Design and Synthesis of Novel Amino and Acetamidoaurones with Antimicrobial Activities

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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 an urgent need with 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** have the highest TI. Finally, screening against a large panel of pathogens confirmed that compounds **10** and **20** possess a large spectrum antimicrobial activity, including on bioweapon BSL3 strains, with MIC values as low as 0.78 µM. These results demonstrate that 5-acetamidoaurones are far more active and safest 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

1.Introduction

The development of novel antibacterial molecules is a major need for the upcoming decades as there is an increasing emergence of multi-drug resistant bacterial strains. This is leading to an elevating mortality rate by infectious disease that can reach more than 10 billion of deaths by 2050 according to WHO. Among these strains, mycobacteria in particular *M. tuberculosis*, the pathogenic agent of tuberculosis, are still responsible in world wild of 10 million new cases by year and has killed almost 1.5 million patients in 2022. More alarming, the number of resistant and ultra-resistant strains 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*

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pneumoniae [1]. 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 of deadly infections, particularly in HIV-infected patients, but also in immunocompetent ones, affecting billions of patients and causing more than 1.5 million death per year [2–5].

Some plant natural 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 cancerology, dermatology, and infectiology [6–8]. 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 [9]. Despite some natural aurones such as cephalocerone [10,11] and hispidol [12] have demonstrated antimicrobial activity, aurones exhibiting natural substitution patterns did not generally lead to the most effective antimicrobial agents. On the other hand, a series of synthetical aurone derivatives showed a strong effect against Gram positive bacteria [13]. Structurally, aurones are characterized by two 6-carbon rings (ring A and B) and a furanone-like third cycle (ring C). Overall, already described scaffold modifications mainly focused on the substitution at the B-ring scaffold, e.g., with the introduction of either ferrocene [14], 5-nitroimidazole [15] or quinolines [16] groups, with a global tendency to retain naturally present hydroxy groups at the A-ring (Figure 1).

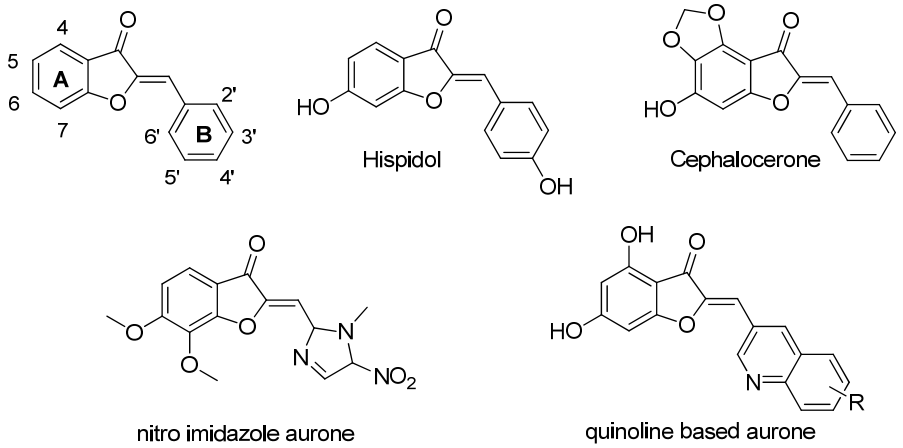
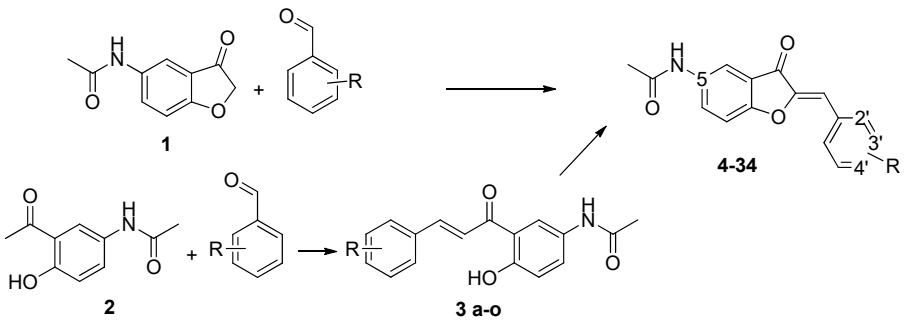


Figure 1. Structure of the aurone scaffold (top left), natural antimicrobial aurones, and examples of synthetic, heavily modified analogues.

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



Scheme 1. Synthetic route of the aurone derivatives. A: benzofuranone (**1**) 1 eq. and various benzaldehydes 1 eq in Choline chloride/Urea (1/2), 80°C, 2h. B: (**2**) 1 eq. and various benzaldehydes 1

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eq. in EtOH, LiOH 3 eq., 90°C, 2h. (**3 a-o**) 1 eq. and Mercuric acetate 1 eq. in pyridine, 110°C, 1h. Acetamido Aurone are converted to their amino analogues in EtOH, 0.5 M HCl, 100°C, 2h.

Table 1. List of the synthetized aurones and their respective substitution on each position.

