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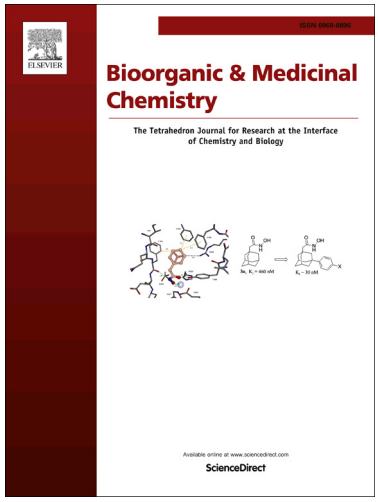

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Graphical Abstract

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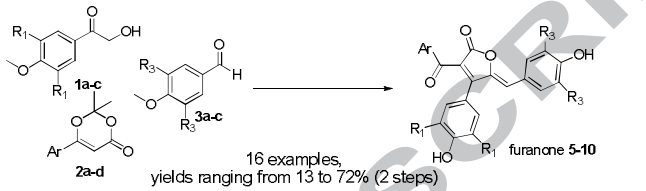
Agathe Boulangé[a], Javier Parraga[b], Abraham Galán[b], Nuria Cabedo[b,c], Stéphane Leleu[a], Maria Jesus Sanz,[d,e] Diego Cortes[b] and Xavier Franck[a,*]



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ABSTRACT

The one-pot multicomponent synthesis of natural butenolides named cadiolides A, B, C and analogues has been realized. The antibacterial structure activity relationship shows that the presence of phenolic hydroxyl groups and the number and position of bromine atoms on the different aromatic rings are important features for antibacterial activity, besides it was demonstrated the tolerance of both benzene and furan ring at position 3 of the butenolide nucleus. Furthermore, none of the most relevant antibacterial compounds showed any cytotoxicity in freshly isolated human neutrophils.

1. Introduction

Natural products are a rich source of chemical diversity by constantly bringing new skeletons to light. This is highly demanded for the development of new drugs and particularly for antibacterial compounds due to worldwide emergence of multidrug resistance. The development of resistance to classic antibiotics has emerged from antibiotic use for both veterinary medicine and human medicine. This reduces the effectiveness of current treatments for infections and becomes a major threat for public health causing a large number of sick people, hospitalizations and deaths.[1] Because of that, new antibacterial molecules are actively sought and this encourages us to devote our research efforts to find new active agents.[2]

Marine butenolides of the cadiolide family, isolated from ascidians or tunicates, have shown wide spectrum antibacterial activities.[3] They are characterized by a tris-aromatic furanone skeleton, where aryl groups are phenols bearing usually one or two bromine atoms. Simpler related bis-aromatic furanones have also been isolated from marine organisms and named rubrolides (Figure 1).[3b,4]

Some members of the cadiolide family, and especially cadiolide E, have shown potent inhibition of Candida albicans isocitrate lyase activity.[3d] However, the structure activity relationship of these natural compounds has not yet been established and may be function of the number and position of bromine atoms on the phenolic rings. We recently developed a one pot multicomponent procedure[5] for the preparation of substituted acylfuranones by condensation of a hydroxyketone **1**, a functionalized dioxinone **2** and an aldehyde **3** or a ketone and now report on the application of this methodology to the synthesis of various cadiolide analogues[5d,e] and their antibacterial activities. The versatility of this method allowed us to easily vary the number and position of bromine atoms on the different aromatic rings by preparing different brominated substrates **1**, **2** and **3** (Scheme 1).

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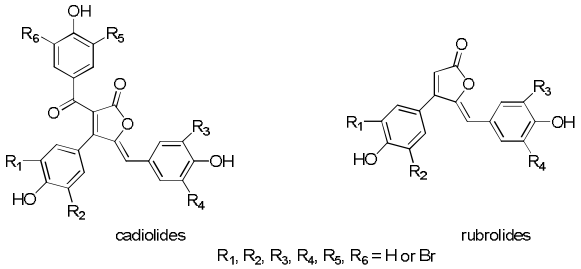
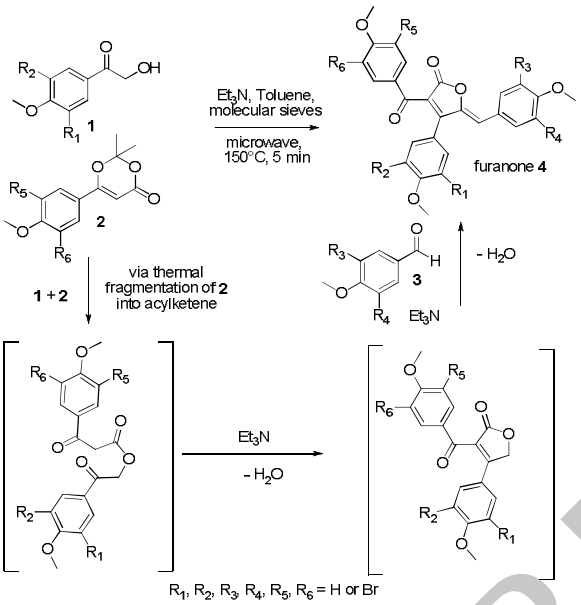


Figure 1. General structures of cadiolides and rubrolides



Scheme 1: Approach to cadiolide analogues

Different aromatic substrates (Figure 2) have then been prepared by mono bromination of the pOMe- or dibromination of pOH-substituted aromatic ring using conventional methods.[6a] Hydroxyketones **1a-1c** have been prepared by bromination of the corresponding acetophenone followed by treatment with sodium formate.[6b] Dioxinones **2a-d** have been synthesized from the corresponding β-ketoesters using our described procedure.[5]

Aldehyde **3a-b** are commercially available and **3c** has been prepared by bromination of pOH-benzaldehyde. Heteroaromatic dioxinone **2d** has also been used in order to determine whether non phenolic substituents were tolerated for biological activity.

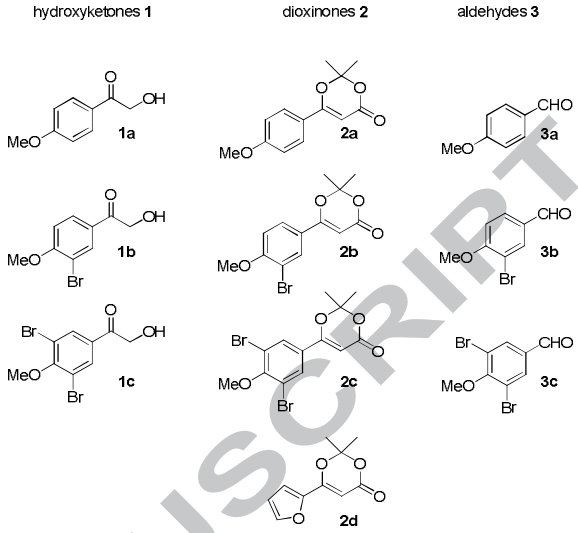
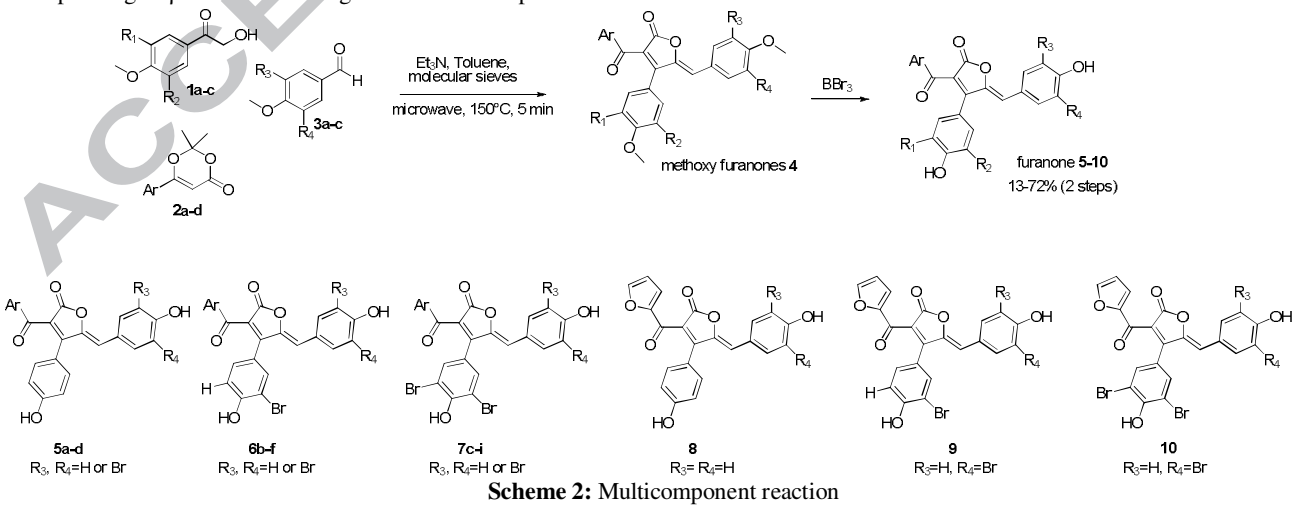


Figure 2: Set of hydroxyketones, dioxinones and aldehydes

Various methoxy-furanones **4** were then prepared by reaction of hydroxyketone **1a-c** with dioxinone **2a-d** and aldehyde **3a-c** at 150°C under microwave irradiation (5 min), in the presence of triethylamine and molecular sieves. Crude reaction mixtures were then subsequently demethylated with BBr_3 to free the phenols and give furanones **5-10**. Yields of **4** usually ranged from 40 to 70% and debromination gave usually good yields of phenols **5-10** (Scheme 2, Table 1). Among them, three natural products were prepared: cadiolide A (**7i**), cadiolide B (**7g**) and cadiolide C (**6f**).



