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Version of Record: https://www.sciencedirect.com/science/article/pii/S0022286021022559
Manuscript_6fb9c831a768c762d00d647186175dc9

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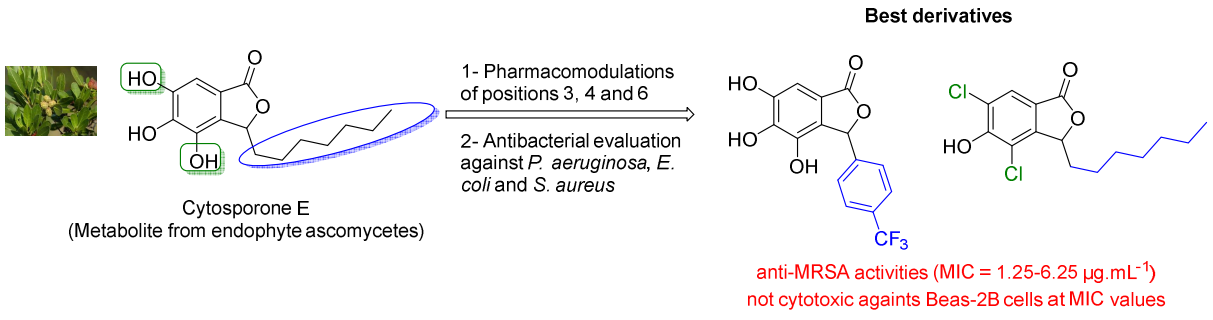
Keywords: natural phthalide, pharmacomodulations, antibacterial activity, cytotoxicity

Abstract: The history of antibacterial discovery has revealed that natural products, especially secondary metabolites, represent privileged chemical matters for the antibacterial discovery. Among these metabolites, several natural phthalides exhibit interesting antibacterial activities, such as cytosporone E isolated from endophyte ascomycetes *Diaporthe* and *Cytospora* sp. We describe herein the synthesis and the pharmacomulations on the positions 3, 4 and 6 of cytosporone E, then the *in vitro* antibacterial evaluation of these compounds against *Staphylococcus aureus* including resistant strains, *Escherichia coli* and *Pseudomonas aeruginosa*. The evaluation of the cytotoxicity of the most active compounds against human bronchial epithelial cells was performed in order to check their safety.

Graphical Abstract:



1. Introduction

The discovery and the improvement of antibiotics has led to make considerably decrease the mortality rate by bacterial infections. However, the over-use of these drugs has resulted in a selection pressure on the bacteria, which have gradually mutated and thus led to the appearance of new generations of multi-resistant bacteria against all families of known antibiotics.[1][2] In spite of the alert launched by the World Health Organization (WHO) and public authorities, the overuse of antibiotics is still topical and multi-resistant bacteria are becoming a worldwide public health problem which keep increasing the next decades if nothing is done. [3] Thus, Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* are two representative pathogens of the multi-antibiotic resistance, which are very often the cause of nosocomial infections. They appeared as respectively high and critical threats according a recent report published by the WHO. [3]

The research of new families of antibiotics, for which the structure can be inspired by natural compounds, is a strategy of interest to solve the problem of multidrug resistant bacteria (MDR).[4] In the last decade, our group developed synthetic pathways for the preparation of natural lactones such as phthalides, an important class of small oxygenated heterocycles which are widely represented in the area of natural compounds.[5][6] Of the known natural phthalides reported in the literature, some of them exhibit interesting antibacterial activities, such as corollosporine,[7] spirolaxine[8] or cytosporone E.[9] It is noteworthy the preparation of analogs of paecilocin A,[10] corollosporine[11] and spirolaxine[12] was performed in order to improve their antibacterial activities (Figure 1).

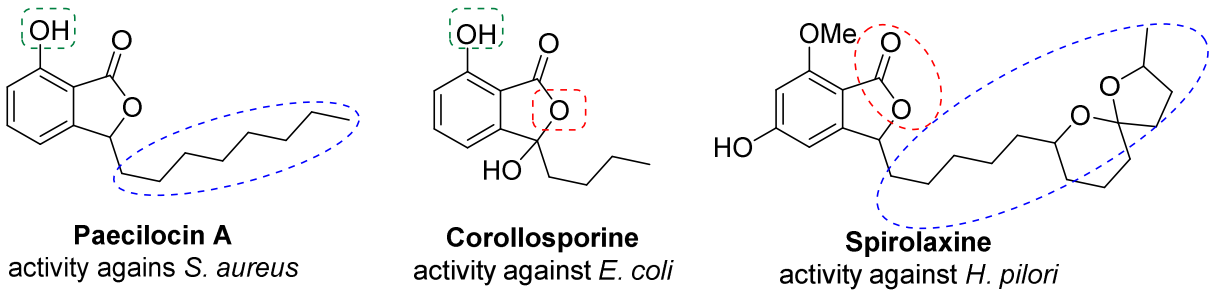


Figure 1: Natural antibacterial phthalides with their reported pharmacomodulations.

The cytosporone E was reported to exhibit antibacterial activities against Gram-positive, Gram-negative and drug-resistant strains[13] with a reasonable cytotoxicity according to the cell line.[13c, 13d] To the best of our knowledge, the preparation of new analogs of this natural compound was never performed in order to improve its antibacterial activities. Considering the natural reported analogs of cytosporone E, the substituent on the position 3 has a strong influence on the biological activities since the introduction of hydroxyl group or the shortening of the heptyl chain to a methyl were deleterious for the antibacterial activity.[13d, 14] We can then assume that certain modifications of the heptyl chain could conversely increase the antibacterial properties. In addition, previous works suggested the hydroxyl on the position 5 is essential for the antibacterial activity since 5-dehydroxycytosporone E did not showed any antimicrobial activity.[9, 15] One can then wonder what would be the impact on the antibacterial properties of the replacement of both hydroxyls on positions 4 and 6 (Figure 2). Besides, it was reported that the absolute configuration of C-3 has no impact on the antibacterial properties of cytosporone E since each enantiomer and the racemic mixture exhibited the same antibacterial activities.[16]

Herein, we report in this paper a short and flexible synthesis of racemic cytosporone E and various analogs pharmacomodulated on the positions 3, 4 and 6, then the *in vitro* antibacterial evaluation of these new compounds against representative bacteria (including resistant strains) and the evaluation of the cytotoxicity of the most active compounds.

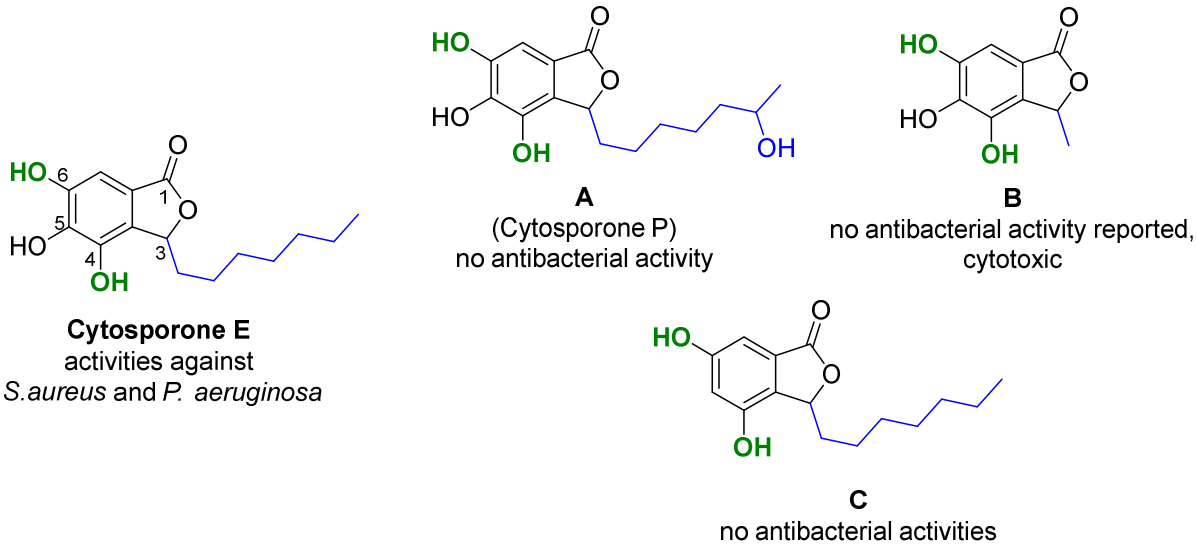
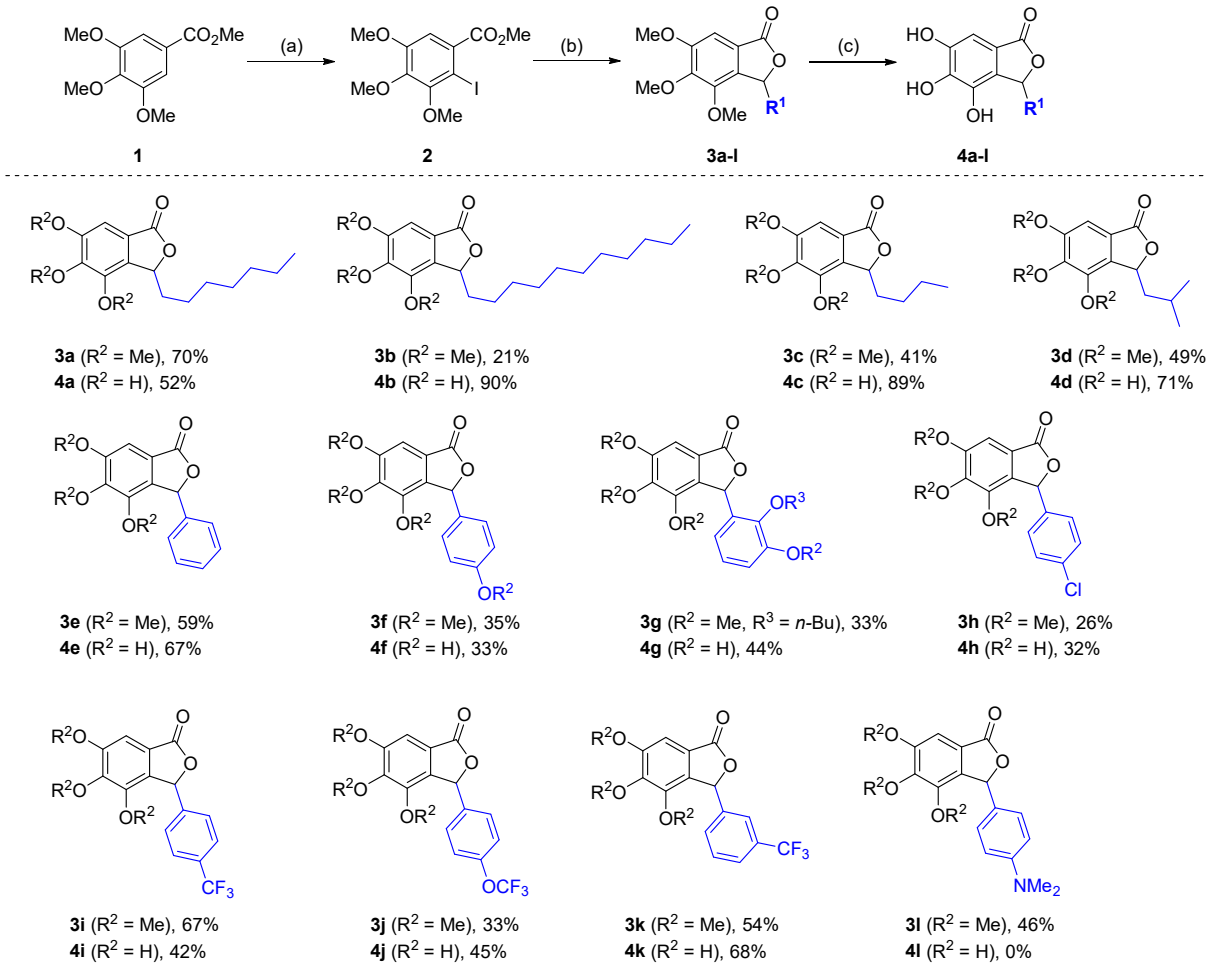


