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antibiotics



Article

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








Dr. Marc Maresca and Dr. Manuel Simões



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Article

Design and Synthesis of Novel Amino and Acetamidoaurones with Antimicrobial Activities

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Abstract: The development of new and effective antimicrobial compounds is urgent due to the emergence of resistant bacteria. Natural plant flavonoids are known to be effective molecules, but their activity and selectivity have to be increased. Based on previous aurone potency, we designed new aurone derivatives bearing acetamido and amino groups at the position 5 of the A ring and managing various monosubstitutions at the B ring. A series of 31 new aurone derivatives were first evaluated for their antimicrobial activity with five derivatives being the most active (compounds **10**, **12**, **15**, **16**, and **20**). The evaluation of their cytotoxicity on human cells and of their therapeutic index (TI) showed that compounds **10** and **20** had the highest TI. Finally, screening against a large panel of pathogens confirmed that compounds **10** and **20** possess large spectrum antimicrobial activity, including on bioweapon BSL3 strains, with MIC values as low as 0.78 μ M. These results demonstrate that 5-acetamidoaurones are far more active and safer compared with 5-aminoaurones, and that benzyloxy and isopropyl substitutions at the B ring are the most promising strategy in the exploration of new antimicrobial aurones.

Keywords: aurone; anti-bacterial agents; cytotoxicity tests; structure-activity relationship



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1. Introduction

The development of novel antibacterial molecules is a major necessity in the upcoming decades, due to the increasing emergence of multi-drug resistant bacterial strains. This is leading to an elevating mortality rate by infectious disease that may reach more than 10 billion deaths by 2050, according to World Health Organization (WHO) [1]. Among these strains, mycobacteria, in particular *M. tuberculosis*, the pathogenic agent of tuberculosis, are still responsible for 10 million new cases every year worldwide and killed almost 1.5 million patients in 2022. More alarmingly, the number of strains resistant and ultra-resistant to cocktails of antibiotics currently used to treat infection is constantly rising. One of the major concerns is about methicillin resistant *Staphylococcus aureus* (MRSA), but also other

Gram-positive and Gram-negative species such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa* or *Klebsiella pneumoniae* [2]. In addition to bacteria, fungi (filamentous fungi such as *Aspergillus fumigatus*, as well as yeasts such as *Candida* species and *Cryptococcus neoformans*) are also responsible for deadly infections, particularly in HIV-infected patients, but also in immunocompetent ones, affecting billions of patients and causing more than 1.5 million deaths per year [3–6].

Some natural plant molecules and/or their derivatives, including flavonoids, have been reported to possess strong antimicrobial activity. For decades, the biological effect of flavonoids has been studied, focusing on the major subclasses such as flavones, flavonols, flavanones and chalcones. However, in the past ten years, the aurone subclass has been demonstrated to display strong biological effects in diverse fields, such as oncology, dermatology, and infectiology [7–9]. The natural occurrences of aurones is limited to a limited number of advanced plant species where they play a variety of key roles as flower pigments, antioxidants and as nectar guides [10]. Although some natural aurones such as cephalocerone [11,12] and hispidol [13] have demonstrated antimicrobial activity, aurones exhibiting natural substitution patterns do not generally lead to the most effective antimicrobial agents. On the other hand, a series of synthetic aurone derivatives showed a strong effect against Gram-positive bacteria [14]. Structurally, aurones are characterized by a benzofuran moiety bearing in position 2 a benzylidene type substituent (rings A, B, and C) (Figure 1). Overall, research on scaffold modifications has mainly focused on the substitution at the B-ring scaffold, e.g., with the introduction of either ferrocene [15], 5-nitroimidazole [16] or quinoline [17] groups, with a global tendency to retain naturally present hydroxy groups at the A-ring.

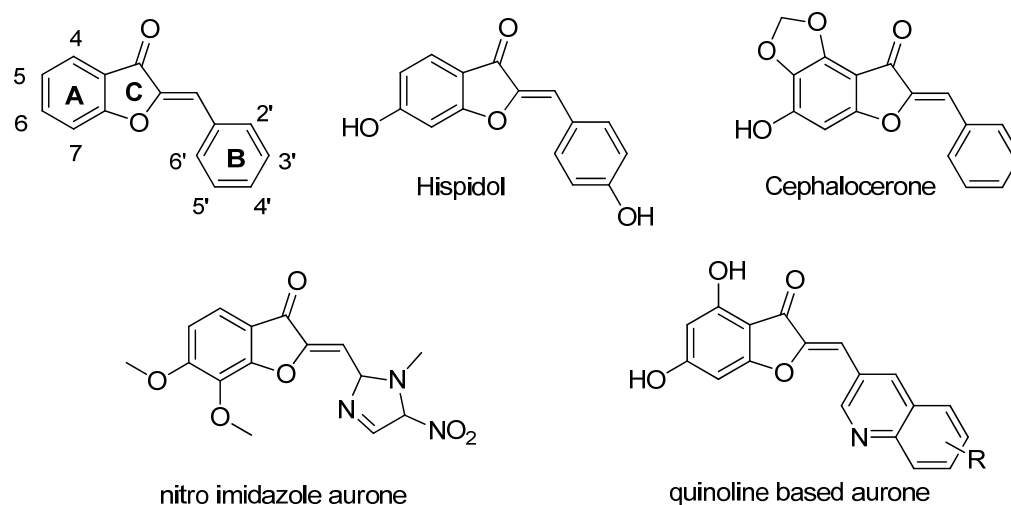


Figure 1. Structure of the aurone scaffold (top left), natural antimicrobial aurones, and examples of synthetic, heavily modified analogues.

In the present study, modifications of the A-ring were performed by substitution with an amino group combined with substitutions of the B-ring (Figure 1). The 31 new aurone derivatives obtained (Table 1) were tested in terms of antibacterial and antifungal activities.

Table 1. List of the synthesized aurones and their respective substitution on each position (OBn: Obenzyl, OPh: Ophenyl, OiPr: Oisopropyl).

Compound	5	2'	3'	4'
4	NHCOCH ₃	OCH ₃	H	H
5	NHCOCH ₃	H	OCH ₃	H
6	NHCOCH ₃	H	H	OCH ₃
7	NH ₂	OCH ₃	H	H

Table 2. Cont.

	Gram-Positive		Gram-Negative		Mycobacteria	Fungi
	<i>B. subtilis</i> ATCC6633	<i>S. aureus</i> ATCC6538P	<i>E. coli</i> ATCC8739	<i>P. aeruginosa</i> ATCC9027	<i>M. smegmatis</i> ATCC700084	<i>C. albicans</i> DSM10697
12	25	100	>100	>100	50	>100
13	100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100
15	50	100	>100	>100	50	>100
16	25	25	25	>100	50	>100
17	>100	>100	>100	>100	>100	>100
18	>100	>100	>100	>100	>100	>100
19	>100	>100	>100	25	>100	>100
20	25	12.5	25	>100	50	50
21	>100	>100	>100	>100	>100	>100
22	>100	>100	>100	>100	>100	>100
23	>100	>100	>100	>100	>100	>100
24	>100	>100	>100	>100	>100	>100
25	50	>100	>100	>100	100	>100
26	50	>100	>100	>100	>100	>100
27	>100	>100	>100	>100	>100	>100
28	>100	>100	>100	>100	>100	>100
29	>100	>100	>100	>100	>100	>100
30	>100	>100	>100	>100	>100	>100
31	50	100	>100	>100	100	>100
32	>100	>100	>100	>100	>100	>100
33	>100	>100	>100	>100	>100	>100
34	50	>100	>100	>100	>100	>100

In this first screening, among the 31 aurone derivatives tested, compounds **10**, **12**, **15**, **16**, and **20** were the most active (Figure 2). Compound **10** gave the lowest MIC values on all micro-organisms tested in Table 2 (i.e., Gram-positive and -negative bacteria, mycobacteria and fungi), with MICs ranging from 3.12 to 50 μ M. Similarly, compound **20** was active on all tested species (MIC ranging from 12.5 to 50 μ M), except *P. aeruginosa* for which MIC was superior to 100 μ M. Compound **16**, although active on *B. subtilis*, *S. aureus*, *E. coli*, and *M. smegmatis* (MIC ranging from 25 to 50 μ M), was however inactive on *P. aeruginosa* and *C. albicans*. Finally, although compounds **12** and **15** were active on tested Gram-positive bacteria and mycobacteria (MIC ranging from 25 to 100 μ M and from 50 to 100 μ M, respectively), they were inactive on tested Gram-negative bacteria and *C. albicans*. Based on MIC values on this first screening, the observed order of antimicrobial activities is as follows: compound **10** > **20** > **12** = **16** > **15**.

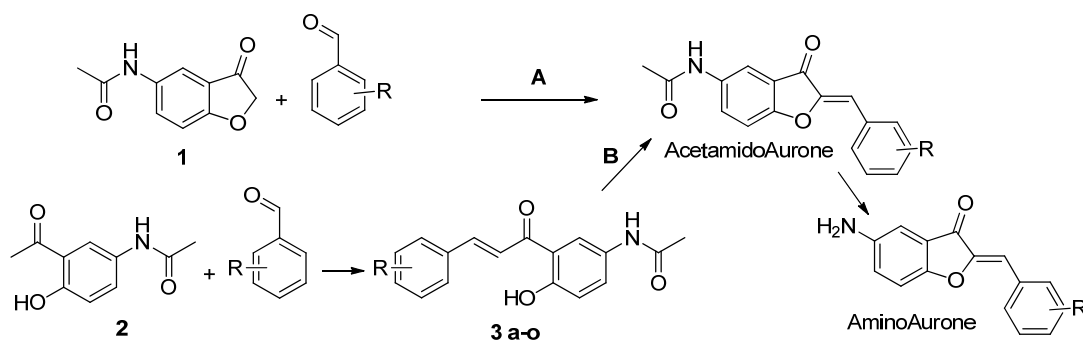


Figure 2. Synthetic route of the aurone derivatives. A: benzofuranone (**1**) 1 eq. and various benzaldehydes 1 eq. in Choline chloride/Urea (1/2), 80 °C, 2 h. B: (**2**) 1 eq. and various benzaldehydes 1 eq. in EtOH, LiOH 3 eq., 90 °C, 2 h. (**3 a-o**) 1 eq. and Mercuric acetate 1 eq. in pyridine, 110 °C, 1 h. Acetamido

Aurone (4–6, 10, 11, 14, 15, 18–20, 24, 25, 29, 31, 32, 34) are converted to their amino analogues (7–9, 12, 13, 16, 17, 21–23, 26–28, 30, 33) in EtOH, 0.5 M HCl, 100 °C, 2 h.

The safety of the five most active derivatives (i.e., compounds 10, 12, 15, 16, and 20) was then evaluated using different human cell types (Figures 3 and 4, Table 3).

