derivatives at 200 mM were prepared in DMSO. Bacteria were diluted in medium to reach bacterial density around 10^5 bacteria / mL. 100 µL per well of bacterial suspension were then added into sterile polypropylene 96 well microplates (Greiner BioOne) already containing 100 µL of medium with increasing concentrations of aurone derivatives obtained by serial dilution (from 0 to 500 µM, 1:2 dilution). Volume of DMSO corresponding to the highest dose of aurone derivatives tested (0.2 % DMSO final concentration) was used as negative control and was found inactive. Plates were incubated at 37 °C for 18–24 h for all bacteria except *C. perfringens* and *M. smegmatis* that where incubated for 24 and 72 hours respectively. All bacterial strains were tested in aerobic conditions except *B. thetaioataomicron, C. botulinum, C. coccoides, C. difficile, C.*

perfringens, *P. acnes*, *M. smegmatis and S. thermophilus* that were tested at 37 °C in an anaerobic cabinet (Coy Laboratory Products, Grass Lake, MI). *H. pylori* and *E. faecalis* were tested in anaerobic conditions generated using micro-anaerobic BD GasPack system. For *C. albicans*, plates were incubated at 35 °C during 24 hours before reading. *A. flavus*, *A. ochraceus*, *F. graminearum*, *F. oxysporum*, *F. verticillioides*, *P. verrucosum* and *S. chartarum* plates were incubated at room temperature for 2 to 5 days before reading. Finally, for *A. niger*, plates were incubated at 35 °C during 48 hours before reading. At the end of the incubation, OD_{600nm} was measured using microplate reader (Synergy Mx, Biotek). The MIC was defined as the lowest concentration of drug that inhibited visible growth of the organism. Experiments were conducted in independent triplicate (n = 3). Antimicrobial activity of aurone derivatives was also evaluated by determination of the minimal bactericidal concentration (MBC), *i.e.* the minimum concentration of the molecule killing 99.9% of the bacteria [24–26]. MBCs were measured by streaking 10 µL of wells with no growth of previously prepared MIC plates on proper agar plates. After incubation in the proper condition, numbers of colonies were counted. Concentrations where ≤ 1 colony grew were considered the MBC.

5.2.3. Cytotoxic assay on human cells

The impact of aurone derivatives on the viability of normal human cells was evaluated using Resazurin assay with some modifications [27]. Normal human cells were preferred to cell lines (*i.e.* cancer cells) as cancer cells and normal cells may display different sensitivity to drugs [28]. Normal human cells used were BEAS-2B (ATCC CRL-9609) and IMR-90 (ATCC CCL-186) corresponding respectively to human normal lung epithelial and lung fibroblast cells. Cells were cultured in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal calf serum (FCS), 1% L-glutamine and 1% antibiotics (all from Invitrogen) and were seeded onto 25 cm^2 flasks maintained in a 5% CO_2 incubator at 37 °C.

Cells grown on 25 cm^2 flasks were detached using trypsin–EDTA solution (from Thermofisher), counted using Mallasez counting chamber and seeded into 96-well cell culture plates (Greiner bio-one) at approximately 10^4 cells per well. The cells were left to grow for 48–72 h at 37 °C in a 5% CO_2 incubator until they reached confluence. Plates were then aspirated and increasing concentrations of aurone derivatives were added to the cells for 48 h at 37 °C in a 5% CO_2 incubator, DMSO (0.2% DMSO final concentration) being used as negative control. At the end of the incubation, wells were emptied and cell viability was evaluated using Resazurin based in vitro toxicity assay kit (from Sigma-Aldrich) following manufacturer's instructions. Briefly, Resazurin stock solution was diluted 1:100 in sterile PBS containing calcium and magnesium (PBS^{++}, pH 7.4) and empty wells were filled with 100 µL of the diluted solution. After 4 h incubation at 37 °C, fluorescence intensity was measured using microplate reader (Synergy Mx, Biotek) with an excitation wavelength of 530 nm and an emission wavelength of 590 nm. The fluorescence values were normalized by the controls (DMSO treated cells) and expressed as percent viability. The IC_{50} values of aurone derivatives on cell viability (*i.e.* the concentration of derivative causing a reduction of 50% of the cell viability) were calculated using GraphPad® Prism 7 software. t-Test and two way ANOVA analyses were used to address the significant differences between mean values with significance set at $p < 0.05$.

