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Original article

Synthesis and biological activities of a series of 4,5-diaryl-3-hydroxy-2(5*H*)-furanones

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Abstract

A series of thirteen 4,5-diaryl-3-hydroxy-2(5*H*)-furanones were synthesized. They were evaluated for their antioxidant potencies and inhibitory properties of 5-lipoxygenase, cyclooxygenases, HIV-1 integrase and PC3 cell proliferation. New hits were discovered either in the anti-proliferation test or in the HIV anti-integrase test.

Keywords: 3-Hydroxy-2-(5*H*)-furanones; Antioxidant; 5-Lipoxygenase; Cyclooxygenases; HIV-1 integrase; PC3 proliferation

1. Introduction

Oxidative stress is common in inflammatory processes of many diseases, including the Chaga's disease [1], heart failure [2], autoimmune rheumatic disease [3], Alzheimer disease [4], asthma, chronic obstructive pulmonary disease [5], and AIDS [6,7]. Reactive oxygen species, either directly or via the formation of lipid peroxidation products, may play a role in enhancing inflammation through the activation of stress kinases (c-jun, p38) and redox-sensitive transcription factors, such as nuclear factor NF-kappaB and activator protein-1. In other respects, lipoxygenases (LOX) constitute a heterogeneous family of lipid-peroxidizing enzymes, widely distributed not only in plants and mammals but also in fungi and invertebrates [8,9]. Leukotrienes are potent mediators of inflammation derived from arachidonic acid through the action of 5-LOX and are also involved in numerous inflammatory diseases and allergic disorders, such as allergy, asthma, arthritis, and psoriasis [10,11]. Several polyphenols display anti-inflammatory properties by different ways: they may inhibit 5-lipoxygenase by interfering with the redox catalytic cycle of the enzyme [12–14], and their antioxidant properties may also modulate cellular signalling inflammatory pathways [15]. In this field, two series of 4,5-diaryl-3-hydroxy-2(5*H*)-furanones with interesting antioxidant and anti-inflammatory properties were elaborated [16,17] and we previously reported the synthesis of a lipophilic analogue of vitamin C, a 4-(4-hydroxyphenyl)-3-hydroxyfuran-2-one [18], which was also found to be a good inhibitor of HIV-1 integrase ($IC_{50} = 3.6$ μM; unpublished data).

On the basis of these results, we elaborated a novel series of 3-hydroxy-2(5*H*)-furanones substituted at 4- and 5- positions by aryl groups. The 4-hydroxyphenyl group at position 4 was maintained in the major part of the series to keep antioxidant potencies as in the lipophilic analogue of vitamin C and we decided to investigate their possible anti-inflammatory

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properties in association with their antioxidant abilities. Keeping in mind the potential anti-integrase properties of these compounds, 4-benzyloxyphenyl, 3,4-dibenzyloxyphenyl and 3,5-dibenzyloxyphenyl groups (as in the HIV-1 integrase inhibitor L-708,906 from Merck Company, Scheme 1) [19] were chosen for position 5. The 4-fluorophenyl group present in the structures of the HIV-1 integrase inhibitors S-1360 from Shionogi Company, L-870,810 (Scheme 1) and L-870,812 from Merck Company [19], was also introduced at positions 4 and 5.

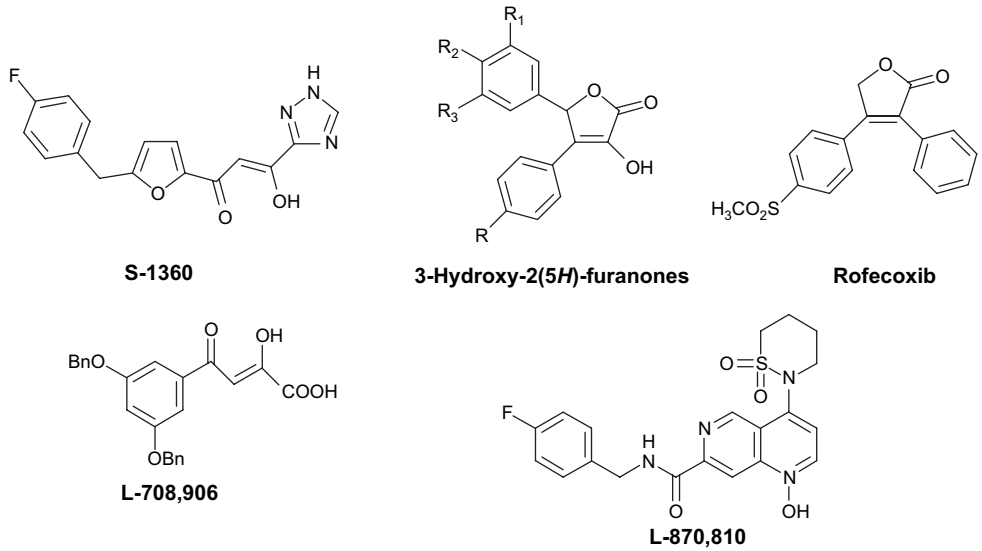
Thirteen molecules were synthesized and herein we present their large physicochemical and pharmacological evaluation. We investigated: (i) their antioxidant properties in the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical and peroxynitrite anion scavenging tests, (ii) their ability to inhibit the 5-lipoxygenase (5-LOX), (iii) their cyclooxygenases (COX-1 and COX-2) inhibitory activities since some cyclooxygenase-2 (COX-2) selective inhibitors like Rofecoxib (Scheme 1) contain a furan-2-one moiety, (iv) their effects on human PC3 cell proliferation since recent data have demonstrated the involvement of COX-2 in both in vitro and in vivo prostate tumor growth rate [20–22] and (v) their inhibitory activities against the catalyzed 3′-processing reaction of wild-type HIV-1 integrase.