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
8	NH_2	H	OCH_3	H
9	NH_2	H	H	OCH_3
10	$NHCOCH_3$	H	Obenzyl	H
11	$NHCOCH_3$	H	H	Obenzyl
12	NH_2	H	Obenzyl	H
13	NH2	H	H	Obenzyl
14	$NHCOCH_3$	H	Ophenyl	H
15	$NHCOCH_3$	H	H	Ophenyl
16	NH_2	H	Ophenyl	H
17	NH_2	H	H	Ophenyl
18	$NHCOCH_3$	Oisopropyl	H	H
19	$NHCOCH_3$	H	Oisopropyl	H
20	$NHCOCH_3$	H	H	Oisopropyl
21	NH_2	Oisopropyl	H	H
22	NH_2	H	Oisopropyl	H
23	NH_2	H	H	Oisopropyl
24	$NHCOCH_3$	F	H	H
25	$NHCOCH_3$	H	F	H
26	NH_2	F	H	H
27	NH_2	H	F	H
28	NH_2	H	H	F
29	$NHCOCH_3$	H	CF_3	H
30	NH_2	H	CF_3	H
31	$NHCOCH_3$	H	H	COOH
32	$NHCOCH_3$	H	OH	H
33	NH_2	H	OH	H
34	$NHCOCH_3$	H	H	H

2. Results

Antimicrobial effect of the aurone derivatives was first evaluated by the determination of their Minimum Inhibitory Concentrations (MIC) on five different bacterial strains representative of Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), mycobacteria (*Mycobacterium smegmatis*), and one fungal strain (*Candida albicans*) (Table 2).

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Table 2. Evaluation of the antimicrobial activities of the 31 newly synthetized aurone derivatives. The antimicrobial activities were determined using a MIC assay on species representative of Gram positive bacteria (*B. subtilis, S. aureus*), Gram negative bacteria (*E. coli*, *P. aeruginosa*), mycobacteria (*M. smegmatis*), and fungi (*C. albicans*). Amphotericin B and gemifloxacin were used as control antimicrobials for fungi and bacteria, respectively. The MIC values are given in μM (n=2-3).

	Gram positive		Gram negative		Mycobacteria	Fungi
	B. subtilis ATCC6633	*S. aureus* ATCC6538P	*E. coli* ATCC8739	*P. aeruginosa* ATCC9027	*M. smegmatis* ATCC700084	*C. albicans* DSM10697
Gemifloxacin	0.03	0.06	0.06	0.25	2	-
Amphotericin B	-	-	-	-	-	0.62
4	50	100	>100	>100	>100	>100
5	>100	100	>100	>100	100	>100
6	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100
8	>100	>100	>100	>100	>100	>100
9	>100	>100	>100	>100	>100	>100
10	3.12	12.5	12.5	25	50	50
11	>100	>100	>100	>100	>100	>100
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

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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 found the more 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 MIC ranging from 3.12 to 50 µM. Similarly, compound **20** was active on all tested species with MIC ranging from 12.5 to 50 µM, except *P. aeruginosa* for which MIC was superior to 100 µM. Compound **16** although active on *B. subtilis*, *S. aureus*, *E. coli*, and *M. smegmatis* (MIC ranging from 25 to 50 µM), was however inactive on *P. aeruginosa* and *C. albicans*. Finally, compounds **12** and **15** found active on tested Gram positive bacteria and mycobacteria (with MIC ranging from 25 to 100 µM and from 50 to 100 µM, respectively) 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** with lowest MIC of 3.12, 12.5, 25 and 50 µM, respectively.

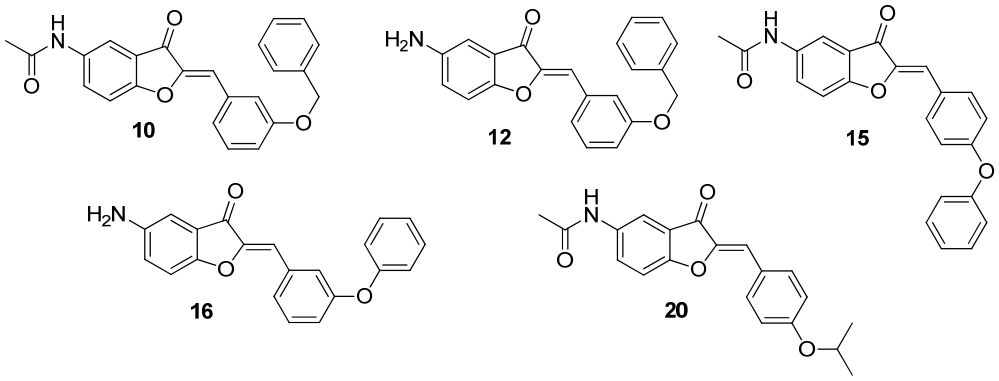

Figure 2. Structures of the more active aurone derivatives identified during the first screening (compounds **10**, **12**, **15**, **16,** and **20**).

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 (Figure 3 and Table 3).

