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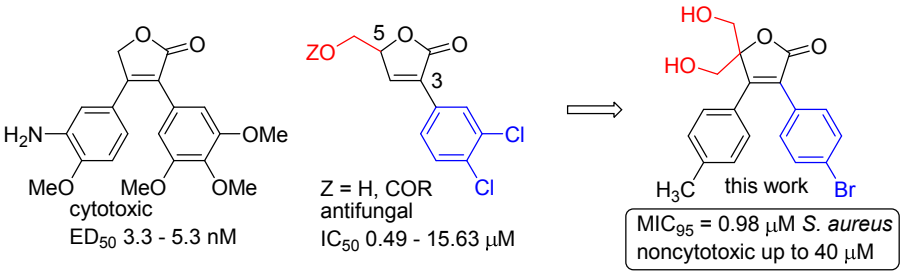
Among 32 novel combretafuranones with substitution related to that of the antifungal 5-acyloxymethyl-3-halofuranones, noncytotoxic antibacterial compounds were identified.



NONTOXIC COMBRETAFURANONE ANALOGUES WITH HIGH IN VITRO ANTIBACTERIAL ACTIVITY

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ABSTRACT: A library of thirty two 3,4-diphenylfuranones related to both combretastatin A-4 and antifungal 5-(acyloxymethyl)-3-(halophenyl)-2,5-dihydrofuran-2-ones was prepared. Cytotoxic effects on a panel of cancer and normal cell lines and antiinfective activity were evaluated, and the data were complemented with tests for the activation of caspase 3 and 7. High cytotoxicity was observed in some of the halogenated analogues, eg. 3-(3,4-dichlorophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one with IC_{50} 0.12 – 0.23 μM, but the compounds were also highly toxic against non-malignant control cells. More importantly, notable antibacterial activity indicating G^+ selectivity has been found in the 3,4-diarylfuranone class of compounds for the first time. Hydroxymethylation of furanone C5 knocked out cytotoxic effects (up to 40 μM) while maintaining significant activity against

Staphylococcus strains in some derivatives. MIC_{95} of the most promising compound, 3-(4-bromophenyl)-5,5-bis(hydroxymethyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one against *S. aureus* strain ATCC 6538 was 0.98 μM (0.38 μg/mL) and 3.9 μM (1.52 μg/mL) after 24 and 48 hours, respectively.

1. Introduction

The *cis*-stilbene fragment is an established privileged structure in medicinal chemistry. The archetypal compound of this family, combretastatin A-4 (CA4, Fig. 1, **1a**), is a well-known suppressor of tubulin assembly *via* interaction with the colchicine binding site, which results in a strong inhibition of cell growth and angiogenesis [1]. In addition, CA-4 also belongs to the family of vascular disrupting agents (VDAs) targeting tumor vasculature as well [2]. Its poor water solubility has been resolved by conversion to a water soluble phosphate prodrug, CA4P [1] (Fig 1, **1b**), which is currently undergoing phase II/III clinical trials in combination with bevacizumab [3]. Another issue of concern often mentioned in literature [4-6] is the tendency of *cis*-stilbenes to isomerize to the more stable *trans* isomers. In response, a huge number of analogs in which the olefinic linker was replaced by a rigid heterocyclic, carbocyclic or aromatic motif (Fig. 1, general structure **2**) allowing to maintain the positions of the two vicinal substituted phenyl rings have been made [7-15]. The syntheses of the compounds are generally straightforward and use low cost chemistry, and, for this reason, novel bioactives of this class could rank among the least expensive anticancer drugs on the market. However, despite a vast number of analogues of type **2** that have been prepared [7-15], with some of them possessing truly amazing low nanomolar biological activities, we are not aware of any synthetic analogue that would have advanced to clinical trials as of this date.

1a Z = H CA4
1b Z = P(O)(OH)$_2$ CA4P

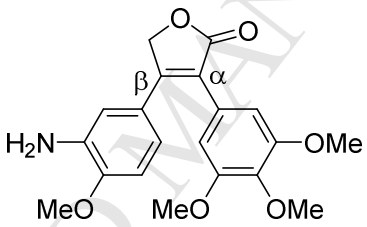
2
X = rigid hetero/carbocyclic linker

Figure 1. CA4, CA4P and their restricted heterocylic analogues.

The design of the restricted heterocyclic analogues usually takes into consideration the SARs developed around the substitution on the phenyl rings [2, 6, 16, 17] . First, the 3,4,5-

trimethoxy substitution on ring A (Fig. 1, **1a**,**b**) seems to be crucial for the activity, even though no conclusive answer to this issue has been given. Second, the presence of the intact 4-methoxy group in ring B is also claimed to be important, while the 3-OH group can be replaced without significant loss of antiproliferative effect [16]. Hence, the substitution of most derivatives prepared to date follows the highly oxygenated substitution pattern of natural combretastatine A4.

However, as early as in 1992, Cushman et al. [18] clearly demonstrated upon extensive screening of a large library of *cis*-stilbenes that the replacement of the 4-methoxyl in the B-ring with SMe, Me, Et and even Pr did not lead to a significant loss of activity. Specifically, the values of ED_{50} for these compounds against the cell lines used remained below 10 nmol/L, an undoubtedly excellent level of activity. Only the change for a butyl group elevated ED_{50} values to a higher, but still interesting low micromolar range. A recent paper [19] also describes 3,4-diaryl-1,2,5-selenadiazoles with excellent activity, and devoid of the 4-OCH_3 group in the B ring.



3 ref 20

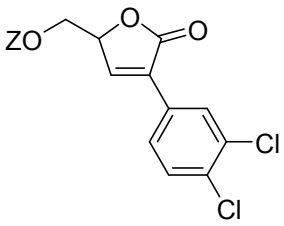
cytotoxic
IC_{50} 3.3 - 5.3 nM

Figure 2. Structures of synthetic furanone analog **3.**

Among the rigid heterocyclic components used, only one example using a 2,5-dihydrofuranone core has been reported. In 2002, Ahn et al. published [20] a small series of 3,4-diphenyl-2,5-dihydrofuran-2-ones. Interestingly, even though the carbonyl group makes the double bond highly polarized, only analogs with the trimethoxyphenyl ring attached to the electron-rich α-position (C3) to carbonyl were prepared. Variation of substitution on the other (C4) phenyl ring gave compounds with activities in low nanomolar range (see structure **3**, Fig. 2), but no mention has been made of their general cellular toxicity.

Our interest in the dihydrofuranone analogs stems from our previous work [21] on the chemistry and antifungal activity of the structurally closely related 3-(substituted phenyl)-5-acyloxymethyl-2,5-dihydrofuran-2-ones derived from the natural product, (-)incrustoporin. We described the beneficial effect of halogens on antifungal activity, and identified

halophenyl (**4a**, **4b**, Fig. 3) analogues, the activities of which were comparable to that of amphotericin B.



4a Z = H ref 21
4b Z = COR
antifungal
IC_{50} 0.49 - 15.63 μM

Figure 3. Structures of halophenyl analogs **4a** and **4b.**

Prompted by the above observations, we became interested in expanding the 3,4-diarylfuranone library by 4-trimethoxyphenyl derivative(s), halophenyl derivatives, and, thirdly, those with a hydroxymethyl at C5. While a cytotoxic effect could be expected in all compounds, we speculated that the addition of halogens and/or the hydroxymethyl group might change the activity in favour of antifungal and/or antimicrobial. Accordingly, the goal of this work was to investigate the impact of the following structural modifications on cytotoxic and/or antiinfective activity of 3,4-diaryl furanones: 1. Attachment of the trimethoxyphenyl moiety to the electron-deficient β-position (C4) to the carbonyl group. 2. Variation of substitution towards halogens and other functional groups that could support antifungal activity. 3. Introduction of hydroxymethyl/acyloxymethyl group(s) to furanone C5. In this paper, we describe the synthesis of a library of such 3,4-diarylfuranones, and their screening for biological activities including cytotoxicity, proapoptotic effects, antifungal and antimicrobial activity.

2. Results and Discussion

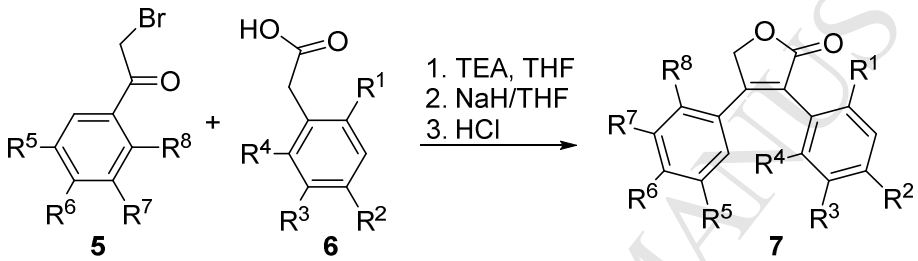
2.1. Experimental Design

Having in mind the goals set forth in the Introduction and taking into consideration commercial availability of substituted acetic acids and acetophenones as the starting materials for the syntheses (*vide infra*), we projected three subseries of diarylfuranones. First, hitherto unknown combretafuranone analogues with the trimethoxyphenyl ring β to carbonyl were planned. Next, taking into account the supportive effect of halogens for antifungal activity of the 3-aryl furanones **4**, the preparation of a wide spectrum of compounds with halogen

substitution was necessary. Finally, the introduction of 5-hydroxymethyl moiety to furanone C5 was intended with a view to exploring the influence of this substitution on the biological activity of the resultant 3,4-diphenylfuranones.