Scheme 2: Multicomponent reaction

2. Bactericidal activity.

A total of 16 substituted furanones **5-10**, including the three natural cadiolides A, B and C, and 14 methoxy-furanones **4** were screened *in vitro* for their antibacterial activity against several human pathogenic bacteria by determination of their minimum inhibitory concentrations (MICs, the lowest concentration of compounds capable of inhibiting the growth of bacteria) and using the 2-fold microtiter method (Table 1, Figure 3).7 Compounds were evaluated against four Gram-positive bacterial strains (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Enterococcus faecalis*) and four Gram-negative (*Salmonella typhi*, *Escherichia coli* 100, *E. coli* 405 and *Erwinia carotovora*). Interestingly, compounds in which the unsaturated butenolide ring is bearing phenolic hydroxyl groups (furanones **5-10**) displayed moderate to significant antibacterial activity against both Gram-positive and Gram-negative strains tested, whereas their methoxy-furanone analogues 4 were completely inactive at the highest concentration tested (125 µg/mL, results not shown). Therefore, the presence of phenolic hydroxyl groups is crucial for antibacterial activity, probably due to their ability to interact with proteins and bacterial cell walls to form complexes.[8] The importance of ionizable phenolic hydroxyl groups to enhance bactericidal activity has been earlier reported.[9] It seems that the phenolic protons can be replaced by another ion (potassium ion or other cation) causing an efflux of cations and influx of protons to the cytoplasm.

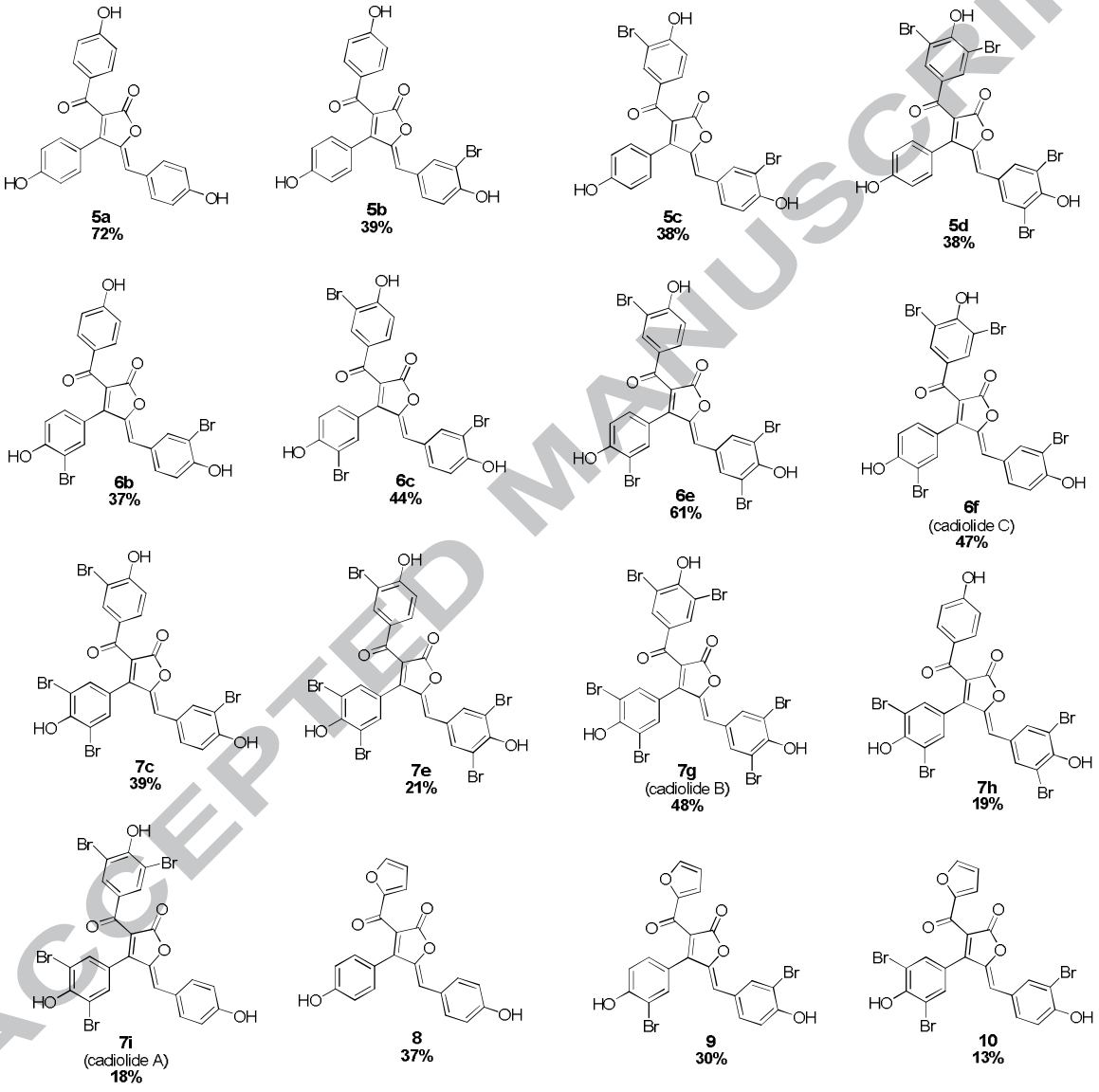


Figure 3: Synthesis of various butenolides **5-10**

Among phenolic analogues, the most potent compounds were the synthetic brominated tris-aromatic furanones **6c**, **7c** and **9**, with a MIC value of 1.95 µg/mL against the following strains tested: *B. cereus, S. aureus, E. faecalis, S. typhi* and *E. coli* 405 (Figure 3, Table 1). The structure-activity relationship (SAR) studies highlighted the importance of both number and location of bromine atoms. Therefore, our initial studies focused on cadiolide analogues bearing a benzoyl substituent attached to position 3 of furanone core showed that 5a analogue devoid of any bromine atom displayed moderate to low activity (MIC from 62.5 to 125 µg/mL). In addition, antibacterial activity dramatically decreased when a single bromine was added on the benzylidene substituent attached to position 5 (compound **5b**). Low activity was also displayed for compound **5c**, with only two bromine atoms located on benzylidene and benzoyl substituents. However, the antibacterial activity was increased when the two

bromine atoms were located one on the benzylidene and one on the phenyl ring, as compound **6b** with MICs ranging from 15.62 to 31.25 μg/mL for *B. cereus, S. aureus, E. faecalis, S. typhi* and *E. coli* 405. However, adding a third bromine atom led to one of the most potent compounds **6c** when a bromine atom was located on each aryl ring. Cadiolide analogues with four bromine atoms resulted in two possible groups of compounds depending on the location of bromine atoms on the aryl rings. The first group had two bromine atoms in one of the aryl rings and a single bromine on each of the other two aryl rings as it occurs for **6e**, **6f** (cadiolide C) and **7c**. Results showed that when the two bromine atoms were on phenyl or benzylidene substituent (**6e** and **7**), higher potency was obtained than when two bromines were on the benzoyl substituent (**6f**, cadiolide C). The second group had two bromine atoms on two aryl rings but the third aryl ring did not have any bromine, like in compounds **5d**, **7i** (cadiolide A) and **7h**. Although the activity of this second group of compounds slightly decreased, they followed the same trend, since **7h** and **7i** (cadiolide A) with the two bromine atoms on phenyl or benzylidene substituent displayed higher potency than on benzoyl substituent (**5d**). A high antibacterial activity was also obtained when a fifth bromine atom was added to yield compound 7e, with MICs ranging from 1.95 to 3.90 μg/mL. However, the antibacterial activity slightly decreased when a sixth bromine was added to give natural product **7g** (cadiolide B) (MICs ranging from 7.81 to 15.62 μg/mL), even though two bromine atoms were located on both benzylidene and phenyl substituents.

In order to improve the antibacterial activity we replaced the benzoyl with furoyl substituent to obtain compounds **8-10**. SAR studies showed that surprisingly compound **8** without any bromine, displayed higher activity than its benzoyl analogue **5a**. The activity increased again when two bromine atoms were added as one on the benzylidene and one on the phenyl ring (compare **9** versus **6b**). In addition, the presence of a third bromine did not seem to affect the activity since compound **10** displayed the same MIC values than compound **9**, which were one of the most potent compounds of this series. Therefore, the replacement of benzoyl by furoyl moiety proved to be advantageous for antibacterial activity in cadiolide analogues.

Table 1. Antibacterial activities.

	MIC (μg.mL-1) 24 h[a]							
Furanone **5-10**	B. cereus	S. aureus	E. faecalis	S. typhi	E. coli 405	E. carotovora	B. subtilis	E. coli 100
5a	62.5	62.5	125.0	125.0	62.5	125.0	> 125.0	62.5
5b	125.0	> 125	> 125	> 125	> 125	> 125	> 125	> 125
5c	3.90	> 125.0	> 125.0	125	125	> 125	> 125	> 125
5d	15.62	15.62	15.62	15.62	15.62	125.0	125.0	> 125
6b	15.62	31.25	31.25	31.25	15.62	125	125	15.62
6c[b]	1.95	1.95	1.95	1.95	1.95	125.0	125.0	> 125
6e	1.95	3.90	1.95	3.90	3.90	125.0	125.0	> 125
6f (cadiolide C)	3.90	3.90	3.90	3.90	3.90	125.0	125.0	> 125
7c[b]	1.95	1.95	1.95	1.95	1.95	125.0	125.0	> 125
7e	3.90	3.90	3.90	3.90	1.95	125.0	125.0	> 125
7g (cadiolide B)	7.81	7.81	7.81	15.62	7.81	> 125	> 125	> 125
7h	3.90	3.90	3.90	3.90	7.81	125.0	125.0	> 125
7i (cadiolide A)	7.81	7.81	7.81	7.81	7.81	125.0	125.0	> 125
8	15.62	15.62	31.25	15.62	31.25	> 125	> 125	31.25
9[b]	1.95	1.95	1.95	1.95	1.95	125.0	125.0	15.62
10[b]	3.90	1.95	1.95	1.95	3.90	125.0	125.0	15.62
tetracycline	3.90	0.24	0.48	7.81	7.81	3.90	0.97	3.90

[a] Doses tested were 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95, 0.97, 0.48 and 0.24 μg/mL. Gram-positive: *Bacillus cereus* (CECT 148), *Staphylococcus aureus* (CECT 86), *Enterococcus faecalis* (CECT 481) and *Bacillus subtilis* (CECT 35); and four Gram-negative: *Salmonella typhi* (CECT 409), *Escherichia coli* 100 (CECT 100), *E. coli* 405 (CECT 405), and *Erwinia carotovora* (CECT 225); [b] For **6c**, IC_{50}= 1.95 μg/mL = 3.06 μM; IC_{50}= 125 μg/mL = 196.21 μM; For **7c**, IC_{50}= 1.95 μg/mL = 2.72 μM; IC_{50}= 125 μg/mL = 174.59 μM; For **9**, IC_{50}= 1.95 μg/mL = 3.66 μM; IC_{50}= 15.62 μg/mL = 29.35 μM; IC_{50}= 125 μg/mL = 234.90 μM; For **10**, IC_{50}= 1.95 μg/mL = 3.19 μM; IC_{50}= 3.90 μg/mL = 6.38 μM; IC_{50}= 15.62 μg/mL = 25.56 μM; IC_{50}= 125 μg/mL = 204.57 μM.