2. Results and discussion

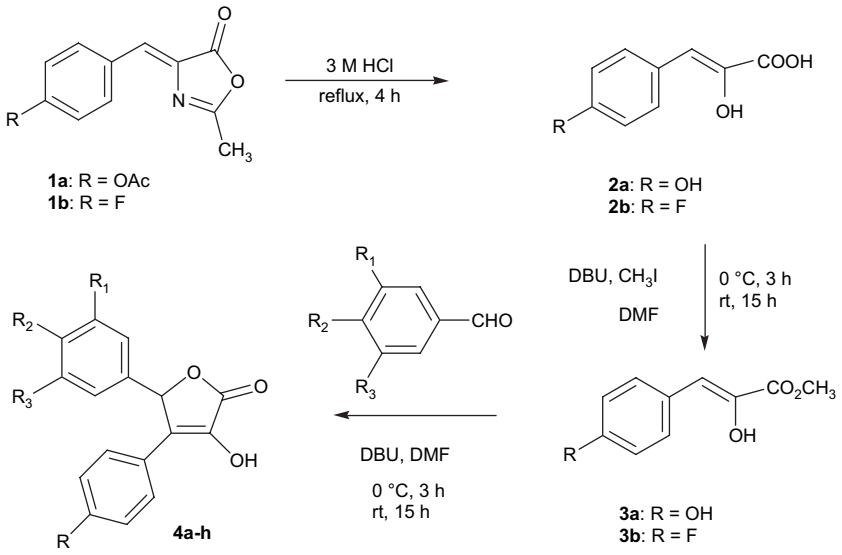
4-Benzylideneoxazol-5(4*H*)-ones **1a**,**b** obtained from 4-hydroxybenzaldehyde and 4-fluorobenzaldehyde by a previously reported Erlenmeyer synthesis [23] were hydrolysed by 3 M HCl to give the corresponding (*Z*)-3-aryl-2-hydroxypropenoic acids **2a**,**b** (Scheme 2) [23]. Methylation in the presence of 1,5-diazabicyclo[5.4.0]undecene (DBU) and methyl iodide according to the method described by Namicki et al. [24] gave the methylarylpyruvates **3a**,**b**. Condensation with the appropriate benzaldehydes in presence of DBU [16,17] gave the corresponding 4,5-diaryl-3-hydroxy-2(5*H*) furanones **4a–h**. Compounds **4i–m** were then obtained by hydrogenolysis of their precursors over Pd/C. We failed to obtain by this method the catechol compound resulting from **4f**.

The antioxidant properties were first expressed by the classical ECR_{50} parameter describing the global reactivity of the tested compound towards the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical. Table 1 shows that the 4-hydroxyphenyl group in position 4 is necessary to confer radical scavenging activity since compounds **4d–f** and **4l**,**m** bearing a 4-fluorophenyl group on the same position were inactive. Compounds **4b**, **4c**, **4j** and **4k** had antioxidant profiles similar to those of reference compounds Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and ascorbate ($ECR_{50} = 0.26$) with a total stoichiometry ($1/2ECR_{50}$), which is about 2. The addition of phenolic or catechol moieties at position 5 did not improve the DPPH scavenging abilities. Furanones effectively protected Evans Blue dye against peroxynitrite-induced oxidation (Table 1). Here again the protecting molecules were the same as in the DPPH test and the substitution by a 4-fluorophenyl group on position 4 was unfavourable (except surprisingly for **4m**). In contrast to the DPPH test, it seems that the presence of (poly)phenolic moieties at position 5 led to increased protective effects when comparing the bleaching inhibitions of compounds **4c**, **4g** and **4h** to those of the respective deprotected homologues **4k**, **4i** and **4j**. The protective effects at 50 μM were in the range 30–45% and largely inferior to those of reference compounds, Trolox (90% bleaching inhibition at 50 μM) and ascorbate (83.6% bleaching inhibition at 50 μM). Only compound **4j** (92%) was as effective as Trolox.

HPLC analysis of calcium ionophore A-stimulated 5-HETE production made assessment of 5-LOX activity in human whole blood samples possible. Only compounds **4g**, **4h** and **4j** inhibited 5-LOX at a 10 μM dose (Table 1) and the experimental results were strongly limited at 100 μM, due to solubility problems. Compounds **4a**, **4b**, **4i**, **4k** and **4m** exhibited



Scheme 1.