Figure 2: Structure and biological activities of cytosporone E and some natural analogs

2. Results and discussion

2.1. Synthesis

The literature abounds with numerous approaches for the preparation of phthalides, which are summarized in two recent reviews. [5] Noteworthy, the synthesis of C_3-functionnalized phthalides based on an iodine-magnesium exchange on *ortho*-iodobenzoate derivatives promoted by *i*PrMgCl.LiCl, followed by a condensation on an aldehyde, was reported as a straightforward and efficient strategy.[10, 17] We considered including this methodology as a key step for the preparation of cytosporone E and its analogues (Scheme 1). Therefore, the *ortho*-iodobenzoate **2** was synthesised from **1** in presence of iodine and a stoichiometric amount of silver trifluoroacetate with 63% yield.[18] A solution of *i*PrMgCl.LiCl in THF was prepared according to the reported procedure,[17b] and used for the magnesium-iodine exchange after titration with standard method. The substrate scope could be extended to aliphatic and various aromatic aldehydes, with average to good overall yields in phthalides **3a-l**. The total demethylation was finally performed with an excess of boron tribromide (BBr_3) in DCM between −20°C and room temperature to afford the trihydroxylated derivatives **4a-k** with modest to good yields ranged from 15 to 90 % (Scheme 1).



3a (R^2 = Me), 70%
4a (R^2 = H), 52%

3b (R^2 = Me), 21%
4b (R^2 = H), 90%

3c (R^2 = Me), 41%
4c (R^2 = H), 89%

3d (R^2 = Me), 49%
4d (R^2 = H), 71%

3e (R^2 = Me), 59%
4e (R^2 = H), 67%

3f (R^2 = Me), 35%
4f (R^2 = H), 33%

3g (R^2 = Me, R^3 = *n*-Bu), 33%
4g (R^2 = H), 44%

3h (R^2 = Me), 26%
4h (R^2 = H), 32%

3i (R^2 = Me), 67%
4i (R^2 = H), 42%

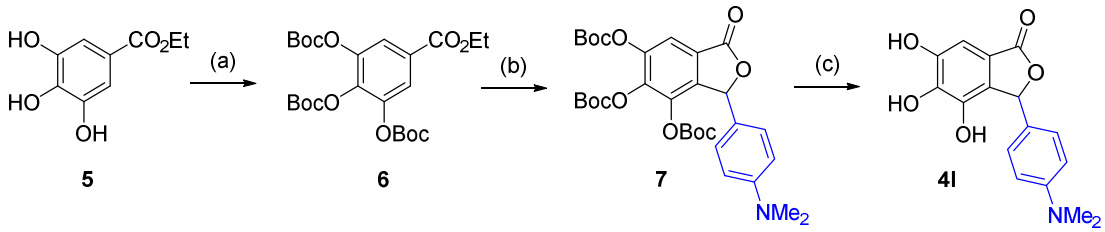
3j (R^2 = Me), 33%
4j (R^2 = H), 45%

3k (R^2 = Me), 54%
4k (R^2 = H), 68%

3l (R^2 = Me), 46%
4l (R^2 = H), 0%

Scheme 1: Synthesis of the cytosporone E and analogs. (a) I_2, CF_3COOAg, $CHCl_3$, rt., 24h, 63%; (b) *i*PrMgCl.LiCl, THF −78 °C, 30 min then R^1CHO −78 °C to rt, overnight, 18-69%; (c) BBr_3 (1M in CH_2Cl_2, 6 to 10 equiv.), CH_2Cl_2, −20°C to rt, overnight. 15-90%.

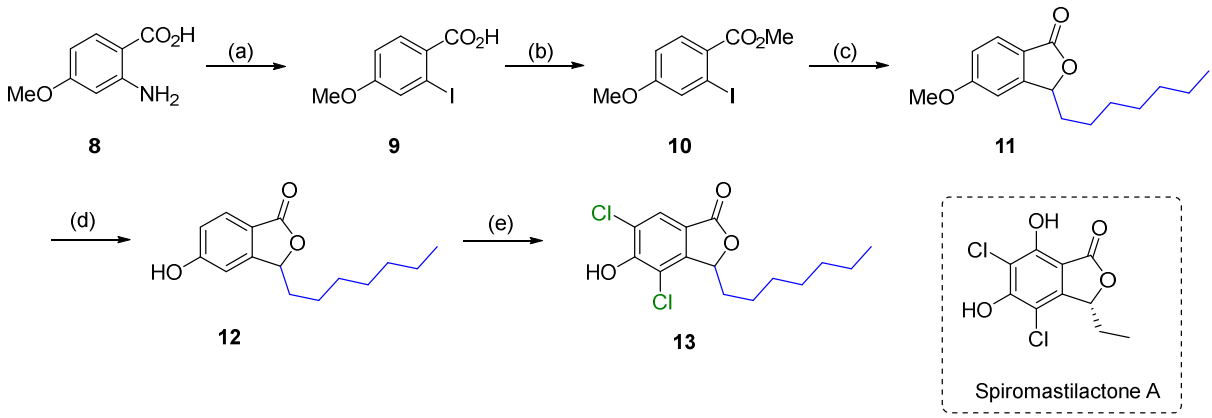
Because of its strong Lewis-acid nature, the boron tribromide mediated demethylation step was not compatible with *p*-dimethylaminophenyl group **3l** (formation of an amino-boron complex). An alternative synthetic pathway for the preparation of **3l** was performed (Scheme 2), based the *ortho*-metalation of activated aromatic ester with TMPMgCl.LiCl.[19] Thus, the hydroxyl functions of ethyl gallate **5** were protected by *t*-butylbenzyloxycarbonyl (Boc), which also plays the role of directing group. The ortho-metallation of **6** was performed with a commercial solution of TMPMgCl.LiCl at –40°C in THF, and was followed by the addition of *p*-dimethylaminobenzaldehyde. The phthalide **7** was obtained with an average yield of 52%, and the Boc-deprotection (TFA, 20°C, 15 min.) afforded the expected analogue **4l**.



5 6 7 4l

Scheme 2: Synthesis of the analog **4l**. (a) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 0°C to rt, overnight, 97%; (b) TMPMgCl.LiCl, THF, –20 °C, 3h then 4-dimethylaminobenzaldehyde, –40 °C, 3h, 52%; (c) CF_3COOH, rt, 15 min, 81%.

We then performed the pharmacomodulation of the C-4 and C-6 hydroxyl substituents of the cytosporone E. The replacement of these hydroxyl by two hydrogen was achieved by the synthesis of the compound **12** (Scheme 3). The latter was prepared in a four step sequence starting from commercial 2-amino-4-methoxybenzoic acid **8** which transformed into the corresponding iodobenzoic acid **9** *via* a Sandmeyer reaction. The acid was then esterified into the corresponding methyl ester **10**, and the phthalide **11** was obtained using the iodine-magnesium exchange strategy with *i*PrMgCl.LiCl and octanal as electrophile. Finally, the demethylation of **11** was performed with an excess of BBr_3 to afford **12**. At this stage, **12** was easily transformed in presence of SO_2Cl_2 in toluene into the corresponding dichlorinated compound **13**, an analogue of spiromastilactone A, a recently discovered natural marine phthalide which exhibit a strong antiviral activity and a weak cytotoxicity.[20]

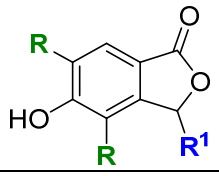


Scheme 3: Synthesis of the analogs **12-13** (a) $NaNO_2$, H_2SO_4, 0°C, 20 min. then KI, rt, 2h, 53%; (b) MeI, DBU, acetonitrile, rt, 1h, 60%; (c) *i*PrMgCl.LiCl, THF −78 °C, 30 min then octanal, −78 °C to rt, overnight, 65%; (d) BBr_3 (1M in CH_2Cl_2, 3 equiv.), CH_2Cl_2, −20°C to rt, overnight. 56%; (e) SO_2Cl_2, *i*Pr_2NH, toluene, 1h, 50°C, 43%.

2.2. Antibacterial activities

The antibacterial activities of **3a**, **4a-l** against Gram-positive *Staphylococcus aureus* (methicillin sensitive strain) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) were evaluated *via* broth microdilution method in accordance with the guidelines of the Clinical and Laboratory Standards Institute.[21] Imipenem and penicillin G were used as positive controls for this antibacterial assays. The Minimal Inhibitory Concentrations (MIC) of these compounds are reported in Table 1.

Table 1. Antibacterial activity of compounds **3a**, **4a-l, 12** and **13** against *S. aureus*, *E. coli* and *P. aeruginosa* by broth microdilution



Entry	Compound			MIC in µg.mL^{-1} (µM) [a]		
		R	R^1	*S. aureus* ATCC29213	*E. coli* ATCC25922	*P. aeruginosa* PAO1
1	**3a**	OMe	*n*-heptyl	>200	>200	NT[b]
2	**4a**	OH	*n*-heptyl	6.25 (22)	>200	50 (176) [c]
3	**4b**	OH	*n*-undecyl	100 (298)	>200	>200

4	**4c**	OH	*n*-butyl	100 (420)	>200	>200
5	**4d**	OH	*sec*-butyl	100 (420)	>200	>200
6	**4e**	OH	C_6H_5	100 (388)	>200	>200
7	**4f**	OH	4-HOC_6H_4	>200	>200	>200
8	**4g**	OH	2,3-HOC_6H_3	>200	>200	>200
9	**4h**	OH	4-ClC_6H_4	100 (342)	>200	>200
10	**4i**	OH	4-$CF_3C_6H_4$	12.5 (38)	>200	>200
11	**4j**	OH	4-$OCF_3C_6H_4$	12.5(37)	>200	>200
12	**4k**	OH	3-$CF_3C_6H_4$	25 (76)	>200	>200
13	**4l**	OH	4-$NMe_2C_6H_4$	>200	>200	>200
14	**12**	H	*n*-heptyl	50 (202)	>200	>200
15	**13**	Cl	*n*-heptyl	12.5 (79)	>200	>200
16	Penicillin G	-	-	2 (12)	NT[b]	NT[b]
17	Imipenem	-	-	NT[b]	NT[b]	8 (26)

[a] Assay experiments were performed in triplicates at three independent times. [b] NT means not tested. [b] MBC = 100 µg.mL^{-1}