2.2. Synthesis

The target 3,4-diphenylfuranones were prepared *via* a modified synthetic route previously employed for the preparation of rofecoxib [22, 23] (Scheme 1). To this end, a substituted phenylacetic acid was esterified with a substituted α-bromoacetophenone, and the resultant ester was subjected to a base-mediated cyclization followed by acidic dehydration. Instead of DBU/DMF used in rofecoxib preparation [23], the cheaper NaH in THF was used in the cyclization step.



Scheme 1. Synthesis of 3,4-diphenylfuranone analogs.

Overviews of furanones with natural combretastatin-like substitution and halogenated derivatives are provided in Tables 1 and 2, respectively. The yields of the compounds exceeded 50 % in all cases.

P[a]	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**Y**[b]
7a	H	OCH_3	H	H	H	OCH_2O		H	65
7b	H	OCH_3	H	H	H	OCH_3	OCH_3	H	63
7c	OCH_3	OCH_3	H	OCH_3	H	OCH_3	OCH_3	OCH_3	60
7d	H	OH	H	H	H	OH	OH	H	70
7e	H	OCH_2O		H	H	OCH_2O		H	69
7f	H	OCH_2O		H	OCH_3	OCH_3	OCH_3	H	60

[a]*product,* [b]*yield (%)*

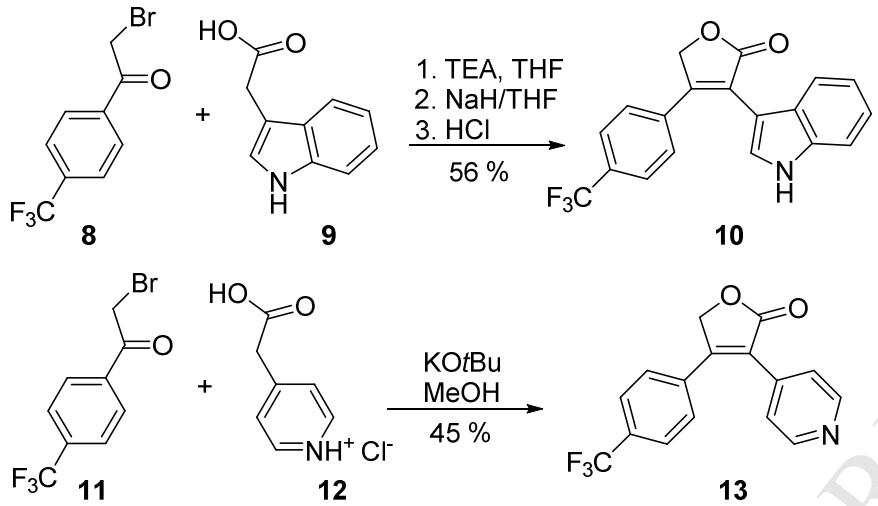
Table 1. Overview of analogs with combretastatin substitution pattern.

P	R1	R2	R3	R4	R5	R6	R7	R8	Y
7g	H	Br	H	H	H	H	H	H	62
7h	H	Br	H	H	H	CH_3	H	H	69
7i	H	Br	H	H	H	CF_3	H	H	57
7j	H	Br	H	H	H	CH_2CH_3	H	H	59
7k	H	H	H	Cl	H	CH_3	H	H	61
7l	H	H	Cl	H	H	CH_3	H	H	62
7m	H	Cl	H	Cl	H	CH_3	H	H	57
7n	H	Cl	Cl	H	H	CH_3	H	H	55
7o	H	F	H	H	H	H	H	H	58
7p	H	F	H	H	H	CH_3	H	H	63
7q	H	H	CF_3	H	H	CH_3	H	H	59
7r	H	H	CF_3	H	H	H	H	H	75
7s	H	CF_3	H	H	H	Cl	H	H	70
7t	H	Br	H	H	H	OCH_3	H	H	65
7u	H	Br	H	H	H	OH	H	H	62
7v	Cl	H	H	Cl	H	OCH_3	H	H	55
7w	H	OCH_3	H	H	H	SO_2CH_3	H	H	50

[a]product, [b]yield (%)

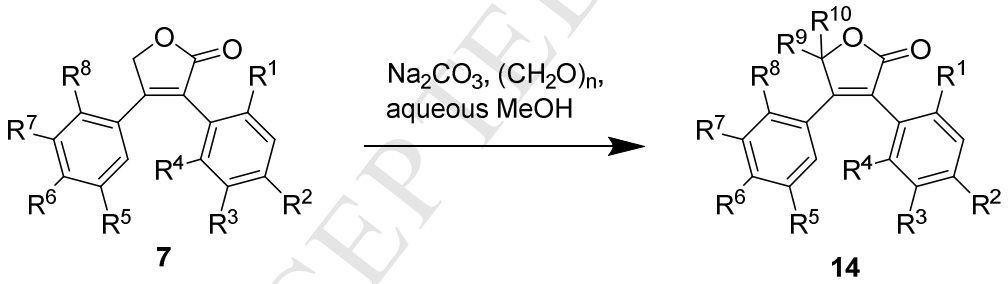
Table 2. Overview of halogenated derivatives.

In addition to diphenyl derivatives **7a-w**, we also prepared analogs **10** and **13** (Scheme 2) in which the C3 phenyl ring was replaced with indolyl and pyridyl moiety. The former was made as shown in Scheme 1, while the latter arose as the sole product from a one pot reaction of pyridine-4-ylacetic acid and 4-trifluoromethyl-α-bromoacetophenone with KO*t*Bu [22] (Scheme 2).



Scheme 2. Synthesis of heterocyclic derivatives **10** and **13**.

Our next task was to develop the route towards the 5-hydroxymethyl derivatives. The structure of furanones **7** offered enolization of γ-position to carbonyl followed by quench with a formaldehyde source. To this end, deprotonization with LDA/THF followed by the addition of paraformaldehyde provided only bis(hydroxymethyl) derivatives, milder conditions using Na_2CO_3 and $(CH_2O)_n$ in aqueous MeOH furnished mixtures of mono- and bishydroxymethylated compounds easily separable by column chromatography. Thus, selected compounds were subjected to this process (Scheme 3, Table 3).



Scheme 3. Hydroxymethylation of furanone C5.

SM[a]	P[b]	R1	R2	R3	R4	R5	R6	R7	R9	R10	Y[c]
7h	**14a**	H	Br	H	H	H	CH_3	H	CH_2OH	H	50
7h	**14b**	H	Br	H	H	H	CH_3	H	CH_2OH	CH_2OH	47
7h	**14c**[d]	H	Br	H	H	H	CH_3	H	CH_2OAc	CH_2OAc	59*
7b	**14d**	H	OCH_3	H	H	H	OCH_3	OCH_3	CH_2OH	H	48
7b	**14e**	H	OCH_3	H	H	H	OCH_3	OCH_3	CH_2OH	CH_2OH	43
	14f	H	H	Cl	Cl	H	Br	H	CH_2OH	CH_2OH	44

	14g	H	H	Cl	Cl	H	OCH_3	H	CH_2OH	CH_2OH	40

[a] *starting material,* [b]*product,* [c]*yield (%),* [d]*obtained by standard acetylation of* ***14b*** *(AcCl, Pyridine, DCM)*

Table 3. γ-Substituted 3,4-diphenylfuranones.

2.3. Screening for Biological Activities

Since some cytotoxicity could be expected with nearly 100 % certainty, screening of the compounds on a panel of cancer and normal cell lines was performed first. For this purpose, two non-malignant cell lines, i.e. retina epithelium cells RPE-1 and fibroblasts BJ, were used. The compounds were tested in dose response, and the incubation lasted for 48 hours. Cell viability assessment was based on the determination of the level of intracellular ATP using a luminescent cell viability assay. The cytotoxic profiles obtained were compared to those determined on cancer cell lines including two leukemia models K562 and HL-60, prostate adenocarcinoma cells PC-3, breast adenocarcinoma cells MCF7, and hepatocellular carcinoma Hep G2 cell line. In order to obtain an idea of the type of cell death, caspase 3/7 activity measurement was carried out. Results for all compounds are summarized in Table 4. In addition, solubility and bioavailability parameters useful for the assessment of the compounds as prospective drugs were calculated, see Table S1 in the Supporting Information (SI).

Firstly, oxygenated derivatives **7a-7f** were tested. No cytotoxic effects on RPE-1 and BJ were found, with the exception of **7e** that slightly decreased the viability of RPE-1. Marginal activity against both leukemic lines was also observed in the same derivative. In the next step, we focused on halogenated derivatives **7g-7s**. Interesting cytotoxic effect in the micromolar range against both leukemic cell lines K562 and HL60 with IC_{50} 4.0 and 9.34 µM, respectively, was detected for the fluorophenyl compound **7p**.