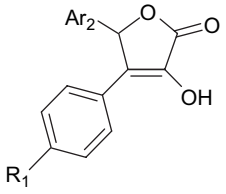
Scheme 2.

poor 5-LOX inhibitory activities (10–30%) even at 100 μM and the most potent inhibitor was compound **4j** (79.5% inhibition). These activities were very modest in regard to that of reference compound, Zileuton (50% inhibition at 0.7 μM).

No compounds displayed COX-2 inhibition and only compounds **4a** and **4b** at 100 μM (32.9% and 16.4% inhibition, respectively) inhibited COX-1 to a lesser extent than reference compound, Celecoxib (50% inhibition at 13.1 μM).

The antiproliferative activity of furanones was evaluated against the human prostate carcinoma PC3 cell line. Compounds bearing benzyloxy or fluorophenyl groups on position 5 were inactive. Compounds **4i**, **4j** and **4l** showed

Table 1
Antioxidant and enzymatic inhibitory activities for test compounds **4a–m**



Entry	R_1	Ar_2	DPPH[a] ECR_{50}	Peroxynitrite % scavenging[b]	5-LOX[c] (10 μM)	5-LOX[d] (100 μM)	HIV-1 Integrase[f] IC_{50} (μM)
4a	–OH	$-C_6H_5$	0.49	29.4	0	18.6	>300
4b	–OH	4-F–C_6H_4	0.27	46.5	0	16.4	>300
4c	–OH	4-OBn–C_6H_4	0.30	0	0	nd[e]	>300
4d	–F	4-OBn–C_6H_4	0	0	0	nd[e]	>300
4e	–F	3,5-diOBn–C_6H_4	0	0	0	nd[e]	30
4f	–F	3,4-diOBn–C_6H_4	0	0	0	nd[e]	10
4g	–OH	3,5-diOBn–C_6H_4	0.65	10.4	32.0	nd[e]	>300
4h	–OH	3,4-diOBn-C_6H_4	0.71	4.90	12.7	nd[e]	1
4i	–OH	3,5-diOH–C_6H_4	0.47	33.1	0	11.6	22
4j	–OH	3,4-diOH–C_6H_4	0.25	92.0	35.4	79.5	9.2
4k	–OH	4-OH–C_6H_4	0.32	44.9	0	27.8	220
4l	–F	4-OH–C_6H_4	0	0	0	0	300
4m	–F	3,5-diOH–C_6H_4	0	40.9	0	10.7	92

[a] Ratio of antioxidant concentration to DPPH concentration producing a 50% decrease in DPPH after 5 min.
[b] Inhibition produced by 50 μM test compound on ONO_2^- induced Blue Evans bleaching.
[c] Inhibition by 10 μM test compound of 5-HETE generation from human whole blood.
[d] Inhibition by 100 μM test compound of 5-HETE generation from human whole blood.
[e] nd = not determined, due to solubility problems.
[f] Inhibition of HIV-1 integrase activity.

Table 2
Activity of compounds **4i–m** on the proliferation of PC3 cancer cell line[a]

Compd	IC_{50} (μM) or % inhibition[b]
Celecoxib	48.0
4i	39%
4j	20%
4k	0%
4l	31%
4m	0%

[a] Activity determined with the MTT method.
[b] Compounds tested at a concentration of 10 μM.

antiproliferative activities ranging between 20 and 30% inhibition at a 10 μM dose (Table 2). These were noticeable potencies when compared to that of reference compound, Celecoxib (50% inhibition at 48 μM).

Inhibition of HIV-1 activity by furanones was very weak with IC_{50} values around 200–300 μM (Table 1) except for compounds **4f**, **4h** and **4j** ($IC_{50} = 10.0$, 1.0 and 9.2 μM, respectively). The good activity of **4j** was expected to be due to its structural similarities with the previously published biscatechols [25] but the presence of a catechol moiety is not required in this series since compound **4f** has a similar micromolar inhibitory activity and compound **4h** is even 10-fold more active than compound **4j**. The structures of **4f** and **4h** may be related to that of a 4-keto-3-hydroxy-furan-2-one, a 3D model pharmacophore discovered by Barreca et al. as a closed structural analogue of the 1,3-diketo acid potent anti-integrase moiety [26]. However compounds **4f** and **4h** lack a second keto group on position 4 and may not be considered as strict diketoacid analogues. We may expect that an additional keto function on position 4 may increase the anti-integrase activities of our compounds although this hypothesis did not recently give potent integrase inhibitors [27]. Furthermore we present the inhibition of the 3′-processing integrase activity but not of the strand transfer one, which is characteristic of the selective inhibition by diketo acids. The inhibition of this strand transfer activity will have to be evaluated.