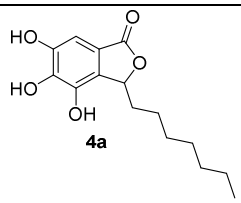
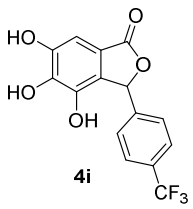
Although the importance of the trihydroxybenzene motif for the antibacterial activity was already assumed,[13a] it was confirmed herein as trimethylated cytosporone E **3a** did not show any antibacterial activity against the three strains (Entry 1). The synthetic cytosporone E **4a** showed a good antibacterial activity against *S. aureus* with a MIC of 6.25 µg.mL^{-1} (22 µM) but no activity against *E. coli* below 200 µg.mL^{-1} (Entry 2). These MIC values were consistent with those previously reported in the literature for the cytosporone E produced from the endophytic fungi. Interestingly, **4a** exhibited a moderate antibacterial activity against *P. aeruginosa*, with a bactericidal mode of action, as the minimal bactericidal concentration (MBC) was found to be at 100 µg.mL^{-1} (MBC/MIC < 4). It is noteworthy the extracted cytosporone E from the fungi did not exhibit activity below 150 µg.mL^{-1} against *P. aeruginosa* according to the literature data. [13b]

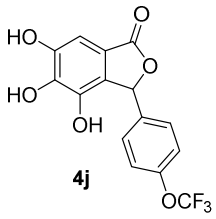
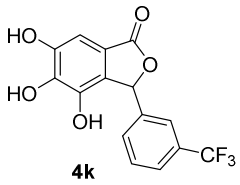
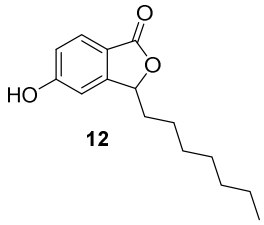
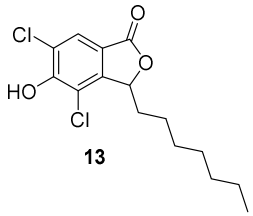
The nature of the substituent on the position 3 (R^1) was found to be essential for the antibacterial activity. While none of the analogs **4b-l** showed antibacterial activity against *E.*

coli and *P. aeruginosa* at concentrations below 200 µg.mL^{-1}, pharmacomodulations of the heptyl chain of **4a** had positive or negative effect on the antibacterial activity against *S. aureus* according to the nature of the substituent. Indeed, the elongation (**4b**), the shortening (**4c**) or the ramification (**4d**) of the alkyl chain were deleterious as the MIC value decreased to 100 µg.mL^{-1} (Entries 3-5). The replacement of the heptyl chain by a benzene ring (**4e**) had also a negative effect on the antibacterial activity, such as *para*-substituted phenyl group with hydroxy (**4f**), chlorine (**4h**) or dimethylamino (**4l**) (Entries 6-9, 13). Interestingly, a good antibacterial activity (MIC = 12.5 µg.mL^{-1}) was obtained with 4-trifluoromethyl (**4i**) and 4-trifluoromethoxy phenyl (**4j**) (Entries 10 and 11). We noticed that the position of the trifluoromethyl substituent on the aromatic ring (**4k**) had a slight effect on the antibacterial activity (Entry 12). The hydrogenated and chlorinated analogs **12** and **13** did not exhibit any activity against the Gram-negative strains. Besides, they were found to exhibit respectively a moderate and a good activity against *S. aureus* (Entries 14 and 15).

The antibacterial activities of compounds **4a**, **4i-k**, **12** and **13** toward methicillin-sensitive *S. aureus* (MSSA ATCC 29213) were then compared to two methicillin-resistant strains of *S. aureus* (MRSA NCTC 12493 and ATCC BAA-1026). The MIC and MBC of this assay are reported in the Table 2.

Table 2. Minimum Inhibitory and Bactericidal Concentrations (in µg.mL^{-1}) of compounds **4a, 4i-k, 12** and **13** against MSSA and MRSA strains

Entry	Compound	MSSA ATCC29213		MRSA NCTC12493		MRSA BAA-1026	
		MIC	MBC	MIC	MBC	MIC	MBC
1	 **4a**	6.25	12.5	**6.25**	12.5	**6.25**	12.5
2	 **4i**	12.5	50	**6.25**	25	**6.25**	25

3	 **4j**	12.5	25	12.5	25	12.5	25
4	 **4k**	25	25	12.5	25	12.5	25
5	 **12**	50	100	12.5	25	12.5	25
6	 **13**	12.5	>100	**1.25**	25	**1.25**	25
7	Penicilin G	2	n.d.	>64	n.d.	>64	n.d.

We observed that **4a** exhibited the same antibacterial activities toward MSSA and MRSA strains with a MIC = 6.25 µg.mL^{-1} and MBC = 12.5 µg.mL^{-1}, such ratio (MBC/MIC = 2) suggested a bactericidal effect (Entry 1). Trifluoromethylated compounds **4i-k** showed similar antibacterial profiles, with no significant difference of activity between the sensitive and the resistant strains (Entries 2-4). **4i** and **4k** were found to be 2 times more active on against MRSA than MSSA. However, we were surprised to observe than **12** and **13** were strongly more active against MRSA strains compared to the sensitive one. Indeed, **12** was eight times less active than **4a** on the MSSA strain, but exhibited the same MIC and MBC values than **4i-k** toward the resistant strains (Entry 5). The chlorinated compound **13** showed a MIC of 12.5 µg.mL^{-1} toward MSSA and was ten times more active on both MRSA strains. The MBC values suggested that **13** was bacteriostatic, unlike the others analogs tested. These MIC values were compared to those obtained with penicillin G, for which the MRSA strains are resistant (MIC > 64 µg.mL^{-1}, Entry 7).

2.3. Cytotoxicity

The cytotoxicity of compounds which exhibited the best antibacterial activities against MRSA (**4a**, **4i-k, 12** and **13**) was evaluated against Beas-2B cells. This human bronchial epithelial cell-line is known to be very sensitive toward exogenous compounds. Starting from a mother solution of each compound diluted in DMSO at 3 $mg.mL^{-1}$, dilutions to 25, 12.5, 6.25, 3.1 $\mu g.mL^{-1}$ (and 1.5 $\mu g.mL^{-1}$ for compound **13**) were made using FK12 medium without FCS. The cells were incubated during 48h in the presence of different concentration of each compound and the cell viability was evaluated by MTS assays. The IC_{50} are reported in the Table 3 and was compared to their MIC against MRSA expressed in µM. While **4j**, **4k** and **12** have IC_{50} values close to their MIC value, compounds **4a**, **4i** and **13** have an interesting biological profile since their IC_{50} value is three to four times higher than their MIC against MRSA.

Table 3. Summary of cytotoxicity against Beas-2B cells and activity against methicillin-resistant *S. aureus* of compounds **4a**, **4i-k**, **12** and **13**

Compound	IC_{50} Beas-2B cells (µM)	MIC against MRSA (µM)
4a	66.4	22.3
4i	87.1	19.2
4j	41.5	36.5
4k	31.3	38.3
12	58.4	50.4
13	15.9	3.9

The cytotoxicity of **4a** and **4i** were then compared to each other at range of concentrations between 25 and 3.1 $\mu g.mL^{-1}$ (Figure 3A). It is noteworthy the cells alone were considered the maximum with 100% cell viability, but due to the sensibility of the cells to the solvent, the control was performed with DMSO at $1/30^{th}$. It has been confirmed that **4a** was not cytotoxic at its MIC value, since more than 80% of cell viability was observed at concentrations below 6.25 µg/mL. **4i** showed a similar profile since more than 80% cell viability was observed at concentrations below 6.25 µg/mL, with a decrease in the cell-toxicity at 25 µg/mL (66% of cell viability against 29% for **4a**).

We finally evaluated the cytotoxicity of **13**, the most promising product under two different conditions (Figure 3B). The first condition consisted to use, as previously, a solution of **13** in DMSO at 3 mg/mL to perform the dilution range (see experimental section). In such conditions, an ambiguity subsisted on the toxicity at it MIC against MRSA (1.25 µg.mL^{-1}) since 74% of cell viability was observed. In this assay, DMSO alone exhibited slight cytotoxicity since 74% of cell viability was observed. In the second condition, the DMSO was replaced by the NADES (NAtural Deep Eutectic Solvent) glycerol/choline chloride to prepare the mother solution of **13** at 3 mg.mL^{-1}. The dilution range was then performed in the same way, and we were pleased to observe more than 80% cell viability at 1.25 µg.mL^{-1} of **13** and no cytotoxic effect of the solvent alone, thus suggesting that 13 is not cytotoxic to its MIC against MRSA.

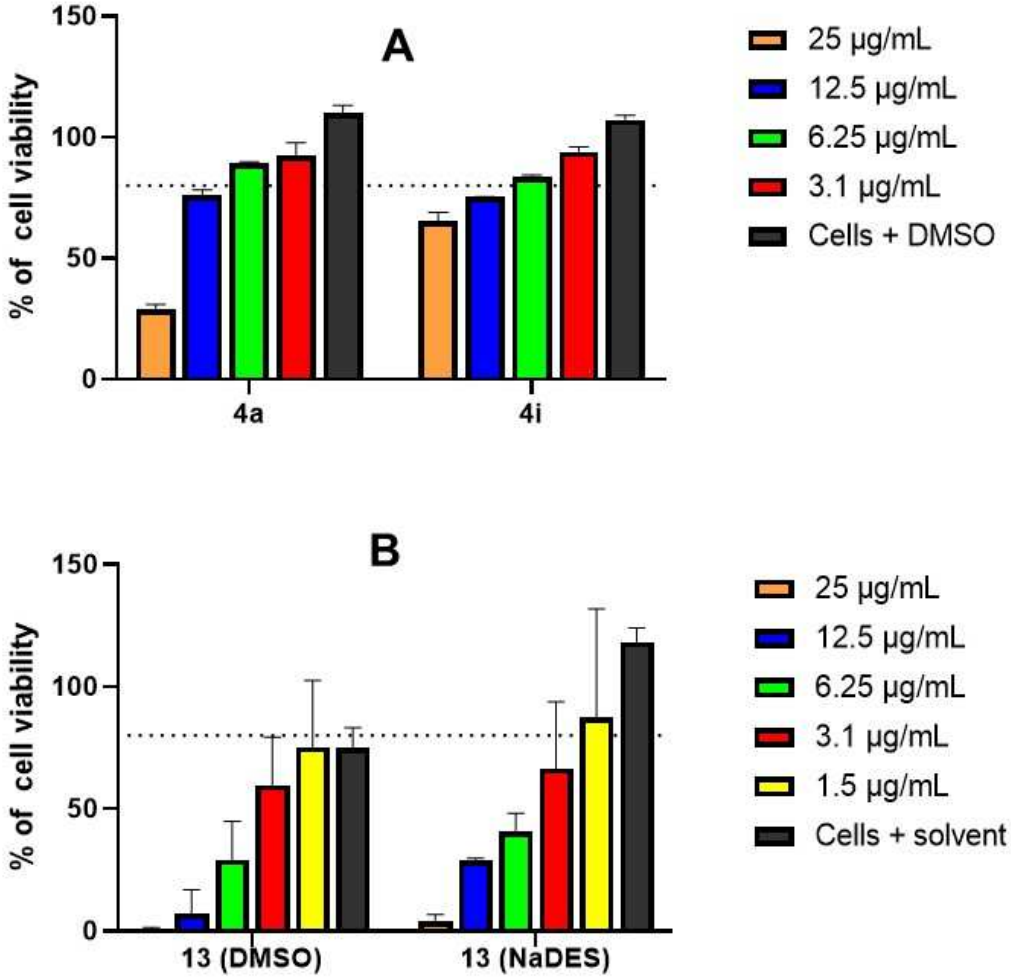


Figure 3: Evaluation of phthalides cytotoxicity on Beas-2B cells by MTS at different concentrations. **A**: Compounds **4a** and **4i** (n = 2). **B**: compound **13** dissolved in DMSO (n= 2) and in NaDES glycerol-choline chloride (n = 3).