	Cytotoxicity [IC_{50}, µM]							**Caspase 3/7 Activity [IC_{50}, µM]**				
	RPE-1	**BJ**	**K562**	**HL-60**	**PC-3**	**MCF7**	**Hep G2**	**RPE-1**	**K562**	**HL-60**	**PC-3**	**Hep G2**
7a	>40	>40	15.65	15.46	>40	>40	>40	>40	>40	>40	>40	>40
7b	>40	>40	>40	>40	15.87	>40	>40	>40	>40	>40	>40	>40
7c	>40	>40	22.81	>40	>40	>40	>40	>40	>40	>40	>40	>40
7d	>40	>40	16.19	>40	>40	>40	>40	>40	>40	>40	>40	>40

7e	35.53	>40	16.01	17.39	>40	>40	>40	>40	>40	>40	>40	>40
7f	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
7g	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
7h	0.97	>40	0.30	0.65	>40	14.80	>40	8.16	0.29	2.77	>40	>40
7i	16.41	>40	>40	>40	>40	6.37	11.36	>40	>40	>40	>40	>40
7j	1.30	>40	0.47	1.02	>40	13.00	>40	>40	>40	>40	>40	>40
7k	2.09	>40	0.78	1.04	>40	>40	>40	13.29	>40	>40	>40	>40
7l	0.88	>40	0.21	0.48	>40	>40	>40	>40	>40	>40	>40	>40
7m	0.91	>40	0.19	0.27	>40	23.92	>40	10.09	>40	0.44	>40	>40
7n	0.54	>40	0.12	0.23	>40	15.40	>40	3.50	>40	0.54	>40	>40
7o	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
7p	>40	>40	4.00	9.34	>40	>40	>40	>40	3.23	7.53	>40	>40
7q	1.33	>40	0.29	0.66	>40	19.06	>40	>40	0.31	2.66	>40	>40
7r	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
7s	14.86	>40	>40	>40	>40	12.34	>40	>40	>40	>40	>40	>40
7t	3.35	>40	0.53	0.83	>40	14.28	>40	56.42	>40	2.18	>40	>40
7u	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
7v	>40	>40	14.43	15.08	>40	>40	>40	>40	16.25	12.50	>40	>40
7w	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
10	0.87	>40	0.26	0.67	>40	>40	>40	1.54	>40	2.94	>40	>40
13	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
14a	>40	>40	31.45	21.32	>40	>40	>40	>40	46.68	>40	>40	>40
14b	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
14c	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
14d	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
14e	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
14f	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
14g	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
Camp	>40	>40	0.09	0.02	0.40	0.34	0.43	27.20	0.711	0.07	>40	4.37

Table 4. Cytotoxic and apoptotic profiling (activation of caspase 3/7) of all compounds. Cell types used: RPE-1: human normal immortalized cells from pigmented epithelium in retina; BJ: human

primary fibroblasts; Hep G2: human hepatocellular carcinoma; K562: human chronic myelogenous leukemia; HL-60: human acute myeloid leukemia; PC-3: human prostate adenocarcinoma; MCF7: human breast adenocarcinoma. Camp= (S)-(+)-Camptothecin.

At the same time, no cytotoxic effect was observed against the other cell lines tested, indicating selectivity of **7p** to leukemia cells. Since the compound also activated caspase 3/7 pathway at nearly the same concentration, triggering of apoptosis can be presumed as the probable reason of cell death (Figure S1 in SI).

Of other compounds, **7v** showed cytotoxic pattern for leukemic cells similar to that of **7p**, albeit its efficiency is lower (see also Figure S2 in SI). On the other hand, high submicromolar cytotoxic activities against both leukemic lines were found for the halogen derivatives **7h**, **7j**, **7k**, **7l**, **7m**, **7n**, **7q** and **7t**. Unfortunately, the compounds were, without exception, also highly cytotoxic against the non-malignant RPE-1 line. As regards heterocyclic isosters **10** and **13**, a notable difference was observed. While the only structural difference was the presence of indolyl or pyridyl moiety at furanone C3, respectively, the former demonstrated cytotoxic effects at submicromolar concentrations (including RPE-1), but the latter was inactive.

Finally, the γ-substituted lactones were investigated without positive results. Only for **14a**, marginal activities resulting in the suppression of leukemic K562 and HL-60 cell growth were detected.

In summary, furanones **7f**, **7g**, **7o**, **7r**, **7u**, **7w**, **13** and **14b-14g** were non-toxic for all cell types up to 40 μM. In addition, the calculations of theoretical solubility and bioavailability gave favourable values (see Table S1 in SI). Thus, given the antimicrobial activities of compounds **7g**, **14a**, **14b**, **14f** and **14g** (vide infra), their further development as potential therapeutic agents is fully justified.

Bacterial strain (incubation period)		**Compound [MIC_{95}, μM]**							
		7a	**7c**	**7g**	**7l**	**14a**	**14b**	**14g**	**Cipr**
SA	24h	7.81	31.25	3.9	1.95	15.62	0.98	7.81	0.98
	48h	15.62	31.25	15.62	1.95	15.62	3.9	15.62	0.98
MRSA	24h	>500	62.5	31.25	15.62	62.5	250	62.5	500
	48h	>500	62.5	62.5	15.62	62.5	500	125	500

SE	24h	125	125	31.25	15.62	62.5	62.5	250	250
	48h	500	125	62.5	15.62	62.5	500	250	250
EF	24h	>500	>500	>1000	>500	500	500	500	0.98
	48h	>500	>500	>1000	>500	>500	>500	>500	0.98
EC	24h	>500	>500	>1000	>500	>500	>500	>500	0.06
	48h	>500	>500	>1000	>500	>500	>500	>500	0.06
KP	24h	>500	>500	>1000	>500	>500	>500	>500	0.12
	48h	>500	>500	>1000	>500	>500	>500	>500	0.12
KP-E	24h	>500	>500	>1000	>500	>500	>500	>500	>500
	48h	>500	>500	>1000	>500	>500	>500	>500	>500
PA	24h	>500	>500	>1000	>500	>500	>500	>500	3.90
	48h	>500	>500	>1000	>500	>500	>500	>500	7.81

Table 5. Antimicrobial activities of selected derivatives, **SA**: *Staphylococcus aureus* ATCC 6538, **MRSA**: *Staphylococcus aureus* HK5996/08, **SE**: *Staphylococcus epidermidis* HK6966/08, **EF**: *Enterococcus* sp. HK14365/08, **EC**: *Escherichia coli* ATCC 8739, **KP**: *Klebsiella pneumoniae* HK11750/08, **KP-E**: *Klebsiella pneumoniae* HK14368/08; and **PA**: *Pseudomonas aeruginosa* ATCC 9027. **Cipr** = Ciprofloxacin

Most of the above substances were subsequently tested for antifungal and antibacterial activity. The former performed on a panel of fungal strains including both *Candida* species and filamentous fungi showed no antifungal effect up to 1 mM concentration. However, antibacterial screening against a panel based on standard reference and antibiotic-resistant strains brought positive results. Of the oxygenated derivatives, compounds **7a** and **7c** exhibited interesting activity against *Staphylococcus aureus* strain ATCC 6538 at the level of minimum inhibitory concentration (MIC_{95}) 7.8-31.2 µM (Table 5, Table S2 in SI).

Even higher activities were observed for the halogenated derivatives with selectivity to *Staphylococcus* strains. Specifically, the lowest MIC_{95} values were found for compounds **7g** and **7l** with MIC_{95} values, 3.9 and 1.9 µM, respectively, after incubation with *Staphylococcus aureus* ATCC 6538 (Table 5, Table S2 in SI). In contrast to **7l**, bromophenyl derivative **7g** is completely non-toxic against human normal cells RPE-1 and BJ up to 40 µM (Table 4), and its activity against the methicillin-resistant clinical isolate is also notable. Other derivatives involved in the study (**7t**, **7v**, **7w**, **10** and **13**) were not active (Table S2 in SI). Some of the

γ-substituted lactones (**14a**, **b**, **f** and **g**) were able to inhibit *Staphylococcus* strains at similar MIC_{95} levels (0.98 - 31.25 μM, Table 5, Table S3 in SI). The best MIC_{95}, comparable to that of the standard ciprofloxacin, 0.98 μM, was found for compound **14b** against *Staphylococcus aureus* ATCC 6538 after 24 hour incubation. Derivatives **14a** and **14g** were also active, but at somewhat higher concentrations than **14b** (Table 5, Table S3 in SI). Considering the nontoxicity of the γ-substituted lactones to the control human cells RPE-1 and BJ, and favourable solubility and bioavailability parameters (Table S1 in SI), the structures are highly promising for further development as novel antibacterials.

3. Conclusion

In summary, the results have shown that placing the 3,4,5-trimethoxyphenyl ring to the β-position of the furanone core completely knocks out antiproliferative effects of the resultant combretafuranone derivatives. Second, substitution of the phenyl ring with halogens (as opposed to the highly oxygenated pattern typical of highly active combretastatin analogues) does not necessarily decrease cytotoxic effects against leukemic cells, but the compounds are also highly cytotoxic against non-malignant control cells. More interestingly, significant antibacterial activity has been uncovered in this class of compounds for the first time. Third, functionalization of furanone C5 with hydroxymethyl groups also leads to compounds without cytotoxic effects. However, a significant antibacterial effect, worthy of further development, is maintained. MIC_{95} of the most interesting derivative, 3-(4-bromophenyl)-5,5-bis(hydroxymethyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one against *S. aureus* strain ATCC 6538 was 0.98 μM (0.38 μg/mL) after 24 hour incubation. This finding is particularly important in view of the current state in the management of bacterial infections, often referred to as the antibiotic resistance crisis.