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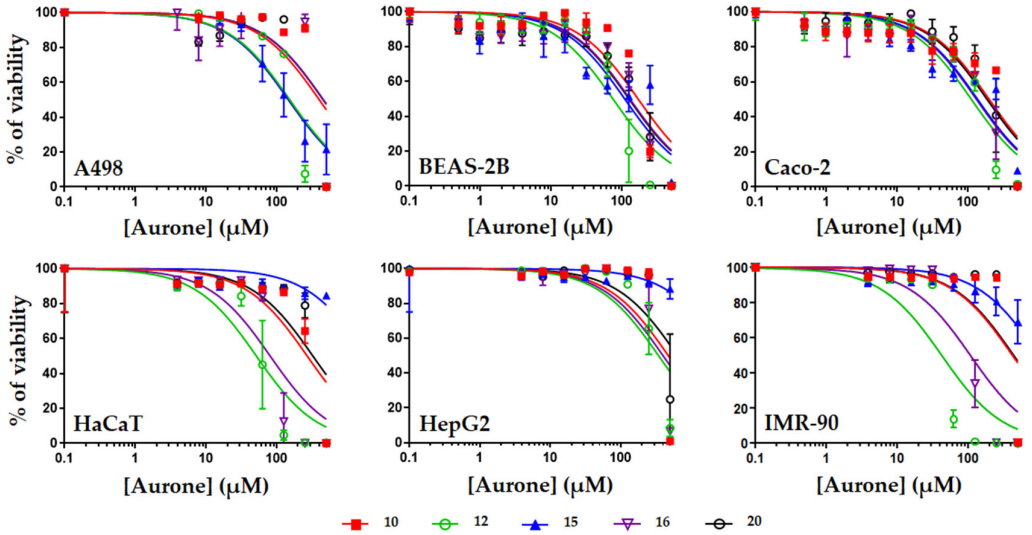

Figure 3. Evaluation of the toxicity of compounds **10**, **12**, **15**, **16,** and **20** on human cells. Human cells corresponding to kidney epithelial cells (A498), lung epithelial cells (BEAS-2B), intestinal epithelial cells (Caco-2), skin cells (HaCaT), liver cells (HepG2), or fibroblasts (IMR-90) were exposed to increasing concentrations of aurones for 48h 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 +/- S.D, n=3).

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

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

Overall, compounds **10**, **12**, **15**, **16**, and **20** were found to be safe with most of their CC_{50} values higher than their MIC ones. Compounds **10** and **20** were found the safest molecules with mean CC_{50} 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 CC_{50} 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 CC_{50} on human cells (Table 3) by their lower MIC (Table 2)), compounds **10** and **20** gave the highest TI values (ranging from 54.1 to 151.4 and 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 1.6 to 18).

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Table 4. Determination of the therapeutic indexes of compounds **10**, **12**, **15**, **16,** and **20**. The therapeutic indexes (TI) of each aurones was calculated by dividing their CC_{50} on human cells (Table 3) by their lower MIC values from Table 2 (i.e. 3.12 µM for compound **10**, 12.5 µM for compound **20**, 25 µM for compounds **12** and **16**, and 50 µM for compound **15**). MIC and CC_{50} are expressed in µM.

Compound	10	12	15	16	20
Lowest MIC	3.12	25	50	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	54.1	1.6	2.19	3.2	10.0
Highest TI	151.4	13.7	>10	18.0	>40

Based on antimicrobial activity and toxicity data, the 2 more active and safest compounds were identified as compounds **10** and **20** that were then tested on a larger panel of bacterial and fungal species in order to evaluate their spectrum of activity (Table 5).

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 Materials and Methods section. The MIC values are given in µM (n=2-3).

	10	20
Gram positive		
Bacillus anthracis (CNR-charbon_04022)	12.5	6.25
Bacillus cereus (DSM31)	12.5	25
Bacillus subtilis (ATCC6633)	3.12	25
Clostridium botulinum (DSM1985)	0.78	3.12
Clostridium difficile (DSM1296)	12.5	3.12
Clostridium perfringens (ATCC13124)	>100	>100
Enterococcus faecalis (DSM2570)	50	100
Enterococcus faecium (DSM20477)	100	25
Listeria monocytogenes (DSM20600)	3.12	6.25
Propionibacterium acnes (ATCC6919)	100	>100
Staphylococcus aureus (ATCC6538P)	12.5	12.5
MRSA (ATCCBAA-1717)	12.5	25
Streptococcus pyogenes (DSM20565)	50	50
Gram negative		
Acinetobacter baumannii (DSM30007)	12.5	25
Brucella melitensis (NR-256)	25	12.5
Enterobacter cloacae (DSM30054)	>100	>100
Escherichia coli (ATCC8739)	12.5	25
Francisella tularensis (NR-643)	50	12.5