In comparison with tetracycline, it was noteworthy that eight of the butenolides tested including cadiolide C were able to inhibit the bacterial growth against *B. cereus*, *S. typhi* and *E. coli* 405 with MIC values lower or in the same order than the reference antibiotic. For instance: **6c**, **7c** and **9** with MIC of 1.95 μg/mL, **6e**, **7e** and **10** with MICs ranging from 1.95 to 3.90 μg/mL, **6f** (cadiolide C) with MIC of 3.90 μg/mL, and **7h** with MICs ranging from 3.90 to 7.81 μg/mL. Compound **7i** (cadiolide A) required the same concentration than tetracycline to inhibit the bacterial growth of *S. typhi* and *E. coli* 405 with MIC of 7.81

μg/mL. Full dibrominated **7g** (cadiolide B) showed the same potency than tetracycline only against *E. coli* 405 (MIC of 7.81 μg/mL), and **5d**, **6b** and **8** exhibited a moderate antibacterial activity with MIC against *B. cereus*, *S. aureus*, *E. faecalis*, *S. typhi* and *E. coli* 405 ranging from 15.62 to 31.25 μg/mL. Curiously, among all the tested compounds, only **5a**, **6b** and the furan analogues **8, 9** and **10** displayed significant activity against *E. coli* 100 with MIC values ranging from 15.62 to 62.5 μg/mL. In general, the presence of free phenolic groups as unique substituents more than bromine atoms on the benzene rings attached to positions 4 and 5 seems to favor the activity against this bacterium.

Some compounds were active against both bacterial strains *B. subtilis* (Gram-positive) and *E. carotovora* (Gram-negative) at 125 μg/mL, for instance the partially bromineated derivatives such as **6c**, **6f** (cadiolide C), **5d**, **7i** (cadiolide A), **7h**, **7c**, **6e**, **7e**, **6b** and the furan analogues **9** and **10**. Among all tested compounds, the less active was **5b** which was able to inhibit the bacterial growth only against *B. cereus* at 125 μg/mL.

3. Cytotoxicity studies.

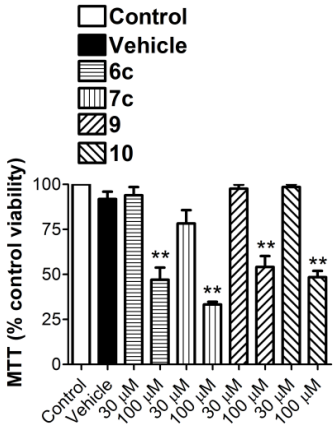


Figure 4: Effect of **6c**, **7c**, **9** and **10** on viability of human neutrophils by MTT assay. Data are presented as mean ± SEM of n=6-9 independent experiments. **p <0.01 relative to the vehicle group.

When testing an antimicrobial activity, it is important to evaluate the lack of toxicity for the host of the synthesized compounds. Therefore, since the compounds 6c, 7c, 9 and 10 exerted the most relevant effects against human pathogens, they were primarily tested at 30 and 100 μM for potential cytotoxic effects in freshly isolated human neutrophils[10] by the use of two different approaches: the MTT (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay[11] and by flow cytometry analysis[12,13] of cell apoptosis and survival.[14] Human neutrophils cell types have already been used for assaying the cytotoxic effects of different semi-synthetic alkaloids with antibacterial activity.[12] All the compounds displayed significant cytotoxicity at 100 μM. However, none of the compounds tested exerted any cytotoxicity at 30 μM on neutrophils (Figure 4). In fact, this dose was 5 to 11-fold higher than the IC_{50} required to kill *S. aureus*, *E. faecalis*, *S. typhi* and *E. coli 405* (IC_{50} values ranged between 2.72 and 6.38 μM, Table 1).

These results were further confirmed when apoptosis, necrosis and survival in this human cell type was determined employing the cytofluorimetric assay. In human neutrophils, most of the compounds tested barely exerted any significant effect on neutrophil apoptosis at the both doses assayed (below the vehicle, Figure 5A). When the survival of human cells was determined, none of the compounds affected this parameter at 30 μM concentration (Figure 5C). In contrast, incubation of the cells with 6c and 9 at 100 μM resulted in 98 and 95% of human neutrophil necrosis respectively (Figure 5B). Compounds 7c and 10 caused a 36 and 57% human neutrophil necrosis when they were tested at 100 μM. Nevertheless, and as found previously for the MTT assay, it is important to note that 30 μM concentration is 5-11 fold higher than the IC_{50} required to kill *S. aureus*, *E. faecalis*, *S. typhi* and *E. coli 405* (Table 1), the most resistant pathogens.

4. Conclusion

In summary, a series of 16 tris-aromatic butenolides including natural cadiolides A, B, C and analogues has been prepared by using a straightforward one-pot multicomponent synthesis (**4** and **5-10**). The antibacterial activity has been evaluated on a panel of pathogenic Gram+ and Gram- bacteria, showing MICs as low as 1.95 $\mu g.mL^{-1}$. The SAR studies showed that phenolic hydroxyl group is necessary but that the biological activity can be dramatically increased by adding bromine atoms on the different aromatic rings with optimal number of 3 to 4 bromine atoms (**6c**, **7c**). We also have shown that a phenolic substituent can be advantageously substituted by a furan ring at position 3 of the butenolide nucleus (**9**, **10**). In addition, none of the most relevant antibacterial compounds showed any cytotoxicity in freshly isolated human neutrophils. The straightforward access to the cadiolide skeleton will easily allow other structural modifications; results will be reported in due course.

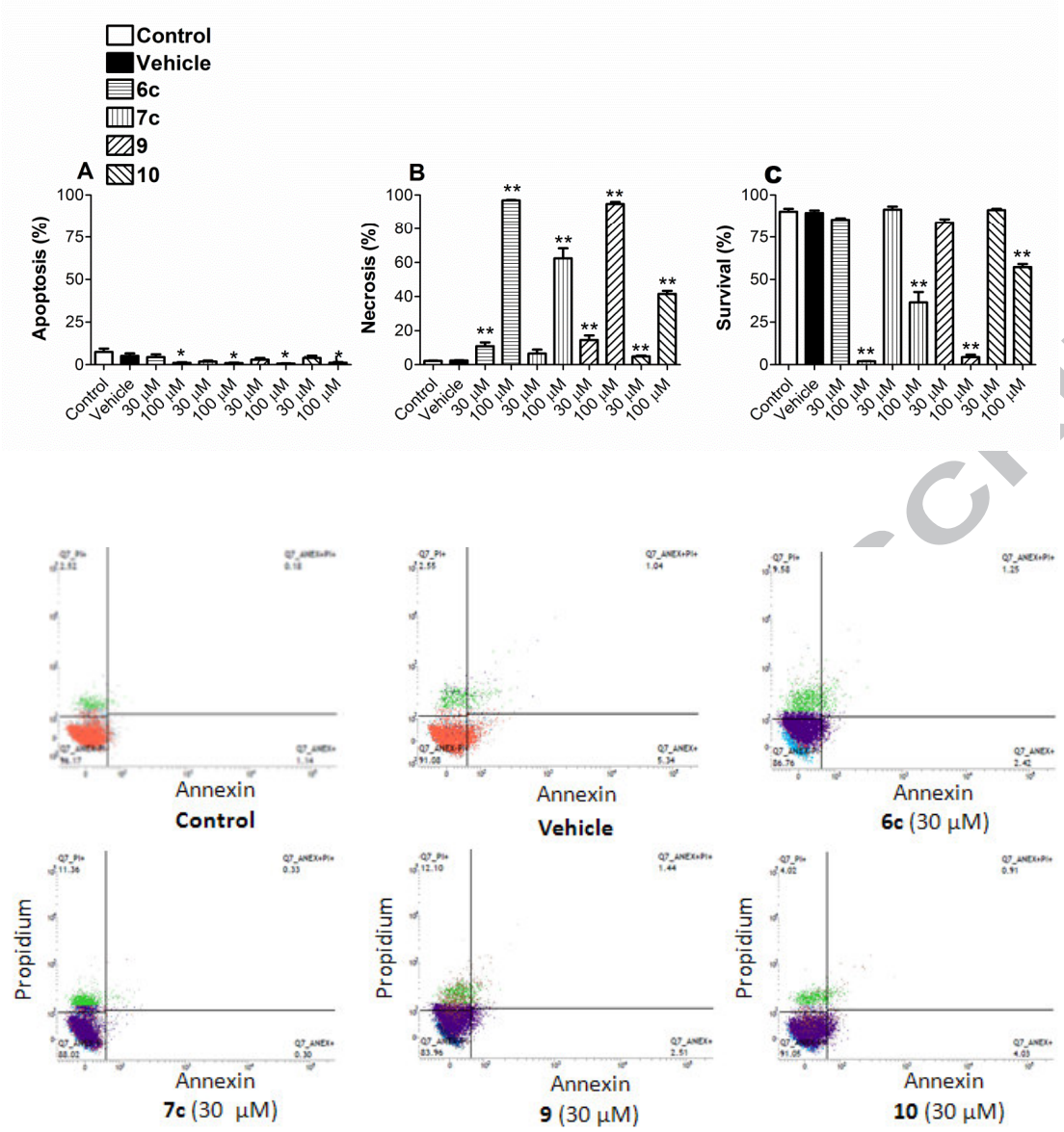


Figure 5: Effect of **6c**, **7c**, **9** and **10** on neutrophil viability by cytofluorometric analysis. Percentage of apoptotic (A), necrotic (B) and survival (C) neutrophils after 24 h incubation with **6c**, **7c**, **9** and **10**. Apoptotic cells were quantified as the percentage of total population of annexin V+, PI– cells, late apoptotic, and/or necrotic cells as annexin V+ and PI+, and viable nonapoptotic cells as annexin V– and PI–. The columns are the mean ± SEM of n=3 independent experiments. Representative flow cytometry panels showing the effects of control, vehicle, **6c**, **7c**, **9** and **10** on neutrophil apoptosis, necrosis and survival have been included. *p <0.05 or **p <0.01 relative to the vehicle group.