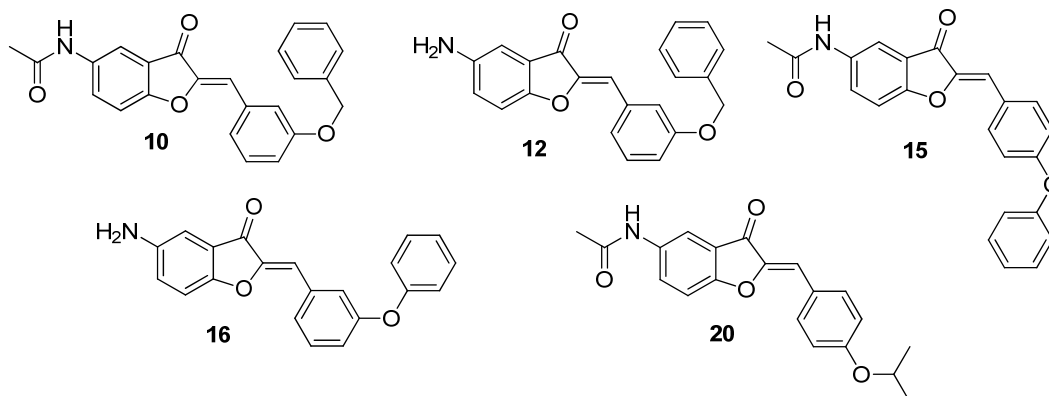


Figure 3. Structures of the more active aurone derivatives identified during the first screening (compounds 10, 12, 15, 16, and 20).

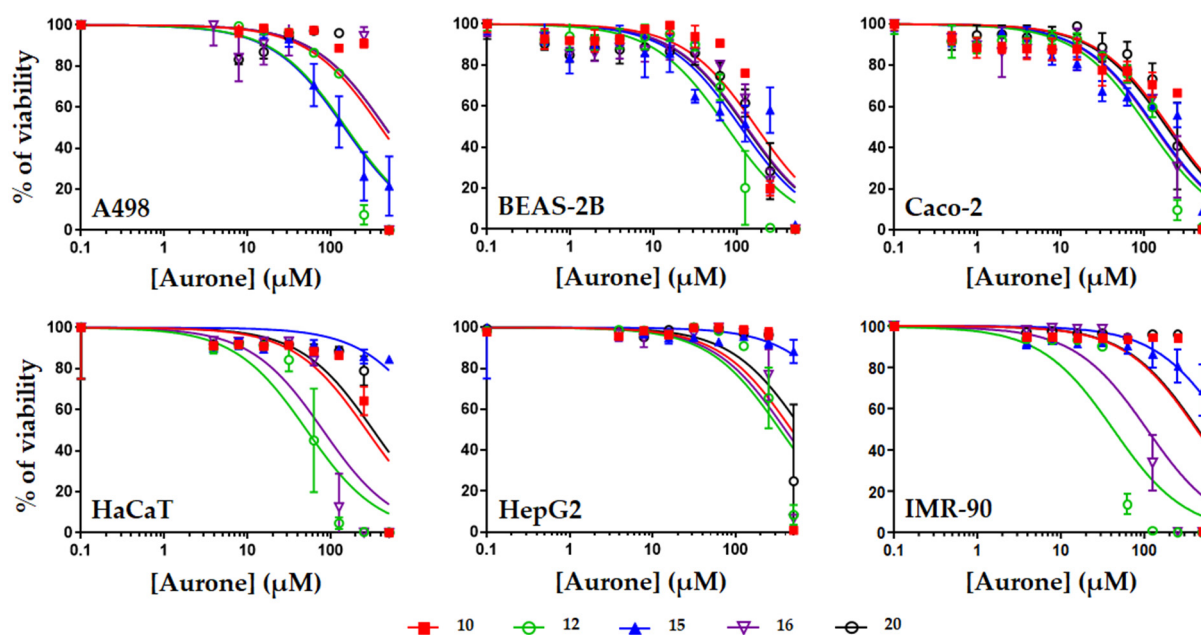


Figure 4. Evaluation of the toxicity of compounds 10, 12, 15, 16, and 20 on human cells. Human cells corresponding to kidney epithelial cells (A498), lung epithelial cells (BEAS-2B), intestinal epithelial cells (Caco-2), skin cells (HaCaT), liver cells (HepG2), or fibroblasts (IMR-90) were exposed to increasing concentrations of aurones for 48 h before measurement of the cell viability using resazurin. Results are expressed as % of cell viability, DMSO alone being used as negative control giving 100% viability. Data were plotted using GraphPad Prism 7 (means \pm S.D, $n = 3$).

Overall, compounds 10, 12, 15, 16, and 20 were found to be safe with most of their CC₅₀ values higher than their MIC ones. Compounds 10 and 20 were the safest molecules with mean CC₅₀ of 321.5 and 305.1 μM (ranging from 169.0 to 472.4 μM and from 125.9 to >500 μM for compounds 10 and 20, respectively). Compounds 12, 15, and 16 were more toxic, with mean CC₅₀ of 129.3, 130.6, and 218.0 μM, respectively. The highest safety of compounds 10 and 20 was further demonstrated when comparing the therapeutic indexes (TI) of the five aurones. Indeed, when calculating the TI of each aurone (by dividing their

CC₅₀ on human cells (Table 3) by their MIC on *S. aureus* (Table 2)), compounds **10** and **20** gave the highest TI values (ranging from 13.5 to 37.7 and from 10.0 to >40, for compounds **10** and **20**, respectively) compared to the TI values of compounds **12**, **15**, and **16** (Table 4) (ranging from 0.4 to 18.0).

Table 3. Determination of the cytotoxic concentrations of compounds **10**, **12**, **15**, **16**, and **20** on human cells. The cytotoxic concentrations 50 (CC₅₀, in μM) (i.e., the concentrations of aurones causing 50% reduction of the cell viability after 48 h exposure) were calculated from Figure 3 using GraphPad Prism 7. Results are expressed as means \pm S.D (μM) ($n = 3$).

Compound	10	12	15	16	20
A498	398.2 \pm 164.6	152.4 \pm 31.4	145.7 \pm 17.3	452.3 \pm 147.9	453.0 \pm 46.4
BEAS-2B	169.0 \pm 28.3	74.6 \pm 12.1	109.5 \pm 17.4	129.6 \pm 18.3	125.9 \pm 17.2
Caco-2	199.6 \pm 33.5	111.7 \pm 15.0	136.8 \pm 7.2	131.7 \pm 18.0	186.5 \pm 27.8
HaCaT	268.5 \pm 51.6	51.3 \pm 9.5	>500	80.4 \pm 17.8	322.9 \pm 73.2
HepG2	472.4 \pm 145.9	343.4 \pm 68.0	>500	397.8 \pm 94.1	>500
IMR-90	421.4 \pm 119.3	42.4 \pm 9.6	>500	116.5 \pm 29.4	437.6 \pm 128
Mean CC ₅₀	321.5	129.3	130.6	218.0	305.1

Table 4. Determination of the therapeutic indexes of compounds **10**, **12**, **15**, **16**, and **20**. The therapeutic indexes (TI) of each aurone was calculated by dividing their CC₅₀ on human cells (Table 3) by their MIC values on *S. aureus* from Table 2. MIC and CC₅₀ are expressed in μM .

Compound	10	12	15	16	20
MIC on <i>S. aureus</i>	12.5	100	100	25	12.5
Lowest CC ₅₀	169.0	42.4	109.5	80.4	125.9
Highest CC ₅₀	472.4	343.4	>500	452.3	>500
Lowest TI	13.5	0.4	1.0	3.2	10.0
Highest TI	37.7	3.4	>5	18.0	>40

Based on antimicrobial activity and toxicity data, the two most active and safest compounds were identified as compounds **10** and **20**, which were then tested on a larger panel of bacterial and fungal species in order to evaluate their spectrum of activity (Table 5).

The results of this second screening confirmed that compounds **10** and **20** are primarily active on Gram-positive bacteria, with MIC values as low as 0.78 and 3.12 μM for compounds **10** and **20**, respectively. Compounds **10** and **20** were particularly active on the various foodborne pathogens. For example, MIC values of 3.12 and 6.25 μM were obtained on *Listeria monocytogenes* for compounds **10** and **20**, respectively. Compounds **10** and **20** gave good activity against Clostridi such as *C. difficile* (MIC values of 12.5 and 3.12 μM for compounds **10** and **20**, respectively) and *C. botulinum* (MIC values of 0.78 and 3.12 μM for compounds **10** and **20**, respectively). In addition, good activities were also observed on the WHO group 3 pathogen *Bacillus anthracis*, responsible for anthrax disease and used as biological weapon (MIC values of 12.5 and 6.25 μM for compounds **10** and **20**, respectively). These MIC values were consistent with the ones obtained on two other *Bacillus* species, i.e., *B. cereus* and *B. subtilis*. On the other hand, *C. perfringens*, *Enterococcus* species, *P. acnes*, and *S. pyogenes* were found to be weakly sensitive to insensitive to compounds **10** and **20** with MIC values from 50 μM to >100 μM . Overall, compound **10** was more active than compound **20** on most tested Gram-positive bacterial strains, except *C. difficile*, *E. faecium*, and *B. anthracis*, for which compound **20** was more active. *S. aureus* and methicillin-resistant *S. aureus* (MRSA) showed good sensitivity with MIC of 12.5–25 μM , showing that resistance to methicillin did not affect the activity of compounds **10** and **20**.

Compounds **10** and **20** were also active on Gram-negative bacteria, with *A. baumannii*, *E. coli*, and *H. pylori* being the more sensitive strains (MIC values as low as 12.5 and 25 μM for compounds **10** and **20**, respectively). *S. enterica*, *S. flexneri*, and *V. alginolyticus* were also found to be sensitive, with MICs ranging from 25 to 50 μM . Although *P. aeruginosa* was sen-

sitive to compound **10** (MIC of 25 μM), it was insensitive to compound **20** (MIC > 100 μM). Good activities were also obtained on the WHO group 3 pathogens *Brucella melitensis*, *Francisella tularensis*, and *Yersinia pestis* (MIC values as low as 12.5 μM). *E. cloacae* and *K. pneumoniae* were insensitive to compounds **10** and **20** (MIC > 100 μM). Compound **10** was more active than compound **20** on most Gram-negative strains tested, except for *B. melitensis* and *F. tularensis*, for which compound **20** gave lower MIC values.

Table 5. Antimicrobial activities of compounds **10** and **20** on various bacterial and fungal species. The antimicrobial activities were determined using MIC assay as described in Section 4. The MIC values are given in μM ($n = 2\text{--}3$).