3. Conclusions

To conclude, we have developed a synthetic pathway for the preparation of racemic cytosporone E **4a** and various analogs (**4b-l**, **12** and **13**) of this natural phthalide pharmacomodulated on positions 3, 4 and 6. All compounds were screened for their antibacterial activities against *S. aureus*, *E. coli* and *P. aeruginosa* using broth microdilution. This study revealed that these analogs did not show any antibacterial activity against the Gram-negative strains. However, several compounds showed significant antibacterial activity against methicillin-sensitive *S. aureus*, and were found to be 2 to 10 times more active on the methicillin-resistant of *S. aureus* compared to the sensitive strain. Among the most active derivatives, **4a**, **4i** and **13** were evaluated for their cytotoxicity against human bronchial epithelial cells at different concentrations and found not toxic at their MIC value. Therefore, these new compounds constitute an interesting line of work in the fight against the antimicrobial resistance.

4. Experimental section

4.1 Chemistry

4.1.1 *General chemical methods*.

All reactions were carried out under argon atmosphere. TLC spots were examined under UV light. The aldehydes were distilled before using. Silica gel (Geduran Si 60, 40-63 µm by Merck) was used for column chromatography. Acetonitrile, dichloromethane and chloroform (stabilized with amylene) were distilled from calcium hydride, tetrahydrofuran and diethyl ether were distilled from sodium/benzophenone. Other chemicals were obtained from commercial sources and were used without further purification.

NMR spectra were obtained at 300 MHz for ^{1}H, 75 MHz for ^{13}C, 282 MHz for ^{19}F with BRUKER AVANCE 300 spectrometer. Chemical shifts are given in parts per million (δ) relative to the residual peak of chloroform (7.26 ppm), dimethylsulfoxide (2.50 ppm), acetone (2.05) or methanol (3.31 ppm). Electrospray ionization mass spectrometry experiments (HRMS) were obtained on a hybrid tandem quadrupole/time-off light (Q-TOF) instrument, equipped with a pneumatically assisted electrospray (Z-spray) ion source (Micromass, Manshester, U.K.) operated in positive mode. The melting points were recorded on a "Stuart™ melting point apparatus SMP3.

4.1.2 *Methyl 2-iodo-3,4,5-trimethoxybenzoate (**2**)[22]*

A dry and argon-flushed round bottom flask was charged with a solution of methyl 3,4,5-trimethoxybenzoate **1** (5.0 g, 22.1 mmol, 1 equiv.) and silver trifluoroacetate (5.1 g, 23.0 mmol, 1.04 equiv.) in dry chloroform (60 mL). Iodine (5.8 g, 23.0 mmol, 1.04 equiv.) was added in small portions over a period of 30 minutes and the dark pink solution was stirred overnight at room temperature. The reaction mixture was filtered over a pad of Celite®, the filtrate was diluted with CH_2Cl_2 (50 mL) and was washed with a 5% aqueous solution of $Na_2S_2O_3$ (100 mL). The aqueous phase was washed with dichloromethane (3 x 50 mL). The combined organic phases were washed with brine (100 mL), dried over $MgSO_4$ and solvent was removed under reduce pressure. The residue was purified by column chromatography on silica gel using CH_2Cl_2 as eluent to afford the iodinated ester **2** (4.39 g, yield = 63%) as a white solid.
^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.17 (s, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.9, 153.9, 153.4, 144.9, 131.0, 110.4, 83.9, 61.1, 60.8, 56.3, 52.5. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{11}H_{14}IO_5$: 352.9886; found. 352.9875.

4.1.3 *Preparation of iPrMgCl·LiCl solution[17b]*

A dry and argon-flushed Schlenk tube was charged with anhydrous lithium chloride (2.1 g, 50 mmol, 1 equiv.) and magnesium turnings (1.3 g, 55 mmol, 1.1 equiv.). The solids were stirred under vaccum for 4 hours at 150°C. After cooling, dry THF (25 mL) was added followed by a solution of *iso*propyl chloride (4.6 mL, 50 mmol, 1 equiv.) in THF (25 mL). This suspension was stirred overnight at room temperature, and the resulting light grey solution was titrated according to Paquette method.[23]

4.1.4 *Typical procedure for the preparation of compounds **3a-l***

A dry and argon-flushed flask equipped with a magnetic stirrer and a septum was charged with a solution of **2** (1 equiv.) in dry THF (10 mL) and was cooled at –78°C. A solution of *i*PrMgCl·LiCl (1.3 equiv.) was added dropwise and the reaction mixture was stirred for 30 minutes at –78°C. The corresponding aldehyde (1.3 equiv.)was added and the reaction mixture was slowly warm to room temperature and stirred overnight. The reaction mixture was hydrolysed with water (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel.

4.1.4.1. *3-Heptyl-4,5,6-trimethoxy*iso*benzofuran-1(3H)-one (**3a**)*

Following the above procedure using **2** (350 mg, 1.0 mmol, 1 equiv.), *i*PrMgCl·LiCl (1.73 mL, 0.75 M solution in THF, 1.3 mmol, 1.3 equiv), octanal (203 µL, 1.3 mmol, 1.3 equiv.) and THF (10 mL). Chromatography on silica gel with Petroleum ether/EtOAc (85:15) as eluent was performed to get the desired compound **3a** (225 mg, yield = 70%) as a white solid. Mp = 57-60°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.10 (s, 1H), 5.44 (dd, *J* = 7.9 Hz, 3.0 Hz, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 2.20-2.12 (m, 1H), 1.74-1.65 (m, 1H), 1.45-1.19 (m, 10H), 0.85 (t, *J* = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.6, 155.5, 147.6, 146.8, 135.4, 121.4, 102.5, 80.2, 61.2, 60.7, 56.4, 33.4, 31.7, 29.2, 29.0, 24.7, 22.6, 14.0.

4.1.4.2. *4,5,6-Trimethoxy-3-undecylisobenzofuran-1(3H)-one (**3b**)*

Following the above procedure using **2** (800 mg, 2.27 mmol, 1 equiv.), *i*PrMgCl·LiCl (2.95 mL, 1 M solution in THF, 2.95 mmol, 1.3 equiv.), dodecanal (654 µL, 2.95 mmol, 1.3 equiv.) and THF (20 mL). Chromatography on silica gel with Petroleum ether/EtOAc (85:15) as eluent was performed to get the desired compound **3b** (180 mg, yield = 21%) as a white solid. Mp = 58-61°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.11 (s, 1H), 5.46 (dd, *J* = 7.9 Hz, 3.0 Hz, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.91 (s, 3H), 2.27-2.07 (m, 1H), 1.80-1.56 (m, 1H), 1.36-1.14 (m, 18H), 0.87 (t, *J* = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.6, 155.5, 147.6, 146.8, 135.4, 121.4, 102.6, 80.2, 61.1, 60.8, 56.4, 33.4, 31.9, 29.59, 29.58, 29.51, 29.4, 29.3, 24.7, 22.7, 14.1. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{22}H_{34}O_5$: 379.24790; found: 397.24753.

4.1.4.3. *3-Butyl-4,5,6-trimethoxy-isobenzofuran-1(3H)-one (**3c**)*

Following the above procedure using **2** (400 mg, 1.14 mmol), *i*PrMgCl·LiCl (3.3 mL, 0.45 M solution in THF, 1.48 mmol, 1.3 equiv.), pentanal (157 µL, 1.48 mmol, 1.3 equiv.) and THF (10 mL). Chromatography on silica gel with Petroleum ether/EtOAc (70:30) as eluent was performed to get the desired compound **3c** (130 mg, yield = 41%) as a white solid. Mp = 75-78°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.10 (s, 1H), 5.45 (dd, *J* = 7.9 Hz, 3.0 Hz, 1H), 3.96 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 2.26-2.10 (m, 1H), 1.78-1.57 (m, 1H), 1.45-1.25 (m, 4H), 0.88 (t, *J* = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.6, 155.5, 147.6, 146.8, 135.4, 121.4, 102.5, 80.1, 61.1, 60.7, 56.3, 33.1, 26.7, 22.3, 13.8. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{15}H_{20}O_5$: 281.13835; found: 281.13790.

4.1.4.4. *3-Isobutyl-4,5,6-trimethoxy-isobenzofuran-1(3H)-one (**3d**)*

Following the above procedure using **2** (800 mg, 2.28 mmol, 1 equiv.), *i*PrMgCl·LiCl (6.6 mL, 0.45 M solution in THF, 2.97 mmol, 1.3 equiv.), *iso*butyraldehyde (314 µL, 2.96 mmol, 1.3

equiv.) and THF (20 mL). Chromatography on silica gel with Petroleum ether/EtOAc (70:30) as eluent was performed to get the desired compound **3d** (310 mg, yield = 49%) as a white solid. Mp = 55-58°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.11 (s, 1H), 5.48 (dd, *J* = 10.1 Hz, 1.7 Hz, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 3.91 (s, 3H), 2.02-1.90 (m, 2H), 1.56-1.48 (m, 1H), 1.06 (d, *J* = 6.0 Hz, 3H), 0.96 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.4, 155.4, 147.5, 146.8, 135.9, 121.1, 102.4, 78.7, 61.0, 60.7, 56.3, 42.9, 25.1, 23.5, 21.6. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{15}H_{20}O_5$: 281.13835; found: 281.13792.

4.1.4.5. *4,5,6-Trimethoxy-3-phenylisobenzofuran-1(3H)-one (**3e**)*

Following the above procedure using **2** (400 mg, 1.14 mmol, 1 equiv.), *i*PrMgCl·LiCl (1.48 mL, 1.0 M solution in THF, 1.48 mmol, 1.3 equiv.), benzaldehyde (150 µL, 1.48 mmol, 1.3 equiv.) and THF (10 mL). Chromatography on silica gel with Petroleum ether/EtOAc (90:10) as eluent was performed to get the desired compound **3e** (200 mg, yield = 59%) as a white solid. Mp = 98-101°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.39-7.32 (m, 3H), 7.29-7.24 (m, 2H), 7.21 (s, 1H), 6.35 (s, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.45 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.4, 155.9, 147.9, 147.5, 136.3, 135.7, 129.2, 128.6 (2C), 127.4 (2C), 120.8, 102.6, 81.2, 61.0, 60.3, 56.4. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{17}H_{16}O_5$: 301.10705; found: 301.10664.

4.1.4.6. *4,5,6-Trimethoxy-3-(4-methoxyphenyl)isobenzofuran-1(3H)-one (**3f**)*

Following the above procedure using **2** (400 mg, 1.14 mmol, 1 equiv.), *i*PrMgCl·LiCl (2.96 mL, 0.5 M solution in THF, 1.48 mmol, 1.3 equiv.), *p*-anisaldehyde (180 µL, 1.48 mmol, 1.3 equiv.) and THF (10 mL). Chromatography on silica gel with Petroleum ether/EtOAc (85:15) as eluent was performed to get the desired compound **3f** (130 mg, yield = 35%) as a white solid. Mp = 95-103°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.19 (s, 1H), 7.17 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 6.32 (s, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.80 (s, 3H), 3.48 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.4, 160.2, 158.8, 147.9, 147.4, 135.5, 128.8 (2C), 128.3, 121.0, 114.0 (2C), 102.6, 81.1, 61.1, 60.4, 56.4, 55.3. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{18}H_{18}O_6$: 331.11761; found: 331.11702.