4. Material and Methods

4.1. Chemistry

All reagents were purchased from Sigma–Aldrich and used without further purification. Solvents (THF, DCM) were distilled prior to use. TLC analyses were performed using Merck TLC Silica gel 60 F254 TLC plates and visualized by UV in combination with staining. Column chromatography was carried out on Merck Silica gel 60 (0.040–0.063 mm). ^{1}H and ^{13}C NMR spectra were recorded with Varian Mercury VxBB 300 or VNMR S500 instruments. The chemical shifts were reported relative to TMS and referenced to the residual

solvent peaks. IR spectra were recorded on a Nicolet 6700 FT-IR equipped with an ATR device. MS data were measured on an Agilent Tech 500 Iontrap spectrometer. HR-MS data were recorded on a QTOF mass spectrometer using the electrospray ionization mode. Calculator Plugins were used for structure property prediction and calculation, Marvin 15.10.26, 2015, ChemAxon (http://www.chemaxon.com).

4.2. General Synthetic Methods

3,4-Diaryl-2,5-dihydrofuran-2-ones **(Method A)**. A substituted 2-bromoketone (1 mmol) and a substituted phenylacetic acid (1 mmol) were dissolved in dry THF (15 mL) and triethylamine (1 mmol) under Ar. The reaction mixture was stirred for 24 hours at room temperature; sodium hydride (60% oil suspension, 1 mmol) was then added, and the resultant mixture stirred for 1 hour. Finally, concentrated aqueous hydrochloric acid was added (to adjust pH to approx. 1), and the mixture stirred for 15 minutes. The reaction mixture was quenched with water (15 mL), and extracted with ethyl acetate (3 × 20 mL). The organic layers were dried over sodium sulfate, and concentrated under reduced pressure. The product was purified by column chromatography using hexane: ethyl acetate 7:3 mixture as a mobile phase.

Demethylation of aromatic methoxy groups (**Method B**). A 3,4-diphenyl furanone (2 mmol) was dissolved in dry DCM (20 mL), and the solution was cooled to -50 °C. Boron tribromide (1M solution in DCM, 6.0 mL) was then added dropwise, the resultant mixture was stirred for 1 hour, and allowed to warm to room temperature. Water (10 mL) was added, and the mixture stirred for 30 min at RT. The reaction mixture was extracted with ethyl acetate (50 mL) and water (100 mL). The organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The product was purified by silica gel column chromatography using hexane: ethyl acetate 50:50 as a mobile phase.

Hydroxymethylation of furanone C5 (**Method C**). A 3,4-diphenyl furanone (2 mmol) and sodium carbonate (2 mmol) were suspended in dry methanol (20 mL) under Ar atmosphere. Paraformaldehyde (2 mmol) was then added, and the resultant mixture stirred for 12 hours at room temperature. The mixture was extracted with ethyl acetate (50 mL) and water (100 mL). The organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The product was purified by silica gel column chromatography using hexane: ethyl acetate 30:70 as a mobile phase.

4-(3,4-methylenedioxophenyl)-3-(4-methoxyphenyl)-2,5-dihydrofuran-2-one (**7a**)

65% yield. Yellow liquid. The title compound was synthesized by Method A using 2-bromo-1-(4-methoxyphenyl)ethan-1-one and 3,4-(methylenedioxy)phenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.45–7.32 (m, 2H), 6.95–6.90 (m, 2H), 6.89–6.86 (m, 1H), 6.81–6.76 (m, 2H), 5.99 (s, 2H), 5.09 (s, 2H), 3.83 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.9, 159.9, 154.2, 149.5, 148.1, 130.6, 124.9, 124.5, 122.4, 122.0, 114.3, 108.8, 107.6, 101.6, 70.5, 55.3; **IR** (ATR) ν_{max} 1057, 1172, 1294, 1307, 1335, 1353, 1379, 1445, 1466, 1489, 1514, 1571, 1605, 1634, 1727, 1747, 2926 cm^{-1}. **LRMS** ($APCI^+$): *m/z* (relative intensity) 311.0 $[M+H]^+$, 292.9 (14); **HRMS** (TOF-ESI+) m/z calcd for $C_{18}H_{14}O_5^+$ 310.0814; found 310.0816. **Anal.** Calc. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55; found: C, 69.72; H, 4.59.

4-(3,4-dimethoxyphenyl)-3-(4-methoxyphenyl)- 2,5-dihydrofuran-2-one (**7b**)

63% yield. Yellow amorphous solid. The title compound was synthesized by Method A using 4-methoxyphenylacyl bromide and 3,4-dimethoxyphenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.46 – 7.38 (m, 2H), 7.00 – 6.91 (m, 3H), 6.89 – 6.82 (m, 2H), 5.16 (s, 2H), 3.90 (s, 3H), 3.83 (s, 3H), 3.59 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.95, 159.84, 154.56, 151.01, 148.91, 130.76, 124.06, 123.48, 122.79, 120.38, 114.14, 111.02, 110.53, 70.30, 55.90, 55.57, 55.29; **IR** (ATR) ν_{max} 1015, 1151, 1179, 1249, 1263, 1329, 1449, 1464, 1507, 1653, 1792, 1844, 1868, 2838, 2935, 3618, 3629 cm^{-1}; **HRMS** (TOF-ESI+) m/z calcd for $C_{19}H_{18}O_5^+$ 327,1227; found 327,1234; **Anal.** Calc. for $C_{19}H_{18}O_5$: C, 69.93; H, 5.56; found: C, 69.94; H, 5.59.

4-(2,3,4-trimethoxyphenyl)-3-(2,4,6-trimethoxyphenyl)-2,5-dihydrofuran-2-one (**7c**)

60% yield. White amorphous solid. The title compound was synthesized by Method A using 2,4,6-trimethoxyphenacyl bromide and 2,3,4-trimethoxyphenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.78 – 7.49 (m, 1H), 6.80 – 6.59 (m, 1H), 6.15 (s, 2H), 5.23 (s, 2H), 4.01 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H), 3.82 (s, 6H), 3.81 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 192.16, 172.07, 160.41, 159.03, 158.06, 154.31, 141.53, 126.00, 122.54, 107.33, 103.86, 90.56, 69.31, 61.25, 60.77, 56.11, 55.81, 55.29, 28.11; **IR** (ATR) ν_{max} 1013, 1043, 1055, 1078, 1104, 1156, 1190, 1205, 1238, 1257, 1289, 1366, 1412, 1438, 1493, 1587, 1676, 1732, 2841, 2945 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (relative intensity) 417.2 $[M+H]^+$; **Anal.** Calc. for $C_{22}H_{24}O_8$: C, 63.45; H, 5.81; found: C, 63.46; H, 5.80.

4-(3,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)- 2,5-dihydrofuran-2-one (**7d**)

70% yield. Yellow liquid. The title compound was synthesized by Method A and subsequently Method B. **^{1}H NMR** (500 MHz, CD_3OD) δ 7.33 – 7.13 (m, 2H), 6.87 – 6.80 (m, 4H), 6.75 – 6.72 (m, 1H), 5.20 (s, 2H); **^{13}C NMR** (125 MHz, CD_3OD) δ 177.01, 158.99, 158.06, 149.38, 146.61, 131.83, 123.89, 123.82, 123.20, 121.26, 116.52, 116.47, 115.71, 72.16; **IR** (ATR) ν_{max} 1043, 1101, 1127, 1164, 1226, 1263, 1437, 1489, 1559, 1570, 1606, 1653, 1716, 1792, 1868, 2853, 2923, 3314, 3566, 3629 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (relative intensity) 345 $[M+H]^+$, 346 (20), 347 (2); **Anal.** Calc. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26; found: C, 67.62; H, 4.29.

3-(3,4-methylenedioxophenyl)-4-(3,4-dimethoxyphenyl))-2,5-dihydrofuran-2-one (**7e**)

69% yield. White amorphous solid. The title compound was synthesized by Method A using 3,4-dimethoxyphenacyl bromide and 3,4-methylenedioxophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 6.99 – 6.91 (m, 3H), 6.90 – 6.83 (m, 3H), 5.98 (s, 2H), 5.15 (s, 2H), 3.91 (s, 3H), 3.64 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.72, 155.06, 151.12, 148.94, 147.88, 147.83, 124.13, 124.03, 123.46, 123.20, 120.54, 111.06, 110.55, 109.77, 108.67, 101.21, 70.27, 55.92, 55.64; **IR** (ATR) ν_{max} 1018, 1071, 1124, 1163, 1250, 1272, 1335, 1441, 1548, 1640, 1728, 1754, 1778, 1820, 1900, 1950, 2031, 2855, 2927, 3019 cm^{-1}; **HRMS** (TOF-ESI+) m/z calcd for $C_{19}H_{16}O_6$+ 341,1020; found 341,1024; **Anal.** Calc. for $C_{19}H_{16}O_6$: C, 67.06; H, 4.74; ; found: C, 67,44 ; H, 5.75.

3-(3,4-methylenedioxophenyl)-4-(3,4,5-trimethoxyphenyl)-2,5-dihydrofuran-2-one (**7f**)

60% yield. White amorphous solid. The title compound was synthesized by Method A using 3,4,5-trimethoxyphenacyl bromide and 3,4-methylenedioxophenylacetic. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.03 – 6.69 (m, 3H), 6.56 (s, 2H), 5.95 (s, 2H), 5.12 (s, 2H), 3.85 (s, 3H), 3.68 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.4, 155.1, 153.4, 147.9, 147.8, 140.1, 125.8, 125.1, 123.8, 123.5, 109.7, 108.6, 104.9, 101.2, 70.4, 60.9, 56.1; **LRMS ($APCI^+$):** *m/z* (rel. intensity) 371 $[M+H]^+$, 372 (20); **IR** (ATR) ν_{max} 1078, 1175, 1199, 1236, 1344, 1368, 1416, 1457, 1494, 1508, 1581, 1635, 1744, 2929 cm^{-1}; **Anal.** Calc. for $C_{20}H_{18}O_7$: C, 64.86; H, 4.90; found: C, 64.90; H, 4.98.