5. Materials and Methods

General: All reactions were carried out under a nitrogen or argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Dry tetrahydrofuran (THF), methylene chloride (CH_2Cl_2), and toluene were obtained by distillation (from CaH_2 for CH_2Cl_2, sodium/benzophenone for THF and from sodium for toluene). Methanol (MeOH), N,N'-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were purchased in anhydrous form and used without further purification. Ethyl acetate (EtOAc), diethyl ether (Et_2O), methylene chloride (CH_2Cl_2), and cyclohexane were purchased at ACS grade quality and used without further purification. Reagents were purchased at the highest commercial quality and used without further purification. Yields refer to chromatographically and spectroscopically (^{1}H NMR and ^{13}C NMR) homogeneous materials. Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck silica gel plates with QF-254 indicator and an ethanolic solution of ammonium molybdate or potassium permanganate and heat as developing agents. E. Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. NMR spectra were recorded on Bruker Avance DMX-300 instrument and calibrated using residual undeuterated solvent as an internal reference (7.26 ppm and 77.16 ppm for ^{1}H and ^{13}C NMR in $CDCl_3$; 2.50 ppm and 39.52 ppm for ^{1}H and ^{13}C NMR in [D_6]DMSO; 2.05 ppm and 29.84 ppm for ^{1}H and ^{13}C NMR in [D_6]Acetone). The following abbreviations were used to describe

the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, pent = pentet, hex = hextet, br = broad. IR spectra were recorded on a Perkin-Elmer Spectrum One FTIR spectrometer with diamond ATR accessory.

UPLC-PDA Analysis. Samples dissolved in MeOH were injected onto a Waters Acquity UPLC system (Milford, MA, USA) equipped with a binary solvent manager, simple manager, column compartment and 2996 PDA detector, connected to Waters Masslynx 4.1 software. The separation was carried out using a Waters BEH C18 column (2.1 x 100 mm, 1.7 µm) at 37 ºC. The optimal chromatographic conditions were established: solvent system, phase A, 0.1% trifluoroacetic acid in water and phase B, 0.1% trifluoroacetic acid in acetonitrile; gradient separation system: 80% A at 0 min for 1 min to 100% B at 5 min for 2 min, then 80% A at 8 min for 2 min; flow rate 0.4 mL/min and injection volume of 2-5 µL.

UPLC-Q-TOF Analysis: High resolution (HRMS) data were recorded on a Waters Xevo quadrupole time-of-flight (Q-TOF) spectrometer (Waters Corp., Milford, MA, USA) coupled to an Acquity UPLC system (Waters Corp., Milford, MA, USA) via an electrospray ionization (ESI) interface. Separation was performed on a Waters Acquity BEH C18 column (50 x 2.1 mm i.d., 1.7 µm). The solvent system consisted of 0.1% formic acid in acetonitrile (phase A) and 0.1% formic acid in ultrapure water (phase B). Gradient conditions were as follows: 20% B at 0 min to 100% A in 3 min, held for 2 min, returned to 20% B in 1 min, and equilibrated for 2 min before the next injection; the flow rate was 0.2 mL/min; the column and sample temperatures were kept at 37ºC and 4ºC, respectively; the sample injection volume was 1 µL. The ESI source was operated in positive ionization mode using leucine-encephaline as reference mass ([M+H]+ ion m/z 556.2771). The capillary and cone voltages were set at 3.0 kV and 45 kV, respectively. The temperature of the source and desolvation was set at 120 ºC and 300 ºC, respectively. The cone and desolvation gas (nitrogen) flows were 500 L h-1 and 50 L h-1. The collision energy was set at 5 eV. ESI data acquisition was collected in Centroid mode in a full scan range from m/z 50-1500 at 0.2 s per scan. All data were acquired using MasslynkTM NT4.1 software (Waters Corp., Milford, MA, USA).

Purity for all final compounds was checked by HPLC and was determined ≥95%, each showed a parent mass ion consistent with the desired structure (HRMS).

General procedure for the synthesis of Dioxinones 2a-d (Procedure A):[5a] To a stirred solution of acid (1 equiv.) in CH_2Cl_2 (for a solution of 0.1 mol.L^{-1}) were added oxalyl chloride [$(COCl)_2$; 1.25 equiv.] and DMF (0.004 equiv.) at room temperature. After 1.5 h, the solvent and the excess oxalyl chloride were removed by evaporation under vacuum. CH_2Cl_2 (for a solution of 0.1 mol L^{-1}), *N,O*-dimethylhydroxylamine (1.4 equiv.), and Et_3N (3 equiv.) were then successively added at room temperature. After 2 h, the reaction was quenched at room temperature with a saturated solution of $NaHCO_3$ and the mixture extracted twice with CH_2Cl_2. The combined organic layers were then washed with a saturated solution of NH_4Cl and brine. The organic layer was then dried with anhydrous $MgSO_4$, filtered, and concentrated under vacuum to afford the crude Weinreb amine.

To a stirred solution of diisopropylamine (DIPA; 3 equiv.) in THF (for a solution of 0.1 mol.L^{-1}) was added *n*-Butyl lithium (*n*BuLi, 1.2 M in hexane, 3.1 equiv.) at –78 °C. After 30 min at 0 °C, the medium was recooled to –78 °C and freshly distilled *t*Bu acetate (3 equiv.) was added. After 30 min at –78 °C, crude Weinreb amide (1 equiv.) was added at this temperature. After 1 h, the reaction was quenched at room temperature with a saturated solution of $NaHCO_3$ and the mixture extracted twice with EtOAc. The combined organic layers were then washed with a saturated solution of NH_4Cl. The organic layer was then dried with anhydrous $MgSO_4$, filtered, and concentrated under vacuum to afford the crude *tert*-butyl ester.

To a stirred solution of *tert*-butyl ester (1 equiv.) in acetone (10 equiv.) were added acetic anhydride (15 equiv.) and sulfuric acid (1 equiv.) at 0 °C. The medium was then warmed slowly to room temperature over 10 min. After 45 min, the reaction was quenched at room temperature with an aqueous solution containing sodium carbonate (30 equiv.) and EtOAc (100 mL) was added. The biphasic medium was then stirred for 40 min (hydrolysis of the remaining acetic anhydride) and the aqueous layer was extracted twice with EtOAc. The combined organic layers were then washed with a saturated solution of NH_4Cl. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated under vacuum. The crude residue was finally purified by flash chromatography silica gel using an appropriate gradient of a cyclohexane/EtOAc mixture as eluent to give the desired dioxinone.

6-(4-methoxyphenyl)-2,2-dimethyl-4*H*-1,3-dioxin-4-one (2a) [87769-44-6]: Following procedure **A**, 4-methoxybenzoic acid (3.41 g, 20 mmol) was used to afford **2a** as yellow solid with 77% yield (3.57 g, 15.3 mmol) after purification by flash chromatography on silica gel (cyclohexane/EtOAc 80:20). mp: 68°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.77 (s, 6H), 3.84 (s, 3H), 5.77 (s, 1H), 6.92 (d, J = 9.0 Hz, 2H), 7.71 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.2, 55.7, 89.5, 106.5, 114.4, 123.5, 128.4, 162.4, 163.0, 165.1; IR (neat): 2999, 2940, 2842, 1721, 1607, 1513, 1363, 1252, 1179, 1027, 804 cm^{-1}; MS (ESI): m/z 235 (M + H$^+$); HRMS (ESI): calcd for $[C_{13}H_{14}O_4 + H]^+$ 235.0970; Found 235.0976.

6-(3-bromo-4-methoxyphenyl)-2,2-dimethyl-4*H*-1,3-dioxin-4-one (2b): Following procedure A, 3-bromo-4-methoxybenzoic acid (4.44 g, 19.2 mmol) was used to afford **2b** as a orange solid in 23% yield (1.37 g, 4.42 mmol) after purification by flash chromatography on silica gel (cyclohexane/EtOAc, 80:20), m.p. 120°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.86 (d, J = 2.1 Hz, 1H), 7.62 (dd, J = 8.7, 2.1 Hz, 1H), 6.93 (d, J = 8.7 Hz, 1H), 5.78 (s, 1H), 3.94 (s, 3H), 1.78 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.6, 161.8, 158.9, 131.5, 127.2, 124.7, 112.3, 111.7, 106.8, 90.4, 56.6, 25.1 ppm. IR (neat): 1713, 1597, 1498, 1357, 1251, 1203, 1037, 1013, 994, 899, 806 cm^{-1}. MS (ESI): *m/z* (%) = 313 (80), 315 (100) [M + H$^+$].