	10	20
Gram-positive		
<i>Bacillus anthracis</i> (CNR-charbon_04022)	12.5	6.25
<i>Bacillus cereus</i> (DSM31)	12.5	25
<i>Bacillus subtilis</i> (ATCC6633)	3.12	25
<i>Clostridium botulinum</i> (DSM1985)	0.78	3.12
<i>Clostridium difficile</i> (DSM1296)	12.5	3.12
<i>Clostridium perfringens</i> (ATCC13124)	>100	>100
<i>Enterococcus faecalis</i> (DSM2570)	50	100
<i>Enterococcus faecium</i> (DSM20477)	100	25
<i>Listeria monocytogenes</i> (DSM20600)	3.12	6.25
<i>Propionibacterium acnes</i> (ATCC6919)	100	>100
<i>Staphylococcus aureus</i> (ATCC6538P)	12.5	12.5
MRSA (ATCCBAA-1717)	12.5	25
<i>Streptococcus pyogenes</i> (DSM20565)	50	50
Gram-negative		
<i>Acinetobacter baumannii</i> (DSM30007)	12.5	25
<i>Brucella melitensis</i> (NR-256)	25	12.5
<i>Enterobacter cloacae</i> (DSM30054)	>100	>100
<i>Escherichia coli</i> (ATCC8739)	12.5	25
<i>Francisella tularensis</i> (NR-643)	50	12.5
<i>Helicobacter pylori</i> (ATCC43504)	12.5	12.5
<i>Klebsiella pneumoniae</i> (DSM26371)	>100	>100
<i>Pseudomonas aeruginosa</i> (ATCC9027)	25	>100
<i>Salmonella enterica</i> (CIP80.39)	25	50
<i>Shigella flexneri</i> (ATCC12022)	25	50
<i>Vibrio alginolyticus</i> (DSM2171)	25	50
<i>Yersinia pestis</i> (NR-641)	12.5	12.5
Mycobacteria		
<i>Mycobacterium abscessus</i> S (CIP 104536 ^T)	>100	>100
<i>Mycobacterium abscessus</i> R (CIP 104536 ^T)	>100	>100
<i>Mycobacterium smegmatis</i> (ATCC700084)	50	50
<i>Mycobacterium tuberculosis</i> H37Rv (mc ² 6230)	>100	>100
Filamentous fungi		
<i>Aspergillus fumigatus</i> (DSM819)	>100	>100
<i>Fusarium oxysporum</i> (DSM62316)	25	25
Yeasts		
<i>Candida albicans</i> (DSM10697)	50	50
<i>Candida auris</i> (DSM21092)	50	12.5
<i>Candida glabrata</i> (DSM11226)	50	50
<i>Candida tropicalis</i> (DSM9419)	100	100
<i>Cryptococcus neoformans</i> (DSM11959)	25	25

Although compounds **10** and **20** were active on *M. smegmatis* (Table 2), they were inactive on tested pathogenic mycobacteria, i.e., *M. abscessus* and *M. tuberculosis*.

Finally, in term of antifungal effect, compounds **10** and **20** were active on the filamentous fungi *F. oxysporum* (MIC of 25 μM) but inactive on another important human pathogen *A. fumigatus*. Antifungal activity was also observed on yeasts, including various *Candida*

species (*C. albicans*, *C. auris*, *C. glabrata*, and *C. tropicalis*) and *Cryptococcus neoformans* with MIC values as low as 25 and 12.5 μM for compounds **10** and **20**, respectively. In most cases, compounds **10** and **20** gave the same MIC values except for *C. auris*, for which compound **20** was more active than compound **10** (MIC of 12.5 and 50 μM , respectively). *C. tropicalis* had a less sensitive MIC value of 100 μM .

The therapeutic indexes (TI) values of compounds **10** and **20** were calculated using the MIC values reported in Table 5 and the CC_{50} on human cells reported in Table 3 (Tables 6 and 7).

Table 6. Safety evaluation for compound **10**. Therapeutic indexes (TI) values were calculated by dividing CC_{50} values by the lowest MIC value (obtained on *C. botulinum*, i.e., 0.78 μM) for compound **10** (Table 5).

	A498	BEAS-2B	Caco-2	HaCaT	HepG-2	IMR-90
CC_{50} (μM)	398.2	169.0	199.6	268.5	472.4	421.4
TI with MIC of 0.78 μM	510.5	216.6	255.8	344.2	605.6	540.2

Table 7. Safety evaluation for compound **20**. Therapeutic indexes (TI) values were calculated by dividing CC_{50} values by the lowest MIC value (obtained on *C. botulinum*, i.e., 3.12 μM) for compound **20** (Table 5).

	A498	BEAS-2B	Caco-2	HaCaT	HepG-2	IMR-90
IC_{50} (μM)	453.0	125.9	186.5	322.9	>500	437.6
TI with MIC of 3.12 μM	145.1	40.3	59.7	103.4	>160.2	140.2

TI values ranged from 216.6 to 605.6 and from 40.3 to >160.2 for compounds **10** and **20**, respectively, confirming that these two aurone derivatives possess good therapeutic values. Compound **10** was the safest in all cases.

3. Discussion

In the present study, 31 new aurone derivatives were synthesized (Figure 5, see Supplementary Materials) and tested in terms of antimicrobial activity against various bacteria and fungi. These new compounds were obtained by the substitution of the aurone scaffold at position 5 by amino and acetamido groups, and through various substitutions at the 2', 3' and 4' positions. The first screening antimicrobial test performed on representative species of bacteria and fungi allowed us to identify compounds **10**, **12**, **15**, **16**, and **20** as the more active aurones. Comparisons between active and inactive structures afforded insightful information to identify the most interesting substitution. Compound **10** can be compared to compound **11**, **12** and **13**. All these compounds are substituted in position 3' or 4' by a benzyloxy group. However, only compound **10** and **12** showed an interesting activity. This suggests that benzyloxy substitution in 3' may be a key element in the activity of the compound. The same methodology could be used to compare compound **20** with other isopropoxy compounds such as **18**, **19**, **21** and **22**. These four last mentioned compounds showed a weak or no activity against tested micro-organisms. Similarly to compound **10**, this could indicate that the 4'-isopropyl substitution may be a far better alternative to the other position and again that the acetamido group is more effective than the amino group in position 5. From the results, it can be suggested that the acetamido group is important for the antibacterial capacity of the aurones. Thus, compounds **12** and **16** are both 5-aminoaurones; compounds **15** and especially compounds **10** and **20** are 5-acetamidoaurones and showed far better antibacterial activity. Moreover, four of these compounds possess a ring substitution in position 3' and 4'. Interestingly, benzyloxy-substituted aurones (i.e., **10** and **12**) seem to only be active in positions 3' when phenyl-substituted aurones (i.e., **15** and **16**) showed activity when substituted both in 3' and 4' position. However, considering the activity of compound **10** compared to **12**, **15** and **16**,

benzyloxy substitution must be considered more promising than phenyl substitution. The other benzyloxy- and phenyl-substituted aurones (i.e., **11**, **13**, **14** and **17**) showed no activity. Isopropoxy-substituted aurones also are an interesting option as shown by compound **20**, which is similar in activity to compound **10**. Again only 4'-isopropyl aurones were active; 3' and 2' were inactive on the varieties of pathogens tested. Methyl (i.e., **4–9**), fluoro (i.e., **24–28**), carboxy (i.e., **31**), trifluoromethyl (i.e., **29** and **30**) and hydroxy (i.e., **32** and **33**) substituted aurones showed no activity and thus should not be considered as privileged substitution in the development of antibacterial aurones. Finally, aurone **34** is also inactive and shows that the 5-acetamido substitution is not enough to produce an antibacterial activity to aurones and that a substitution on the B-ring is mandatory. These five aurones were then tested in term of toxicity against various human cell types. The toxicity data demonstrated that compounds **10** and **20** were the safest ones compared to compounds **12**, **15**, and **16**. Again, 5-acetamido aurones seem to be more interesting as they are safer on human cells than 5-aminoaurones. Compounds **10** and **20** were then further tested on a larger panel of pathogens, including Gram-positive and Gram-negative bacteria. In this second screening, these compounds showed an interesting activity as antimicrobials against Gram-positive strains such as *S. aureus*, methicillin-resistant *S. aureus*, *L. monocytogenes*, *B. subtilis* and *C. difficile* and Gram-negative strains such as *E. coli*, *A. baumannii* and *H. pylori*. The two selected compounds shared some structural similarities, such as the 5-acetamido substitution. Out of all the compounds, 3'-benzyloxy and 4'-isopropoxy were the most promising substitutions. Compared to previously described aurones active on various Gram-positive bacteria but only two Gram-negative strains (i.e., *H. pylori* or *V. alginolyticus*) [14], compounds **10** and **20** were active on a large number of Gram-positive and -negative bacteria as well as fungi. Structurally, the most active compound synthesized by Olleik et al. [14] was a 5,7-dihydroxyaurone substituted in 4' by a benzyloxy group and in 3' by a methoxy group. Again, this shows the promising nature of the benzyloxy substitution and that hydroxy aurones, vastly found in nature, are far less active than synthetic aurones such as amino and acetamidoaurones.

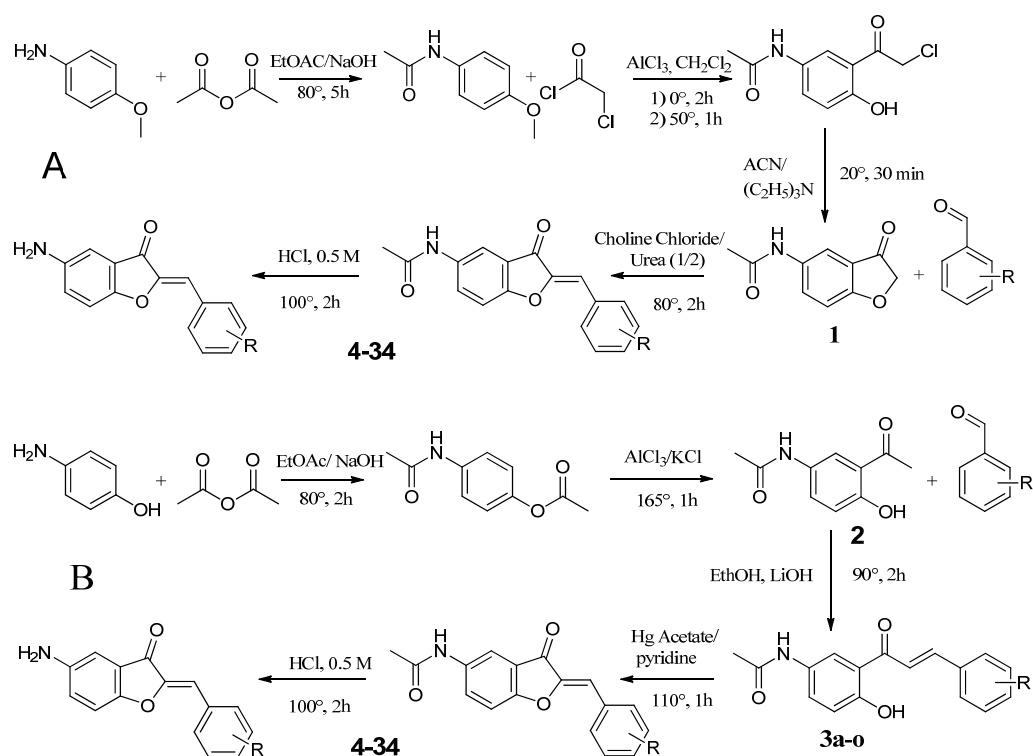


Figure 5. Route A and B used for the synthesis of 5-amino and 5-acetamido aurones.

4. Materials and Methods

4.1. Biology

4.1.1. Microorganism Strains and Growth Conditions

Bacterial and fungi strains used in this study, except when mentioned, were obtained from either the American Type Culture Collection (ATCC), the German Leibniz Institute (DSMZ), or the French Pasteur Institute (CIP) and correspond to reference strains. They were maintained on agar plates using appropriate media and culture conditions (in terms of temperature and aerobic/microaerobic/anaerobic condition) as previously described [14,18]. Regarding BSL-3 strains, *Bacillus anthracis*, *Francisella tularensis*, and *Brucella melitensis* strains were maintained on Chocolate agar PolyViteX (Biomérieux) agar at 37 °C, and at 26 °C for *Yersinia pestis* [19,20]. Regarding mycobacteria, *M. smegmatis* mc²155 (ATCC700084) was grown in Middlebrook 7H9 complete medium containing 0.05% Tween-80 and 0.2% Glycerol (7H9-TG) and *M. abscessus* (CIP104536T) S and R morphotypes, were cultured in 7H9-TG containing 10% BBL™ Middlebrook OADC Enrichment (7H9-TG^{OADC}) at 37 °C under stirring (200 rpm). *M. tuberculosis* mc²6230, a derivative of H37Rv which contains a deletion of the RD1 region and *panCD*, resulting in a pan(−) phenotype, was grown in 7H9-TG^{OADC} supplemented with 24 µg/mL D-panthothenate (Sigma-Aldrich, Lyon, France). Cultures were kept at 37 °C without shaking.