3-(4-bromophenyl)-4-phenyl-2,5-dihydrofuran-2-one (**7g**)

62% yield. White amorphous solid. The title compound was synthesized by Method A using phenylacyl bromide and 4-bromophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.54–7.49 (m, 2H), 7.47–7.41 (m, 1H), 7.41–7.36 (m, 2H), 7.35–7.30 (m, 4H), 5.17 (s, 2H); **^{13}C NMR**

(125 MHz, $CDCl_3$) δ 173.0, 156.7, 131.9, 130.9, 130.8, 130.5, 129.1, 129.0, 127.4, 125.0, 123.1, 70.6; **IR** (ATR) ν_{max} 1086, 1099, 1158, 1230, 1338, 1368, 1395, 1452, 1485, 1497, 1575, 1589, 1652, 1746, 3075 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (rel. intensity) 315 $[M+H]^+$, 317 (97), 236 (50); **HRMS** (TOF-ESI+**)** m/z calcd for $C_{16}H_{11}BrO_2$+ 315.0015; found 315.0030; **Anal.** Calc. for $C_{18}H_{14}O_5$: C, 60.98; H, 3.52; found: C, 60.69; H, 3.50.

3-(4-bromophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**7h**)

69% yield. Yellow amorphous solid. The title compound was synthesized by Method A using 4-methylphenacyl bromide and 4-bromophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.54–7.48 (m, 2H), 7.36–7.30 (m, 2H), 7.24 – 7.20 (m, 2H), 7.20 – 7.15 (m, 2H), 5.15 (s, 2H), 2.38 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.2 156.8, 141.5, 131.9, 130.9, 129.8, 129.3, 127.6, 127.3, 124.2, 123.2, 70.6, 21.5; **IR** (ATR) ν_{max} 1064, 1112, 1125, 1186, 1241, 1337, 1367, 1379, 1450, 1488, 1514, 1586, 1609, 1650, 1752, 2920 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (relative intensity) 329 $[M+H]^+$, 332 (18); **HRMS** (TOF-ESI+) m/z calcd for $C_{17}H_{13}BrO_2^+$ 329.0170; found 329.0182; **Anal.** Calc. for $C_{17}H_{13}BrO_2$: C, 62.03; H, 3.98; found: C, 62.15; H, 4.02.

3-(4-bromophenyl)-4-(4-(trifluoromethyl)phenyl) -2,5-dihydrofuran-2-one (**7i**)

57% yield of yellow amorphous solid . The title compound was synthesized by Method A using phenacyl bromide and phenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.71 – 7.61 (m, 2H), 7.57 – 7.49 (m, 2H), 7.48 – 7.39 (m, 2H), 7.34 – 7.28 (m, 2H), 5.18 (s, 2H). **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.5, 154.9, 134.3, 132.6 (q, J = 33.02 Hz), 132.3, 130.9, 128.4, 128.0, 127.2, 126.3 (q, J = 3.42 Hz), 123.9, 123.6 (q, J = 272.55 Hz), 70.7. **LRMS** ($APCI^+$): *m/z* (relative intensity) 383.1 $[M+H]^+$; 385 (97). **Anal.** Calc. for $C_{17}H_{10}BrF_3O_2$: C, 53.29; H, 2.63; found: C, 53.15; H, 2.52.

3-(4-bromophenyl)-4-(4-ethylphenyl)- 2,5-dihydrofuran-2-one (**7j**)

59% yield. Yellow amorphous solid. The title compound was synthesized by Method A using 4ethylphenacyl bromide and 4-bromophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.56 – 7.47 (m, 2H), 7.36 – 7.31 (m, 2H), 7.26 – 7.23 (m, 2H), 7.22 – 7.18 (m, 2H), 5.16 (s, 2H), 2.75 – 2.60 (m, 2H), 1.25 (t, J = 7.6 Hz, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.17, 156.74, 147.64, 131.88, 130.92, 130.91, 129.29, 128.60, 127.77, 127.40, 122.97, 70.59, 28.73, 15.07; **IR** (ATR) ν_{max} 1055, 1067, 1133, 1167, 1191, 1340, 1390, 1366, 1434, 1458, 1484, 1519, 1559, 1609, 1647, 1698, 1737, 2933, 2969 cm^{-1}; **HRMS** (TOF-ESI+) m/z calcd for

$C_{18}H_{15}BrO_2$+ 343,0328; found 343,0329; **Anal.** Calc. for $C_{18}H_{15}BrO_2$: C, 62.99; H, 4.41; found: C, 62.95; H, 4.37.

3-(2-chlorophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**7k**)

61% yield. White amorphous solid. The title compound was synthesized by Method A using 4-methylphenacyl bromide and 2-chlorophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.54 – 7.46 (m, 1H), 7.43 – 7.32 (m, 2H), 7.34 – 7.28 (m, 1H), 7.16 – 7.08 (m, 4H), 5.31 (d, *J* = 12.3 Hz, 2H), 2.35 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.92, 157.65, 141.75, 133.91, 131.28, 130.35, 130.19, 130.07, 129.74, 127.58, 127.28, 127.00, 123.54, 70.52, 21.49; **IR** (ATR) ν_{max} 1036, 1059, 1128, 1164, 1337, 1364, 1438, 1472, 1516, 1611, 1651, 1750 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (relative intensity) 285.2 $[M+H]^+$ **HRMS** (TOF-ESI+) m/z calcd for $C_{17}H_{13}ClO_2$+ 285,0677; found 285,0682; **Anal.** Calc. for $C_{17}H_{13}ClO_2$: C, 71.71; H, 4.60; found: C, 71.75; H, 4.65.

3-(3-chlorophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**7l**)

62% yield. White amorphous solid. The title compound was synthesized by Method A using 4-methylphenacyl bromide and 3-chlorophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.48–7.45 (m, 2H), 7.38 –7.29 (m, 4H), 7.23 – 7.15 (m, 2H), 5.18 (s, 2H), 2.38 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.07, 157.22, 141.59, 134.57, 132.22, 129.93, 129.83, 129.27, 128.87, 127.42, 127.37, 124.02, 70.55, 21.49, 21.48; **IR** (ATR) ν_{max} 1023, 1038, 1063, 1103, 1158, 1236, 1338, 1358, 1434, 1477, 1560, 1612, 1652, 1764, 2913 cm^{-1}; **HRMS** (TOF-ESI+) m/z calcd for $C_{17}H_{13}ClO_2$+ 285,0677; found 285,0686; **Anal.** Calc. for $C_{17}H_{13}ClO_2$: C, 71.71; H, 4.60; found: C, 71.78; H, 4.66.

3-(2,4-dichlorophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**7m**)

57% yield. White amorphous solid. The title compound was synthesized by Method A using 4-methylphenacyl bromide and 2,4-dichlorophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.52 (d, J = 2.0 Hz, 1H), 7.38 – 7.31 (m, 1H), 7.28 – 7.24 (m, 1H), 7.18 – 7.14 (m, 2H), 7.13 – 7.09 (m, 2H), 5.55 – 5.04 (m, 2H), 2.37 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.61, 158.32, 142.04, 135.52, 134.76, 132.17, 130.04, 129.84, 128.92, 127.73, 127.35, 126.95, 122.44, 70.58, 21.49; **IR** (ATR) ν_{max} 1044, 1062, 1070, 1163, 1188, 1337, 1368, 1476, 1558, 1610, 1646, 1683, 1746, 1792, 1844, 1868, 1922, 2929, 3674 cm^{-1}; **HRMS** (TOF-ESI+) m/z calcd for $C_{17}H_{12}Cl_2O_2$+ 319,0287; found 319,0295; **Anal.** Calc. for $C_{17}H_{12}Cl_2O_2$: C, 63.97; H, 3.79; found: C, 64,27 ; H, 3,77.

3-(3,4-dichlorophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**7n**)

55% yield. White amorphous solid. The title compound was synthesized by Method A using 4-methylphenacyl bromide and 3,4-dichlorophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.60 (d, J = 2.0 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 7.33 – 7.24 (m, 1H), 7.24 – 7.16 (m, 4H), 5.17 (s, 2H), 2.39 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.79, 157.67, 141.79, 132.92, 132.89, 131.10, 130.63, 130.33, 129.92, 128.62, 127.30, 127.19, 122.97, 70.61, 21.48; **IR** (ATR) ν_{max} 1070, 1137, 1166, 1190, 1230, 1321, 1344, 1368, 1437, 1457, 1473, 1489, 1517, 1541, 1558, 1610, 1637, 1652, 1684, 1717, 1737, 1934, 3082 cm^{-1}; **HRMS** (TOF-ESI+) m/z calcd for $C_{17}H_{22}Cl_2O2+$ 319,0287; found 319,0294; **Anal.** Calc. for $C_{17}H_{22}Cl_2O_2$: C, 63.97; H, 3.79; found: C, 63,64 ; H, 3,77.