3. Conclusion

We synthesized and investigated the pharmacological properties of a series of 4,5-diaryl-3-hydroxy-2(5*H*)-furanones. Compounds with a 4-hydroxyphenyl group on position 4, and particularly compound **4j**, displayed vitamin C-like antioxidant behaviours like the original lipophilic analogue of vitamin C [18]. They did not inhibit COX-1 and COX-2 cyclooxygenases because they lack a phenyl group at position 2 and an alkylsulfonyl(or sulfonamido)phenyl group at position 4 present in the COX-2 selective Rofecoxib. They had no or low potency as 5-LOX inhibitors, when compared to Zileuton. Three compounds were found to be moderate inhibitors of HIV-1 integrase activity. New hits like **4h** in the HIV anti-integrase test and **4i** (or **4l**) in the antiproliferation test were discovered and are worth being pharmacologically evaluated in more detail.

4. Experimental

4.1. Pharmacological assays

4.1.1. HPLC analysis of arachidonic acid metabolites in whole blood [28]

Fresh blood was collected in heparinized tubes from normal volunteers. Then 1 mL aliquots were transferred to heparinized tubes preloaded with either 4 μL of vehicle (DMSO) or 4 μL of test compounds and incubated for 15 min at 37 °C. Then, 40 μM calcium ionophore A 23187 alone or in combination with LPS (500 μg) was added. Incubation was carried out at 37 °C for 30 min and 20 h at 37 °C to stimulate 5-LOX and COX-1 pathways, respectively. For the COX-2 pathway, samples stimulated with A 23187 + LPS were incubated for 20 h. Eicosanoids were extracted from the samples into ethyl acetate. Samples were then evaporated to dryness under nitrogen, re-suspended in the mobile phase to precipitate proteins and then centrifuged. The supernatant was directly analysed by HPLC using Hypersil ODS 3 μM columns (12.5 mm × 0.46 mm), a methanol/water/acetic acid (80:20:0.08) mobile phase at a flow rate of 1 mL/min and UV detection firstly at 270 nm and 12 min later at 245 nm.

4.1.2. Cell culture and cell proliferation assay

Human prostate PC3 cancer cells were grown at 37 °C in RPMI 1640 medium supplemented with 10% fetal calf serum, in a humidified incubator containing 5% CO_2. In the cell proliferation assay, cells were plated (1.8×104 cells/well) on 24-well plates. After 3 days, the cell medium was changed to serum-free medium, and the cells were starved for 24 h for culture synchronization. Cells were then incubated in culture medium that contained various concentrations of test compounds, each dissolved in less than 0.1% DMSO. After incubating for 72 h, cell growth was estimated by the colorimetric MTT test.

4.1.3. HIV-1 integrase assay

Oligonucleotides were purchased from Eurogentec and further purified on 18% acrylamide/urea denaturing gel. U5B: GTGTGGAAAATCTCTAGCA; U5A: 59-ACTGCTAGA GATTTTCCACAC. Wild-type HIV-1 integrase was purified as described previously [29]. 3′-Processing assay was performed in a reaction volume of 20 μL containing 0.025 pmol of labelled U5A/U5B double-stranded DNA substrate and 1 pmol of integrase in buffer [20 mM Hepes (pH 7.2), 10 mM $MgCl_2$, 25 mM NaCl, and 1 mM DTT]. Products were separated on an 18% acrylamide/urea denaturing gel and quantified on a phosphoimager using ImageQuant software (Amersham Pharmacia Biotech).

4.2. Antioxidant assays

4.2.1. Reaction with DPPH

All spectrophotometric measurements were performed with a Kontron Uvikon 932 spectrophotometer. Calibration curves for 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical were

carried out in triplicate and the DPPH concentration (μM) was calculated from the following equation:

$$A_{515} = 0.0087 \times C_{DPPH} - 0.0097$$

Stock solutions (0.01 M) of antioxidants were prepared in *N*,*N*-dimethylformamide (DMF) and rapidly mixed (volumes from 1.5 to 270 μL) with an ethanol solution of DPPH (final volume 3.0 mL). Initial concentrations of DPPH were around 50 μM. The decrease in absorbance at 515 nm was recorded every 0.02 min. The effective concentration ratio (ECR_{50}) is the ratio of antioxidant concentration to DPPH concentration producing a 50% decrease in DPPH after 6 min of reaction. Antioxidant reference compounds like Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) and ascorbic acid were used.

4.2.2. Prevention of peroxynitrite-mediated oxidation

Peroxynitrite synthesis was carried out as previously reported [30]. Experiments were conducted at room temperature in a 50 mM phosphate-buffered saline containing 0.1 mM diethylenetriaminepentaacetic acid, 90 mM NaCl and 5 mM KCl, pH 7.4. Blanks using DMF alone in the absence of test compound and peroxynitrite allowed to degrade for 5 min in phosphate-buffered saline, pH 7.4, were also run. There was no interference of DMF and degraded peroxynitrite with the oxidative modification of Evans Blue dye. Test compound, Evans Blue (17.5 μM) and peroxynitrite (335 μM) were rapidly mixed and the peroxynitrite-induced bleaching of Evans Blue dye was measured at 608 nm ($\epsilon = 70000\ M^{-1}\,cm^{-1}$). The effects are expressed as the concentration giving 50% inhibition of the oxidation of Evans Blue (IC_{50}).