4.1.2. Antimicrobial Activity Assay

The antimicrobial activity of aurones on BSL2 bacteria and fungi was evaluated through determination of the Minimum Inhibitory Concentration (MIC) using two-fold serial dilutions in liquid media following the National Committee of Clinical Laboratory Standards (NCCLS, 1997) as previously described [14,18,21,22]. For BSL-3 bacteria, the MIC of aurones was determined following the Clinical and Laboratory Standards Institute (CLSI) recommendations as previously described [23]. For determining the antimycobacterial activity of the different aurones, the microdilution method was used in sterile 96-well flat-bottom Greiner Bio-One polypropylene microplates with lid (Thermo Fisher Scientific) using the resazurin microtiter assay (REMA) as previously described [24,25]. The concentration of aurones leading to 90% inhibition of mycobacteria growth was defined as the MIC. All experiments were performed independently at least three times.

4.1.3. Cytotoxic Assays

The impact of aurones on the viability of human cells were evaluated as previously described [18,22,26]. Human cells used were kidney epithelial cell line A498 (ATCC® HTB-44), normal lung epithelial cells BEAS-2B (ATCC® CRL-9609), intestinal cell line Caco-2 (ATCC® HTB-37), normal epidermal keratinocytes (HaCaT) (from Creative Bioarray, Shirley, NY, USA), liver cell line HepG2 (ATCC® HB-8065), and normal lung fibroblasts IMR-90 (ATCC® CCL186). Cells were cultured in DMEM supplemented with 10% fetal calf serum (FCS), 1% l-glutamine, and 1% antibiotics (all from Invitrogen (Carlsbad, CA, USA)). Cells were routinely grown on 25 cm² flasks and maintained in a 5% CO₂ incubator at 37 °C. For toxicity assays, human cells grown on 25 cm² flasks were detached using trypsin-EDTA solution (from ThermoFisher, Waltham, MA, USA), counted using Malassez counting chamber, diluted in appropriate culture media, and seeded into 96-well cell culture plates (Greiner bio-one, Paris, France) at approximately 10⁴ cells per well. The cells were left to grow for 48–72 h at 37 °C in a 5% CO₂ incubator until confluence. Media from wells was then aspirated and cells were treated with 100 µL of appropriate culture media containing increasing concentrations of tested aurones (from 0 to 400 µM, 1:2 serial dilutions). Volume of DMSO corresponding to 400 µM of aurones was used as negative control and was found not toxic. The plates were then incubated at 37 °C for 48 h. Resazurin-based in vitro toxicity assay kit (from Sigma-Aldrich, Lyon, France) was then used to assess the viability of the cells following manufacturer's instructions. Briefly, resazurin stock solution was diluted 1:10 in sterile PBS containing calcium and magnesium (PBS⁺⁺, pH 7.4). Plates were aspirated and 100 µL of the diluted solution was added per well. After 2 h incubation at

37 °C, fluorescence intensity was measured using a microplate reader (Biotek, Synergy Mx, Colmar, France) (excitation wavelength of 530 nm/emission wavelength of 590 nm). The fluorescence values were normalized by the controls (DMSO treated cells) and expressed as percentage of cell viability. The CC₅₀ values of aurones (i.e., the concentrations causing a reduction of 50% of the cell viability) were calculated using GraphPad® Prism 7 software (San Diego, CA, USA). Experiments were conducted in triplicate ($n = 3$).

4.2. Chemistry

¹H and ¹³C NMR spectra were recorded on a Bruker Avance III nanobay—300 MHz instrument (Bruker, Bremen, Germany, 300 MHz for ¹H, 75 MHz for ¹³C). Chemical shifts are reported in ppm relative to the solvent in which the spectrum was recorded [¹H: δ (d₆-DMSO) = 2.50 ppm, δ (CDCl₃) = 7.27 ppm; ¹³C: δ (d₆-DMSO) = 39.52 ppm, δ (CDCl₃) = 77.16 ppm], full spectra are presented in Supplementary Materials. Combustion analyses were performed at the analysis facilities of Spectropole (<https://fr-chimie.univ-amu.fr/spectropole>, accessed on 20 December 2023) with a Thermo Finnigan (San Jose, CA, USA) EA 1112 apparatus; all compounds had purity higher than 95%. Microwave-assisted reactions were performed in a CEM Discover microwave reactor with a focused field (CEM Corporation, Matthews, NC, USA). Silica gel F-254 plates (0.25 mm; Merck, Darmstadt, Germany) 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.

4.2.1. Synthesis Route A

Synthesis of N-(4-methoxyphenyl)acetamide (**1a**)

In a solution of 8 g of *m*-anisidine and 2.5 eq. of NaOH in 50 mL of ethyl acetate, 6.6 g of acetic anhydride (1.5 eq.) was added dropwise. When the addition was completed, the mixture was heated at 80 °C for 5 h. The solution was cooled and filtered. Under pressure, the solvent was removed, the obtained product was dissolved in ethanol and hexane to precipitate, to obtain 5 g of **1a**.

Synthesis of 2-Chloro-1-[2-hydroxy-5-[(1-hydroxyethyl)amino]cyclohexyl]ethan-1-one (**2a**)

A total of 5 g of **1a** and 18 g of AlCl₃ were dissolved in 30 mL of dichloromethane then at 0 °C, 3.5 eq. of chloroacetyl chloride was added dropwise. When the addition was completed, the solution was heated up to 50 °C for 1 h. The mixture was poured on ice and extracted with ethyl acetate. EtOAc was removed under pressure to obtain **2a**.

Synthesis of N-(3-oxo-2,3-dihydro-1-benzofuran-5-yl)acetamide (**3a**)

In a flask, 2 g of **2a** and 1.5 eq. of triethylamine were added to 20 mL of acetonitrile; the solution reacted at 25 °C for 12 h. Solvent was removed under pressure. The left-over mixture was dissolved in EtOAc, washed several times with water then extracted. EtOAc was removed under pressure to obtain **3a**.

Synthesis of Substituted 5-Acetamidoaurones

In a flask, 1 mmol of **3a** and 1 mmol of the corresponding benzaldehyde were dissolved in 10 mL of choline chloride/urea. Three drops of 50% KOH solution were added. The mixture was heated at 80 °C for 2 h. Water and HCl were added, then the precipitate was filtered and washed several times with ether to obtain acetamido substituted aurones.

Synthesis of Substituted 5-Aminoaurone

A total of 10 mmol of acetamido aurones was added to a mixture of EtOH (20 mL) and 0.5 M HCl (5 mL). The solution was refluxed for 2 h. Upon cooling, the solvent was removed under vacuum and the residue obtained was poured onto iced water (100 mL). The resulting solution was neutralized with NH₄OH 16% until pH = 7. The precipitate formed was collected by filtration and washed with excess cold water.

4.2.2. Synthesis Route B

Synthesis of 4-Acetamidophenyl Acetate (**1b**)

To a solution of 8 g of 4-aminophenol and 2.5 eq. of NaOH in 50 mL of ethyl acetate, 26.5 g of acetic anhydride (3.5 eq.) was added dropwise. When the addition was completed, the solution was heated at 80 °C for 5 h. Upon cooling, the mixture was filtered. Solvent was removed under pressure; the product was recrystallized in ethanol and hexane to obtain 5 g of **1b**.

Synthesis of N-(3-acetyl-4-hydroxyphenyl)acetamide (**2b**)

To a solution of **1b** (5 g, 26 mmol), 15 g of AlCl₃ (113 mmol, 4 eq.) and 1.9 g of KCl (26 mmol, 1 eq.) were added. The mixture was then heated a 165 °C for 1 h until a brown paste appeared. Upon cooling, ice cold water was added (300 mL) and the mixture was filtered to obtain 2 g of a beige powder (**2b**).

Synthesis of Substituted of 5-Acetamidochalcones (**3b**)

In a flask, 193 mg of **2b** (0.001 mmol), 1 eq. of chosen benzaldehyde and 188 mg of LiOH (16 eq., 0.016 mmol) were dissolved in ethanol (20 mL). The mixture was heated for 2 h at 90 °C. The solvent was then removed under pressure, cold water and HCl were added, and the precipitate was filtered to obtain the desired chalcone (**3b**).

Synthesis of Substituted 5-Acetamidoaurones

To a mixture of chosen **3b** chalcone in pyridine (20 mL), 1 eq. of mercury acetate was added. The solution was heated for 1 h at 110 °C. Water and HCl were added. The precipitate was filtered and washed several times with ice cold water to get rid of the mercury. Obtention of a powder red-yellow powder depended the substitution.

Synthesis of Substituted 5-Aminoaurone

For synthesis of 5-aminoaurone see Section Synthesis of Substituted 5-Aminoaurone.

Yield: 83%; mp: 234.8 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.15 (s, 1H, NH), 8.20–8.18 (dd, 1H, J = 1.2;7.8 Hz, C-H_{6'}), 8.10 (d, 1H, J = 1.9 Hz, C-H₄), 7.83–7.80 (dd, 1H, J = 2.2;8.9 Hz, C-H₆), 7.52–7.49 (d, 1H, J = 8.9 Hz, C-H₇), 7.46 (dt, 1H, J = 7.1 Hz, C-H_{4'}), 7.19 (s, 1H, C-H₁₀), 7.16–7.09 (m, 2H, C-H_{3',5'}), 3.91 (s, 3H, OCH₃), 2.07 (s, 3H, NHCOCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.53(C-3), 168.41(CO), 161.21(C-8), 158.32(C-2'), 146.75(C-2), 135.53(C-5), 131.99(C-4'), 131.11(C-6'), 128.71(C-6), 120.89(C-1'), 120.68(C-5'), 120.08(C-9), 113.31(C-3'), 113.15(C-7), 111.57(C-10), 105.47(C-4), 55.84(OCH₃), 23.83(CH₃). Elemental analysis calcd (%) for C₁₈H₁₅NO₄: C, 69.89; H, 4.89; N, 4.53; found C, 69.87; H, 4.91; N, 4.52. *m/z*: 309.1001 (100.0%).

(Z)-N-(2-(3-methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (**5**):

Yield: 71%; mp: 204.2 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.16 (s, 1H, NH), 8.10 (d, 1H, J = 2 Hz, C-H₄), 7.83–7.80 (dd, 1H, J = 2.2;8.9 Hz, C-H₆), 7.60–7.55 (m, 2H, C-H_{2',4'}), 7.54–7.51 (d, 1H, J = 8.9 Hz, C-H₇), 7.43 (dt, 1H, J = 8.0 Hz, C-H_{5'}), 7.06–7.03 (dd, 1H, J = 2.6;8.2 Hz, C-H_{6'}), 6.90 (s, 1H, C-H₁₀), 7.16–7.09 (m, 2H, C-H_{3',5'}), 3.82 (s, 3H, OCH₃), 2.07 (s, 3H, NHCOCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.68(C-3), 168.42(CO), 161.33(C-8), 159.42(C-3'), 146.88(C-2), 135.58(C-5), 133.08(C-6'), 130(C-2'), 128.82(C-6), 123.74(C-1'), 120.59(C-9), 116.57(C-6'), 115.76(C-4'), 113.35(C-7), 113.15(C-4), 112.07(C-10), 55.15(OCH₃), 23.83(CH₃). Elemental analysis calcd (%) for C₁₈H₁₅NO₄: C, 69.89; H, 4.89; N, 4.53; found C, 69.84; H, 4.88; N, 4.53. *m/z*: 309.1001 (100.0%).