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Helicobacter pylori (ATCC43504)	12.5	12.5
Klebsiella pneumonia (DSM26371)	>100	>100
Pseudomonas aeruginosa (ATCC9027)	25	>100
Salmonella enterica (CIP80.39)	25	50
Shigella flexneri (ATCC12022)	25	50
Vibrio alginolyticus (DSM2171)	25	50
Yersinia pestis (NR-641)	12.5	12.5
Mycobacteria		
Mycobacterium abscessus S (CIP 104536^T)	>100	>100
Mycobacterium abscessus R(CIP 104536^T)	>100	>100
Mycobacterium smegmatis (ATCC700084)	50	50
Mycobacterium tuberculosis H37Rv (mc^{2}6230)	>100	>100
Filamentous fungi		
Aspergillus fumigatus (DSM819)	>100	>100
Fusarium oxysporum (DSM62316)	25	25
Yeasts		
Candida albicans (DSM10697)	50	50
Candida auris (DSM21092)	50	12.5
Candida glabrata (DSM11226)	50	50
Candida tropicalis (DSM9419)	100	100
Cryptococcus neoformans (DSM11959)	25	25

he 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 food-born pathogens *Listeria monocytogenes* (MIC values of 3.12 and 6.25 µM for compounds **10** and **20**, respectively), *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). *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**. 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), similar MIC being obtained on two other *Bacillus* species, i.e. *B. cereus* and *B. subtilis*. On the opposite, *C. perfringens*, *Enterococcus* species, *P. acnes*, and *S. pyogenes* were found weakly sensitive to insensitive to compounds **10** and **20** with MIC values from 50 µM to > 100 µM. Compound **10** was found 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.

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Compounds **10** and **20** were also found active on Gram negative bacteria, *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 sensitive with MIC ranging from 25 to 50 µM. Although *P. aeruginosa* was sensitive to compound **10** (MIC of 25 µM), it is 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 found 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.

Although compounds **10** and **20** were found 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 found 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 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* was found the less sensitive with 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 bacteria and/or fungi for compound **10**, i.e. 0.78 µM (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 bacteria and/or fungi for compound **20,** i.e. 3.12 µM (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** being found the safest in all cases.

3. Discussion

In the present study, 31 new aurone derivative compounds were synthetized and tested in term 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′, 4′ and 5′ positions. The first screening antimicrobial test performed on representative species of bacteria and fungi allowed 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 could indicate that

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benzyloxy substitution in 3′ is a key element in the activity of the compound. The same methodology could be used to compare compound 20 with other isopropyl compound such as 18, 19, 21 and 22. These four last mentioned compounds showed a weak or no activity against any pathogens. Similarly to compound 10, this could indicate that the 4′-isopropyl substitution is a far better alternative to other position and again that acetamido group is more effective than amino group in position 5. On the behalf of the results, it can be accepted that the acetamido groups is essential 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 4 of these compounds possess a ring substitution in position 3′ and 4′. Interestingly, benzyloxy-substituted aurones (i.e., 10 and 12) seems 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 compound 10 activity compared to 12, 15 and 16 activities, benzyloxy substitution must be considered more promising than phenyl ones. The other benzyloxy and phenyl-substituted aurones (i.e., 11, 13, 14 and 17) showed no activity. Isopropyl-substituted aurones also are an interesting option as shown by compound 20 which is similarly active as compound 10. Again only 4′-isopropyl aurones is active, 3′ and 2′ are inactive on the variety of pathogen 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, aurones 34 is also inactive and attests that the 5-acetamido substitution appears as 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 seems 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 structure similarity such as the 5-acetamido substitution. Out of all the compounds, the 3′-benzyloxy and 4′ benzyloxy were found as the most promising substitutions. Compared to previously described aurones active on various Gram positive bacteria but only two Gram negative (i.e. *H. pylori* or *V. alginolyticus*) [13], compounds 10 and 20 were found active on a large number of Gram positive and negative bacteria as well as fungi. Structurally, the most active compound synthetized by Olleik et al [13] 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.

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 plate using appropriate media and culture conditions (in term of temperature and aerobic/microaerobic/anaerobic condition) as previously described [13,17]. Regarding BSL-3 strains, *Bacillus anthracis*, *Francisella tularensis*, and *Brucella melitensis* strains were maintained on Chocolate agar PolyViteX (Biomerieux) agar at 37°C, and at 26°C for *Yersinia pestis* [18,19]. 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

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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). 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 [13,17,20,21]. For BSL-3 bacteria, the MIC of aurones was determined following the Clinical and Laboratory Standards Institut (CLSI) recommendations as previously described [22]. 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 [23,24]. 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 [17,21,25]. 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 11967, 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 cm^2 flasks and maintained in a 5% CO_2 incubator at 37 ◦C. For toxicity assay, human cells grown on 25 cm^2 flasks were detached using trypsin-EDTA solution (from Thermofisher), 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 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 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 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 CC_{50} 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 ^{13}C NMR spectra were recorded on a Bruker Avance III nanobay - 300MHz instrument (Bruker, Bremen, Germany, 300 MHz for ^1H, 75 MHz for ^{13}C). Chemical shifts are reported in ppm relative to the solvent in which the spectrum was recorded [^1H: 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]. Combustion analyses were performed at the analysis facilities of Spectropole (https://fr-chimie.univ-amu.fr/spectropole) with a

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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.