4.3. Chemistry

All reagents and solvents were purchased from Aldrich-Chimie (Saint-Quentin-Fallavier, France) of ACS reagent grade and were used as provided. TLC analyses were performed on plastic sheets precoated with silica gel 60F254 (Merck). SiO_2, 200–400 mesh (Merck) was used for column chromatography. NMR spectra were obtained on an AC 200 Bruker spectrometer in the appropriate solvent with TMS as internal reference. Melting points were obtained on a Reichert Thermopan melting point apparatus, equipped with a microscope and are uncorrected. Mass spectra were recorded on a Voyager DE STR mass spectrometer (Applied Biosystems) (MALDI-TOF). HRMS were obtained on an APEX Qe 9.4 T Bruker Deltonics spectrometer.

4.3.1. Synthesis of (Z)-4-benzylideneoxazol-5(4H)-ones *1a*,*b*: general procedure

4-Hydroxybenzaldehyde or 4-fluorobenzaldehyde (100 mmol), *N*-acetylglycine (14.04 g; 120 mmol), anhydrous NaOAc (10.66 g; 130 mmol) and Ac_2O (51 g; 500 mmol) were mixed and stirred at 100 °C for 5 h. The mixture was allowed to cool at room temperature. Then ice water (50 mL) was added. The resulting precipitate was filtered, washed with 50% aq EtOH (4 × 20 mL) and dried at 60 °C to give **1a** or **1b**.

Compound **1a**: 95% yield (lit. [31] 90%); yellow solid; mp 134–135 °C (lit. [31] 134–136 °C); ^{1}H NMR (DMSO-d_6, 200 MHz) 2.26 (s, 3H), 2.37 (s, 3H), 7.10 (s, 1H), 7.23 (d, 2H, $^3J = 8.80$ Hz), 8.23 (d, 2H, $^3J = 8.80$ Hz); **1b**: 88% yield; yellow solid; mp 150–152 °C (lit. [32] 154 °C); ^{1}H NMR (DMSO-d_6, 200 MHz) 2.38 (s, 3H), 7.24 (s, 1H), 7.35 (t, 2H, $^3J_{H-H} = {}^3J_{H-F} = 8.90$ Hz), 8.21 (dd, 1H, $^3J_{H-H} = 8.90$ Hz, $^4J_{H-F} = 6.10$ Hz); ^{13}C NMR (DMSO-d_6, 50 MHz) 15.3, 116.2 ($^2J_{C-F} = 21.60$ Hz), 128.5, 129.8 ($^4J_{C-F} = 3.35$ Hz), 132.2 ($^5J_{C-F} = 2.60$ Hz), 134.4 ($^3J_{C-F} = 8.90$ Hz), 163.4 ($^1J_{C-F} = 251.20$ Hz), 166.8 ($^6J_{C-F} = 1.50$ Hz), 167.3.

4.3.2. Synthesis of (Z)-3-aryl-2-hydroxypropenoic acids *2a*,*b*: general procedure

A solution of the 4-benzylideneoxazol-5-one **1a**,**b** (10 mmol) in 3 N HCl (60 mL) was refluxed for 4 h. After filtration of the hot mixture, cooling at room temperature and filtration gave the pyruvic acids **2a**,**b**.

Compound **2a**: 87% yield; red solid; mp 207 °C dec (lit. [31] 207 °C dec); ^{1}H NMR (DMSO-d_6, 200 MHz) 6.35 (s, 1H), 6.75 (d, 2H, $^3J = 8.20$ Hz), 7.21 (d, 2H, $^3J = 8.20$ Hz), 8.81 (s, 1H), 9.60 (s, 1H). **2b**: 78% yield; yellow solid; mp 163–164 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 6.42 (s, 1H), 7.20 (t, 2H, $^3J_{H-H} = {}^3J_{H-F} = 8.90$ Hz), 7.82 (dd, 2H, $^3J_{H-H} = 8.90$ Hz, $^4J_{H-F} = 5.80$ Hz), 9.30 (s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) 109.8 ($^5J_{C-F} = 1.10$ Hz), 116.0 ($^2J_{C-F} = 21.40$ Hz), 130.1 ($^4J_{C-F} = 3.30$ Hz), 132.5 ($^3J_{C-F} = 8.40$ Hz), 141.2 ($^6J_{C-F} = 2.50$ Hz), 162.8 ($^1J_{C-F} = 246.0$ Hz), 167.0.

4.3.3. Synthesis of methyl (Z)-3-aryl-2-hydroxypropenoate *3a*,*b*: general procedure

The pyruvic acid **2a**,**b** (13.7 mmol) was dissolved in dry DMF (80 mL) at 0 °C. DBU (2.28 mL, 15.2 mmol) and methyl iodide (4.75 mL, 76.3 mmol) were successively added and the solution was stirred at 0 °C for 3 h and then overnight at room temperature. The solution was poured into a mixture of AcOEt (10 mL) and 1 M HCl (50 mL). The organic layer was separated and the aqueous layer was extracted four times with AcOEt (15 mL). The combined organic layers were washed with H_2O (20 mL) and dried over Na_2SO_4. Evaporation of the volatiles in vacuo afforded the methyl pyruvates **3a**,**b**.