3-(4-fluorophenyl)-4-phenyl-2,5-dihydrofuran-2-one (**7o**)

58% yield. White amorphous solid. The title compound was synthesized by Method A using phenacyl bromide and 4-fluorophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.47 – 7.40 (m, 3H), 7.40 – 7.34 (m, 2H), 7.34 – 7.30 (m, 2H), 7.12 – 7.03 (m, 2H), 5.18 (s, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.4, 163.2 (d, J = 249.0 Hz), 156.4, 131.3 (d, J = 8.3 Hz), 130.8, 130.8, 129.2, 127.6, 126.2 (d, J = 3.5 Hz), 125.3, 115.9 (d, J = 21.6 Hz), 70.8; **IR** (ATR) ν_{max} 1002, 1028, 1067, 1085, 1100, 1163, 1217, 1338, 1365, 1408, 1447, 1498, 1510, 1541, 1575, 1596, 1640, 1707, 2922, 3051 cm^{-1}; **HRMS (APCI^{+})**: *m/z* (rel. Intensity) 255.1 [M+H]+.

3-(4-fluorophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**7p**)

63% yield. White amorphous solid. The title compound was synthesized by Method A using 4-methylphenacyl bromide and 4-fluorophenylacetic acid. The spectral data were in accordance with those in the literature [24]. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.47 – 7.40 (m, 2H), 7.24 – 7.14 (m, 4H), 7.08 (s, 2H), 5.16 (s, 2H), 2.38 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.6, 163.0 (d, J = 248.8 Hz), 156.5, 141.4, 131.3 (d, J = 8.2 Hz), 129.9, 127.9, 127.5, 126.5 (d, J = 3.4 Hz), 124.5, 115.9 (d, J = 21.7 Hz), 70.7, 21.6; **IR** (ATR) ν_{max} 1020, 1068, 1117, 1129, 1193, 1337, 1364, 1407, 1444, 1505, 1518, 1598, 1611, 1645, 1708, 1745, 1915, 2864, 2922, 3067 cm^{-1}. **LRMS** (APCI^{+}): *m/z* (relative intensity) 269.1 $[M+H]^{+}$; 267 (5); **Anal.** Calc. for $C_{17}H_{13}FO_2$: C, 76.11; H, 4.88; found: C, 76.12 ; H, 4.90.

4-(4-methylphenyl)-3-(3-(trifluoromethyl)phenyl)- 2,5-dihydrofuran-2-one (**7q**)

59% yield. White amorphous solid. The title compound was synthesized by Method A using 3-(trifluoromethyl)phenacyl bromide and 4-methylphenylacetic acid. **^{1}H NMR** (500 MHz,

$CDCl_3$) δ 7.79 – 7.71 (m, 1H), 7.67 – 7.58 (m, 2H), 7.56 – 7.44 (m, 1H), 7.24 – 7.07 (m, 4H), 5.20 (s, 2H), 2.38 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.2, 157.8, 141.9, 132.9, 131.4, 131.2 (q, J = 32.55), 130.3, 129.3, 127.5, 127.4, 126.5 – 126.3 (m), 126.0 (q, J = 3.7 Hz), 124.0 (q, J = 272.47 Hz), 70.8 (t, J = 5.91), 21.6; **IR** (ATR) ν_{max} 1074; 1128; 1135; 1157; 1180; 1280; 1322; 1348; 1445; 1611; 1649; 1703; 1741; 2926; 3070 cm^{-1}; **HRMS** (TOF-ESI+) m/z calcd for $C_{18}H_{13}F_3O_2$+ 319,0940; found 319,0952; **LRMS** ($APCI^+$): *m/z* (relative intensity) 319.5 $[M+H]^+$; 320 (20); **Anal.** Calc. for $C_{18}H_{13}F_3O_2$: C, 67.92; H, 4.12; found: C, 67.94; H, 4.20.

4-phenyl-3-[3-(trifluoromethyl)phenyl]-2,5-dihydrofuran-2-one (**7r**)

75% yield. Yellow liquid. The title compound was synthesized by Method A using 3-(trifluoromethyl)phenacyl bromide and phenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.75 – 7.70 (m, 1H), 7.70 – 7.60 (m, 2H), 7.56 – 7.48 (m, 1H), 7.48 – 7.42 (m, 1H), 7.41 – 7.36 (m, 2H), 7.34 – 7.28 (m, 2H), 5.22 (s, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.0. 157.8. 132.8, 131.3 (q, J = 32.64 Hz) 131.2, 131.1, 130.4, 129.4, 127.6, 126.5 – 126.3 (q≈m, J = 1.71 Hz), 125.7 (q, J = 3.80 Hz), 124.9, 123.9 (q, J = 272.57 Hz), 70.9 (t, J = 4.98 Hz); **LRMS** ($APCI^+$): *m/z* (relative intensity) 305.5 $[M+H]^+$; **Anal.** Calc. for $C_{17}H_{11}F_3O_2$: C, 67.11; H, 3.64; found: C, 67.15; H, 3.72.

4-(3-chlorophenyl)-3-[4-(trifluoromethyl)phenyl]- 2,5-dihydrofuran-2-one (**7s**)

70% yield. White liquid. The title compound was synthesized by Method A using 4-(trifloromethyl)phenacyl bromide and 3-chlorophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.65 (d, J = 8.3 Hz, 2H), 7.48 – 7.42 (m, 3H), 7.41 – 7.36 (m, 1H), 7.35 – 7.30 (m, 1H), 7.29 – 7.23 (m, 1H), 5.20 (s, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.4, 155.5, 135.0, 134.0 (q≈d , J = 1.3 Hz), 132.6 (q, J = 33.0 Hz), 131.3, 130.3, 129.6, 129.3,128.1, 127.5, 127.0, 126.3 (q, J = 3.76 Hz), 123.6 (q, J = 272.6), 70.6; **LRMS** ($APCI^+$): *m/z* (relative intensity) 339.4 $[M+H]^+$; **HRMS** (TOF-ESI+) m/z calcd for $C_{17}H_{10}ClF_3O_2$+ 339,0394; found 339,0396.

3-(4-bromophenyl)-4-(4-methoxyphenyl)-2,5-dihydrofuran-2-one (**7t**)

65% yield. White amorphous solid. The title compound was synthesized by Method A using 4-bromophenacyl bromide and 4-methoxyphenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.53 (m, 2H), 7.31 (m, 4H), 6.87 (m, 2H), 5.15 (s, 2H), 3.83 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$ δ δ 173.3, 161.6, 156.3, 131.9, 130.9, 129.5, 129.1, 123.0, 122.9, 122.7, 114.5, 70.4,

55.4; **IR** (ATR) ν_{max} 1165, 1100, 1165, 1181, 1261, 1306, 1369, 1426, 1454, 1488, 1518, 1571, 1605, 1641, 1727, 1915, 2852, 2925 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (relative intensity) 345 $[M+H]^+$, 348 (20), 349 (1,5); **HRMS** (TOF-ESI+) m/z calcd for $C_{17}H_{13}BrO_3^+$ 345,0131; found 345,0130; **Anal**. Calc. for C17H13BrO3: C, 59.15; H, 3.80; found: C, 59.37; H, 3.78.

3-(4-bromophenyl)-4-(4-hydroxyphenyl)- 2,5-dihydrofuran-2-one (**7u**)

62% yield. White liquid. The title compound was synthesized by Method A and subsequently Method B. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.61 – 7.48 (m, 2H), 7.35 – 7.28 (m, 2H), 7.29 – 7.22 (m, 2H), 6.84 – 6.70 (m, 2H), 5.28 (s, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 176.00, 161.60, 159.99, 132.94, 132.42, 131.72, 130.70, 123.56, 122.84, 122.70, 116.85, 72.27; **IR** (ATR) ν_{max} 1037, 1043, 1064, 1171, 1251, 1344, 1396, 1540, 1608, 1636, 1709, 1792, 1869, 3237 cm^{-1}; **HRMS** (TOF-ESI+) m/z calcd for $C_{16}H_{11}BrO_3$+ 330,9964; found 330,9961; **Anal**. Calc. for $C_{16}H_{11}BrO_3$: C, 58.03; H, 3.35; found: C, 58.12; H, 3.42.

3-(2,6-dichlorophenyl)-4-(4-methoxyphenyl)-2,5-dihydrofuran-2-one (**7v**)

55% yield. Yellow amorphous solid. The title compound was synthesized by Method A using 4-methoxyphenacyl bromide and 2,6-dichlorophenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.45 – 7.41 (m, 2H), 7.36 – 7.29 (m, 1H), 7.19 – 7.13 (m, 2H), 6.91 – 6.73 (m, 2H), 5.35 (s, 2H), 3.81 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.0, 162.1, 158.2, 135.7, 130.6, 130.0, 128.5, 128.4, 122.7, 120.2, 114.7, 70.6, 55.4; **IR** (ATR) ν_{max} 1028, 1063, 1098, 1181, 1261, 1335, 1431, 1518, 1606, 1650, 1711, 1748, 2850, 2928, 3075 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (relative intensity) 345 [M+H]+ , 348 (20), 349 (1,5); **Anal**. Calc. for $C_{17}H_{12}Cl_2O_3$: C, 60.92; H, 3.61; found: C, 60.99; H, 3.66.