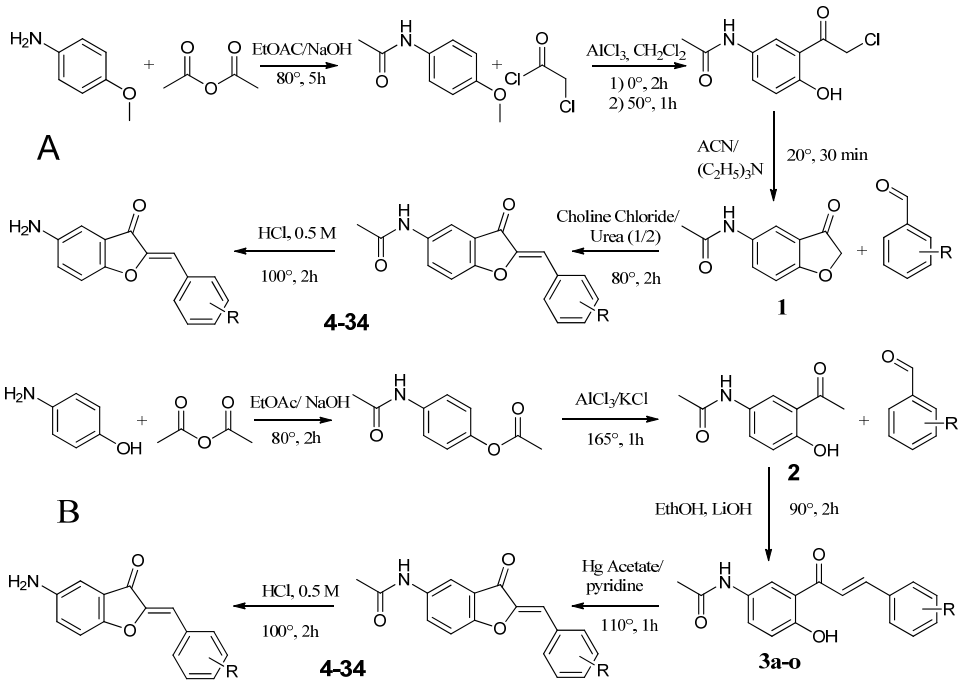

Figure 3. Route A and B used for the synthesis of 5-amino and 5-acetamido aurones.

4.2.1. Synthesis route A

4.2.1.1. 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 add dropwise 6.6g of acetic anhydride (1.5 eq). When the addition is complete, heat the solution at 80° for 5h. Let the solution cool and filter it. Under pressure remove the solvent, dissolve the product in ethanol and hexane to obtain 5 g of **1a**.

4.2.1.2. Synthesis of 2-chloro-1-{2-hydroxy-5-[(1-hydroxyethyl)amino]cyclohexyl}ethan-1-one (2a)

In a solution of 5g of **1a** and 18 g of $AlCl_3$ dissolved in 30 ml of dichloromethane add dropwise at 0°, 3.5 eq of chloroacetyl chloride. When the addition is complete heat the solution up to 50° for 1h. Pour the mixture on ice and extract with ethyl acetate. Remove EtOAc under pressure to obtain **2a**.

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

In a flask, add 2g of **2a** and 1.5 eq of triethylamine in 20 ml of acetonitrile, let react at 25° for 12h. evaporate under pressure the solvent, extract with ethyl acetate. Remove EtOAc under pressure to obtain **3a**.

4.2.1.4. Synthesis of substituted 5-acetamidoaurones

In a flask add 1mmol of **3a** and 1mmol of the corresponding benzaldehyde, dissolved in 10 ml of Choline Chloride/ Urea. Ad 3 drop of 50% KOH solution. Heat at 80° for 2h. Add water and HCl, filter the precipitate, wash it with ether to obtain acetamido substituted aurones.

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4.2.1.5. Synthesis of substituted 5-aminoaurone

10 mmol of acetamido aurones were added to a mixture of EtOH (20 mL) and conc. H2SO4 (5 mL). The solution was refluxed for 2h. 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

4.2.2.1. 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 add dropwise 26.5 g of acetic anhydride (3.5 eq). When the addition is complete, heat the solution at 80° for 5h. Let the solution cold and filter it. Under pressure remove the solvent, dissolve the product in ethanol and hexane to obtain 5 g of **1b**.

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

To a solution of **1b** (5g, 26 mmol), is added 15g of $AlCl_3$ (113 mmol, 4 eq) and 1.9 g of KCl (26 mmol, 1eq). The mixture is then heated a 165° for 1h until a brown paste appear. Let it cool, then ad cold water (300 ml), obtention of 2g of a beige powder (**2b**).

4.2.2.3. 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) are dissolved in ethanol (20 ml). The mixture is heated for 2h at 90°. The solvent is then removed under pressure, cold water and HCl are added, and the precipitate is filtered to obtain the desired chalcone (**3b**).

4.2.2.4. Synthesis of substituted 5-acetamidoaurones

To a mixture of chosen **3b** chalcone in pyridine (20 ml), is added 1 eq of mercury acetate. The solution is heated for 1h bat 110°. Water and HCl are added. The precipitate was filtrated and washed several times with cold water to get rid of the mercury. Obtention of a powder.