Compound **3a**: 92% yield; red solid; mp 115–116 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 3.77 (s, 3H), 6.75 (d, 2H, $^3J = 8.60$ Hz), 7.62 (d, 2H, $^3J = 8.60$ Hz), 9.10 (s, 1H), 9.65 (s, 1H). **3b**: 83% yield; beige solid; mp 138–140 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 3.80 (s, 3H), 6.45 (s, 1H), 7.19 (t, 2H, $^3J_{H-H} = {}^3J_{H-F} = 8.90$ Hz), 7.85 (dd, 2H, $^3J_{H-H} = 8.90$ Hz, $^4J_{H-F} = 5.80$ Hz), 9.61 (s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) 52.8, 109.7 ($^5J_{C-F} = 0.70$ Hz), 115.6 ($^2J_{C-F} = 21.10$ Hz), 130.2 ($^4J_{C-F} = 3.20$ Hz), 131.5 ($^3J_{C-F} = 7.90$ Hz), 140.9 ($^6J_{C-F} = 2.60$ Hz), 158.8 ($^1J_{C-F} = 244.4$ Hz), 165.2.

4.3.4. Synthesis of the 4,5-diaryl-3-hydroxy-2(5H) furanones *4a*–*h*: general procedure

To a cold solution of methyl pyruvate (5.27 mmol) and appropriate benzaldehyde (1.3 equiv) in dry DMF (25 mL) was added DBU (0.8 mL, 5.35 mmol). The mixture was stirred

for 3 h at 0 °C and then overnight at room temperature. The solution was poured into a mixture of AcOEt (10 mL) and 1 M HCl (50 mL). The organic layer was separated and the aqueous layer was extracted several times with AcOEt. The combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. The oily residue was then purified by column chromatography (AcOEt/hexane, 30:70) for compounds **4b**, **4d**, **4e** and **4f**. For the other ones **4a**, **4c**, **4g** and **4h**, the oily residue was dissolved in aqueous 2 M NaOH and, after filtration of the insoluble, the solution was acidified to pH 1.0 with 6 M HCl. The precipitate was filtered and dried at room temperature.

4.3.4.1. 3-Hydroxy-4-(4-hydroxyphenyl)-5-phenyl-2(5H)-furanone ***4a***. Yield 30% (lit. [16] 21%); brown solid; mp 253–255 °C (lit. [16] 258 °C); ^{1}H NMR (DMSO-d_6, 200 MHz) 6.46 (s, 1H), 6.71 (d, 2H, $^3J = 8.90$ Hz), 7.35–7.37 (m, 5H), 7.46 (d, 2H, $^3J = 8.90$ Hz), 9.79 (s, 1H), 10.61 (s, 1H).

4.3.4.2. 5-(4-Fluorophenyl)-3-hydroxy-4-(4-hydroxyphenyl)-2(5H)-furanone ***4b***. Yield 17%; yellow solid; mp 270–271 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 6.49 (s, 1H), 6.71 (d, 2H, $^3J_{H-H} = 8.90$ Hz), 7.19 (t, 2H, $^3J_{H-H} = {}^3J_{H-F} = 8.60$ Hz), 7.40 (m, 4H), 9.79 (s, 1H), 10.64 (s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) 78.6 (C_5), 115.3, 115.8 ($^2J_{C-F} = 21.60$ Hz), 121.3, 128.91 (C_4), 128.92, 129.5 ($^3J_{C-F} = 8.60$ Hz), 133.2 ($^4J_{C-F} = 3.00$ Hz), 136.5 (C_3), 157.7(C–OH), 162.3, 169.4 (C_2); MS (MALDI-TOF): *m/z* (%) = 287 ([MH$^+$], 78), 269 (100), 257 (11), 241 (20), 229 (16); HRMS (nanoESI-FR-ICR) calcd for $C_{16}H_{11}FO_4$ 286.06414, found 286.06475.

4.3.4.3. 5-(4-Benzyloxyphenyl)-3-hydroxy-4-(4-hydroxyphenyl)-2(5H)-furanone ***4c***. Yield 21%; pink solid; mp 268–270 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 5.06 (s, 2H), 6.40 (s, 1H), 6.72 (d, 2H, $^3J = 8.50$ Hz), 6.99 (d, 2H, $^3J = 8.20$ Hz), 7.28 (d, 2H, $^3J = 8.50$ Hz), 7.41 (m, 7H); ^{13}C NMR (DMSO-d_6, 50 MHz) 69.2 (CH_2), 79.2 (C_5), 115.0, 115.3, 121.6, 122.7, 127.8, 128.4, 128.96, 128.99 (C_4), 129.1, 129.2, 136.4 (C_3), 136.8, 157.7 (C–OH), 158.9 (C–OBn), 169.3 (C_2); MS (MALDI-TOF): *m/z* (%) = 375 ([MH$^+$], 30), 309 (100), 231 (50); HRMS (nanoESI-FR-ICR) calcd for $C_{23}H_{18}O_5$ 374.11543, found 374.11517.