5.2.4. Membrane permeability assay

Bacterial membrane permeabilization was evaluated using the cell-impermeable DNA/RNA probe propidium iodide as previously explained [24,29]. Logarithmic growing bacterial suspension was prepared from overnight bacterial suspension by 1 in 10 dilution. After 3 h incubation at 37 °C, 200 rpm, bacterial suspensions were centrifuged for 5 min at 3000×g. Cell pellets were then resuspended in sterile PBS at about 10^9 bacteria/mL. Propidium iodide (solution at 1 mg/mL from Sigma Aldrich) was then added to the suspension at a final

concentration of 60 µM. 100 µL of this suspension were then transferred into 96-well black plates already containing 100 µL of medium with increasing concentrations of aurone derivatives obtained by serial dilution (1:2 dilution). Volume of DMSO (vehicle) corresponding to the highest doses of derivatives was used as negative control and was found inactive. Kinetics of fluorescence variations (excitation at 530 nm and emission at 590 nm) were then recorded over 240 min of incubation at 37 °C in anaerobic condition using a microplate reader (Biotek, Synergy Mx), cetyl trimethylammonium bromide (CTAB) at 300 µM and Nisin at 5-times its MIC value being used as positive controls giving 100% permeabilization. Results were expressed in % of permeability. All experiments were done in triplicate.

Notes

The authors declare no competing financial interests.

Acknowledgements

This work has been partially supported by Labex ARCANE and CBH-EUR-GS (ANR-17-EURE-0003). We thank the AVB platform (iSm2 CNRS UMR 7313, Marseille) for its support in growing fungal strains used in this study.

References

[1] F. Farhadi, B. Khameneh, M. Iranshahi, M. Iranshahy, Antibacterial activity of flavonoids and their structure-activity relationship: an update review, Phytother. Res., in press (doi: 10.1002/ptr.6208).

[2] B. Boucherle, M. Peuchmaur, A. Boumendjel, R. Haudecoeur, Occurrences, biosynthesis and properties of aurones as high-end evolutionary products, Phytochemistry 142 (2017) 92–111.

[3] A. Boumendjel, Aurones: a subclass of flavones with promising biological potential, Curr. Med. Chem. 10 (2003) 2621–2630.

[4] R. Haudecoeur, A. Boumendjel, Recent advances in the medicinal chemistry of aurones, Curr. Med. Chem. 19 (2012) 2861–2875.

[5] P.W. Pare, N. Dmitrieva, T.J. Mabry, Phytoalexin aurone induced in *Cephalocereus senilis* liquid suspension culture, Phytochemistry 30 (1991) 1133–1135.

[6] P.W. Pare, C.F. Mischke, R. Edwards, R.A. Dixon, H.A. Norman, T.J. Mabry, Induction of phenylpropanoid pathway enzymes in elicitor-treated cultures of *Cephalocereus senilis*, Phytochemistry 31 (1992) 149–153.

[7] M.A. Farag, B.E. Deavours, A. de Fatima, M. Naoumkina, R.A. Dixon, L.W. Sumner, Integrated metabolite and transcript profiling identify a biosynthetic mechanism for hispidol in *Medicago truncatula* cell cultures, Plant Physiol. 151 (2009) 1096–1113.

[8] W.L.A. Chu, F.R. Jensen, T.B. Jensen, J.B. McAlpine, B. Soklide, A.M. Santana-Sorensen, S. Ratnayake, J.B. Jiang, C. Noble, A.M. Stafford, Substituted aurone derivatives, US Patent 6,307,070 (2001).