4.1.4.7.*4,5,6-3-(2-Butoxy-3-methoxyphenyl)-4,5,6-trimethoyisobenzofuran-1(3H)-one (**3g**)*

Following the above procedure using **2** (500 mg, 1.44 mmol, 1 equiv.), *i*PrMgCl·LiCl (3.74 mL, 0.5 M solution in THF, 1.87 mmol, 1.3 equiv.), 2-butoxy-3-methoxybenzaldehyde (389

mg, 1.87 mmol, 1.3 equiv.) and THF (20 mL). Chromatography on silica gel with Petroleum ether/Et_2O (85:15) as eluent was performed to get the desired compound **112g** (240 mg, yield = 42%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.20 (s, 1H), 6.99-6.88 (m, 2H), 6.83 (s, 1H), 6.41 (dd, *J* = 7.3 Hz, 1.8 Hz, 1H), 4.14 (dd, *J* = 6.7 Hz, 2.3 Hz, 1H), 3.98 (dd, *J* = 6.9 Hz, 2.2 Hz, 1H),3.94 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.50 (s, 3H), 1.82-1.71 (m, 2H), 1.57-1.42 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.83, 155.81, 153.06, 147.98, 147.64, 147.36, 135.44, 130.07, 124.04, 121.84, 119.87, 113.45, 102.60, 76.17, 74.04, 61.22, 60.32, 56.59, 55.99, 32.39, 19.31, 14.06. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{22}H_{29}O_7$: 403.17513; found: 403.17465.

4.1.4.8. *3-(4-chlorophenyl)-4,5,6-trimethoxyisobenzofuran-1(3H)-one (**3h**)*

Following the above procedure using **2** (400 mg, 1.14 mmol, 1 equiv.), *i*PrMgCl·LiCl (2.96 mL, 0.5 M solution in THF, 1.48 mmol, 1.3 equiv.), 4-chlorobenzaldehyde (207 µL, 1.48 mmol, 1.3 equiv.) and THF (10 mL). Chromatography on silica gel with Petroleum ether/EtOAc (80:20) as eluent was performed to get the desired compound **3h** (100 mg, yield = 26%) as a bright yellow solid. Mp = 82-85°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.34 (d, *J* = 8.3 Hz, 2H), 7.24-7.18 (m, 3H), 6.31 (s, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.53 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.2, 161.3, 156.2, 147.8, 147.5, 134.9, 128.9 (2C), 128.7 (2C), 128.3, 120.7, 102.6, 80.3, 61.1, 60.5, 56.5. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{17}H_{15}$ $^{35}ClO_5$: 335.06808; found: 335.06755.

4.1.4.9 *4,5,6-Trimethoxy-3(4-(trifluoromethyl)phenyl)isobenzofuran-1(3H)-one (**3i**)*

Following the above procedure using **2** (500 mg, 1.48 mmol, 1 euiv.), *i*PrMgCl·LiCl (3.85 mL, 0.5 M solution in THF, 1.93 mmol, 1.3 equiv.), 4-(trifluoromethyl)-benzaldehyde (263 µL, 1.93 mmol, 1.3 equiv.) and THF (20 mL). Chromatography on silica gel with Petroleum ether/EtOAc (70:30) as eluent was performed to get the desired compound **3i** (380 mg, yield = 70%) as a white solid. Mp = 104-107°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.63 (d, *J* = 8.2 Hz, 2H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.20 (s, 1H), 6.38 (s, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 3.54 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.0, 156.2, 147.7, 147.3, 140.3, 134.8, 130.7 (q, *J* = 32 Hz), 127.6 (2C), 125.6 (q, *J* = 3.2 Hz, 2C), 122.9 (q, *J* = 270 Hz) 120.4, 102.6, 80.0, 61.0, 60.3, 56.4. ^{19}F NMR (282 MHz, $CDCl_3$) δ = –62.7. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{18}H_{15}$ F_3O_5: 369.09443; found: 369.09414.

4.1.4.10. *4,5,6-Trimethoxy-3-(4-(trifluromethoxy)phenyl)isobenzofuran-1(3H)-one (**3j**)*

Following the above procedure using **2** (500 mg, 1.44 mmol, 1 equiv.), *i*PrMgCl·LiCl (3.74 mL, 0.5 M solution in THF, 1.87 mmol, 1.3 equiv.), 4-(trifluoromethoxy)-benzaldehyde (262 µL, 1.84 mmol, 1.3 equiv.) and THF (20 mL). Chromatography on silica gel with Petroleum ether/EtOAc (80:20) as eluent was performed to get the desired compound **3j** (330 mg, yield = 60%) as a white solid. Mp = 97-100°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.32 (d, *J* = 8 Hz, 2H), 7.25-7.17 (m, 3H), 6.34 (s, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.53 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.1, 156.2, 149.6 (q, *J* = 3.2 Hz), 147.8, 147.4, 135.2, 135.1, 128.9 (2C), 121.0 (2C), 120.6, 120.4 (q, *J* = 256 Hz), 102.7, 80.1, 61.1, 60.3, 56.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ = –57.9. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{18}H_{15}F_3O_6$: 385.08935; found: 385.08886.

4.1.4.11. *4,5,6-Trimethoxy-3-(3-(trifluromethyl)phenyl)isobenzofuran-1(3H)-one (**3k**)*

Following the above procedure using **2** (830 mg, 2.35 mmol, 1 equiv.), *i*PrMgCl·LiCl (6.12 mL, 0.7 M solution in THF, 3.06 mmol, 1.3 equiv.), 3-(trifluromethyl)benzaldehyde (409 µL, 3.06 mmol, 1.3 equiv.) and THF (20 mL). Chromatography on silica gel with Petroleum ether/EtOAc (80:20) as eluent was performed to get the desired compound **3k** (510 mg, yield = 54%) as a yellow solid. Mp = 86-89°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.64-7.55 (m, 2H), 7.52-7.44 (m, 2H), 7.21 (s, 1H), 6.38 (s, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.52 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.1, 156.3, 147.8, 147.5, 137.6, 134.8, 131.1 (q, *J* = 32.5 Hz), 130.4, 129.2, 125.8 (q, *J* = 3.7 Hz), 124.2 (q, *J* = 3.9 Hz), 124.1 (q, *J* = 270 Hz), 120.5, 102.7, 80.1, 61.1, 60.3, 56.5. ^{19}F NMR (282 MHz, $CDCl_3$) δ = –62.7. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{18}H_{15}F_3O_5$: 369.09443; found: 369.09406.

4.1.4.12. *3-(4-(Dimethylamino)phenyl)-4,5,6-trimethoxyisobenzofuran-1(3H)-one (**3l**)*

Following the above procedure using **2** (400 mg, 1.14 mmol, 1 equiv.), *i*PrMgCl·LiCl (2.96 mL, 0.5 M solution in THF, 1.48 mmol, 1.3 equiv.), 4-(dimethylamino)benzaldehyde (222 mg, 1.48 mmol, 1.3 equiv.) and THF (10 mL). Chromatography on silica gel with Petroleum ether/EtOAc (80:20) as eluent was performed to get the desired compound **3h** (180 mg, yield = 46%) as a yellow solid. Mp = 113-116°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.19 (s, 1H), 7.08 (d, *J* = 8.8 Hz, 2H), 6.66 (d, *J* = 8.8 Hz, 2H), 6.31 (s, 1H), 3.94 (s, 3H), 3.91(s, 3H), 3.50 (s, 3H), 2.95 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.7, 155.6, 151.1, 148.0, 147.3, 135.6,

128.6 (2C), 123.2, 121.4, 112.1 (2C), 102.5, 81.8, 61.1, 60.4, 56.4, 40.4 (2C). HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{19}H_{22}NO_5$: 344.1493; found: 344.1488.

4.1.5. *Typical procedure for the preparation of compounds **4a-k***

A dry and argon-flushed flask equipped with a magnetic stirrer and a septum was charged with a solution of phthalide **3a-k** (1 equiv.) in dry CH_2Cl_2 (5 mL) and was cooled at −30°C. A solution of BBr_3 (6 equiv.) was added and the reaction mixture was stirred overnight. The reaction mixture was hydrolysed with water (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel.

4.1.5.1. *3-Heptyl-4,5,6-trihydroxyisobenzofuran-1(3H)-one (**4a**)*

Following the above procedure using **3a** (322 mg, 1.0 mmol, 1 equiv.), BBr_3 (6 mL, 1 M in CH_2Cl_2, 6 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (95:5) as eluent was performed to get the desired compound **4a**, (45 mg, yield = 52%) as a white solid. . Mp = 170-173°C. ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.03 (s, 1H), 9.39 (s, 1H), 6.83 (s, 1H), 5.44 (dd, *J* = 7.7 Hz, 3.0 Hz, 1H), 2.30-2.22 (m, 1H), 1.78-1.65 (m, 1H), 1.45-1.20 (m, 10H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 170.2, 146.8, 139.7, 138.8, 129.5, 117.1, 102.0, 79.2, 32.9, 31.6, 29.2, 29.0, 24.5, 22.4, 13.4.

4.1.5.2. *4,5,6-trihydroxy-3-undecylisonenzofuran-1(3H)-one (**4b**)*

Following the above procedure using **3b** (96 mg, 0.25 mmol, 1 equiv.), BBr_3 (1.5 mL, 1 M in CH_2Cl_2,1.5 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (95:5) as eluent was performed to get the desired compound **4b** (80 mg, yield = 90%) as a white solid. Mp = 157-160°C. ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.02 (s, 1H), 8.33 (s, 1H), 8.25(s, 1H), 6.83 (s, 1H), 5.43 (dd, *J* = 7.7 Hz, 3.0 Hz, 1H), 2.32-2.18 (m, 1H), 1.77-1.65 (m, 1H), 1.45-1.23 (m, 18H), 0.87 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 170.1, 146.8, 139.7, 138.7, 129.5, 117.2, 102.0, 79.2, 33.0, 31.7, 29.5 (2C), 29.4, 29.33, 29.2 (2C), 24.6, 22.4, 13.4. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{19}H_{28}O_5$: 337.20095; found: 337.20048.

4.1.5.3. 3-*butyl-4,5,6-trihydroxyisobenzofuran-1(3H)-one (**4c**)*

Following the above procedure using **3c** (70 mg, 0.25 mmol, 1 equiv.), BBr_3 (1.5 mL, 1 M in CH_2Cl_2, 1.5 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (95:5) as eluent was performed to get the desired compound **4c** (54 mg, yield = 89%) as a white solid. Mp = 132-135°C. ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.09 (s, 1H), 8.39 (s, 1H), 8.29 (s, 1H), 6.83 (s, 1H), 5.44 (dd, *J* = 7.6, 2.9 Hz, 1H), 2.25 (m, 1H), 1.71 (m, 1H), 1.32 (m, 4H), 0.90 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 170.3, 146.9, 139.7, 138.9, 129.4, 117.0, 102.0, 79.3, 32.6, 26.7, 22.2, 13.4. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{12}H_{14}O_5$: 239.09140; found: 239.09094.