(Z)-N-(2-(4-methoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (**6**):

Yield: 91%; mp: 252 °C [1]; ¹H NMR (300 MHz, DMSO-d₆): δ 10.17 (s, 1H, NH), 8.10 (d, 1H, J = 2 Hz, C-H₄), 7.96–7.93 (d, 2H, J = 7.9 Hz, C-H_{2',6'}), 7.81–7.78 (dd, 1H, J = 2.2;8.9 Hz, C-H₆), 7.50–7.47 (d, 1H, J = 8.9 Hz, C-H₇), 7.08–7.06 (d, 2H, J = 8.0 Hz, C-H_{3',5'}), 6.91 (s, 1H, C-H₁₀), 3.83 (s, 3H, OCH₃), 2.07 (s, 3H, NHCOCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.37(C-3), 168.46(CO), 161.06(C-8), 161.00(C-4'), 145.75(C-2), 135.47(C-5), 133.40(C-2',6'), 128.51(C-6), 124.47(C-1'), 120.98(C-9), 114.71(C-3'-5'), 113.30(C-7), 113.07(C-4), 112.74(C-10),

55.40(OCH₃), 23.91(CH₃). Elemental analysis calcd (%) for C₁₈H₁₅NO₄: C, 69.89; H, 4.89; N, 4.53; found C, 69.78; H, 4.87; N, 4.48. *m/z*: 309.1001 (100.0%).

(Z)-5-amino-2-(2-methoxybenzylidene)benzofuran-3(2H)-one (7):

Yield: 22%; mp: 189.3 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 8.18–8.16 (dd, 1H, *J* = 1.6;7.8 Hz, C-H_{6'}), 7.43 (dt, 1H, *J* = 1.5;8.5 Hz, C-H_{4'}), 7.26–7.23 (d, 1H, *J* = 8.8 Hz, C-H₇), 7.14–7.11 (m, 2H, C-H_{3',5'}), 7.10 (s, 1H, C-H₁₀), 7.06–7.03 (dd, 1H, *J* = 2.5;8.8 Hz, C-H₆), 6.84 (d, 1H, *J* = 2.4 Hz, C-H₄), 5.23 (bs, 2H, NH₂), 3.90 (s, 3H, OCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 184.09(C-3), 158.13(C-2'), 158.00(C-8), 147.05(C-2), 145.56(C-5), 131.55(C-4'), 130.98(C-6'), 124.55(C-6), 121.01(C-9), 120.84(C-5'), 120.41(C-1'), 113.14(C-7), 111.47(C-10), 105.44(C-3'), 104.18(C-4), 55.79(OCH₃). Elemental analysis calcd (%) for C₁₆H₁₃NO₃: C, 71.90; H, 4.90; N, 5.24; found C, 71.85; H, 4.95; N, 5.21. *m/z*: 267.0895 (100.0%).

(Z)-5-amino-2-(3-methoxybenzylidene)benzofuran-3(2H)-one (8):

Yield: 50%; mp: 190 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 7.59–7.57 (d, 1H, *J* = 7.7 Hz, C-H_{6'}), 7.54 (d, 1H, *J* = 2.4 Hz, C-H₄), 7.49–7.46 (d, 1H, *J* = 8.8 Hz, C-H₇), 7.42 (dt, 1H, *J* = 8.2 Hz, C-H_{5'}), 7.38–7.35 (dd, 1H, *J* = 2.2;8.8 Hz, C-H₆), 7.24 (d, 1H, *J* = 2.11 Hz, C-H_{2'}), 7.06–7.03 (dd, 1H, *J* = 1.9;8.1 Hz, C-H_{4'}), 6.88 (s, 1H, C-H₁₀), 3.82 (s, 3H, OCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.6 (C-3), 160.79 (C-8), 159.42 (C-3'), 146.96 (C-2), 137.91 (C-5'), 133.13 (C-1'), 129.99 (C-5'), 127.97(C-6), 123.71 (C-7), 121.18 (C-9), 116.54 (C-6'), 115.72 (C-4'), 113.87 (C-4), 111.88 (C-2'), 111.18 (C-10), 55.16 (OCH₃). Elemental analysis calcd (%) for C₁₆H₁₃NO₃: C, 71.90; H, 4.90; N, 5.24; found C, 71.92; H, 4.92; N, 5.28. *m/z*: 267.0895 (100.0%).

(Z)-5-amino-2-(4-methoxybenzylidene)benzofuran-3(2H)-one (9):

Yield: 86%; mp: 110.4 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 7.95–7.92 (d, 2H, *J* = 8.1 Hz, C-H_{2'}), 7.31–7.28 (d, 1H, *J* = 8.8 Hz, C-H₇), 7.13–7.10 (dd, 1H, *J* = 2.2;8.8 Hz, C-H₆), 7.09–7.06 (d, 2H, *J* = 8.1 Hz, C-H_{3'}), 6.93 (d, 1H, *J* = 2.4 Hz, C-H₄), 6.83 (s, 1H, C-H₁₀), 3.82 (s, 3H, OCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.77 (C-4), 160.61 (C-4'), 158.51 (C-8), 145.94 (C-5), 143.60 (C-2), 133.16 (C-2'), 125.22 (C-1'), 124.69 (C-6), 121.35 (C-9), 114.65 (C-3'), 113.30 (C-4), 111.68 (C-10), 106.77 (C-7), 55.37 (OCH₃). Elemental analysis calcd (%) for C₁₆H₁₃NO₃: C, 71.90; H, 4.90; N, 5.24; found C, 71.88; H, 4.97; N, 5.30. *m/z*: 267.0895 (100.0%).

(Z)-N-(2-(3-(benzyloxy)benzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (10):

Yield: 80%; mp: 204.5 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.16 (s, 1H, NH), 8.10 (d, 1H, *J* = 2 Hz, C-H₄), 7.84–7.80 (dd, 1H, *J* = 2.2;8.9 Hz, C-H₆), 7.63 (bs, 1H, C-H_{2'}), 7.58–7.32 (m, 8H), 7.14–7.11 (dd, 1H, *J* = 7.9 Hz, C-H_{4'}), 6.89 (s, 1H, C-H₁₀), 5.18 (m, 2H, CH₂), 2.07 (s, 3H, NHCOCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.74 (C-4), 168.51 (CO), 161.36 (C-8), 158.53 (C-3'), 146.92 (C-2), 136.87 (C-1bn), 135.64 (C-5), 133.14 (C-5'), 130.1 (C-6'), 128.87 (C-6), 128.48 (C-3bn), 127.94 (C-4bn), 127.83 (C-2bn), 124.14 (C-1'), 120.62 (C-9), 117.27 (C-4'), 116.75 (C-2'), 113.45 (C-7), 113.17 (C-4), 112.09 (C-10), 69.34 (CH₂), 23.91 (CH₃). Elemental analysis calcd (%) for C₂₄H₁₉NO₄: C, 74.79; H, 4.97; N, 3.63; found C, 74.74; H, 5.01; N, 3.60. *m/z*: 385.13141 (100.0%).

(Z)-N-(2-(4-(benzyloxy)benzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (11):

Yield: 72%; mp: 212.8 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.14 (s, 1H, NH), 8.09 (d, 1H, *J* = 2.02 Hz, C-H₄), 7.96–7.93 (d, 2H, *J* = 8.8 Hz, C-H_{2'}), 7.82–7.79 (dd, 1H, *J* = 2.2;8.9 Hz, C-H₆), 7.50–7.34 (m, 6H, C-H_{7, bn}), 7.16–7.13 (d, 2H, *J* = 8.8 Hz, C-H_{3'}), 6.90 (s, 1H, C-H₁₀), 5.18 (m, 2H, CH₂), 2.07 (s, 3H, NHCOCH₃).

¹³C NMR (75 MHz, DMSO-d₆): δ 183.37 (C-3), 168.48 (CO), 161.06 (C-8), 159.93 (C-4'), 145.75 (C-2), 136.58 (C-1bn), 135.45 (C-5), 133.39 (C-2'), 128.56 (C-1'), 128.49 (C-3bn), 128 (C-4bn), 127.84 (C-2bn), 124.64 (C-6), 120.95 (C-9), 115.52 (C-3'), 113.33 (C-7), 113.08 (C-4), 112.68 (C-10), 69.44 (CH₂), 23.9 (CH₃). Elemental analysis calcd (%) for C₂₄H₁₉NO₄: C, 74.79; H, 4.97; N, 3.63; found C, 74.77; H, 4.96; N, 3.61. *m/z*: 385.13141 (100.0%).

(Z)-5-amino-2-(3-(benzyloxy)benzylidene)benzofuran-3(2H)-one (12):

Yield: 80%; mp: 132.6 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 7.61 (bs, 1H, C-H_{2'}), 7.55–7.53 (d, 1H, *J* = 8.8 Hz, C-H₇), 7.50–7.48 (d, 2H, C-H_{2bn}), 7.41 (dt, 2H, C-H_{3bn}), 7.36–7.33 (m, 2H, C-H_{5', 4bn}), 7.11–7.09 (dd, 1H, *J* = 7.9 Hz, C-H₆), 7.06–7.04 (dd, 1H, *J* = 7.9 Hz, C-H_{4'}),

6.83 (d, 1H, $J = 2$ Hz, C-H₄), 6.77 (s, 1H, C-H₁₀), 5.26 (bs, 2H, NH₂), 5.17 (m, 2H, CH₂). ¹³C NMR (75 MHz, DMSO-d₆): δ 184.24 (C-3), 158.48 (C-3'), 158.14 (C-8), 147.21 (C-2), 145.63 (C-5), 136.87 (C-1bn), 133.43 (C-1'), 129.97 (C-5'), 128.42 (C-3bn), 127.86 (C-4bn), 127.75 (C-2bn), 124.71 (C-6), 123.89 (C-7), 120.91 (C-9), 117.04 (C-4'), 116.4 (C-2'), 113.21 (C-6'), 110.73 (C-10), 105.45 (C-4), 69.31 (CH₂). Elemental analysis calcd (%) for C₂₂H₁₇NO₃: C, 76.95; H, 4.99; N, 4.08; found C, 76.88; H, 5.01; N, 4.04. m/z : 343.12084 (100.0%).