6-(3,5-dibromo-4-methoxyphenyl)-2,2-dimethyl-4*H*-1,3-dioxin-4-one (2c): Following procedure A, 3,5-dibromo-4-methoxybenzoic acid (4.90 g, 15.8 mmol) was used to afford **2c** as a orange solid in 74% yield (4.57 g, 11.7 mmol) after purification by flash chromatography on silica gel (cyclohexane/EtOAc, 90:10), m.p. 127°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80 (s, 2H), 5.81 (s, 1H), 3.91 (s, 3H), 1.78 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.8, 161.0, 157.2, 130.6, 129.6, 118.9, 107.2, 92.4, 60.9, 25.1 ppm. IR (neat): 1716, 1616, 1381, 1340, 1260, 1199, 984, 904, 830, 743 cm^{-1}. MS (ESI): *m/z* (%) = 391 (65), 393 (100), 395 (50) [M + H$^+$].

6-(furan-2-yl)-2,2-dimethyl-4H-1,3-dioxin-4-one (2d) [1293991-93-1]. Following procedure A, 2-furoic acid (2.24 g, 20 mmol) was used to afford **2d** as red oil with 57% yield (2.20 g, 11.3 mmol) after purification by flash chromatography on silica gel (cyclohexane/EtOAc 80:20). 1H NMR (300 MHz, CDCl3): δ 1.71 (s, 6H), 5.74 (s, 1H), 6.49 (dd, J = 1.7, 3.6 Hz, 1H), 6.86 (d, J = 3.6 Hz, 1H), 7.53 (brs, 1H); 13C NMR (75 MHz, CDCl3): δ 25.0, 89.6, 107.0, 112.5, 114.2, 146.2, 146.3,

156.4, 161.6; IR (neat): 3134, 3000, 2945, 1727, 1679, 1641, 1470, 1361, 1278, 1205, 1017 cm-1; MS (ESI): m/z 195 (M + H).

3-bromo-4-methoxyacetophenone (11b) [35310-75-9]:[6a] Bromine (0.54 mL, 10.5 mmol) in acetic acid (1.7 mL) was added dropwise to a mixture of 4-methoxyacetophenone (758 mg, 5 mmol) and sodium acetate (1.28 g, 15.5 mmol) in acid acetic (10 mL) at room temperature over 10 min. The reaction mixture was stirred for 1 h at room temperature. H_2O (30 mL) was added and the solid was filtered, washed with H_2O and dried under high vacuum to afford 3-bromo-4-methoxyacetophenone **11b** as a white solid in 88% yield (1.01 g, 4.4 mmol). M.p. 86 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.17 (s, 1H), 7.91 (d, J = 8.7 Hz, 1H), 6.93 (d, J = 8.7 Hz, 1H), 3.97 (s, 3H), 2.56 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 195.8, 159.7, 134.0, 131.4, 129.6, 112.0, 111.2, 56.6, 26.5 ppm. IR (neat): 1670, 1590, 1260, 1230, 1043, 1012, 817, 590 cm^{-1}. MS (APCI): *m/z* (%) = 228 (100), 229 (10) [M - H$^+$].

3,5-dibromo-4-methoxyacetophenone (11c) [79324-79-1]: Bromine (1.63 mL, 31.5 mmol) in acetic acid (5 mL) was added dropwise to a mixture of 4-hydroxyacetophenone (2.08 g, 15 mmol) and sodium acetate (3.85 g, 46.5 mmol) in acid acetic (35 mL) at room temperature over 20 min. The reaction mixture was stirred for 1 h at room temperature. H_2O (75 mL) was added and the solid was filtered, washed with H_2O and dried under high vacuum to afford 3,5-dibromo-4-hydroxyacetophenone **12** as a white solid. To a stirred solution of **12** in DMF (50 mL) was added K_2CO_3 (4.15 g, 30 mmol) in portions, followed by the addition of MeI (3.9 mL, 60 mmol), and the mixture was stirred at room temperature for 24 h. The reaction mixture was taken in EtOAc (100 mL) and washed successively with H_2O (50 mL), 1N HCl (50 mL), saturated solution of $NaHCO_3$ (50 mL), and brine (2 x 50 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under vacuum to afford 3,5-bromo-4-methoxyacetophenone **11c** as a white solid in 96% yield (4.45 g, 14.4 mmol). M.p. 83 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.08 (s, 2H), 3.93 (s, 3H), 2.56 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 194.7, 158.2, 135.2, 133.0, 118.8, 60.9, 26.6 ppm. IR (neat): 1684, 1380, 1349, 1263, 979, 736, 592 cm^{-1}. MS (APCI): *m/z* (%) = 308 (55), 228 (100) [M - H$^+$].

General Procedure for the synthesis of α-bromo-ketones 13b-c (Procedure B): To a stirred solution of acetophenones **11b-c** (1 equiv.) in THF (30 mL) was added trimethylphenylammonium tribromide (1.05 equiv.) at 0 °C. The reaction mixture was stirred at room temperature for 12 h. The solid was filtered, and to the filtrate was added EtOAc (20 mL). The organic layer was washed successively with H_2O (20 mL) and brine (20 mL). The organic layer was then dried over Na_2SO_4, filtered and concentrated under vacuum. The crude residue was then purified by flash chromatography on silica gel using an appropriate gradient of cyclohexane/EtOAc as eluent to give the desired α-bromo-ketone **13b-c**.

2-bromo-1-(3-bromo-4-methoxyphenyl)ethanone (13b) [6096-83-9]: Following procedure B, 3-bromo-4-methoxyacetophenone **11b** (1.01 g, 4.4 mmol) was used to afford **13b** as a white solid in 89% yield (1.20 g, 3.9 mmol) after purification by flash chromatography on silica gel (cyclohexane/EtOAc, 90:10), m.p. 113 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.19 (d, J = 2.1 Hz, 1H), 7.94 (dd, J = 8.7, 2.1 Hz, 1H), 6.95 (d, J = 8.7 Hz, 1H), 4.37 (s, 2H), 3.98 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 189.1, 160.4, 134.5, 130.5, 128.0, 112.4, 111.4, 56.7, 30.4 ppm. IR (neat): 1688, 1586, 1493, 1272, 1247, 1180, 1045, 1002, 813, 660, 606, 586 cm^{-1}. MS (APCI): *m/z* (%) = 307 (40), 228 (100) [M - H$^+$].

2-bromo-1-(3,5-dibromo-4-methoxyphenyl)ethanone [1563037-34-2] (13c): Following procedure B, 3,5-dibromo-4-methoxyacetophenone **11c** (4.36 g, 14.2 mmol) was used to afford **13c** as a white solid in 99% yield (5.42 g, 14.0 mmol) after purification by flash chromatography on silica gel (cyclohexane/EtOAc, 90:10), m.p. 101 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.12 (s, 2H), 4.36 (s, 2H), 3.96 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.3, 158.9, 133.6, 132.0, 119.0, 61.0, 30.0 ppm. IR (neat): 1698, 1469, 1377, 1273, 1178, 985, 851, 735, 608 cm^{-1}.

General Procedure for the Synthesis of α-hydroxy-ketones 1a-c (Procedure C): Sodium formate (3 equiv.) was stirred in ethanol (0.1 mol.L^{-1}) for 15 min and α-bromo ketone **13** (1 equiv.) was then added. The mixture was stirred at 70 °C overnight. The solution was filtered hot and concentrated under vacuum. The crude residue was then purified by flash chromatography on silica gel using an appropriate gradient of cyclohexane/EtOAc as eluent to give the desired α-hydroxy-ketone **1a-c**.

2-hydroxy-1-(3-bromo-4-methoxyphenyl)--ethanone (1b) [927802-90-2]: Following procedure C, 2-bromo-1-(3-bromo-4-methoxyphenyl)ethanone **13b** (1.16 g, 3.8 mmol) was used to afford **1b** as a white solid in 58% yield (533 mg, 2.2 mmol) after purification by flash chromatography on silica gel (cyclohexane/EtOAc, 80:20), m.p. 115 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.12 (d, J = 2.1 Hz, 1H), 7.87 (dd, J = 8.7, 2.1 Hz, 1H), 6.96 (d, J = 8.7 Hz, 1H), 4.80 (d, J = 3.6 Hz, 2H), 3.98 (s, 3H), 3.47 (t, J = 3.6 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 196.1, 160.6, 133.3, 129.0, 127.5, 112.5, 111.5, 65.2, 56.7 ppm. IR (neat): 3389, 1671, 1590, 1414, 1256, 1209, 1108, 1049, 1002, 916, 676, 622, 597 cm^{-1}.

2-hydroxy-1-(3,5-dibromo-4-methoxyphenyl)-ethanone (1c): Following procedure C, 2-bromo-1-(3,5-di bromo-4-methoxyphenyl)ethanone **13c** (5.41 g, 14 mmol) was used to afford **1c** as a white solid in 60% yield (2.71 g, 8.4 mmol) after purification by flash chromatography on silica gel (cyclohexane/EtOAc, 80:20), m.p. 124 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.06 (s, 2H), 4.81 (s, 2H), 3.96 (s, 3H), 3.34 (brs, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 195.6, 159.2, 132.4, 131.5, 119.3, 65.6, 61.0 ppm. IR (neat): 3248, 1695, 1262, 1234, 981, 736 cm^{-1}.

3,5-dibromo-4-methoxybenzaldehyde (3c) [108940-96-1]: Bromine (1.63 mL, 31.5 mmol) in acetic acid (5 mL) was added dropwise to a mixture of 4-hydroxybenzaldehyde (1.85 g, 15 mmol) and sodium acetate (3.85 g, 46.5 mmol) in acid acetic (35 mL) at room temperature over 20 min. The reaction mixture was stirred for 1 h at room temperature. H_2O (75 mL) was added and the solid was filtered, washed with H_2O and dried under high vacuum to afford 3,5-dibromo-4-hydroxybenzaldehyde **14** as a white solid.