[9] J.R. Pires, C. Saito, S.L. Gomes, A.M. Giesbrecht, A.T. Amaral, Investigation of 5-nitrofuran derivatives: synthesis, antibacterial activity, and quantitative structure-activity relationships, J. Med. Chem. 44 (2001) 3673–3681.

[10] N. Hadj-esfandiari, L. Navidpour, H. Shadnia, M. Amini, N. Samadi, M.A. Faramarzi, A. Shafiee, Synthesis, antibacterial activity, and quantitative structure-activity relationships of new (*Z*)-2-(nitroimidazolylmethylene)-3(2*H*)-benzofuranone derivatives, Bioorg. Med. Chem. Lett. 17 (2007) 6354–6363.

[11] K.N. Tiwari, J.-P. Monserrat, A. Hequet, C. Ganem-Elbaz, T. Cresteil, G. Jaouen, A. Vessières, E.A. Hillard, C. Jolivalt, In vitro inhibitory properties of ferrocene-substituted chalcones and aurones on bacterial and human cell cultures, Dalton Trans. 41 (2012) 6451–6457.

[12] B.P. Bandgar, S.A. Patil, B.L. Korbad, S.C. Biradar, S.N. Nile, C.N. Khobragade, Synthesis and biological evaluation of a novel series of 2,2-bisaminomethylated aurone analogues as anti-inflammatory and antimicrobial agents, Eur. J. Med. Chem. 45 (2010) 3223–3227.

[13] D. Ashok, R.S. Kumar, D.M. Gandhi, A. Jayashree, Solvent-free microwave-assisted synthesis and biological evaluation of aurones and flavanones based on 2,2-dimethylchroman-4-one, Chem. Heterocycl. Comp. 52 (2016) 453–459.

[14] H.H. Jardosh, M.P. Patel, Antimicrobial and antioxidant evaluation of new quinolone based aurone analogs, Arab. J. Chem. 10 (2017) S3781–S3791.

[15] S. Abdeen, T. Kunkle, N. Salim, A.-M. Ray, N. Mammadova, C. Summers, M. Stevens, A.J. Ambrose, Y. Park, P.G. Schultz, A.L. Horwich, Q.Q. Hoang, E. Chapman, S.M. Johnson, Sulfonamido-2-arylbenzoxazole GroEL/ES inhibitors as potent antibacterials against Methicillin-Resistant *Staphylococcus aureus* (MRSA), J. Med. Chem. 61 (2018) 7345–7357.

[16] R. Haudecoeur, A. Ahmed-Belkacem, W. Yi, A. Fortuné, R. Brillet, C. Belle, E. Nicolle, C. Pallier, J.-M. Pawlotsky, A. Boumendjel, Discovery of naturally occurring aurones that are potent allosteric inhibitors of hepatitis C virus RNA-dependent RNA polymerase, J. Med. Chem. 54 (2011) 5395–5402.

[17] L.E. Alcaraz, S.E. Blanco, O.N. Puig, F. Tomas, F.H. Ferretti, Antibacterial activity of flavonoids against methicillin-resistant *Staphylococcus aureus* strains, J. Theo. Biol. 205 (2000) 231–240.

[18] T.P.T. Cushnie, A.J. Lamb, Recent advances in understanding the antibacterial properties of flavonoids, Int. J. Antimicrob. Agents 39 (2011) 99–107.

[19] J. Echeverria, J. Opazo, L. Mendoza, A. Urzua, M. Wilkens, Structure-activity and lipophilicity relationships of selected antibacterial natural flavones and flavanones of Chilean flora, Molecules 22 (2017) 608.

[20] S. Okombi, D. Rival, S. Bonnet, A.-M. Mariotte, E. Perrier, A. Boumendjel, Discovery of benzylidenebenzofuran-3(2*H*)-one (aurones) as inhibitors of tyrosinase derived from human melanocytes, J. Med. Chem. 49 (2006) 329–333.