4.1.5.4. *4,5,6-trihydroxy-3-isobutylisobenzofuran-1(3H)-one (**4d**)*

Following the above procedure using **3d** (100 mg, 0.36 mmol, 1 equiv.), BBr_3 (2.16 mL, 1 M in CH_2Cl_2, 2.16 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (95:5) as eluent was performed to get the desired compound **4d** (60 mg, yield = 71%) as a white solid. Mp = 197-200°C. ^{1}H NMR (300 MHz, acetone-d_6) δ 9.07 (s, 1H), 8.39 (s, 1H), 8.29 (s, 1H), 6.83 (s, 1H), 5.47 (dd, *J* = 9.9, 2.5 Hz, 1H), 2.13 (m, 1H), 1.84 (m, 1H), 1.48 (m, 1H), 1.04 (d, *J* = 6.6 Hz, 3H), 0.96 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 170.1, 146.8, 139.7, 138.8, 130.1, 116.8, 102.0, 77.9, 42.4, 25.1, 23.0, 21.3. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{12}H_{14}O_5$: 239.09140; found: 239.0909.

4.1.5.5. *4,5,6-trihydroxy-3-phenylisobenzofuran-1(3H)-one (**4e**)*

Following the above procedure using **3e** (120 mg, 0.40 mmol, 1 equiv.), BBr_3 (2.4 mL, 1 M in CH_2Cl_2, 2.4 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Filtration over a plug of silica using ethyl acetate as eluant was performed to get the desired compound **4e** (70 mg, yield = 67%) as black solid. Mp = 234-237°C. ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.12 (s, 1H), 8.40 (s, 1H), 8.20 (s, 1H), 7.38-7.31 (m, 5H), 6.93 (s, 1H), 6.40 (s, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 170.6, 147.8, 145.0, 140.8, 138.0, 129.2, 129.0 (2C), 128.1 (2C), 117.4, 102.6, 81.0. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{14}H_{10}O_5$: 259.06010; found: 259.05973.

4.1.5.6. *4,5,6-trihydroxy-3-(4-hydroxyphenyl)isobenzofuran-1(3H)-one* (***4f***)

Following the above procedure using **3f** (100 mg, 0.33 mmol, 1 equiv.), BBr_3 (1.98 mL, 1 M in CH_2Cl_2, 1.98 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Filtration over a plug of silica using ethyl acetate as eluant was performed to get the desired compound **4f** (33 mg, yield = 33%) as dark solid. Mp > 250°C (decomposition). ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.12 (s, 1H), 8.40 (s,

1H), 8.20 (s, 1H), 7.38-7.31 (m, 5H), 6.93 (s, 1H), 6.40 (s, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 170.2, 157.9, 147.4, 140.2, 139.5, 129.1 (2C), 129.0, 128.2, 116.7, 115.2 (2C), 101.8, 80.4. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{14}H_{11}O_6$: 275.0550; found: 275.0546.

4.1.5.7. *3-(2,3-dihydroxyphenyl)-4,5,6-trihydroxyisobenzofuran-1(3H)-one* (**4g**)

Following the above procedure using **3g** (100 mg, 0.25 mmol, 1 equiv.), BBr_3 (2 mL, 1 M in CH_2Cl_2, 2 mmol, 8 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (90:10) as eluent was performed to get the desired compound **4g** (30 mg, yield = 44%) as dark brown solid.

Mp = 180-190°C. ^{1}H NMR (300 MHz, acetone-d_6) δ = 8.45 (s, 5H), 6.92 (s, 1H), 6.86 (dd, *J* = 7.9 Hz, 1.5 Hz, 1H), 6.78 (s, 1H), 6.67 (t, *J* = 7.9 Hz, 1H), 6.43 (dd, *J* = 7.9 Hz, 1.5 Hz, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 171.3, 148.1, 145.8, 144.2, 140.6, 139.8, 129.5, 124.9, 120.8, 118.8, 118.0, 116.3, 103.2, 75.9. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{14}H_{10}O_7$: 29104993; found: 291.04940.

4.1.5.8. *3-(4-(chlorophenyl)-4,5,6-trihydroxy-isobenzofuran-1(3H)-one* (**4h**)

Following the above procedure using **3h** (100 mg, 0.29 mmol, 1 equiv.), BBr_3 (1.74 mL, 1 M in CH_2Cl_2, 1.74 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (95:5) as eluent was performed to get the desired compound **127l** (27mg, yield = 32%) as a brownish white solid. Mp = 185-188°C. ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.43 (br s, 1H), 8.52-8.31 (br s, 2H), 7.42 (d, *J* = 8.7 Hz, 2H), 7.35 (d, *J* = 8.7 Hz, 2H), 6.92 (s, 1H), 6.41 (s, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 170.0, 147.5, 140.1, 139.4, 136.4, 133.9, 129.3 (2C), 128.6, 128.5 (2C), 116.5, 102.1, 79.4. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{14}H_{10}ClO_5$: 293,0211; found: 293.0217.

4.1.5.9. *4,5,6-trihydroxy-3(4-(trifluoromethyl)phenyl)isobenzofuran-1(3H)-one* (***4i***)

Following the above procedure using **3i** (160 mg, 0.43 mmol, 1 equiv.), BBr_3 (2.58 mL, 1 M in CH_2Cl_2, 2.58 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (95:5) as eluent was performed to get the desired compound **4i** (60 mg, yield = 42%) as brownish white solid. Mp = 233-236°C. ^{1}H NMR (300 MHz, methanol-d_4) δ = 7.66 (d, *J* = 8.2 Hz, 2H), 7.49 (d, *J* = 8.1 Hz, 2H), 6.89 (s, 1H), 6.46 (s, 1H). ^{13}C NMR (75 MHz, methanol-d_4) δ = 170.8, 146.6, 140.0, 139.0, 138.0, 129.1 (q, *J* = 32.3 Hz), 127.4, 126.6 (2C), 123.8 (q, *J* = 3.8 Hz, 2C), 122.9 (q, *J* = 270.0 Hz), 114.0, 100.4, 79.0. ^{19}F NMR

(282 MHz, methanol-d_4) δ = −64.1. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{15}H_9F_3O_5$: 327.04748; found: 327.04696.

4.1.5.10. *4,5,6-Trihydroxy-3(4-(trifluoromethoxy)phenyl)isobenzofuran-1(3H)-one* (***4j***):

Following the above procedure using **3j** (150 mg, 0.39 mmol, 1 equiv.), BBr_3 (2.34 mL, 1 M in CH_2Cl_2, 2.34 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (95:5) as eluent was performed to get the desired compound **4j** (60 mg, yield = 45%) as a white solid.

Mp = 155-158°C. ^{1}H NMR (300 MHz, acetone-d_6) δ = 8.64 (s, 3H), 7.48 (d, *J* = 8.7 Hz, 2H), 7.35 (d, *J* = 8.7 Hz, 2H), 6.93 (s, 1H), 6.47 (s, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 169.8, 149.1 (q, *J* = 2.2 Hz), 147.4, 140.1, 139.3, 136.7, 129.4 (2C), 128.7, 121.0 (2C), 120.5 (*J* = 254 Hz), 116.6, 102.2, 79.3. ^{19}F NMR (282 MHz, acetone-d_6) δ = −58.6. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{15}H_9F_3O_6$: 343.04240; found: 343.04186.

4.1.5.11**.** *4,5,6-trihydroxy-3(3-(trifluoromethyl)phenyl)isobenzofuran-1(3H)-one* (***4k***)

Following the above procedure using **3k** (282 mg, 0.77 mmol, 1 equiv.), BBr_3 (4.62 mL, 1 M in CH_2Cl_2, 2.62 mmol, 6 equiv.) and CH_2Cl_2 (5 mL). Chromatography on silica gel with dichloromethane/methanol (90:10) as eluent was performed to get the desired compound **4k** (170 mg, yield = 68%) as brown solid. Mp = 233-236°C. ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.20 (br s, 1H), 8.48 (br s, 1H), 8.28 (br s, 1H), 7.75-7.69 (m, 2H), 7.66-7.62 (m, 2H), 6.95 (s, 1H), 6.55 (s, 1H). ^{13}C NMR (75 MHz, acetone-d_6) δ = 169.8, 147.4, 140.0, 139.1, 138.9, 132.1, 130.2 (q, *J* = 32 Hz), 129.5, 128.4, 125.4 (q, *J* = 3.7 Hz), 124.3 (q, *J* = 270.0 Hz), 124.2 (*J*= 3.7 Hz), 116.5, 102.3, 79.3. ^{19}F NMR (282 MHz, acetone-d_6) δ = −63.1. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{15}H_9F_3O_5$: 327.04748; found: 327.04700.

4.1.6. *Ethyl 3,4,5-tri((tert-butoxycarbonyl)oxy benzoate (**6**).*

A dry and argon-flushed round bottom flask was charged with a solution of ethyl gallate **5** (2.97 g, 15 mmol, 1 equiv.) in dried dichloromethane (50 mL) and stirred at 0°C, then trimethylamine (1.5 mL, 20 mmol, 1.3 equiv.), DMAP (244 mg, 2 mmol, 0.1 equiv.) and Boc_2O (11.7g, 54 mmol, 3.6 equiv.) were added. The reaction mixture was stirred overnight at room temperature and saturated aqueous solution of NH_4Cl (40 mL) was added. The aqueous phase was washed

with dichloromethane (3 x 100 mL). The combined organic phases were washed with brine (100 mL), dried over $MgSO_4$ and solvent was removed under reduce pressure. The residue was purified by column chromatography on silica gel using petroleum ether/diethyl ether (85:15) as eluent to afford the compound **6** (7.2 g, yield = 97%) as a white solid. Mp = 77-80°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.84 (s, 1H), 4.36 (q, *J* = 7.2 Hz, 2H), 1.69-1.46 (m, 27H), 1.37 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 164.5 150.1, 148.8, 143.7, 138.9, 128.2, 121.7, 84.5, 84.4, 61.6, 27.6, 25.5, 14.3.

4.1.7. *Tri-tert-butyl(3-(4-(dimethylamino)phenyl)-1-oxo-1,3-dihydroisobenzofuran-4,5,6-triyl)tricarbonate* (***7***)

A dry and argon-flushed round bottom flask was charged with a solution of **6** (400mg, 0.8 mmol, 1 equiv.) in dry THF (4 mL). The mixture was stirred under argon atmosphere at –20°C and TMP.MgCl.LiCl (0.88 mL, 0.88 mmol, 1.1 equiv.) was added dropwise. The reaction mixture was stirred for 3 hours at –40°C and 4-(dimethyl amino)benzaldehyde (180 mg, 1.2 mmol, 1.5 equiv.) was added. The reaction mixture was stirred for 3 hours at –40°C and saturated aqueous solution of NH_4Cl (30 mL) was added at –40°C. The aqueous phase was washed with dichloromethane (3 x 30 mL). The combined organic phases were washed with brine (50 mL), dried over $MgSO_4$ and solvent was removed under reduce pressure. The residue was purified by flash chromatography using petroleum ether: diethyl ether (80:20) as eluent to afford compound **7** (250 mg, yield= 52%) as a white solid. Mp = 110-113°C^{1}H NMR (300 MHz, $CDCl_3$) δ= 7.78 (s, 1H), 7.04(d, *J* = 8.8 Hz, 2H), 6.62(d, *J* = 8.8 Hz, 2H), 6.42 (s, 1H), 2.94 (s, 6H), 1.56 (s, 9H), 1.52 (s, 9H), 1.36 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ= 169.6, 151.4, 149.9, 148.6, 148.5, 145.2, 140.3, 139.4, 138.8, 129.1 (2C), 124.6, 120.5, 117.1, 112.2 (2C), 84.9, 84.8, 84.7, 82.3, 40.3 (2C), 27.6 (3C), 27.5 (3C), 27.4 (3C). HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{31}H_{39}NO_{11}$: 602.25959; found: 602.25876.