4.2.2.5. Synthesis of substituted 5-aminoaurone

For synthesis of 5-aminoaurone see section 2.2.1.5.

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

Yield: 83%; mp: 234.8 °C; ^{1}H NMR (300MHz, DMSO-d_6): 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-H_4), 7.83-7.80 (dd, 1H, J=2.2;8.9 Hz, C-H_6), 7.52-7.49 (d, 1H, J=8.9 Hz, C-H_7), 7.46 (dt, 1H, J=7.1 Hz, C-$H_{4'}$), 7.19 (s, 1H, C-H_{10}), 7.16-7.09 (m, 2H, C-$H_{3',5'}$), 3.91 (s, 3H, OCH_3), 2.07 (s, 3H, $NHCOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): 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(OCH_3), 23.83(CH_3). Elemental analysis calcd (%) for $C_{18}H_{15}NO_4$: 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; ^{1}H NMR (300MHz, DMSO-d_6): δ 10.16 (s, 1H, NH), 8.10 (d, 1H, J=2 Hz, C-H_4), 7.83-7.80 (dd, 1H, J=2.2;8.9 Hz, C-H_6), 7.60-7.55 (m, 2H, C-$H_{2',4'}$), 7.54-7.51 (d, 1H, J=8.9 Hz, C-H_7), 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-H_{10}), 7.16-7.09 (m, 2H, C-$H_{3',5'}$), 3.82 (s, 3H, OCH_3), 2.07 (s, 3H, $NHCOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): 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'),

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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(CH_3). Elemental analysis calcd (%) for $C_{18}H_{15}NO_4$: 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]; ^{1}H NMR (300MHz, DMSO-d_6): δ 10.17 (s, 1H, NH), 8.10 (d, 1H, J=2 Hz, C-H_4), 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-H_6), 7.50-7.47 (d, 1H, J=8.9 Hz, C-H_7), 7.08-7.06 (d, 2H, J=8.0 Hz, C-$H_{3',5'}$), 6.91 (s, 1H, C-H_{10}), 3.83 (s, 3H, OCH_3), 2.07 (s, 3H, $NHCOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{18}H_{15}NO_4$: 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; ^{1}H NMR (300MHz, DMSO-d_6): 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-H_7), 7.14-7.11 (m, 2H, C-$H_{3',5'}$), 7.10 (s, 1H, C-H_{10}), 7.06-7.03 (dd, 1H, J=2.5;8.8 Hz, C-H_6), 6.84 (d, 1H, J=2.4 Hz, C-H_4), 5.23 (bs, 2H, NH_2), 3.90 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 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(OCH_3). Elemental analysis calcd (%) for $C_{16}H_{13}NO_3$: 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; ^{1}H NMR (300MHz, DMSO-d_6): δ 7.59-7.57 (d, 1H, J=7.7 Hz, C-$H_{6'}$), 7.54 (d, 1H, J=2.4 Hz, C-H_4), 7.49-7.46 (d, 1H, J=8.8 Hz, C-H_7), 7.42 (dt, 1H, J=8.2 Hz, C-$H_{5'}$), 7.38-7.35 (dd, 1H, J=2.2;8.8 Hz, C-H_6), 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-H_{10}), 3.82 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 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 (OCH_3). Elemental analysis calcd (%) for $C_{16}H_{13}NO_3$: 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; ^{1}H NMR (300MHz, DMSO-d_6): 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-H_7), 7.13-7.10 (dd, 1H, J=2.2;8.8 Hz, C-H_6), 7.09-7.06 (d, 2H, J=8.1 Hz, C-$H_{3'}$), 6.93 (d, 1H, J=2.4 Hz, C-H_4), 6.83 (s, 1H, C-H_{10}), 3.82 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6): 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 (OCH_3). Elemental analysis calcd (%) for $C_{16}H_{13}NO_3$: 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; ^{1}H NMR (300MHz, DMSO-d_6): δ 10.16 (s, 1H, NH), 8.10 (d, 1H, J=2 Hz, C-H_4), 7.84-7.80 (dd, 1H, J=2.2;8.9 Hz, C-H_6), 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-H_{10}), 5.18 (m, 2H, CH_2), 2.07 (s, 3H, $NHCOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{24}H_{19}NO_4$: 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):

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Yield: 72%; mp: 212.8 °C; ^{1}H NMR (300MHz, DMSO-d_6): δ 10.14 (s, 1H, NH), 8.09 (d, 1H, J=2.02 Hz, C-H_4), 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-H_6), 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-H_{10}), 5.18 (m, 2H, CH_2), 2.07 (s, 3H, $NHCOCH_3$).