To a stirred solution of **14** in DMF (50 mL) was added K_2CO_3 (4.15 g, 30 mmol) in portions, followed by the addition of MeI (3.9 mL, 60 mmol), and the mixture was stirred at room temperature for 24 h. The reaction mixture was taken in EtOAc (100 mL) and washed successively with H_2O (50 mL), 1M HCl (50 mL), saturated solution of $NaHCO_3$ (50 mL), and brine (2 x 50 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by chromatography on silica gel by using cyclohexane/EtOAc (90:10) as eluent to give 3,5-dibromo-4-methoxybenzaldehyde **3c** as a white solid in 82% yield (3.62 g, 12.3 mmol). M.p. 97 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.86 (s, 1H), 8.02 (s, 2H), 3.96 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 188.6, 159.3, 134.4,

134.1, 119.5, 61.0 ppm. IR (neat): 1685, 1545, 1468, 1365, 1258, 1186, 979, 877, 730, 653, 531 cm^{-1}.

General Procedure for the Synthesis of Cadiolide analogues 5-10: Toluene (for a solution of 1.0 $mol.L^{-1}$), Et_3N (2.0 equiv.) and the appropriate α-hydroxy-ketone **1a-c** (1 equiv.), dioxinone **2a-d** (2 equiv.) and aldehyde **3a-c** (1 equiv.) were added to a 15 mL tube flushed with argon. The tube was sealed and then heated in a microwave reactor. Over 1.5 min, the temperature was raised to 150 °C, held for 5 min, and then cooled for 10 min. The reaction was quenched at room temperature with 1M HCl and the mixture extracted with EtOAc. The combined organic layers were then dried with Na_2SO_4, filtered and concentrated under vacuum. Filtration through a plug of silica and elution with (cyclohexane/EtOAc, 80:20) afforded a mixture of bis-methoxylated and mono-methoxylated acylfuranones **4**, which was used directly without further purification (partial de-methoxylation resulted during the microwave-assisted multi-component recation).

To a stirred solution of bis-methoxylated and mono-methoxylated acylfuranones **4** (obtained above) in CH_2Cl_2 (5 mL) was added BBr_3 (10 equiv.) at -78 °C. The mixture was then slowly warmed to room temperature over 20 h. The reaction was quenched at –78 °C with MeOH then at room temperature with H_2O and the mixture extracted twice with EtOAc. The combined organic layers were then dried with Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash chromatography on silica gel using a gradient of EtOAc/MeOH (100:0 to 95:5) to give cadiolide analogues **5-10**.

(*Z*)-3-(4-hydroxybenzoyl)-5-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)furan-2(5*H*)-one (5a): Following procedure D, *α*-hydroxy-ketone **1a** (83 mg, 0.5 mmol), dioxinone **2a** (234 mg, 1.0 mmol) and 4-methoxybenzaldehyde **3a** (0.06 mL, 0.5 mmol) were used to afford **5a** as yellow solid in 72% yield (142 mg, 0.36 mmol). Analyses are similar to those described in the literature.[5d] M.p. 262 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.69 (brs, 1H), 10.28 (brs, 1H), 10.15 (brs, 1H), 7.74 (d, *J* = 8.7 Hz, 2H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.22 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 2H), 6.33 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 188.0, 166.1, 163.2, 159.6, 159.5, 155.9, 144.6, 133.0, 132.1, 130.8, 127.4, 124.0, 121.4, 119.3, 116.4, 116.1, 115.8, 115.5 ppm. HRMS (ESI): calculated for $C_{24}H_{17}O_6$ 401.1025 found 401.1016.

(*Z*)-5-(3-bromo-4-hydroxybenzylidene)-3-(4-hydroxybenzoyl)-4-(4-hydroxyphenyl)furan-2(5*H*)-one (5b): Following procedure D, *α*-hydroxy-ketone **1a** (41 mg, 0.25 mmol), dioxinone **2a** (117 mg, 0.5 mmol) and 3-bromo-4-methoxybenzaldehyde **3b** (54 mg, 0.25 mmol) were used to afford **5b** as yellow solid in 39% yield (43 mg, 0.09 mmol). M.p. 159 ºC. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.03 (brs, 1H), 10.61 (brs, 1H), 10.09 (brs, 1H), 8.08 (d, *J* = 2.1 Hz, 1H), 7.76 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 2H), 7.23 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 1H), 6.81 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 6.36 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 188.3, 166.3, 163.5, 160.0, 155.9, 145.7, 135.1, 132.6, 132.2, 131.2, 127.7, 126.1, 122.4, 119.6, 117.1, 116.1, 115.8, 114.9, 110.3 ppm. IR (neat): υ = 3233, 1729, 1576, 1557, 1501, 1440, 1410, 1371, 1274, 1221, 1152, 1041, 863, 841 cm^{-1}. HRMS (ESI): calculated for $C_{24}H_{16}BrO_6$ 479.0130 found 479.0116.

(*Z*)-3-(3-bromo-4-hydroxybenzoyl)-5-(3-bromo-4-hydroxybenzylidene)-4-(4-hydroxyphenyl)furan-2(5*H*)-one (5c): Following procedure D, *α*-hydroxy-ketone **1a** (41 mg, 0.25 mmol), dioxinone **2b** (156 mg, 0.5 mmol) and 3-bromo-4-methoxybenzaldehyde **3b** (54 mg, 0.25 mmol) were used to afford **5c** as yellow solid in 38% yield (52 mg, 0.09 mmol). M.p. 219 ºC. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.10 (brs, 1H), 10.62 (brs, 1H), 10.11 (brs, 1H), 8.09 (d, *J* = 2.1 Hz, 1H), 7.95 (d, *J* = 2.2 Hz, 1H), 7.76 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.69 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.25 (d, *J* = 8.8 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 1H), 6.93 (d, *J* = 8.7 Hz, 1H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.37 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): 187.4, 166.2, 160.1, 159.8, 157.1, 156.1, 145.8, 135.8, 133.4, 132.5, 132.3, 131.4, 129.0, 126.1, 121.5, 119.5, 117.1, 116.1, 115.5, 110.3, 110.0 ppm. IR (neat): υ = 3336, 1747, 1714, 1584, 1556, 1504, 1409, 1383, 1303, 1218, 1179, 1150, 1081, 848, 825 cm^{-1}. HRMS (ESI): calculated for $C_{24}H_{15}Br_2O_6$ 558.9217 found 558.9201.

(*Z*)-3-(3,5-dibromo-4-hydroxybenzoyl)-5-(3,5-dibromo-4-hydroxybenzylidene)-4-(4-hydroxyphenyl)furan-2(5*H*)-one (5d): Following procedure D, α-hydroxy-ketone **1a** (44 mg, 0.26 mmol), dioxinone **2c** (204 mg, 0.52 mmol) and 3,5-dibromo-4-methoxybenzaldehyde **3c** (76 mg, 0.26 mmol) were used to afford **5d** as a yellow solid in 38% yield (70 mg, 0.01 mmol). Decomp. 150 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.15 (brs, 1H), 8.12 (s, 2H), 7.97 (s, 2H), 7.21 (d, *J* = 8.7 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.42 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 186.0, 165.6, 159.9, 157.7, 156.0, 152.1, 146.6, 134.7, 133.9, 131.3, 129.5, 127.6, 121.0, 119.0, 115.9, 113.7, 112.1, 111.5 ppm. IR (neat): 3208, 1748, 1578, 1476, 1376, 1297, 1152, 981, 679 cm^{-1}; MS (ESI): *m*/*z* (%) = 719 (15), 718 (19), 717 (70), 716 (30), 715 (100), 714 (22), 713 (60), 711 (15). HRMS (ESI): calculated for $C_{24}H_{13}Br_4O_6$ 716.7407 found 716.7396.

(*Z*)-5-(3-bromo-4-hydroxybenzylidene)-4-(3-bromo-4-hydroxyphenyl)-3-(4-hydroxybenzoyl)furan-2(5*H*)-one (6b): Following procedure D, *α*-hydroxy-ketone **1b** (61 mg, 0.25 mmol), dioxinone **2a** (117 mg, 0.5 mmol) and 3-bromo-4-methoxybenzaldehyde **3b** (54 mg, 0.25 mmol) were used to afford **6b** as yellow solid in 37% yield (51 mg, 0.09 mmol). M.p. 148 ºC. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.92 (brs, 1H), 10.71 (brs, 1H), 8.30 (brs, 1H), 8.09 (d, *J* = 1.9 Hz, 1H), 7.76 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.49 (d, *J* = 2.1 Hz, 1H), 7.25 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.03 (d, *J* = 8.6 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.79 (d, *J* = 8.8 Hz, 2H), 6.35 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): 187.9, 166.1, 163.6, 156.5, 156.1, 154.9, 145.8, 135.9, 133.8, 132.6, 132.3, 130.3, 127.7, 126.0, 121.0, 117.1, 116.8, 115.9, 115.2, 110.3, 109.9 ppm. IR (neat): υ = 3160, 1732, 1561, 1548, 1504, 1406, 1370, 1287, 1225, 1161, 1042, 866, 848 cm^{-1}. HRMS (ESI): calculated for $C_{24}H_{15}Br_2O_6$ 558.9217 found 558.9219.

(*Z*)-3-(3-bromo-4-hydroxybenzoyl)-5-(3-bromo-4-hydroxybenzylidene)-4-(3-bromo-4-hydroxyphenyl)furan-2(5*H*)-one (6c): Following procedure D, *α*-hydroxy-ketone **1b** (123 mg, 0.5 mmol), dioxinone **2b** (313 mg, 1.0 mmol) and aldehyde **3b** (110 mg, 0.5 mmol) were used to afford **6c** as an orange solid in 44% yield (158 mg, 0.22 mmol). Decomp. 98 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.47 (brs, 1H), 11.06 (brs, 1H), 10.93 (brs, 1H), 8.08 (d, *J* = 2.1 Hz, 1H), 7.97 (d, *J* = 2.1 Hz, 1H), 7.75 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.70 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.50 (d, *J* = 2.1 Hz, 1H), 7.23 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.05 (d, *J* = 8.7 Hz, 1H), 6.98 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 8.7 Hz, 1H), 6.36 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 186.7, 165.7, 159.5, 156.2, 155.9, 155.7, 145.4, 135.7, 135.0, 133.7, 132.1, 131.0, 130.2, 128.7, 125.8, 122.0, 120.7, 116.8, 116.5, 116.0, 115.5, 110.0, 109.7 (2C) ppm. IR (neat): 2925, 1745, 1555, 1495, 1367, 1291, 974, 820 cm^{-1}; MS (ESI): *m*/*z* (%) = 641 (39), 640 (22), 639 (100), 638 (32), 637 (58), 636 (30).