4.1.8. *3-(4-(dimethylamino)phenyl)-4,5,6-trihydroxyisobenzofuran-1(3H)-one* (***4l***).

A dry and argon-flushed flask equipped with a magnetic stirrer and a septum was charged with a solution of **7** (200 mg, 0.33 mmol, 1 equiv.) in trifluoroacetic acid (3 mL) and stirred for 15 minutes. Excess trifluoroacetic acid was evaporated. Reaction mixture was filtered through a pad of silica gel. Compound **4l** (80 mg, yield= 81%) was obtained as a black solid. Mp = 120-123°C. ^{1}H NMR (300 MHz, acetone-*d*$_6$) δ= 8.15 (s, 3H), 7.18 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.7 Hz, 3H), 6.33 (s, 1H), 3.01 (s, 6H). ^{13}C NMR (75 MHz, acetone-*d*$_6$) δ= 171.0, 149.6, 148.2,

141.0, 139.9, 130.0, 129.8, 129.7, 118.0, 115.8, 102.9, 81.2, 42.5. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{16}H_{15}NO_5$: 302.10230; found: 302.10189.

4.1.9. *2-Iodo-4-methoxybenzoic acid* (***9***).[24] A dry and argon-flushed three-neck round bottom flask equipped with a magnetic stirrer, condenser, a bubbler and a bromine funnel was charged with a 50% aqueous solution of sulfuric acid (75 mL) and 4-methoxyantranilic acid **8** (6 g, 36 mmol, 1 equiv.) at 0°C. A solution of sodium nitrite (2.75 g, 30 mmol, 1.1 equiv.) in water (10 mL) was added dropwise and the temperature was kept below 5°C. After 10 minutes of stirring at 0°C, a solution of potassium iodide (8.25 g, 50 mmol, 1.4 equiv.) in water (20 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 hours then heated to 70°C until the nitrogen release was ceased. The reaction mixture was cooled to room temperature and then extracted with ethyl acetate (3 x 50 mL). The organic phases were combined and washed with water (2 x 50 mL) and brine (1 x 50mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford **9** (5.32 g, yield = 53%) as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.05 (d, *J* = 8.9 Hz, 1H), 7.58 (d, *J* = 2.5 Hz, 1H), 6.94 (dd, *J* = 8.9 Hz, 2.5 Hz, 1H), 3.87 (d, *J* = 7.8 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d6) δ = 166.9, 161.4, 132.1, 131.3, 127.2, 113.7, 96.0, 55.7.

4.1.10. *Methyl-2-iodo-4-methoxybenzoate* (***10***).[25] A dry and argon-flushed round bottom flask equipped with a magnetic stirrer and a septum was charged with a solution of **9** (5 g, 18 mmol, 1 equiv.) in dry acetonitrile (40 mL). Iodomethane (1.46 mL, 23.4 mmol, 1.3 equiv.) and DBU (3.95 mL, 19.8 mmol, 1.1 equiv.) were successively added and the reaction mixture was stirred overnight at room temperature. The solvent was removed and the residue was diluted in ethyl acetate (50 mL). The organic phase was washed with water (3 x 50 mL), a aqueous solution of HCl 1 M (2 x 50mL), a aqeous saturated solution of $NaHCO_3$ (2 x 50mL) and brine (50mL). The organic phase was the dried over $MgSO_4$, filtered and solvent was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (95:5) as eluent to afford **10** (3.11 g, yield = 60%) as clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.85 (d, *J* = 8.8 Hz, 1H), 7.52 (d, *J* = 2.5 Hz, 1H), 6.90 (dd, *J* = 8.8, 2.6 Hz, 1H), 3.89 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 166.0, 162.0, 132.5, 131.6, 127.0, 113.6, 95.6, 55.6, 52.1.

4.1.11. *3-Heptyl-5-methoxyisobenzofuran-1(3H)-one* (***11***). A dry and argon-flushed flask equipped with a magnetic stirrer and a septum was charged with a solution of **10** (500 mg,

1.7 mmol, 1 equiv.) in dry THF (10 mL) and was cooled at −78°C. A solution of *i*PrMgCl·LiCl (2.35 mL, 0.94 M solution in THF, 2.2 mmol, 1.3 equiv) was added dropwise and the reaction mixture was stirred for 30 minutes at −78°C. Then octanal (345 µL, 2.2 mmol, 1.3 equiv.) was added and the reaction mixture was slowly warm to room temperature and stirred overnight. The reaction mixture was hydrolysed with water (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (90:10) as eluent to afford **11** (230 mg, yield = 52%) as clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.76 (d, *J* = 8.5 Hz, 1H), 6.98 (dd, *J* = 8.5 Hz, 2.1 Hz, 1H), 6.81 (d, *J* = 2.1 Hz, 1H), 5.35 (dd, *J* = 7.9 Hz, 4.0 Hz, 1H), 3.87 (s, 3H), 1.96 (d, *J* = 4.1 Hz, 2H), 1.70 (d, *J* = 8.1 Hz, 2H), 1.35-1.15 (m, 8H), 0.83 (d, *J* = 7.0 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.8, 165.0, 153.3, 127.6, 118.9, 116.5, 106.3, 81.1, 56.2, 35.2, 32.1, 29.7, 29.5, 25.2, 23.0, 14.5. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{16}H_{22}O_3$: 236.1642; found. 263.1645.

4.1.12. 3-Heptyl-5-hydroxylisobenzofuran-1(3H)-one (**12**). A dry and argon-flushed flask equipped with a magnetic stirrer and a septum was charged with a solution of **11** (191 mg, 0.73 mmol, 1 equiv.) in dry CH_2Cl_2 (10 mL) and was cooled at −40°C. A solution of BBr_3 (1.46 mL, 1 M in CH_2Cl_2, 1.46 mmol, 2 equiv.) was added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was hydrolysed with water (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate (60:40) as eluent to afford **12** (102 mg, yield = 56 %) as white solid. Mp = 83-86°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.76 (d, *J* = 8.3 Hz, 1H), 6.97 (d, *J* = 8.3 Hz, 1H), 6.85 (s, 1H), 6.33 (s, 1H), 5.39 (dd, *J* = 7.7 Hz, 4.1 Hz, 1H), 2.07-1.94 (m, 1H), 1.80-1.68 (m, 1H), 1.53-1.40 (m, 2H), 1.40-1.20 (m, 8H), 0.87 (t, *J* = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 171.1, 161.5, 153.2, 127.6, 118.3, 117.3, 108.1, 81.0, 34.7, 31.7, 29.3, 29.1, 24.7, 22.6, 14.1. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{15}H_{20}O_3$: 249.1485; found. 249.1489.

4.1.13. *4,6-Dichloro-3-heptyl-5-hydroxylisobenzofuran-1(3H)-one* (***13***). A dry and argon-flushed flask equipped with a magnetic stirrer and a septum was charged with a solution of **12** (60 mg, 0.24 mmol, 1 equiv.) in toluene (5 mL), di*iso*propylamine (1 µL, 0.007 mmol,

0.03 equiv.) and sulfuryl chloride (36 µL, 0.44 mmol, 1.8 equiv.) were added successively. The reaction mixture was stirred at 70°C for 1 hour, then cooled to room temperature and hydrolysed with methanol (5 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using dichloromethane (100%) as eluent to afford **13** (26 mg, yield = 43%) as white solid. Mp = 119-122°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.83 (s, 1H), 6.54 (s, 1H), 5.49 (dd, *J* = 7.7 Hz, 2.6 Hz, 1H), 2.40-2.25 (m, 2H), 1.87-1.74 (m, 2H), 1.40-1.15 (m, 8H), 0.86 (t, *J* = 6.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 168.2, 152.7, 147.4, 125.1, 123.0, 120.1, 115.1, 80.6, 32.0, 31.7, 29.1, 29.0, 24.4, 22.6, 14.1. HRMS (ESI): *m/z* calcd for $[M+H]^+$ $C_{15}H_{18}Cl_2O_3$: 317.0706; found. 317.0712.

4.2. Biology

4.2.1. *Antibacterial assays*

Tested compounds were dissolve in DMSO to get a mother solution of 3 mg/mL and then further dilutions were made in Mueller Hinton (MH) to get a range of tested concentration (200, 100, 50, 25, 12.5, 6.25. 3.125 µg.mL^{-1}). Broth microdilution method and numerations were used for the determination of minimal inhibitory and bactericidal concentrations (MIC and MBC).

4.2.1.1. *Bacterial culture*

S. aureus and *E. coli* strains were cultured on Luria-Bertani (LB) plates overnight. Several isolated colonies were suspended in 5 mL of MH media and grown overnight at 37°C, under agitation (200 rpm). Optical density (OD) was measured and then several dilutions of this fresh suspension were prepared in MH medium to obtain 2.10^5 CFU/mL (CFU for colony forming unit). The inoculum size was verified by plating 5-fold dilutions on LB plates and incubating overnight at 37°C for CFU counts.

Subculturing of PAO1 Lux strain was performed on TSA agar from an aliquot stored at −80°C. After one night at 37°C, few colonies were suspended in 3 mL of LB medium and incubated at 37°C, with agitation at 200 rpm, overnight. The next day, several dilutions were made in LB medium to obtain OD between 0.3-0.6. This dilution was then incubated at 37°C, with agitation for approximately 3 hours, then it was centrifuged at 3000 *g* for 10 minutes, at 6°C. Then the bacterial pellet was suspended in MH and adjusted to an OD of 0.4, corresponding to 2.10^8 CFU/mL, which was then diluted to obtain a bacterial suspension containing 2.10^5 CFU/mL

The inoculum size was verified by plating 100 µL 3-fold dilutions on TSA agars and incubating overnight at 37°C for CFU counts.

4.2.1.2. *MIC and MBC evaluation*

Hundred microliter/well of the bacterial suspension was inoculated into 96-well microtiter plate (Greiner Bio-One; Cellstar/*S. aureus* and *E. coli*), (Fisher Scientific, Illkirch-Graffenstaden, France/*PAO*1). 100 µL/well of MH (control) or molecules was added in duplicate for each condition. The microtiter plate was incubated in Eon thermo-regulated spectrophotometer plate reader (BioTek Instruments / *S. aureus* and *E. coli*), (Tecan, Lyon, France / *PAO*1) for 24 h at 37°C in ambient air with continuous shaking. The absorbance at OD 600 nm was read at 30 min intervals. After MIC determination, corresponding to the first well without bacterial growth, the MBC was evaluated. For that purpose, 50 µL of each well were 5-fold diluted and each of dilutions were plated on a LB agar plate, which was then incubated at 37°C for 24 h. The MBCs were recorded as the lowest dilution that produced a ≥ 99.99% reduction in growth (≥4-log10 reduction in CFU/mL) in comparison to the initial inoculum ~~(Figure 32)~~. This test were performed in triplicate at three independent times.