3-(4-methoxyphenyl)-4-[4-(methylsulfonyl)phenyl]-2,5-dihydrofuran-2-one (**7w**)

50% yield. White amorphous solid. The title compound was synthesized by Method A using 4-methylsulfonylphenacyl bromide and 4-methoxyphenylacetic acid. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.04 – 7.89 (m, 2H), 7.62 – 7.49 (m, 2H), 7.44 – 7.32 (m, 2H), 6.98 – 6.65 (m, 2H), 5.16 (s, 2H), 3.84 (s, 3H), 3.08 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.7, 160.5, 151.8, 141.7, 136.7, 130.6, 128.5, 128.4, 128.1, 121.2, 114.4, 70.3, 55.3, 44.3; **IR** (ATR) ν_{max} 1024; 1114; 1126; 1178; 1253; 1287; 1310; 1365; 1458; 1541; 1607; 1753; 1922; 2894; 2952; 2958; 3009 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (relative intensity) 345 $[M+H]^+$, 346 (20), 347 (2); **Anal**. Calc. for $C_{18}H_{16}O_5S$: C, 62.78; H, 4.68; found: C, 62.84; H, 4.73.

3-(pyridin-4-yl)-4-[4-(trifluoromethyl)phenyl]- 2,5-dihydrofuran-2-one (**13**)

45% yield. White amorphous solid. 4-pyridylacetic acid hydrochloride (1 mmol) was dissolved in dry methanol (10 mL), and potassium *tert*-butoxide (2 mmol) was added. The resultant mixture was stirred for 15 minutes under Ar atmosphere. The solvent was then removed, and the remaining solid redissolved in dry DMF (10 mL). 4-(Trifluoromethyl)phenacyl bromide (1 mmol) was added to the solution, and the reaction mixture was stirred for 1 hour. The mixture was extracted with ethyl acetate (50 mL) and water (100 mL). The organic layer was dried over sodium sulfate, and the solvent removed. The product was purified by silica gel column chromatography using hexane: ethyl acetate 40:60 as a mobile phase. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.71 – 8.59 (m, 2H), 7.75 – 7.60 (m, 2H), 7.46 – 7.39 (m, 2H), 7.36 – 7.32 (m, 2H), 5.19 (s, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.4, 157.2, 150.4, 137.8, 133.8, 133.5, 133.2, 132.9, 132.7, 129.1, 127.9, 126.7, 126.4, 126.4, 126.4, 126.3, 125.9, 124.5, 123.5, 122.4, 70.7; **HRMS** (TOF-ESI+) m/z calcd for $C_{16}H_{10}F_3NO_2$+ 306,0736; found 306,0742; **LRMS** (APCI$^+$): *m/z* (relative intensity) 306.4 $[M+H]^+$; **Anal**. Calc. for $C_{16}H_{10}F_3NO_2$: C, 50.03; H, 2.36; N, 3.65; found: C, 50.04; H, 2.39, N, 3.57.

3-(1H-indol-3-yl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**10**)

56% yield. White amorphous solid. The title compound was synthesized by Method A using 4-methylphenacyl bromide and indol-3-yl acetic acid. **^{1}H NMR** (500 MHz, acetone) δ 10.67 (s, 1H), 7.79 – 7.73 (m, 1H), 7.52 – 7.46 (m, 1H), 7.42 – 7.36 (m, 2H), 7.25 – 6.99 (m, 3H), 6.90 – 6.74 (m, 2H), 5.34 (s, 2H), 2.33 (s, 3H); **^{13}C NMR** (125 MHz, acetone) δ 174.60, 153.15, 140.86, 137.49, 130.37, 129.96, 128.34, 127.85, 125.82, 122.48, 121.63, 120.03, 119.85, 112.57, 106.45, 71.27, 21.37; **IR** (ATR) ν_{max} 1108; 1246; 1295; 1342; 1350; 1380; 1438; 1459; 1506; 1559; 1616; 1652; 1683; 2928; 3277 cm^{-1}. **LRMS** (APCI$^+$): *m/z* (relative intensity) 290.2 $[M+H]^+$; 291.1 (20). **Anal**. Calc. for $C_{19}H_{15}NO_2$: C, 78.87; H, 5.23; N, 4.84; found: C, 79.86; H, 5.25; N, 4.82. **HRMS** (TOF-ESI+) m/z calcd for $C_{19}H_{15}NO_2^+$ 290,1176; found 290,1181.

3-(4-bromophenyl)-5-(hydroxymethyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**14a**)

50% yield. Yellow amorphous solid. The title compound was synthesized by Method A and subsequently by Method C. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.53 – 7.39 (m, 2H), 7.36 – 7.28 (m, 2H), 7.24 – 7.10 (m, 4H), 5.60 – 5.46 (m, 1H), 4.02 (dd, *J* = 12.5, 2.7 Hz, 1H), 3.68 (dd, *J* = 12.5, 4.8 Hz, 1H), 2.38 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.1, 157.9, 141.0, 131.7,

130.8, 129.9, 128.8, 127.8, 127.5, 126.1, 123.0, 82.6, 62.4, 21.4; **IR (ATR) ν_{max}** 1039, 1105, 1072, 1170, 1233, 1334, 1356, 1396, 1418, 1456, 1473, 1507, 1540, 1653, 1684, 1699, 1736, 2855, 2926, 3402 cm^{-1}; **LRMS** ($APCI^+$): *m/z* (relative intensity) 359 $[M+H]^+$, 361 (97), 362 (20), 360 (17); **Anal**. Calc. for $C_{18}H_{15}BrO_3$: C, 60.19; H, 4.21; found: C, 60.24; H, 4.25; **HRMS** (TOF-ESI+) m/z calcd for $C_{18}H_{15}BrO_3^+$ 359,0277; found 359,0283.

3-(4-bromophenyl)-5,5-bis(hydroxymethyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**14b**) 47% overall yield White liquid. The title compound was synthesized by Method A and subsequently by Method C. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.40 – 7.34 (m, 2H), 7.32 – 7.17 (m, 6H), 3.89 (d, *J* = 12.3 Hz, 2H), 3.73 (d, *J* = 12.3 Hz, 2H), 2.34 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 173.6, 163.0, 140.7, 132.2, 132.1, 130.7, 130.5, 130.2, 130.1, 129.1, 123.4, 94.3, 62.1, 21.4; **LRMS** ($APCI^+$): *m/z* (relative intensity) 389 $[M+H]^+$, 391 (97), 391 (17); **HRMS** (TOF-ESI+) m/z calcd for $C_{19}H_{17}BrO_4$+ 389,0383; found 389,0393; **Anal**. Calc. for $C_{19}H_{17}BrO_4$: C, 58.63; H, 4.40; found: C, 58.71; H, 4.49.

5,5-bis(acetoxymethyl)-3-(4-bromophenyl)-4-(4-methylphenyl)-2,5-dihydrofuran-2-one (**14c**) 59% overall yield. White liquid. Lactone **14b** (0.5 mmol) was dissolved in dry DCM (10 mL). Pyridine (1,5 mmol), and acetyl chloride (1,5 mmol) were added to the solution. After 1 hour, the solution was diluted with water and stirred for 30 min at RT. The resultant mixture was extracted with ethyl acetate (50 mL) and water (100 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The product was purified by column chromatography using hexane: ethyl acetate 70:30 mixture as a mobile phase. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.44 – 7.37 (m, 2H), 7.32 – 7.25 (m, 2H), 7.25 – 7.19 (m, 2H), 7.05 – 6.98 (m, 2H), 4.55 (d, *J* = 12.1 Hz, 2H), 4.30 (d, *J* = 12.1 Hz, 2H), 2.38 (s, 3H), 2.05 (s, 6H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.05, 169.94, 158.28, 140.25, 131.57, 130.65, 130.24, 129.05, 128.01, 127.75, 127.45, 123.38, 86.28, 62.76, 21.32, 20.51; **Anal**. Calc. for $C_{23}H_{21}BrO_6$: C, 58.37; H, 4.47; found: C, 58.40; H, 4.48; **HRMS** (TOF-ESI+) m/z calcd for $C_{23}H_{21}BrO_6$+ 472,0500; found 472,0610.

4-(3,4-dimethoxyphenyl)-5-(hydroxymethyl)-3-(4-methoxyphenyl)- 2,5-dihydrofuran-2-one (**14d**)

48% overall yield. White amorphous solid. The title compound was synthesized by Method A and subsequently by Method C using paraformaldehyde. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.44 – 7.36 (m, 2H), 6.95 – 6.85 (m, 4H), 6.81 – 6.77 (m, 1H), 5.55 – 5.40 (m, 2H), 4.08 – 4.00 (m,

2H), 3.91 (s, 3H), 3.82 (s, 3H), 3.73 – 3.66 (m, 2H), 3.62 (s, 3H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.72, 159.84, 155.29, 150.74, 149.09, 130.70, 125.99, 123.29, 122.28, 121.00, 113.97, 111.22, 111.13, 82.15, 63.15, 55.91, 55.68, 55.27;. **HRMS** (TOF-ESI+) m/z calcd for $C_{20}H_{20}O_6$+ 356,1300; found 356,1300; **Anal.** Calc. for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66 found: C 68.00; H, 5.67.