4.3.4.4. 5-(4-Benzyloxyphenyl)-4-(4-fluorophenyl)-3-hydroxy-2(5H)-furanone ***4d***. Yield 16%; white solid; mp 198–199 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 5.06 (s, 2H), 6.50 (s, 1H), 6.99 (d, 2H, $^3J_{H-H} = 8.60$ Hz), 7.19 (t, 2H, $^3J_{H-H} = {}^3J_{H-F} = 8.60$ Hz), 7.30 (d, 2H, $^3J_{H-H} = 8.60$ Hz), 7.40 (m, 5H), 7.64 (dd, 2H, $^3J_{H-H} = 8.90$ Hz, $^4J_{H-F} = 5.40$ Hz), 11.07 (s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) 69.3 (CH_2), 79.4 (C_5), 115.1, 115.5 ($^2J_{C-F} = 21.60$ Hz), 126.9 (C_4, $^5J_{C-F} = 0.80$ Hz), 127.2 ($^4J_{C-F} = 3.30$ Hz), 127.7, 127.9, 128.4, 129.3, 129.6 ($^3J_{C-F} = 8.20$ Hz), 136.8, 138.5 (C_3, $^6J_{C-F} = 1.90$ Hz), 159.0 (C–OBn), 161.5 ($^1J_{C-F} = 249.50$ Hz), 169.0 (C_2); MS (MALDI-TOF): *m/z* (%) = 377 ([MH$^+$], 100), 359 (18), 331 (20), 313 (13), 263 (14), 232 (17); HRMS (nanoESI-FR-ICR) calcd for $C_{23}H_{17}FO_4$ 376.11109, found 376.11113.

4.3.4.5. 5-(3,5-Dibenzyloxyphenyl)-4-(4-fluorophenyl)-3-hydroxy-2(5H)-furanone ***4e***. Yield 12%; white solid; mp 159–160 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 4.98 (s, 4H), 6.06 (s, 1H), 6.57 (d, 2H, $^4J_{H-H} = 2.20$ Hz), 6.63 (t, 1H, $^4J_{H-H} = 2.20$ Hz), 6.99 (t, 2H, $^3J_{H-H} = {}^3J_{H-F} = 8.90$ Hz), 7.36 (m, 10H), 7.56 (dd, 2H, $^3J_{H-H} = 8.90$ Hz, $^4J_{H-F} = 5.40$ Hz); ^{13}C NMR (DMSO-d_6, 50 MHz) 70.2 (CH_2), 81.6 (C_5), 103.2, 107.4, 115.7 ($^2J_{C-F} = 21.60$ Hz), 126.1 ($^4J_{C-F} = 3.30$ Hz), 127.4 (C_4, $^5J_{C-F} = 1.10$ Hz), 127.5, 128.1, 128.5, 129.7 ($^3J_{C-F} = 8.20$ Hz), 136.3, 137.2, 137.5 (C_3, $^6J_{C-F} = 2.20$ Hz), 160.4 (C–OBn), 162.6 ($^1J_{C-F} = 250.90$ Hz), 170.2 (C_2); MS (MALDI-TOF): *m/z* (%) = 483 ([MH$^+$], 100), 309 (11), 287 (9), 231 (8); HRMS (nanoESI-FR-ICR) calcd for $C_{30}H_{23}FO_5$ 482.15295, found 482.15304.

4.3.4.6. 5-(3,4-Dibenzyloxyphenyl)-4-(4-fluorophenyl)-3-hydroxy-2(5H)-furanone ***4f***. Yield 10%; white solid; mp 155–157 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 5.08 (s, 4H), 6.41 (s, 1H), 6.89 (dd, 1H, $^3J_{H-H} = 8.20$ Hz, $^4J_{H-H} = 1.80$ Hz), 7.03 (d, 1H, $^3J_{H-H} = 8.20$ Hz), 7.08 (d, 1H, $^4J_{H-H} = 1.80$ Hz), 7.16 (t, 2H, $^3J_{H-H} = {}^3J_{H-F} = 8.90$ Hz), 7.30–7.40 (m, 10H), 7.60 (dd, 2H, $^3J_{H-H} = 8.90$ Hz, $^4J_{H-F} = 5.50$ Hz); ^{13}C NMR (DMSO-d_6, 50 MHz) 69.3 (CH_2), 70.2 (CH_2), 79.6 (C_5), 114.19, 114.25, 115.5 ($^2J_{C-F} = 21.50$ Hz), 121.0, 126.7 (C_4), 127.2 ($^3J_{C-F} = 3.30$ Hz), 127.5, 127.6, 127.76, 127.79, 128.30, 128.36, 128.9, 129.5 ($^3J_{C-F} = 7.80$ Hz), 137.0, 138.5 (C_3, $^6J_{C-F} = 1.50$ Hz), 148.1 (C–OBn), 149.1 (C–OBn), 161.5 ($^1J_{C-F} = 246.20$ Hz), 169.0 (C_2); HRMS (nanoESI-FR-ICR) calcd for $C_{30}H_{23}FO_5$ 482.15295, found 482.15374.