(Z)-5-amino-2-(4-(benzyloxy)benzylidene)benzofuran-3(2H)-one (13):

Yield: 80%; mp: 141.6 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 7.97–7.95 (d, 2H, C-H_{2',6'}, $J = 8.8$ Hz), 7.49–7.47 (m, 3H, C-H), 7.41–7.39 (m, 4H, C-H_{bn}), 7.28 (s, 1H, C-H₄), 7.17–7.15 (d, 2H, C-H_{3',5'}, $J = 8.8$ Hz), 6.92 (s, 1H, C-H₁₀), 5.20 (s, 2H, CH₂). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.16 (C-3), 160.82 (C-4'), 159.91 (C-8), 145.76 (C-2), 136.55 (C-5), 133.36 (C-1'), 128.46 (C-3bn, C-5bn), 128.20 (C-1bn), 127.92 (C-4bn), 127.81 (C-2bn, C-6bn), 124.64 (C-6), 121.55 (C-9), 115.5 (C-2', C-6'), 113.90 (C-7), 112.58 (C-4), 111.79 (C-10), 69.42 (CH₂). (CH₃). Elemental analysis calcd (%) for C₂₂H₁₇NO₃: C, 76.95; H, 4.99; N, 4.08; found C, 76.77; H, 4.98; N, 4.06. m/z : 343.13141 (100.0%).

(Z)-N-(3-oxo-2-(3-phenoxybenzylidene)-2,3-dihydrobenzofuran-5-yl)acetamide (14):

Yield: 96%; mp: 196.4 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.16 (s, 1H, NH), 8.10 (d, 1H, $J = 2$ Hz, C-H₄), 7.83–7.79 (dd, 1H, $J = 2.2; 8.9$ Hz, C-H₆), 7.75–7.72 (d, 1H, $J = 7.9$ Hz, C-H_{6'}), 7.67 (bs, 1H, C-H_{2'}), 7.51 (dt, 1H, $J = 7.4$ Hz, C-H_{5'}), 7.44–7.41 (m, 3H, C-H_{4',8'}), 7.20 (t, 1H, $J = 7.4$ Hz, C-H_{10'}), 7.10–7.07 (d, 3H, C-H_{9',7'}), 6.93 (s, 1H, C-H₁₀), 2.07 (s, 3H, NHCOCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.72 (C-3), 168.52 (CO), 161.30 (C-8), 157.14 (C-7'), 156.19 (C-3'), 147.11 (C-2), 135.68 (C-5), 133.78 (C-1'), 130.60 (C-6), 130.17 (C-9'), 128.92 (C-5'), 126.54 (C-9), 123.88 (C-4), 120.62 (C-6'), 120.57 (C-10'), 119.97 (C-4'), 118.99 (C-8'), 113.30 (C-2'), 113.21 (C-7), 111.41 (C-10), 23.91 (CH₃). Elemental analysis calcd (%) for C₂₃H₁₇NO₄: C, 74.38; H, 4.61; N, 3.77; found C, 74.33; H, 4.63; N, 3.71. m/z : 371.11576 (100.0%).

(Z)-N-(3-oxo-2-(4-phenoxybenzylidene)-2,3-dihydrobenzofuran-5-yl)acetamide (15):

Yield: 67%; mp: 213.5 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.15 (s, 1H, NH), 8.11 (d, 1H, $J = 2$ Hz, C-H₄), 8.03–8.00 (d, 2H, $J = 7.9$ Hz, C-H_{2'}), 7.83–7.79 (dd, 1H, $J = 2.2; 8.9$ Hz, C-H₆), 7.50–7.42 (m, 3H, C-H_{7,7'}), 7.22 (t, 1H, $J = 7.4$ Hz, C-H_{8'}), 7.12–7.09 (m, 4H, C-H_{3',6'}), 6.94 (s, 1H, C-H₁₀), 2.07 (s, 3H, NHCOCH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.43 (C-3), 168.38 (CO), 161.14 (C-8), 158.41 (C-1''), 155.43 (C-4'), 146.18 (C-2), 135.5 (C-5), 133.43 (C-3''), 130.15 (C-2'), 128.67 (C-6), 126.79 (C-1'), 124.27 (C-4''), 120.76 (C-9), 119.41 (C-3'), 118.28 (C-2''), 113.24 (C-7), 113.11 (C-4), 111.83 (C-10), 23.82 (CH₃). Elemental analysis calcd (%) for C₂₃H₁₇NO₄: C, 74.38; H, 4.61; N, 3.77; found C, 74.35; H, 4.67; N, 3.73. m/z : 371.11576 (100.0%).

(Z)-5-amino-2-(3-phenoxybenzylidene)benzofuran-3(2H)-one (16):

Yield: 51%; mp: 145.6 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 7.71–7.69 (d, 1H, $J = 7.9$ Hz, C-H_{6'}), 7.64 (bs, 1H, C-H_{2'}), 7.51–7.49 (d, 1H, $J = 7.9$ Hz, C-H₆), 7.44 (dt, 2H, C-H_{9'}), 7.22–7.15 (m, 2H, C-H_{4,5'}), 7.09–7.04 (m, 4H, C-H_{7,9',10'}), 6.83 (d, 1H, $J = 2.02$ Hz, C-H₄), 6.77 (s, 1H, C-H₁₀), 5.41 (bs, 2H, NH₂). ¹³C NMR (75 MHz, DMSO-d₆): δ 184.24(C-3), 158.13(C-7'), 157.1(C-3'), 156.21(C-8), 147.41(C-2), 145.56(C-5), 134.09(C-1'), 130.52(C-5'), 130.15(C-9'), 126.34(C-6), 124.82(C-9), 123.81(C-6'), 120.88(C-10'), 120.43(C-4'), 119.65(C-4), 118.95(C-8'), 113.13(C-8), 110.13(C-10), 105.61(C-7). Elemental analysis calcd (%) for C₂₁H₁₅NO₃: C, 76.58; H, 4.59; N, 4.25; found C, 76.56; H, 4.61; N, 4.22. m/z : 329.10519 (100.0%).

(Z)-5-amino-2-(4-phenoxybenzylidene)benzofuran-3(2H)-one (17):

Yield: 76%; mp: 170.6 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 7.99–7.96 (d, 2H, $J = 8.6$ Hz, C-H_{3'}), 7.44 (dt, 2H, $J = 7.7$ Hz, C-H_{7'}), 7.24–7.21 (m, 2H, C-H_{7,8'}), 7.10–7.02 (m, 5H, C-H_{6,2',6'}), 6.83 (bs, 2H, C-H_{4,10}), 5.25 (bs, 2H, NH₂). ¹³C NMR (75 MHz, DMSO-d₆): δ 184.11 (C-3), 158.16 (C-5'), 158.02 (C-8), 155.58 (C-4'), 146.56 (C-2), 145.6 (C-5), 133.27 (C-7'), 130.24 (C-2'), 127.21 (C-1'), 124.62 (C-6), 124.28 (C-8'), 121.15 (C-9), 119.41 (C-3'), 118.39 (C-6'), 113.17 (C-4), 110.62 (C-10), 105.45 (C-7). Elemental analysis calcd (%) for C₂₁H₁₅NO₃: C, 76.58; H, 4.59; N, 4.25; found C, 76.43; H, 4.64; N, 4.54. m/z : 329.10519 (100.0%).

(Z)-N-(2-(2-isopropoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (18):

Yield: 93%; mp: 230 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.15 (s, 1H, NH), 8.21–8.19 (d, 1H, $J = 1.2$; 7.8 Hz, C- $\text{H}_{6'}$), 8.12 (bs, 1H, C- H_4), 7.81–7.78 (d, 1H, $J = 8.7$ Hz, C- H_6), 7.52–7.49 (d, 1H, $J = 8.9$ Hz, C- H_7), 7.42 (dt, 1H, $J = 7.7$ Hz, C- $\text{H}_{4'}$), 7.20 (s, 1H, C- H_{10}), 7.16–7.13 (d, 1H, $J = 8.3$ Hz, C- $\text{H}_{3'}$), 7.08 (dt, 1H, $J = 7.6$ Hz, C- $\text{H}_{5'}$), 4.75 (q, 1H, $J = 5.9$; 11.9 Hz, C- H_{isop}), 2.07 (s, 3H, NHCOCH_3), 1.35–1.33 (d, 6H, $J = 5.8$ Hz, C- $\text{H}_{3_{\text{isop}}}$). ^{13}C NMR (75 MHz, DMSO- d_6): δ 183.52 (C-3), 168.39 (CO), 161.15 (C-8), 156.76 (C-2'), 146.69 (C-2), 135.5 (C-5), 131.85 (C-6'), 131.38 (C-4'), 128.64 (C-6), 121.03 (C-9), 120.73 (C-1'), 120.69 (C-5'), 113.85 (C-7), 113.28 (C-4), 113.11 (C-10), 105.92 (C-3'), 70.49 (CHiPr), 23.83 (CH3), 21.74 (CH3iPr). Elemental analysis calcd (%) for $\text{C}_{20}\text{H}_{19}\text{NO}_4$: C, 71.20; H, 5.68; N, 4.15; found C, 71.14; H, 5.67; N, 4.12. m/z : 337.13 (100.0%).

(Z)-N-(2-(3-isopropoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (19):

Yield: 91%; mp: 167 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.19 (s, 1H, NH), 8.12 (d, 1H, $J = 2$ Hz, C- H_4), 7.83–7.80 (dd, 1H, $J = 2.2$; 8.9 Hz, C- H_6), 7.57–7.52 (m, 3H, C- $\text{H}_{2',4',7}$), 7.40 (dt, 1H, $J = 8.0$ Hz, C- $\text{H}_{5'}$), 7.04–7.01 (dd, 1H, $J = 2.6$; 8.2 Hz, C- $\text{H}_{6'}$), 6.91 (s, 1H, C- H_{10}), 4.68 (q, 1H, $J = 5.9$; 11.9 Hz, C- H_{isop}), 2.07 (s, 3H, NHCOCH_3), 1.31–1.29 (d, 6H, $J = 5.8$ Hz, C- $\text{H}_{3_{\text{isop}}}$). ^{13}C NMR (75 MHz, DMSO- d_6): δ 183.73 (C-3), 168.49 (CO), 161.35 (C-8), 157.66 (C-3'), 146.87 (C-2), 135.62 (C-5), 133.17 (C-1'), 130.1 (C-5'), 128.86 (C-6), 123.56 (C-6'), 120.64 (C-9), 118.25 (C-2'), 117.32 (C-3'), 113.41 (C-7), 113.17 (C-4), 112.28 (C-10), 69.35 (CHiPr), 23.88 (CH3), 21.77 (CH3iPr). Elemental analysis calcd (%) for $\text{C}_{20}\text{H}_{19}\text{NO}_4$: C, 71.20; H, 5.68; N, 4.15; found C, 71.18; H, 5.66; N, 4.16. m/z : 337.13 (100.0%).

(Z)-N-(2-(4-isopropoxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (20):

Yield: 93%; mp: 200.2 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.14 (s, 1H, NH), 8.10 (d, 1H, $J = 1.9$ Hz, C- H_4), 7.94–7.91 (d, 2H, $J = 8.8$ Hz, C- $\text{H}_{2'}$), 7.82–7.78 (dd, 1H, $J = 2.2$; 8.8 Hz, C- H_6), 7.51–7.48 (d, 1H, $J = 8.89$ Hz, C- H_7), 7.06–7.03 (d, 2H, $J = 8.8$ Hz, C- $\text{H}_{3'}$), 6.90 (s, 1H, C- H_{10}), 4.72 (q, 1H, $J = 5.9$; 11.9 Hz, C- H_{isop}), 2.07 (s, 3H, NHCOCH_3), 1.30–1.28 (d, 6H, $J = 5.8$ Hz, C- $\text{H}_{3_{\text{isop}}}$). ^{13}C NMR (75 MHz, DMSO- d_6): δ 183.25 (C-3), 168.39 (CO), 160.98 (C-8), 159.16 (C-4'), 145.6 (C-2), 135.39 (C-5), 133.41 (C-2'), 128.48 (C-6), 124.02 (C-1'), 120.94 (C-9), 115.94 (C-3'), 113.2 (C-7), 113.06 (C-4), 112.77 (C-10), 69.5 (CHiPr), 23.83 (CH3), 21.68 (CH3iPr). Elemental analysis calcd (%) for $\text{C}_{20}\text{H}_{19}\text{NO}_4$: C, 71.20; H, 5.68; N, 4.15; found C, 71.18; H, 5.69; N, 4.12. m/z : 337.13 (100.0%).