4-(3,4-dimethoxyphenyl)-5,5-bis(hydroxymethyl)-3-(4-methoxyphenyl)- 2,5-dihydrofuran-2-one (**14e**)

43% overall yield. Yellow amorphous solid. The title compound was synthesized by Method A and subsequently by Method C using paraformaldehyde. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.46 – 7.32 (m, 2H), 6.90 – 6.87 (m, 2H), 6.83 – 6.74 (m, 3H), 4.02 – 3.95 (m, 2H), 3.90 (s, 3H), 3.87 – 3.79 (m, 2H), 3.77 (s, 3H), 3.73 (s, 3H)**. ^{13}C NMR** (125 MHz, $CDCl_3$) δ 172.48, 159.67, 158.21, 149.75, 149.39, 130.50, 128.73, 124.19, 121.99, 120.66, 113.62, 111.55, 111.16, 91.98, 62.54, 55.90, 55.84, 55.17. **HRMS** (TOF-ESI+) m/z calcd for $C_{21}H_{22}O_7$+ 386,1400; found 386,1400. **Anal.** Calc. for $C_{21}H_{22}O_7$: C, 65.28; H, 5.74 found: C 65.30; H, 5.75.

4-(4-bromophenyl)-3-(3,4-dichlorophenyl)-5,5-bis(hydroxymethyl)-2,5-dihydrofuran-2-one (**14f**)

44% overall yield. Yellow amorphous solid. The title compound was synthesized by Method A and subsequently by Method C using paraformaldehyde. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.55 – 7.46 (m, 1H), 7.45 – 7.36 (m, 2H), 7.31 – 7.19 (m, 3H), 7.17 – 7.06 (m, 1H), 4.02 – 3.88 (m, 2H), 3.79 – 3.68 (m, 2H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 170.9, 157.6, 134.2, 133.9, 131.7, 131.5, 131.4, 130.6, 130.3, 129.6, 127.5, 127.4, 123.6, 92.0, 61.7; **LRMS** ($APCI^+$): *m/z* (relative intensity) 443.0 $[M+H]^+$, 447 (70); **IR** (ATR) ν_{max} 1013, 1030, 1080, 1137, 1159, 1202, 1271, 1296, 1321, 1352, 1375, 1403, 1448, 1469, 1485, 1497, 1572, 1585, 1641, 1658, 1721, 1758, 1819, 1839, 1852, 1903, 1962 cm^{-1}; **Anal.** Calc. for $C_{18}H_{13}BrCl_2O_4$: C, 48.68; H, 2.95; found: C, 48.73; H, 3.09.

3-(3,4-dichlorophenyl)-5,5-bis(hydroxymethyl)-4-(4-methoxyphenyl)- 2,5-dihydrofuran-2-one (**14g**)

40% overall yield. White amorphous solid. The title compound was synthesized by Method A and subsequently by Method C using paraformaldehyde. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.67 – 7.53 (m, 1H), 7.30 – 7.23 (m, 1H), 7.22 – 7.16 (m, 2H), 7.16 – 7.07 (m, 1H), 6.98 – 6.86 (m,

2H), 4.02 – 3.92 (m, 2H), 3.85 – 3.75 (m, 5H), 3.07 (bs, 1H); **^{13}C NMR** (125 MHz, $CDCl_3$) δ 171.90, 162.04, 160.65, 132.69, 132.41, 130.95, 130.13, 129.57, 129.27, 128.46, 127.24, 122.84, 114.86, 92.84, 62.05, 55.32; **IR** (ATR) ν_{max} 1030, 1053, 1092, 1179, 1252, 1292, 1349, 1473, 1512, 1570, 1607, 1645, 1736, 2840, 2934, 3401 cm^{-1}; **Anal.** Calc. for $C_{19}H_{16}Cl_2O_5$: C, 57.74; H, 4.08; found: C, 57.78; H, 4.13.

4.3. Biological testing

4.3.1. Cell Lines

The cell lines used throughout this study are included in the standard testing panel at CZ OPENSCREEN, and cover several major oncological diseases. Cellular profiling was carried out on a panel of cell lines of diverse tissue origin: RPE-1 – human normal immortalized cells from pigmented epithelium in retina; BJ – human primary fibroblasts; Hep G2 – human hepatocellular carcinoma; K562 – human chronic myelogenous leukemia; HL-60 – human acute myeloid leukemia; PC-3 – human prostate adenocarcinoma; MCF7 – human breast adenocarcinoma.

4.3.2. Cell Culture

Cell lines were purchased from the American Type Culture Collection (ATCC). The cells were propagated in a monolayer in the cell culture medium supplemented with 10% fetal bovine serum, 2 mM glutamax, 1 mM non-essential amino acids (NEAA) and penicillin/streptomycin (Thermo Fisher Scientific, MA, USA) and incubated in a 5% CO_2 humidified atmosphere at 37 °C. DMEM was used as a culture medium for RPE-1, Hep G2 and BJ cells, F-12K for PC3 and IMDM for K562 and HL-60. BJ cells were propagated in the medium supplemented with 10 ng/ml of bFGF.

4.3.3. Cell Viability and Apoptosis Assay

Cells were propagated in the cell growth medium to the amount needed for the experiment. The cells were harvested, counted and diluted in the growth medium to the final concentration of $0.1x10^6$/ml and dispensed with a liquid dispenser Multidrop (Thermo Fisher Scientific, MA, USA), to the cell culture treated, white solid 1536-well plates (Corning Inc., NY, USA) at 500 cells/well in 5 µl of the total media volume. The test compounds were diluted in DMSO and transferred to the cells using acoustic dispenser Echo 520 (Labcyte) integrated in the fully automated robotic HTS station Cell∷Explorer (Perkin Elmer). The compounds were

tested at 12 different concentration points in the concentration range from 40 µM to 1 nM, in triplicates. Cell viability was assessed after 48h incubation with the compounds by determining the level of intracellular ATP using the ATPlite™ (Perkin Elmer, USA) luminescent assay. Induction of apoptosis was assessed after 24h of incubation with the compounds by measuring the activity of caspase-3 and -7 using Caspase-Glo® 3/7 luminescent assay (Promega, USA). In both cases, luciferase signal was measured on the multimode plate reader Envision (Perkin Elmer, USA). The data were collected and processed with an in-house developed LIMS system ScreenX. Here, the data were processed, normalized, and ED_{50} values were calculated using a nonlinear regression function (dose response, variable slope).

4.3.4. Antimicrobial Activity Testing

Antifungal activities of the compounds were evaluated on a panel of four ATCC strains (*Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258) and eight clinical isolates of yeasts (*Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporon asahii* 1188) and filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. Three ATCC strains were used as the quality control strains. All the isolates were maintained on Sabouraud dextrose agar prior to being tested.

Minimum inhibitory concentrations (MICs) were determined by modified CLSI standard of microdilution format of the M27-A3 and M38-A2 documents [25, 26]. Dimethyl sulfoxide (100 %) served as a diluent for all compounds; the final concentration did not exceed 2 %. RPMI 1640 (Sevapharma, Prague) medium supplemented with *L*-glutamine and buffered with 0.165 M morpholinepropanesulfonic acid (Serva) to pH 7.0 by 10 M NaOH was used as the test medium. The wells of the microdilution tray contained 200 µl of the RPMI 1640 medium with 2-fold serial dilutions of the compounds (2000 to 0.488 µmol/l for the new compounds) and 10 µl of inoculum suspension. Fungal inoculum in RPMI 1640 was prepared to give a final concentration of $5 \times 10^3 \pm 0.2$ cfu.ml^{-1}. The trays were incubated at 35°C and MICs were read visually after 24 h and 48 h. The MIC values for the dermatophytic strain (*T. mentagrophytes*) were determined after 72 h and 120 h. The MICs were defined as 80 % inhibition (IC_{80}) of the growth of control for yeasts and as 50 % inhibition (IC_{50}) of the

growth of control for filamentous fungi. MICs were determined twice and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

In vitro antibacterial activities [27] of the compounds were evaluated on a panel of three ATCC strains (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) and five clinical isolates (*Staphylococcus aureus* MRSA HK5996/08, *Staphylococcus epidermidis* HK6966/08, *Enterococcus* sp. HK14365/08, *Klebsiella pneumoniae* HK11750/08, *Klebsiella pneumoniae* ESBL HK14368/08) from the collection of fungal strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. The above-mentioned ATCC strains also served as the quality control strains. All the isolates were maintained on Mueller-Hinton agar prior to being tested.

Dimethyl sulfoxide (100 %) served as a diluent for all compounds; the final concentration did not exceed 2 %. Mueller-Hinton agar (MH, HiMedia, Čadersky-Envitek, Czech Republic) buffered to pH 7.4 (±0.2) was used as the test medium. The wells of the microdilution tray contained 200 µl of the Mueller-Hinton medium with 2-fold serial dilutions of the compounds (2000 to 0.488 µmol/l) and 10 µl of inoculum suspension. Inoculum in MH medium was prepared to give a final concentration of 0.5 McFarland scale (1.5×10^8 cfu.ml^{-1}). The trays were incubated at 37°C and MICs were read visually after 24 h and 48 h. The MICs were defined as 95 % inhibition of the growth of control. MICs were determined twice, and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

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Appendix A. Supplementary data

Supplementary data (Tables S1-S2 and Figures S1-S2) related to this article can be found at...

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- Library of 32 novel combretafuranones was synthesized
- Their in vitro cytotoxicity and antiinfective activity was evaluated
- Compounds with submicromolar cytotoxicity were also toxic to nonmalignant cells
- Significant antibacterial activity combined with low toxicity was unveiled