HRMS (ESI): calculated for $C_{24}H_{14}Br_3O_6$ 636.8322 found 636.8324.

(*Z*)-3-(3-bromo-4-hydroxybenzoyl)-4-(3-bromo-4-hydroxyphenyl)-5-(3,5-dibromo-4-hydroxybenzylidene)furan-2(5*H*)-one (6e): Following procedure D, α-hydroxy-ketone **1b** (123 mg, 0.5 mmol), dioxinone **2b** (313 mg, 1.0 mmol) and 3,5-dibromo-4-methoxybenzaldehyde **3c** (147 mg, 0.5 mmol) were used to afford **6e** as a brown solid in 61% yield (220 mg, 0.31 mmol). Decomp. 182 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.48 (brs, 1H), 10.94 (brs, 1H), 10.65 (brs, 1H), 8.10 (s, 2H), 7.97 (d, *J* = 2.0 Hz, 1H), 7.70 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.49 (d, *J* = 2.0 Hz, 1H), 7.22 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.97 (d, *J* = 8.5 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 6.39 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 186.6, 165.6, 159.7, 156.4, 155.6, 152.1, 146.5, 135.2, 134.8, 133.8, 131.1, 130.3, 128.6, 127.6, 122.8, 120.6, 116.6, 116.2, 113.6, 112.1 (2C), 109.8 ppm. IR (neat): 3188, 1737, 1476, 1403, 1367, 1296, 1191, 978, 708, 635, 545 cm^{-1}. MS (ESI): *m/z* (%) = 719 (26), 718 (21), 717 (100), 716 (55), 715 (96), 714 (30), 713 (80), 711 (27). HRMS (ESI): calculated for $C_{24}H_{13}Br_4O_6$ 716.7407 found 716.7411.

Cadiolide C: (*Z*)-5-(3-bromo-4-hydroxybenzylidene)-4-(3-bromo-4-hydroxyphenyl)-3-(3,5-dibromo-4-hydroxybenzoyl)furan-2(5*H*)-one [1414518-14-1] (6f): Following procedure D, *α*-hydroxy-ketone **1b** (123 mg, 0.5 mmol), dioxinone **2c** (392 mg, 1.0 mmol) and 3-bromo-4-methoxybenzaldehyde **3b** (110 mg, 0.5 mmol) were used to afford **6f** as an orange solid in 47% yield (168 mg, 0.24 mmol). Decomp. 116 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.11 (brs, 1H), 10.95 (brs, 1H), 8.09 (s, 1H), 7.96 (s, 2H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.50 (s, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 6.38 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 185.7, 165.6, 156.9, 156.2, 156.0, 155.7, 145.5, 135.8, 133.8, 132.2, 130.3, 129.8, 125.8, 121.0, 120.7, 116.8, 116.4, 116.0, 111.4 (2C), 110.0, 109.7 ppm. IR (neat): 3073, 1739, 1547, 1400, 1295, 979, 685 cm^{-1}; MS (ESI): *m/z* (%) = 719 (25), 718 (24), 717 (70), 716 (30), 715 (100), 714 (22), 713 (72), 711 (17). HRMS (ESI): calculated for $C_{24}H_{13}Br_4O_6$ 716.7407 found 716.7406.

(*Z*)-3-(3-bromo-4-hydroxybenzoyl)-5-(3-bromo-4-hydroxybenzylidene)-4-(3,5-dibromo-4-hydroxyphenyl)furan-2(5*H*)-one (7c): Following procedure D, α-hydroxy-ketone **1c** (162 mg, 0.5 mmol), dioxinone **2b** (313 mg, 1.0 mmol) and 3-bromo-4-methoxybenzaldehyde **3b** (110 mg, 0.5 mmol) were used to afford **7c** as an orange solid in 39% yield (140 mg, 0.2 mmol). Decomp. 110 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.48 (brs, 1H), 11.11 (brs, 1H), 8.09 (d, *J* = 2.1 Hz, 1H), 7.97 (d, *J* = 2.1 Hz, 1H), 7.78 (dd, *J* = 2.1, 8.7 Hz, 1H), 7.70 (dd, *J* = 2.1, 8.7 Hz, 1H), 7.55 (s, 2H), 7.06 (d, *J* = 8.7 Hz, 1H), 6.96 (d, *J* = 8.7 Hz, 1H), 6.38 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 186.2, 165.6, 159.5, 156.0, 154.8, 152.8, 145.4, 135.9, 135.2, 132.9, 132.2, 130.8, 128.6, 125.7, 122.8, 122.5, 116.8, 116.0, 115.8, 111.8, 110.0, 109.6 ppm. IR (neat): 2919, 1746, 1592, 1296, 1164, 1022, 978, 824, 744, 634 cm^{-1}. MS (ESI): *m/z* (%) = 719 (20), 718 (18), 717 (65), 716 (27), 715 (100), 714 (16), 713 (61), 711 (16). HRMS (ESI): calculated for $C_{24}H_{13}Br_4O_6$ 716.7407 found 716.7400.

(*Z*)-3-(3-bromo-4-hydroxybenzoyl)-5-(3,5-dibromo-4-hydroxybenzylidene)-4-(3,5-dibromo-4-hydroxyphenyl)furan-2(5*H*)-one (7e): Following procedure D, α-hydroxy-ketone **1c** (162 mg, 0.5 mmol), dioxinone **2b** (313 mg, 1.0 mmol) and 3,5-dibromo-4-methoxybenzaldehyde **3c** (147 mg, 0.5 mmol) were used to afford **7e** as a brown solid in 21% yield (85 mg, 0.11 mmol). Decomp. 110 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.51 (brs, 1H), 10.61 (brs, 1H), 8.13 (s, 2H), 8.0 (s, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.55 (s, 2H), 6.96 (d, *J* = 8.6 Hz, 1H), 6.42 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 186.1, 165.3, 159.7, 154.6, 152.6, 152.2, 146.4, 135.3, 134.7, 133.4, 133.0, 130.8, 128.5, 127.5, 123.6, 122.3, 116.1, 113.7, 112.1, 111.9, 111.4, 109.6 ppm. IR (neat): 3201, 1754, 1589, 1476, 1406, 1296, 1194, 1152, 1021, 977, 816, 740, 634 cm^{-1}. MS (ESI): *m/z* (%) = 799 (15), 798 (20), 797 (53), 796 (26), 795 (96), 794 (32), 793 (100), 791 (51), 789 (12). HRMS (ESI): calculated for $C_{24}H_{12}Br_5O_6$ 794.6512 found 794.6512.

Cadiolide B: (*Z*)-3-(3,5-dibromo-4-hydroxybenzoyl)-5-(3,5-dibromo-4-hydroxybenzylidene)-4-(3,5-dibromo-4-hydroxyphenyl)furan-2(5*H*)-one [206763-42-0]: (7g) To a solution of acylfuranone **5a** (26 mg, 0.065 mmol) in dioxane-H_2O (2 mL, 1:1) was added dropwise a brominating agent (0.69 mL) prepared from Br_2 (0.03 mL) and KBr (150 mg) in H_2O (1 mL). The mixture was stirred for 24h, treated with brine, and extracted with Et_2O. The organic layer was washed with brine then with $Na_2S_2O_3$. The combined organic layer was then dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product was chromatographied on silica gel using a gradient of EtOAc/MeOH mixture (100:0 to 90:10) to give Cadiolide **B** as a black solid with 67% yield (38 mg, 0.044 mmol). Analyses are similar to those described in the literature.[3a,b] ^{1}H NMR (300 MHz, [D$_6$]DMSO): 8.12 (s, 2H), 7.91 (s, 2H), 7.55 (s, 2H), 6.40 (s, 1H) ppm^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 184.3, 165.3, 156.2, 154.8, 152.6, 146.2, 134.8, 133.7, 133.0, 130.2, 127.1, 122.8, 122.3, 114.0, 112.2, 112.0, 111.8, 109.7 ppm. MS (ESI): m/z 879 (8), 878 (14), 877 (30), 874 (78), 873 (100), 871 (84), 868 (30), 867 (6). HRMS (ESI): calculated for $C_{24}H_{11}Br_6O_6$ 874.5590 found 874.5598.

(*Z*)-5-(3,5-dibromo-4-hydroxybenzylidene)-4-(3,5-dibromo-4-hydroxyphenyl)-3-(4-hydroxybenzoyl)furan-2(5*H*)-one (7h): Following procedure D, *α*-hydroxy-ketone **1c** (162 mg, 0.5 mmol), dioxinone **2a** (234 mg, 1.0 mmol) and 3,5-dibromo-4-methoxybenzaldehyde **3c** (147 mg, 0.5 mmol) were used to afford **7h** as a brown solid in 19% yield (69 mg, 0.96 mmol). Decomp. 128 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.67 (brs, 1H), 8.13 (s, 1H), 7.75 (d, *J* = 8.7 Hz, 2H), 7.55 (s, 2H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.41 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 187.0, 165.4, 163.4, 153.4, 152.6, 152.2, 146.2, 134.6, 132.8, 132.4, 127.3 (2C), 124.4, 122.3, 115.5, 113.2, 112.1, 111.8 ppm. IR (neat): 3073, 1753, 1575, 1476, 1291, 1246, 1161, 974, 874, 741, 620 cm^{-1}. MS (ESI): *m/z* (%) = 719 (20), 718 (23), 717 (85), 716 (28), 715 (100), 714 (24), 713 (74), 711 (30). HRMS (ESI): calculated for $C_{24}H_{13}Br_4O_6$ 716.7407 found 716.7394.