(Z)-5-amino-2-(2-isopropoxybenzylidene)benzofuran-3(2H)-one (21):

Yield: 68%; mp: 126.7 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 8.19–8.17 (d, 1H, $J = 6.9$ Hz, C- $\text{H}_{6'}$), 7.39 (t, 1H, $J = 7.3$ Hz, C- $\text{H}_{4'}$), 7.26–7.24 (d, 1H, $J = 8.7$ Hz, C- H_7), 7.14 (d, 1H, C- $\text{H}_{3'}$), 7.11 (bs, 2H, NH2), 7.08–7.06 (d, 1H, C- $\text{H}_{5'}$), 7.06–7.04 (dd, 1H, $J = 2.2$; 7.7 Hz, C- H_6), 6.95 (s, 1H, C- H_{10}), 6.85 (d, 1H, $J = 2.2$ Hz, C- H_4), 4.73 (q, 1H, $J = 5.9$; 11.9 Hz, C- H_{isop}), 1.34–1.32 (d, 6H, $J = 5.8$ Hz, C- $\text{H}_{3_{\text{isop}}}$). ^{13}C NMR (75 MHz, DMSO- d_6): 184.16 (CO), 158.14 (C-8), 156.62 (C-2'), 147.02 (C-2), 145.24 (C-5), 131.55 (C-6'), 131.33 (C-4'), 124.76 (C-6), 121.4 (C-9), 121.11 (C-5'), 120.72 (C-1'), 113.85 (C-7), 113.23 (C-10), 105.77 (C-3'), 104.8 (C-4), 70.46 (CHiPr), 21.82 (CH3iPr). Elemental analysis calcd (%) for $\text{C}_{20}\text{H}_{19}\text{NO}_4$: C, 71.20; H, 5.68; N, 4.15; found C, 71.24; H, 5.74; N, 4.18. m/z : 337.13 (100.0%).

(Z)-5-amino-2-(3-isopropoxybenzylidene)benzofuran-3(2H)-one (22):

Yield: 51%; mp: 198.4 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 7.57–7.54 (d, 1H, $J = 7.8$ Hz, C- $\text{H}_{6'}$), 7.51 (bs, 1H, C- $\text{H}_{2'}$), 7.51–7.48 (d, 1H, $J = 8.6$ Hz, C- H_7), 7.43–7.40 (d, 2H, $J = 7.8$ Hz, C- $\text{H}_{4'}$), 7.41 (dt, 1H, $J = 7.8$ Hz, C- $\text{H}_{5'}$), 7.27 (d, 1H, $J = 1.9$ Hz, C- H_4), 7.04–7.01 (dd, 1H, $J = 2.0$; 8.0 Hz, C- H_6), 6.89 (s, 1H, C- H_{10}), 4.68 (q, 1H, $J = 5.9$; 11.9 Hz, C- H_{isop}), 1.31–1.29 (d, 6H, $J = 5.8$ Hz, C- $\text{H}_{3_{\text{isop}}}$). ^{13}C NMR (75 MHz, DMSO- d_6): δ 183.65 (C-3), 160.93 (C-8), 157.67 (C-3'), 146.95 (C-2), 137.64 (C-5), 133.24 (C-1'), 130.11 (C-5'), 128.17 (C-6), 123.56 (C-6'), 121.25 (C-9), 118.24 (C-2'), 117.28 (C-3'), 113.96 (C-7), 112.14 (C-4), 111.46 (C-10), 69.35 (CHiPr), 21.79 (CH3iPr). Elemental analysis calcd (%) for $\text{C}_{10}\text{H}_{17}\text{NO}_3$: C, 71.20; H, 5.68; N, 4.15; found C, 71.18; H, 5.65; N, 4.12. m/z : 295.12 (100.0%).

(Z)-5-amino-2-(4-isopropoxybenzylidene)benzofuran-3(2H)-one (23):

Yield: 50%; mp: >350 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 7.97–7.95 (d, 2H, $J = 8.8$ Hz, C- $\text{H}_{2'}$), 7.66–7.64 (m, 3H, C- $\text{H}_{4,6,7}$), 7.07–7.05 (d, 2H, $J = 8.8$ Hz, C- $\text{H}_{3'}$), 6.98 (s, 1H, C- H_{10}). ^{13}C NMR (75 MHz, DMSO- d_6): δ 183.22 (C-3), 160.22 (C-8), 159.12 (C-4'), 145.7 (C-2),

133.38 (C-2'), 138.33 (C-5), 127.35 (C-7), 124.1 (C-1'), 121.52 (C-9), 115.94 (C-3'), 113.69 (C-6), 112.51 (C-4), 110.58 (C-10), 69.49 (CH₂iPr), 21.68 (CH₃iPr). Elemental analysis calcd (%) for C₁₀H₁₇NO₃: C, 71.20; H, 5.68; N, 4.15; found C, 71.15; H, 5.66; N, 4.18. *m/z*: 295.12 (100.0%).

(Z)-N-(2-(2-fluorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (24):

Yield: 68%; mp: 226 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.17 (s, 1H, NH), 8.25 (t, 1H, *J* = 7.8 Hz, C-H_{2'}), 8.12 (d, 1H, *J* = 2.2 Hz, C-H₄), 7.85–7.82 (dd, 1H, *J* = 2.5, 8.7 Hz, C-H_{6'}), 7.54–7.51 (m, 2H, C-H_{4',7'}), 7.41–7.39 (d, 1H, C-H_{3'}), 7.35 (dt, 1H, C-H_{5'}), 6.90 (s, 1H, C-H₁₀), 2.08 (s, 3H, CH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.5 (C-3), 168.44 (CO), 164.9–161.44 (C-2', *J* = 260 Hz), 161.41 (C-8), 147.71 (C-2), 135.78 (C-5), 132.24–132.12 (C-4', *J* = 8.8 Hz), 131.31 (C-6'), 129.02 (C-6), 125.11 (C-5', *J* = 3.3 Hz), 120.38 (C-9), 119.67–119.52 (C-1', *J* = 11 Hz), 115.93–115.64 (C-3', *J* = 22 Hz), 113.38 (C-7), 113.25 (C-4), 102.1–102.0 (C-10, *J* = 7.7 Hz), 23.83 (CH₃). Elemental analysis calcd (%) for C₁₇H₁₂FNO₃: C, 68.68; H, 4.07; N, 4.71; found C, 68.65; H, 4.01; N, 4.65. *m/z*: 297.08 (100.0%).

(Z)-N-(2-(3-fluorobenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (25):

Yield: 82%; mp: 243.6 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.22 (s, 1H, NH), 8.12 (d, 1H, *J* = 2.1 Hz, C-H₄), 7.83–7.79 (m, 3H, C-H_{6,2',6'}), 7.59–7.51 (m, 2H, C-H_{4',7'}), 7.31–7.39 (dt, 1H, *J* = 2.1, 8.4 Hz, C-H_{5'}), 6.59 (s, 1H, C-H₁₀), 2.07 (s, 3H, CH₃). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.83 (C-3), 168.6 (CO), 163.85–160.62 (C-3', *J* = 244 Hz), 161.48 (C-8), 147.37 (C-2), 135.77 (C-5), 134.3–134.19 (C-1', *J* = 8.25 Hz), 131.08–130.97 (C-5', *J* = 8.25 Hz), 129.05 (C-6), 127.62–127.58 (C-6', *J* = 2.75 Hz), 120.53 (C-9), 117.45–117.15 (C-4', *J* = 22.56 Hz), 117.02–116.73 (C-2', *J* = 21.5 Hz), 113.53 (C-7), 113.25 (C-4), 110.69–110.66 (C-10, *J* = 2.75 Hz), 23.92 (CH₃). Elemental analysis calcd (%) for C₁₇H₁₂FNO₃: C, 68.68; H, 4.07; N, 4.71; found C, 68.58; H, 4.12; N, 4.73. *m/z*: 297.08 (100.0%).

(Z)-5-amino-2-(2-fluorobenzylidene)benzofuran-3(2H)-one (26):

Yield: 62%; mp: 161.3 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 8.23 (dt, 1H, *J* = 1.65, 7.8 Hz, C-H_{6'}), 7.51–7.47 (m, 1H, C-H_{4'}), 7.37 (t, 1H, C-H_{3'}), 7.34 (dt, 1H, C-H_{5'}), 7.28–7.25 (d, 1H, *J* = 8.8 Hz, C-H₇), 7.09–7.05 (dd, 1H, *J* = 2.5, 8.7 Hz, C-H₆), 6.86 (d, 1H, *J* = 2.4 Hz, C-H₄), 6.81 (s, 1H, C-H₁₀), 5.28 (bs, 2H, NH₂). ¹³C NMR (75 MHz, DMSO-d₆): δ 184.05 (C-3), 164.75–161.71 (C-2', *J* = 260 Hz), 158.19 (C-8), 148.04 (C-2), 145.83 (C-5), 131.84–131.73 (C-4', *J* = 8 Hz), 131.2 (C-6'), 125.11–125.06 (C-5', *J* = 3 Hz), 124.84 (C-6), 120.69 (C-9), 119.97–119.81 (C-1', *J* = 12 Hz), 115.85–115.56 (C-3'), 113.22 (C-7), 105.54 (C-4), 100.82–100.72 (C-10).

Elemental analysis calcd (%) for C₁₅H₁₀FNO₂: C, 70.58; H, 3.95; N, 5.49; found C, 70.44; H, 3.99; N, 5.32. *m/z*: 255.07 (100.0%).

(Z)-5-amino-2-(3-fluorobenzylidene)benzofuran-3(2H)-one (27):

Yield: 85%; mp: 159.7 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 7.80–7.78 (m, 2H, C-H_{2',4'}), 7.57–7.50 (dt, 1H, C-H_{5'}), 7.32–7.27 (m, 2H, C-H_{7,6'}), 7.10–7.07 (dd, 1H, *J* = 2.5, 8.7 Hz, C-H₆), 6.87 (d, 1H, *J* = 2.4 Hz, C-H₄), 6.85 (s, 1H, C-H₁₀). ¹³C NMR (75 MHz, DMSO-d₆): δ 184.26 (C-3), 163.81–160.58 (C-3', *J* = 244 Hz), 158.49 (C-8), 147.63 (C-2), 145.01 (C-5), 134.58–134.47 (C-1', *J* = 8.25 Hz), 130.85 (C-5'), 127.34 (C-6), 125.18 (C-6'), 120.84 (C-9), 117.22–116.92 (C-4', *J* = 22.5 Hz), 116.64–116.36 (C-2'), 113.38 (C-7), 109.49–109.45 (C-10, *J* = 2.75 Hz), 106.08 (C-4). Elemental analysis calcd (%) for C₁₅H₁₀FNO₂: C, 70.58; H, 3.95; N, 5.49; found C, 70.66; H, 4.08; N, 5.31. *m/z*: 255.07 (100.0%).