4.2.2. *Cytotoxicity assays*

4.2.2.1. Culture, maintenance and preparation of Beas-2B cells

The Beas-2B line is a line of human bronchial epithelial cells transformed with the SV40 virus. These cells are adherent and become immortal. A culture medium F12Mix+Glutamax (Ref 31765-027 Gibco) was used, to which was added Fetal Bovine Serum (FBS) at 10% final as well as Penicillin-Streptomycin (P/S) at 1% final. This medium was called complete F12K medium. The cells were stored in complete F12K culture medium to which was added 10% DMSO, at −196°C. For their preparation, the cells were first thawed rapidly at 37°C with stirring. They were then diluted in 10 mL of complete medium before centrifugation at 150 g for 5 minutes, at 20°C. Once the supernatant has been removed, the cells were cultured in 75 cm² dishes in the presence of 15 mL of complete medium, then incubated at 37°C under a humid atmosphere in the presence of 5% CO_2. After 48 hours of incubation, the medium was replaced with new complete medium. The medium was removed as the cell were at confluence and the cells were detached from the dish by the action of trypsin, an endoprotease which hydrolyzes peptide bonds. After rinsing the cell layer with 2 mL of trypsin, 3 mL were added and the flask was incubated 1 at 2 minutes at 37°C, under a humid atmosphere in the presence of 5% CO_2. After cell detachement the action of the trypsin was neutralized by the addition of 7 mL of

complete medium. The cells were then transferred to a Falcon tube and 100 µL are taken in order to determine the total number of cells by the Malassez cell counting technique. Centrifugation of the tube was carried out for 5 minutes at 150 g, at 20°C. The supernatant has been removed and the cells were placed in culture at a rate of 300,000 in 15 mL of complete medium in a 75 cm² dish. The dish was then incubated at 37°C in a humid atmosphere in the presence of 5% CO_2.

4.2.2.2. Principle of the MTS test[26]

Cellular cytotoxicity is linked to cell survival. The latter can be evaluated by a colorimetric method based on the detection of the conversion of a tetrazolium derivative (MTS) by cells metabolically active, CellTiter 96® Aqueous One Solution Cell Proliferation Assay Promega was used. Succinate dehydrogenases in cells reduce the yellow tetrazolium compound MTS, also called (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, yellow into purple formazan, soluble in the culture medium. Thus, after incubation for 1 hour, the intensity of the purple color is directly proportional to the number of living cells. A determination was carried out by measuring the absorbance of the formazan at 490 nm. The cytotoxicity was calculated as following: Cell viability (%) = [OD of exposed cells / (OD of control cells)] x 100. A compound was described as non-cytotoxic when the resulting cell viability is greater than 80%. The cells were seeded in a 96-well plate at a rate of 10 000 cells in 150 µL of medium. Some wells were not inoculated to serve as a positive control (control cells) for MTS. The cells were incubated for 48 hours in a humid atmosphere in the presence of 5% CO_2, at 37°C. After incubation and verification of the confluence of the cells, the wells were washed with 200 µL of medium without FBS, then 100 µL of each compound were deposited per well, at a rate of 6 wells per compound (or dilution) tested. The MTS was diluted to 1/6 in FK12 without FBS and 100 µL were deposited in each well. The plate was then incubated for 1 hour in a humid atmosphere in the presence of 5% CO_2, at 37°C. After the incubation was completed, the plate was placed in the Tecan spectrometer to measure the absorbance of the wells at 490 nm. The results were normalized against the control MTS and expressed as a percentage. This test was perfomed in duplicata at two independent times

Acknowledgements

We acknowledge Prof. Leslie Delaye-Boudescoque for her helpful discussion and for providing the NaDES solvent. We would also like to thank Dr. Frédéric Montigny (University of Tours)

for mass spectra and HRMS and the French Ministry for Research and Innovation for the financial support.

References and notes

[1] C. L. Ventola. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277-283.
[2] (a) S. B. Levy, B. Marshall, *Nat. Med.* **2004**, *10*, S122-S129 http://doi.org/10.1038/nm1145; (b) A. P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, D. L. Monnet, *Clinical Microbiology and Infection* **2012**, *18*, 268-281 http://doi.org/10.1111/j.1469-0691.2011.03570.x
[3] E. Tacconelli, E. Carrara, A. Savoldi, S. Harbarth, M. Mendelson, D. L. Monnet, C. Pulcini, G. Kahlmeter, J. Kluytmans, Y. Carmeli, M. Ouellette, K. Outterson, J. Patel, M. Cavaleri, E. M. Cox, C. R. Houchens, M. L. Grayson, P. Hansen, N. Singh, U. Theuretzbacher, N. Magrini, W. H. O. P. P. L. W. Group, *Lancet. Infect. Dis.* **2018**, *18*, 318-327 http://doi.org/10.1016/S1473-3099(17)30753-3
[4] (a) S. Gatadi, J. Gour, S. Nanduri, *Biorg. Med. Chem.* **2019**, *27*, 3760-3774 http://doi.org/10.1016/j.bmc.2019.07.023; (b) C. G. Bologa, O. Ursu, T. I. Oprea, C. E. Melançon, G. P. Tegos, *Curr. Opin. Pharmacol.* **2013**, *13*, 678-687 http://doi.org/10.1016/j.coph.2013.07.002; (c) R. H. Baltz, V. Miao, S. K. Wrigley, *Nat. Prod. Rep.* **2005**, *22*, http://doi.org/10.1039/b416648p; (d) Q. Kong, Y. Yang, *Bioorg. Med. Chem.* **2021**, *35*, http://doi.org/10.1016/j.bmcl.2021.127799
[5] (a) R. Karmakar, P. Pahari, D. Mal, *Chem.Rev.* **2014**, *114*, 6213-6284 http://doi.org/10.1021/cr400524q; (b) A. Awasthi, M. Singh, G. Rathee, R. Chandra, *RSC Advances* **2020**, *10*, 12626-12652 http://doi.org/10.1039/d0ra00701c
[6] A. D. Kinghorn, H. Falk, S. Gibbons, Y. Asakawa & J. K. Liu (Eds.). (2017). Progress in the chemistry of organic natural products.
[7] K. Liberra, R. Jansen, U. Lindequist, *Pharmazie* **1998**, *53*, 578-581 http://doi.org/10.1002/chin.199847275
[8] K. A. Dekker, T. Inagaki, T. D. Gootz, K. Kaneda, E. Nomura, T. Sakakibara, S. Sakemi, Y. Sugie, Y. Yamauchi, N. Yoshikawa, N. Kojima, *J. Antibiot.* **1997**, *50*, 833-839 http://doi.org/10.7164/antibiotics.50.833
[9] S. F. Brady, M. M. Wagenaar, M. P. Singh, J. E. Janso, J. Clardy, *Org. Lett.* **2000**, *2*, 4043-4046 http://doi.org/10.1021/ol006680s
[10] W. Ibraheem, Q. Wils, E. Camiade, E. Ahmed, J. Thibonnet, E. Thiery, J. Petrignet, *Tetrahedron Lett.* **2021**, *67*, http://doi.org/10.1016/j.tetlet.2021.152888
[11] (a) H. Neumann, D. Strübing, M. Lalk, S. Klaus, S. Hübner, A. Spannenberg, U. Lindequist, M. Beller, *Org. Biomol. Chem.* **2006**, *4*, 1365 http://doi.org/10.1039/b517101f; (b) A. Mikolasch, S. Hessel, M. G. Salazar, H. Neumann, K. Manda, D. Gordes, E. Schmidt, K. Thurow, E. Hammer, U. Lindequist, M. Beller, F. Schauer, *Chem. Pharm. Bull.* **2008**, *56*, 781-786 http://doi.org/10.1248/cpb.56.781.
[12] (a) F. J. Radcliff, J. D. Fraser, Z. E. Wilson, A. M. Heapy, J. E. Robinson, C. J. Bryant, C. L. Flowers, M. A. Brimble, *Bioorg. Med. Chem.* **2008**, *16*, 6179-6185 http://doi.org/10.1016/j.bmc.2008.04.037; (b) Z. E. Wilson, A. M. Heapy, M. A. Brimble, *Tetrahedron* **2007**, *63*, 5379-5385 http://doi.org/10.1016/j.tet.2007.04.067; (c) I. Dimitrov, D. P. Furkert, J. D. Fraser, F. J. Radcliff, O. Finch, M. A. Brimble, *MedChemComm* **2012**, *3*, 938 http://doi.org/10.1039/c2md00314g
[13] (a) J. D. Hall, N. W. Duncan-Gould, N. A. Siddiqi, J. N. Kelly, L. A. Hoeferlin, S. J. Morrison, J. K. Wyatt, *Bioorg. Med. Chem.* **2005**, *13*, 1409-1413 http://doi.org/10.1016/j.bmc.2004.10.018; (b) M. P. Singh, J. E. Janso, S. F. Brady, *Mar. Drugs* **2007**, *5*, 71-84 http://doi.org/10.3390/md503071; (c) J. Beau, N. Mahid, W. N. Burda, L. Harrington, L. N. Shaw, T. Mutka, D. E. Kyle, B. Barisic, A. van Olphen, B. J. Baker, *Mar. Drugs* **2012**, *10*, 762-774 http://doi.org/10.3390/md10040762; (d) J. Kornsakulkarn, W. Somyong, S. Supothina, N. Boonyuen, C. Thongpanchang, *Tetrahedron* **2015**, *71*, 9112-9116 http://doi.org/10.1016/j.tet.2015.10.015

[14] E. L. Mullady, W. P. Millett, H.-D. Yoo, A. S. Weiskopf, J. Chen, D. DiTullio, V. Knight-Connoni, D. E. Hughes, W. E. Pierceall, *J. Nat. Prod.* **2004**, *67*, 2086-2089 http://doi.org/10.1021/np040123n
[15] T. Takano, T. Koseki, H. Koyama, Y. Shiono, *Nat. Prod. Commun.* **2014**, *9*, http://doi.org/10.1177/1934578x1400900723
[16] T. Ohzeki, K. Mori, *Biosci. Biotechnol. Biochem.* **2003**, *67*, 2584-2590 http://doi.org/10.1271/bbb.67.2584
[17] (a) J. T. Kuethe, K. M. Maloney, *Tetrahedron* **2013**, *69*, 5248-5258 http://doi.org/10.1016/j.tet.2013.02.051; (b) A. Krasovskiy, P. Knochel, *Angew. Chem. Int. Ed.* **2004**, *43*, 3333-3336 http://doi.org/10.1002/anie.200454084
[18] K. Ohba, Y. Koga, S. Nomura, M. Nakata, *Tetrahedron Lett.* **2015**, *56*, 1007-1010 http://doi.org/10.1016/j.tetlet.2015.01.054
[19] W. Lin, O. Baron, P. Knochel, *Org. Lett.* **2006**, *8*, 5673-5676 http://doi.org/10.1021/ol0625536
[20] S. Niu, L. Si, D. Liu, A. Zhou, Z. Zhang, Z. Shao, S. Wang, L. Zhang, D. Zhou, W. Lin, *Eur. J. Med. Chem.* **2016**, *108*, 229-244 http://doi.org/10.1016/j.ejmech.2015.09.037
[21] Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI document M07–A9 2012 Wayne, PA.
[22] K. Ohba, Y. Koga, S. Nomura, M. Nakata, *Tetrahedron Lett.* **2015**, *56*, 1007-1010 http://doi.org/10.1016/j.tetlet.2015.01.054
[23] H.-S. Lin, L. A. Paquette, *Synth. Commun.* **1994**, *24*, 2503-2506 http://doi.org/10.1080/00397919408010560
[24] A. D. Marchese, T. Adrianov, M. F. Köllen, B. Mirabi, M. Lautens, *ACS Catalysis* **2021**, *11*, 925-931 http://doi.org/10.1021/acscatal.0c04956
[25] K. Vong, T. Yamamoto, T.-c. Chang, K. Tanaka, *Chem. Sci.* **2020**, *11*, 10928-10933 http://doi.org/10.1039/d0sc04329j
[26] A. H. Cory, T. C. Owen, J. A. Barltrop, J. G. Cory, *Cancer Commun.* **1991**, *3*, 207-212 http://doi.org/10.3727/095535491820873191