[21] C. Dubois, R. Haudecoeur, M. Orio, C. Belle, C. Bochot, A. Boumendjel, R. Hardré, H. Jamet, M. Réglier, Versatile effects of aurone structure on mushroom tyrosinase activity, ChemBioChem 13 (2012) 559–565.

[22] R. Haudecoeur, A. Gouron, C. Dubois, H. Jamet, M. Lightbody, R. Hardré, A. Milet, E. Bergantino, L. Bubacco, C. Belle, M. Réglier, A. Boumendjel, Investigation of binding-site homology between mushroom and bacterial tyrosinases by using aurones as effectors, ChemBioChem 15 (2014) 1325–1333.

[23] L. Lunven, H. Bonnet, S. Yahiaoui, W. Yi, L. Da Costa, M. Peuchmaur, A. Boumendjel, S. Chierici, Disruption of fibers from the tau model AcPHF6 by naturally occurring aurones and synthetic analogues, ACS Chem. Neurosci. 7 (2016) 995–1003.

[24] L.B. Oyama, S.E. Girdwood, A. Cookson, N. Fernandez-Fuentes, F. Privé, H.E. Vallin, T. Wilkinson, P. Golyshin, O. Golyshina, R. Mikut, K. Hilpert, M. Wooton, J.E. Edwards, M. Maresca, J. Perrier, F.T. Lundy, Y. Luo, M. Zhou, M. Hess, H.C. Mantovani, C. Creevey, S.A. Huws, The rumen microbiome: an underexplored resource for novel antimicrobial discovery, NPJ Biofilms Microbiomes 3 (2017) 33.

[25] A. Tardy, J.-C. Honoré, J. Tran, D. Siri, B. Favaloro, V. Delplace, I. Bataille, D. Letourneur, J. Perrier, C. Nicoletti, M. Maresca, C. Lefay, D. Gigmes, J. Nicolas, Y. Guillaneuf, Radical copolymerization of vinyl ethers and cyclic ketene acetals as a versatile platform to design functional polyesters. Angew. Chem. Int. Ed. 56 (2017) 16515–16520.

[26] B.T. Benkhaled, S. Hadiouch, H. Olleik, J. Perrier, C. Ysacco, Y. Guillaneuf, D. Gigmes, M. Maresca, C. Lefay, Elaboration of antimicrobial polymeric materials by dispersion of well-defined amphiphilic methacrylic SG1-based copolymers. Polym. Chem. 9 (2018) 3127–3141.

[27] H. Razafimanjato, N. Garmy, X.J. Guo, K. Varini, C. Di Scala, E. Di Pasquale, N. Taïeb, M. Maresca, The food-associated fungal toxin ochratoxin A inhibits the absorption of glutamate by astrocytes through a decrease in cell surface expression of the excitatory amino acid transporters GLAST and GLT-1. Neurotoxicology 31 (2010) 475–484.

[28] C. Borie, S. Mondal, T. Arif, M. Briand, H. Lingua, F. Dumur, D. Gigmes, P. Stocker, B. Barbarat, V. Robert, C. Nicoletti, D. Olive, M. Maresca, M. Nechab, Enediynes bearing polyfluoroaryl sulfoxide as new antiproliferative agents with dual targeting of microtubules and DNA, Eur. J. Med. Chem. 148 (2018) 306–313.

[29] E. Di Pasquale, C. Salmi, J.M. Brunel, P. Sanchez, J. Fantini, M. Maresca, Biophysical studies of the interaction of squalamine and other cationic amphiphilic molecules with bacterial and eukaryotic membranes. Importance of the distribution coefficient in membrane selectivity, Chem. Phys. Lipids 163 (2010) 131–140.

- 34 aurones are synthesized and evaluated against a full panel of micro-organisms.
- Aurone scaffold is a promising platform for anti-Gram-positive bacteria agents.
- Excellent therapeutic indexes are found for three molecules.
- A selective inhibition of two strains among Gram-negative strains are recorded.
- Preliminary assays suggest that aurones could target the bacterial membrane.