^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{24}H_{19}NO_4$: 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; ^{1}H NMR (300MHz, DMSO-d_6): δ 7.61 (bs, 1H, C-$H_{2'}$), 7.55-7.53 (d, 1H, J=8.8 Hz, C-H_7), 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-H_6), 7.06-7.04 (dd, 1H, J=7.9 Hz, C-$H_{4'}$), 6.83 (d, 1H, J=2 Hz, C-H_4), 6.77 (s, 1H, C-H_{10}), 5.26 (bs, 2H, NH_2), 5.17 (m, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{22}H_{17}NO_3$: 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; ^{1}H NMR (300MHz, DMSO-d_6): 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-H_4), 7.17-7.15 (d, 2H, C-$H_{3'-5}$, J=8.8 Hz), 6.92 (s, 1H, C-H_{10}), 5.20 (s, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 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 (CH_2). (CH3). Elemental analysis calcd (%) for $C_{22}H_{17}NO_3$: 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%). (r

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

Yield: 96%; mp: 196.4 °C; ^{1}H NMR (300MHz, DMSO-d_6): δ 10.16 (s, 1H, NH), 8.10 (d, 1H, J=2 Hz, C-H_4), 7.83-7.79 (dd, 1H, J=2.2;8.9 Hz, C-H_6), 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-H_{10}), 2.07 (s, 3H, $NHCOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{23}H_{17}NO_4$: 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; ^{1}H NMR (300MHz, DMSO-d_6): δ 10.15 (s, 1H, NH), 8.11 (d, 1H, J=2 Hz, C-H_4), 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-H_6), 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-H_{10}), 2.07 (s, 3H, $NHCOCH_3$). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{23}H_{17}NO_4$: 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; ^{1}H NMR (300MHz, DMSO-d_6): 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-H_6), 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-H_4), 6.77 (s, 1H, C-H_{10}), 5.41 (bs, 2H, NH_2). ^{13}C NMR

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(75 MHz, DMSO-d_6): 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 $C_{21}H_{15}NO_3$: 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; ^{1}H NMR (300MHz, DMSO-d_6): 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, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6): 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 $C_{21}H_{15}NO_3$: 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; ^{1}H NMR (300MHz, DMSO-d_6): δ 10.15 (s, 1H, NH), 8.21-8.19 (d, 1H, J=1.2;7.8 Hz, C-$H_{6'}$), 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-$H_{4'}$), 7.20 (s, 1H, C-H_{10}), 7.16-7.13 (d, 1H, J=8.3 Hz, C-$H_{3'}$), 7.08 (dt, 1H, J=7.6 Hz, C-$H_{5'}$), 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-$H3_{isop}$). ^{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 $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15; found C, 71.14; H, 5.67; N, 4.12. m/z: 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; ^{1}H NMR (300MHz, 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-$H_{2',4',7}$), 7.40 (dt, 1H, J=8.0 Hz, C-$H_{5'}$), 7.04-7.01 (dd, 1H, J=2.6;8.2 Hz, C-$H_{6'}$), 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-$H3_{isop}$). ^{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 $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15; found C, 71.18; H, 5.66; N, 4.16. m/z: 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; ^{1}H NMR (300MHz, 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-$H_{2'}$), 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-$H_{3'}$), 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-$H3_{isop}$). ^{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 $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15; found C, 71.18; H, 5.69; N, 4.12. m/z: m/z: 337,13 (100,0%).

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

Yield: 68%; mp: 126.7 °C; ^{1}H NMR (300MHz, DMSO-d_6): δ 8.19-8.17 (d, 1H, J=6.9 Hz, C-$H_{6'}$), 7.39 (t, 1H, J=7.3 Hz, C-$H_{4'}$), 7.26-7.24 (d, 1H, J=8.7 Hz, C-H_7), 7.14 (d, 1H, C-$H_{3'}$), 7.11 (bs, 2H, NH2), 7.08-7.06 (d, 1H, C-$H_{5'}$), 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-$H3_{isop}$). ^{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 $C_{20}H_{19}NO_4$: C, 71.20; H, 5.68; N, 4.15; found C, 71.24; H, 5.74; N, 4.18. m/z: m/z: 337,13 (100,0%)

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

Yield: 51%; mp: 198.4 °C; ^{1}H NMR (300MHz, DMSO-d$_6$): δ 7.57-7.54 (d, 1H, J=7.8 Hz, C-H$_{6'}$), 7.51 (bs , 1H, C-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-H$_{4'}$), 7.41 (dt, 1H, J=7.8 Hz, C-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-H3$_{isop}$). ^{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 C$_{10}$H$_{17}$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; ^{1}H NMR (300MHz, DMSO-d$_6$): δ 7.97-7.95 (d, 2H, J=8.8 Hz, C-H$_{2'}$), 7.66-7.64 (m, 3H, C-H$_{4,6,7}$), 7.07-7.05 (d, 2H, J=8.8 Hz, C-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 C$_{10}$H$_{17}$NO$_3$: 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; ^{1}H NMR (300MHz, DMSO-d$_6$): 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-H$_4$), 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-H$_{10}$), 2.08 (s, 3H, CH3). ^{13}C NMR (75 MHz, DMSO-d$_6$): δ 183.5 (C-3), 168.44 (CO), 164.9-161.44 (C-2', J=260Hz), 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 C$_{17}$H$_{12}$FNO$_3$: 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; ^{1}H NMR (300MHz, DMSO-d$_6$): δ 10.22 (s, 1h, NH), 8.12 (d, 1H, J=2.1 Hz, C-H$_4$), 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-H$_{10}$), 2.07 (s, 3H, CH3). ^{13}C NMR (75 MHz, DMSO-d$_6$): 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 C$_{17}$H$_{12}$FNO$_3$: 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; ^{1}H NMR (300MHz, DMSO-d$_6$): 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-H$_7$), 7.09-7.05 (dd, 1H, J=2.5, 8.7 Hz, C-H$_6$), 6.86 (d, 1H, J=2.4Hz, C-H$_4$), 6.81 (s, 1H, C-H$_{10}$), 5.28 (bs, 2H, NH$_2$). ^{13}C NMR (75 MHz, DMSO-d$_6$): 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= 12Hz), 115.85-115.56 (C-3'), 113.22 (C-7), 105.54 (C-4), 100.82-100.72 (C-10).