Cadiolide A: (*Z*)-3-(3,5-dibromo-4-hydroxybenzoyl)-4-(3,5-dibromo-4-hydroxyphenyl)-5-(4-hydroxybenzylidene)furan-2(5*H*)-one [206763-40-8]: (7i) Following procedure D, *α*-hydroxy-ketone **1c** (162 mg, 0.5 mmol), dioxinone **2c** (392 mg, 1.0 mmol) and *p*-methoxybenzaldehyde **3a** (0.06 mL, 0.5 mmol) were used to afford **7i** as an orange solid in 18% yield (64 mg, 0.09 mmol). Decomp. 168 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.56 (brs, 1H), 10.37 (brs, 1H), 7.93 (s, 2H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.56 (s, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.37 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 185.3, 165.5, 159.9, 156.0, 155.6, 152.4, 144.6, 133.7, 133.6, 133.0, 129.8, 124.1, 122.7, 121.1, 118.3, 116.2, 111.7, 111.3 ppm. IR (neat): 3073, 1749, 1597, 1538, 1293, 1170, 987, 740 cm^{-1}. MS (ESI): *m/z* (%) = 719 (20), 718 (20), 717 (62), 716 (26), 715 (100), 714 (22), 713 (60), 711 (13). HRMS (ESI): calculated for $C_{24}H_{13}Br_4O_6$ 716.7407 found 716.7402.

(*Z*)-3-(furan-2-carbonyl)-5-(4-hydroxybenzylidene)-4-(4-hydroxyphenyl)furan-2(5*H*)-one (8): Following procedure D, α-hydroxy-ketone **1a** (166 mg, 1.0 mmol), dioxinone **2d** (388 mg, 2.0 mmol) and 4-methoxybenzaldehyde **3a** (0.11 mL, 1.0 mmol) were used to afford **8** as a yellow solid in 37% yield (143 mg, 0.37 mmol). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.24 (brs, 1H), 10.11 (brs, 1H), 7.97 (d, *J* = 1.8 Hz, 1H), 7.75 (d, *J* = 9.0 Hz, 2H), 7.35 (d, *J* = 3.6 Hz, 1H), 7.28 (dd, *J* = 6.9, 1.8Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.85 (d, *J* = 6.9, 9.0 Hz, 2H), 6.64 (dd, *J* = 3.6, 1.8 Hz, 1H), 6.37 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 176.0, 165.8, 159.8, 159.6, 157.5, 151.3, 149.3, 144.5, 133.3, 131.0, 124.1, 122.6, 119.9, 119.3, 117.6, 116.2, 115.9, 113.0 ppm. MS (ESI): *m*/*z* = 373 [M – H]$^+$. HRMS (ESI): calculated for $C_{22}H_{15}O_6$ 375.0869 found 375.0857.

(*Z*)-5-(3-bromo-4-hydroxybenzylidene)-4-(3-bromo-4-hydroxyphenyl)-3-(furan-2-carbonyl)furan-2(5*H*)-one (9): Following procedure D, α-hydroxy-ketone **1b** (61 mg, 0.25 mmol), dioxinone **2d** (97 mg, 0.5 mmol) and 3-bromo-4-methoxybenzaldehyde **3b** (54 mg, 0.25 mmol) were used to afford **9** as a brown solid in 30% yield (40 mg, 0.075 mmol). M.p. = 246 °C. ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 11.05 (brs, 1H), 10.98 (brs, 1H), 8.10 (d, *J* = 2.1 Hz, 1H), 8.02 (d, *J* = 0.6 Hz, 1H), 7.78 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.55 (d, *J* = 2.1 Hz, 1H), 7.46 (dd, *J* = 3.9, 0.9 Hz, 1H), 7.29 (dd, *J* = 8.4, 2.1 Hz, 1H), 7.04 (t, *J* = 7.8 Hz, 2H), 6.69 (dd, *J* = 3.9, 1.8 Hz, 1H), 6.39 (s, 1H) ppm. ^{13}C NMR (75 MHz, [D$_6$]Acetone): δ = 176.3, 166.1, 156.84, 156.81, 152.8, 149.5, 147.0, 136.8, 134.8, 132.9, 131.1, 127.6, 123.5, 122.6, 117.6, 117.3, 116.2, 113.6, 110.8, 110.6 ppm. IR (neat): 2289, 1743, 1600, 1543, 1496, 1453, 1398, 1299, 1182, 986, 822, 772, 671, 587, 529 cm^{-1}. MS (ESI): *m*/*z* = 531 [M – H]$^+$. HRMS (ESI): calculated for $C_{22}H_{12}Br_2O_6Na$ 552.8898 found 552.8903.

(*Z*)-5-(3-bromo-4-hydroxybenzylidene)-4-(3,5-dibromo-4-hydroxyphenyl)-3-(furan-2-carbonyl)furan-2(5*H*)-one (10): Following procedure D, α-hydroxy-ketone **1c** (81 mg, 0.25 mmol), dioxinone **2d** (97 mg, 0.5 mmol) and 3-bromo-4-methoxybenzaldehyde **3b** (54 mg, 0.25 mmol) were used to afford **10** as a brown solid in 13% yield (20 mg, 0.03 mmol). M.p. > 266 °C. ^{1}H NMR (300 MHz, [D$_6$]Acetone): δ = 8.13 (d, *J*=0.7 Hz, 1H), 7.86 (d, *J*=0.7 Hz, 1H), 7.82 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.73 (s, 2H), 7.46 (dd, *J* = 3.7, 0.7 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 6.67 (dd, *J* = 3.7, 1.7 Hz, 1H), 6.47 (s, 1H), 5.63 (s, residual CH_2Cl_2) ppm. ^{13}C NMR (75 MHz, [D$_6$]Acetone): δ = 175.9, 166.0, 156.7, 155.8, 153.6, 152.8, 149.7, 146.9, 136.9, 134.0, 133.0, 127.5, 124.1, 123.8, 122.8, 117.7, 116.6, 113.7, 111.7, 111.0 ppm. IR (neat): 3077, 1736, 1643, 1591, 1561, 1461, 1416, 1331, 1277, 1166, 830, 807, 755, 655, 561 cm^{-1}. MS (ESI): *m*/*z* = 610 [M – H]$^+$. HRMS (ESI): calculated for $C_{22}H_{12}Br_3O_6$ 608.8184 found 608.8180.

Antibacterial activity. The following eight bacteria were provided by the Spanish Type Culture Collection (CECT): *Bacillus cereus* (CECT 148), *Staphylococcus aureus* (CECT 86), *Enterococcus faecalis* (CECT 481), *Bacillus subtilis* (CECT 35), *Salmonella typhi* (CECT 409), *Escherichia coli 405* (CECT 405), *Escherichia coli 100* (CECT 100) and *Erwinia carotovora* (CECT 225). The minimum inhibitory concentration (MIC) was determined as the lowest compound concentration required to completely inhibit growth of bacteria, by the 2-fold microtiter broth dilution method and according to CLSI guidelines.[7] Each compound was dissolved in dimethyl sulfoxide (DMSO), and ten 2-fold serial dilutions of the stock were prepared. Stock suspensions of bacteria were prepared to OD_{620}= 0.001 in Nutrient Broth (Difco). Then, this suspension of bacteria (100 µL) was mixed with serial dilutions of each compound (100 µL) in each well of a 96-well of polypropylene microplates and incubated at 28ºC or 37ºC, depending on each strain growth conditions overnight (18 h). After, bacteria growth was determined turbidimetrically at OD_{620} in each well using a microplate reader (Thermo Scientific Multiskan FC multimode). Each experiment was performed by triplicate. The antibacterial activity was expressed as the MIC range and defined as the lowest concentration of test compounds that inhibited bacterial growth. Tetracycline hydrochloride was used as a reference compound.

Cytotoxicity studies. The cytotoxicity of the most active compounds, **6c**, **7c**, **9** and **10** was determined at 30 and 100 µM on freshly isolated human neutrophils by the use of two different approaches: the MTT colorimetric assay and by flow cytometry analysis of cell apoptosis and survival.[14] Human neutrophils were obtained from buffy coats of healthy donors by Ficoll-Hypaque density gradient centrifugation as described.[10]

MTT assay: The viability of neutrophils was determined using the previously described MTT (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay.[11] 100 µL of neutrophils suspension in supplemented RPMI medium (2 x 10^5 cells/mL) was added to each well of a 96-well microtiter plate. Cells were incubated in the absence or presence of compounds at 37 ºC for 24 h. MTT was freshly prepared at 2 mg/mL in PBS. 100 µL of MTT solution was added to each well and incubated at 37°C for another 3 h. The supernatants were discarded and 200 µL of DMSO was added to each well to dissolve the formazan. The optical densities at dual wavelengths (560 and 630 nm) were determined in a spectrophotometer (Infinite M200, Tecan, Mannedorf, Switzerland).

Cytofluorometric Analysis of Apoptosis, Necrosis and Survival: freshly isolated neutrophils were resuspended in supplemented RPMI medium at 2 x 10^6 cells/mL. 25 µL were cultured in a 96-well plate containing 200 µL of supplemented RPMI medium for 24 h in the absence or presence of the compounds as described previously.[12] Assessment of apoptosis was performed by flow cytometry using annexin V-FITC and propidium iodide (PI). The protocol indicated by the manufacturer (FITC Annexin V Apoptosis Detection Kit I; BD Biosciences) was used as outlined previously.[13] Cells (1 x 10^4) were analyzed in a BD FACSVerse Flow Cytometer (BD Biosciences, San Jose, CA) and differentiated as early or viable apoptotic (annexin V+ and PI-), late apoptotic and/or necrotic (annexin V+ and PI+), and viable nonapoptotic (annexin V- and PI-) cells.

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Supplementary Material

^{1}H and ^{13}C NMR spectra.