(Z)-5-amino-2-(4-fluorobenzylidene)benzofuran-3(2H)-one (28):

Yield: 82%; mp: 164.4 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 8.09–8.04 (dd, 2H, *J* = 7.8 Hz, C-H_{2'}), 7.81–7.78 (d, 1H, C-H_{4'}), 7.57–7.54 (d, 1H, *J* = 8.4 Hz, C-H₇), 7.36 (t, 2H, C-H_{3'}), 7.33 (d, 1H, C-H₄), 6.98 (s, 1H, C-H₁₀). ¹³C NMR (75 MHz, DMSO-d₆): δ 183.5 (C-4), 165.39 (C-8), 164.38–161.07 (C-4', *J* = 250 Hz), 145.91 (C-2), 137.62 (C-5), 133.73–133.62 (C-2', *J* = 9 Hz), 128.55 (C-1', *J* = 3 Hz), 124.24 (C-6), 123.95 (C-7), 120.81 (C-9), 116.26–115.97 (C-3', *J* = 22 Hz), 113.14 (C-4), 111.04 (C-10). Elemental analysis calcd (%) for C₁₅H₁₀FNO₂: C, 70.58; H, 3.95; N, 5.49; found C, 70.52; H, 4.07; N, 5.23. *m/z*: 255.06956 (100.0%).

(Z)-N-(3-oxo-2-(3-(trifluoromethyl)benzylidene)-2,3-dihydrobenzofuran-5-yl)acetamide (29):

Yield: 82%; mp: 252.1 °C; ¹H NMR (300 MHz, DMSO-d₆): δ 10.20 (bs, 1H, NH), 8.30–8.28 (m, 2H, C-H_{2',4'}), 8.13 (d, 1H, *J* = 2.1 Hz, C-H₄), 7.85–7.81 (dd, 1H, *J* = 2.3, 8.8 Hz,

C-H₆), 7.79–7.72 (m, 2H, C-H_{5',6'}), 7.56–7.53 (d, 1H, *J* = 8.9 Hz, C-H₇), 7.06 (s, 1H, C-H₁₀), 2.07 (s, 3H, CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 183.81 (C-3), 168.54 (CO), 161.44 (C-8), 147.58 (C-2), 135.82 (C-5), 134.74 (C-1'), 133.09 (C-6), 130.15 (C-6'), 129.94 (C-5'), 129.63–129.03 (C-3', *J* = 31.7 Hz), 127.46 (C-4'), 126.17 (C-2'), 125.32–122.61 (CF₃, *J* = 270 Hz), 120.48 (C-9), 113.5 (C-7), 113.23 (C-4), 110.18 (C-10), 23.91 (CH₃). Elemental analysis calcd (%) for C₁₈H₁₂F₃NO₃: C, 62.25; H, 3.48; N, 4.03; found C, 62.09; H, 3.54; N, 3.98. *m/z*: 347.07693 (100.0%).

(Z)-5-amino-2-(3-(trifluoromethyl)benzylidene)benzofuran-3(2H)-one (30):

Yield: 82%; mp: >350 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.31–8.29 (m, 2H, C-H_{2',4'}), 7.80–7.73 (m, 2H, C-H_{6',5'}), 7.52–7.49 (d, 1H, *J* = 8.9 Hz, C-H₇), 7.40–7.36 (dd, 1H, *J* = 2.3, 8.9 Hz, C-H₇), 7.26 (s, 1H, C-H₄), 7.05 (s, 1H, C-H₁₀). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 183.7 (C-3), 161.09 (C-8), 147.65 (C-2), 134.75 (C-1'), 133.13 (C-5), 130.16 (C-6'), 130.01–129.59 (C-3', *J* = 31 Hz), 128.44 (C-7), 127.48 (C-4'), 127.43 (C-2'), 126.11–122.78 (CF₃, *J* = 250 Hz), 121.1 (C-9), 114.05 (C-6), 111.68 (C-4), 110.05 (C-10). Elemental analysis calcd (%) for C₁₆H₁₀F₃NO₂: C, 62.96; H, 3.30; N, 4.59; found C, 63.11; H, 3.35; N, 4.55. *m/z*: 305.06636 (100.0%).

(Z)-4-((5-acetamido-3-oxobenzofuran-2(3H)-ylidene)methyl)benzoic acid (31):

Yield: 71%; mp: 165.3 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.28 (s, 1H, NH), 8.14 (d, 1H, *J* = 1.9 Hz, C-H₄), 8.10–8.07 (d, 2H, *J* = 8.8 Hz, C-H₂'), 8.05–8.02 (d, 2H, *J* = 8.8 Hz, C-H₃'), 7.87–7.83 (dd, 1H, *J* = 2.2;8.8 Hz, C-H₆), 7.55–7.53 (d, 1H, *J* = 8.89 Hz, C-H₇), 6.98 (s, 1H, C-H₁₀), 2.08 (s, 3H, NHCOCH₃). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 183.85 (C-3), 168.54 (CO), 166.77 (COOH), 161.47 (C-8), 147.69 (C-2), 136.08 (C-1'), 135.82 (C-5), 131.35 (C-4'), 131.23 (C-3'), 129.74 (C-2'), 129.04 (C-6), 120.47 (C-9), 113.44 (C-7), 113.25 (C-4), 110.61 (C-10), 23.89 (CH₃). Elemental analysis calcd (%) for C₁₈H₁₃NO₅: C, 66.87; H, 4.05; N, 4.33; found C, 66.85; H, 4.12; N, 4.27. *m/z*: 323.07937 (100.0%).

(Z)-N-(7-nitro-3-oxo-2-(3-phenoxybenzylidene)-2,3-dihydrobenzofuran-5-yl)acetamide (32):

Yield: 91%; mp: 230.3 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.51 (bs, 1H, NH), 8.71 (d, 1H, *J* = 2.2 Hz, C-H₆), 8.35 (d, 1H, *J* = 2.2 Hz, C-H₄), 7.89–7.86 (d, 2H, *J* = 7.8 Hz, C-H_{2''}), 7.56 (t, 1H, *J* = 8.16 Hz, C-H₅'), 7.42 (dt, 2H, C-H_{3''}), 7.20–7.17 (d, 1H, *J* = 7.3 Hz, C-H_{4'}), 7.14 (s, 1H, C-H₂'), 7.09–7.06 (m, 2H, C-H_{10,6'}), 2.11 (s, 3H, CH₃). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 178.66 (C-3), 169.01 (CO), 157.07 (C-1''), 156.36 (C-3'), 153.21 (C-8), 146.13 (C-2), 143.98 (C-7), 135.31 (C-5), 133.21 (C-1'), 130.64 (C-5'), 130.09 (C-3''), 127.01 (C-6), 124.60 (C-9), 123.69 (C-4''), 121.59 (C-6'), 120.86 (C-4), 119.76 (C-4'), 119.35 (C-2'), 118.66 (C-2''), 113.73 (C-10), 23.86 (CH₃). Elemental analysis calcd (%) for C₂₃H₁₆N₂O₆: C, 66.34; H, 3.87; N, 6.73; found C, 66.21; H, 3.74; N, 6.71. *m/z*: 416.10084 (100.0%).

(Z)-N-(2-(3-hydroxybenzylidene)-3-oxo-2,3-dihydrobenzofuran-5-yl)acetamide (33):

Yield: 75%; mp: >350 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.15 (s, 1H, NH), 9.67 (bs, 1H, OH), 8.11–8.10 (d, 1H, *J* = 2.2 Hz, C-H₄), 7.83–7.80 (dd, 1H, *J* = 2.2;8.8 Hz, C-H₆), 7.50–7.47 (d, 1H, *J* = 8.9 Hz, C-H₇), 7.42 (d, 1H, C-H_{2'}), 7.40–7.38 (d, 1H, *J* = 7.8 Hz, C-H_{6'}), 7.30 (t, 1H, *J* = 7.8 Hz, C-H_{5'}), 6.89–6.86 (dd, 1H, C-H_{4'}), 6.82 (s, 1H, C-H₁₀), 2.07 (s, 3H, C-H₃'). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 183.65 (C-3), 168.41 (CO), 161.27 (C-8), 157.58 (C-3'), 146.69 (C-2), 135.54 (C-5), 132.9 (C-1'), 129.89 (C-5'), 128.8 (C-6), 122.64 (C-6'), 120.65 (C-9), 117.53 (C-3'), 117.46 (C-2'), 113.21 (C-7), 113.16 (C-4), 112.47 (C-10), 23.83 (CH₃). Elemental analysis calcd (%) for C₁₇H₁₃NO₄: C, 69.15; H, 4.44; N, 4.74; found C, 69.01; H, 4.48; N, 4.69. *m/z*: 295.08 (100.0%).

(Z)-5-amino-2-(3-hydroxybenzylidene)benzofuran-3(2H)-one (34):

Yield: 95%; mp: 225.2 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 9.63 (bs, 1H, OH), 7.39 (d, 1H, C-H₂'), 7.37–7.34 (d, 1H, *J* = 7.8 Hz, C-H_{6'}), 7.28 (t, 1H, *J* = 7.8 Hz, C-H_{5'}), 7.25–7.22 (d, *J* = 8.8 Hz, C-H₇), 7.07–7.04 (dd, 1H, *J* = 2.2;8.5 Hz, C-H_{4'}), 6.86–6.83 (m, 2H, C-H_{4,6}), 6.70 (s, 1H, C-H₁₀), 5.24 (bs, 2H, NH₂). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 184.22 (C-3), 158.08 (C-8), 157.53 (C-3'), 146.99 (C-2), 145.57 (C-5), 133.21 (C-1'), 129.82 (C-5'), 124.66 (C-6), 122.42 (C-6'), 120.98 (C-9), 117.34 (C-3'), 117.1 (C-2'), 113.06 (C-7), 111.17 (C-4), 105.46 (C-10). Elemental analysis calcd (%) for C₁₅H₁₁NO₃: C, 71.14; H, 4.38; N, 5.53; found C, 71.21; H, 4.34; N, 5.49. *m/z*: 253.07 (100.0%).

5. Conclusions

In the present study, 31 new aurone derivative compounds were synthesized. These new compounds were obtained by the substitution of the aurone scaffold at position 5 by amino and acetamido groups, and through various substitutions at the 2', 3' and 4' positions. Antimicrobial testing identified two of these compounds, i.e., **10** and **20**, as the most active on both Gram-positive and -negative bacteria with MIC values as low as 0.78 μ M. These were also the safest regarding human cells. The two selected compounds shared some structural similarity with the 5-acetamido substitution. The SAR study from this work correlates with the results previously obtained by Olleik et al. [14] showing that benzyloxy and isopropoxy lead to interesting activities in aurone scaffolds with substitution on the A ring with amino or acetamido groups, improving the activity compared to the natural OH group. Taken together, these results confirm that the aurone scaffold is a promising structure that could be the starting point for the design of new antibacterial agents by diversifying the substitution pattern on A and B rings altogether.

6. Patents

Aurone derivatives and uses thereof for controlling bacteria and/or fungi. PCT/EP2021/069047. BOLLA Jean Michel, MARESCA Marc, NEULAT-RIPOLL Fabienne, OLLEIK Hamza, PERRIER-VIRET Josette, PIQUE Valérie. ROBIN Maxime.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antibiotics13040300/s1>, File S1: NMR Spectra of compounds 4–34.

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