Elemental analysis calcd (%) for C$_{15}$H$_{10}$FNO$_2$: 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; ^{1}H NMR (300MHz, DMSO-d$_6$): 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-H$_6$), 6.87 (d, 1H, J=2.4Hz,

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C-H4), 6.85 (s, 1H, C-H10). ^{13}C NMR (75 MHz, DMSO-d6): δ 184.26(C-3), 163.81-160.58 (C-3', J=244Hz), 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 $C_{15}H_{10}FNO_2$: 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; ^{1}H NMR (300MHz, DMSO-d6): δ 8.09-8.04 (dd, 2H, J=7.8 Hz, C-H2'), 7.81-7. 78 (d, 1H, C-H4'), 7.57-7.54 (d, 1H, J=8.4 Hz, C-H7), 7.36 (t, 2H, C-H3'), 7.33 (d, 1H, C-H4), 6.98 (s, 1H, C-H10). ^{13}C NMR (75 MHz, DMSO-d6): δ 183.5 (C-4), 165.39 (C-8), 164.38-161.07 (C-4', J=250Hz), 145.91 (C-2), 137.62 (C-5), 133.73-133.62 (C-2', J=9Hz), 128.55 (C-1', J=3Hz), 124.24 (C-6), 123.95 (C-7), 120.81 (C-9), 116.26-115.97 (C-3', J=22Hz), 113.14 (C-4), 111.04 (C-10). Elemental analysis calcd (%) for $C_{15}H_{10}FNO_2$: 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; ^{1}H NMR (300MHz, DMSO-d6): δ 10.20 (bs, 1H, NH), 8.30-8.28 (m, 2H, C-H2',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-H5',6'), 7.56-7.53 (d, 1H, J=8.9 Hz, C-H7), 7.06 (s, 1H, C-H10), 2.07 (s, 3H, CH3). ^{13}C NMR (75 MHz, DMSO-d6): 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 $C_{18}H_{12}F_3NO_3$: 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; ^{1}H NMR (300MHz, DMSO-d6): δ 8.31-8.29 (m, 2H, C-H2',4'), 7.80-7.73 (m, 2H, C-H6',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). ^{13}C NMR (75 MHz, DMSO-d6): 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=250Hz), 121.1 (C-9), 114.05 (C-6), 111.68 (C-4), 110.05 (C-10). Elemental analysis calcd (%) for $C_{16}H_{10}F_3NO_2$: 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; ^{1}H NMR (300MHz, DMSO-d6): 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). ^{13}C NMR (75 MHz, DMSO-d6): 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 $C_{18}H_{13}NO_5$: 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; ^{1}H NMR (300MHz, 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-H2''), 7.56 (t, 1H, *J*=8.16 Hz, C-H5'), 7.42 (dt, 2H, C-H3''), 7.20-7.17 (d, 1H, *J*=7.3 Hz, C-H4'), 7.14 (s, 1H, C-H2'), 7.09-7.06 (m, 2H, C-H10, 6'), 2.11 (s, 3H, CH3). ^{13}C 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 $C_{23}H_{16}N_2O_6$: 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):

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Yield: 75%; mp: >350 °C; ^{1}H NMR (300MHz, 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-H_4), 7.83-7.80 (dd, 1H, J=2.2;8.8 Hz, C-H_6), 7.50-7.47 (d, 1H, J=8.9 Hz, C-H_7), 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-H_{10}), 2.07 (s, 3H, C-$H_{3'}$). ^{13}C 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 $C_{17}H_{13}NO_4$: 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; ^{1}H NMR (300MHz, DMSO-d_6): δ 9.63 (bs, 1H, OH), 7.39 (d, 1H, C-$H_{2'}$), 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-H_7), 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-H_{10}), 5.24 (bs, 2H, NH_2). ^{13}C 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 $C_{15}H_{11}NO_3$: 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 synthetized. 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′, 4′ and 5′ 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 μM and also the safest regarding human cells. The two selected compound shared some structure similarity such as the 5-acetamido substitution. The SAR study from this work correlates the previous one of Olleik and al [13] showing that benzyloxy and isopropyloxy gives some interesting activity to aurone scaffold with substitution on A ring with amino or acetamido group improving the activity compared to 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 ring all together.

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.

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