4.3.4.7. 5-(3,5-Dibenzyloxyphenyl)-3-hydroxy-4-(4-hydroxyphenyl)-2(5H)-furanone ***4g***. Yield 20%; beige solid; mp 216–217 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 5.04 (s, 4H), 6.34 (s, 1H), 6.67 (d, 2H, $^4J = 2.20$ Hz), 6.74 (t, 2H, $^3J = 8.60$ Hz), 7.35 (m, 10H), 9.80 (s, 1H), 10.58 (s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) 69.4 (CH_2), 79.3 (C_5), 102.2, 106.9, 115.3, 121.5 (C_4), 127.8, 127.9, 128.9, 129.0, 136.4, 136.6 (C_3), 139.2 (C–OBn), 159.7 (C–OH), 169.2 (C_2); MS (MALDI-TOF): *m/z* (%) = 481 ([MH$^+$], 100), 423 (18), 287 (5); HRMS (nanoESI-FR-ICR) calcd for $C_{30}H_{24}O_6$ 480.15729, found 480.15813.

4.3.4.8. 5-(3,4-Dibenzyloxyphenyl)-3-hydroxy-4-(4-hydroxyphenyl)-2(5H)-furanone ***4h***. Yield 29%; brown solid; mp 218–220 °C; ^{1}H NMR (DMSO-d_6, 200 MHz) 5.08 (s, 2H), 6.33 (s, 1H), 6.71 (d, 2H, $^3J = 8.20$ Hz), 6.88 (d, 1H, $^3J = 8.20$ Hz, $^4J = 1.80$ Hz), 7.03 (d, 1H, $^3J = 8.20$ Hz), 7.10 (d, 1H, $^4J = 1.80$ Hz), 7.25–7.30 (m, 12H), 7.80 (s, 1H), 9.82 (s, 1H); ^{13}C NMR (DMSO-d_6, 50 MHz) 69.9 (CH_2), 70.2 (CH_2), 79.5 (C_5), 114.2, 114.4, 115.3, 120.8, 121.5, 127.5, 127.71, 127.73, 128.3, 128.8 (C_4), 129.0, 129.5, 136.4 (C_3), 136.9, 137.0, 148.0 (C–OBn), 148.9 (C–OBn), 157.6 (C–OH), 169.3(C_2); MS (MALDI-TOF): *m/z* (%) = 481

([MH^+], 100), 463 (43), 439 (38), 423 (33), 407 (21), 362 (15), 335 (28), 287 (19), 229 (18); HRMS (nanoESI-FR-ICR) calcd for $C_{30}H_{24}O_6$ 480.15729, found 480.15668.

*4.3.5. Synthesis of the 4,5-diaryl-3-hydroxy-2(5H) furanones **4i–m**: general procedure*

Pd/C (10%; 0.091 g/mmol OBn group) was added to the furanone dissolved in THF/EtOH and the solution was submitted to a hydrogen atmosphere for 5 h. After filtration of the catalyst, the solvent was evaporated in vacuo. Purification by column chromatography (AcOEt/hexane, 50:50) and crystallization from ethyl ether afforded the deprotected furanones.

*4.3.5.1. 5-(3,5-Dihydroxyphenyl)-3-hydroxy-4-(4-hydroxyphenyl)-2(5H)-furanone **4i***. Yield 82%; brown solid; mp 270–272 °C; ^{1}H NMR (acetone-d_6, 200 MHz) 6.10 (s, 1H), 6.33 (m, 1H), 6.38 (m, 2H), 6.78 (d, 2H, $^3J = 7.90$ Hz), 7.53 (d, 2H, $^3J = 7.90$ Hz); ^{13}C NMR (acetone-d_6, 50 MHz) 80.5 (C_5), 103.5, 106.5, 115.4, 122.3 (C_4), 128.8, 129.5, 136.5, 139.1 (C_3), 157.8 (C–OH), 158.8 (C–OH), 169.3 (C_2); MS (MALDI-TOF): *m/z* (%) = 301 ([MH^+], 28), 265 (9), 231 (17); HRMS (nanoESI-FR-ICR) calcd for $C_{16}H_{12}O_6$ 300.06339, found 300.06288.

*4.3.5.2. 5-(3,4-Dihydroxyphenyl)-3-hydroxy-4-(4-hydroxyphenyl)-2(5H)-furanone **4j***. Yield 86%; black solid; mp 273–274 °C; ^{1}H NMR (acetone-d_6, 200 MHz) 6.17 (s, 1H), 6.77 (m, 5H), 7.54 (dd, 2H, $^3J = 6.75$ Hz, $^4J = 2.05$ Hz), 8.01 (s, 1H), 8.13 (s, 1H), 8.72 (s, 1H), 9.07 (s, 1H); ^{13}C NMR (acetone-d_6, 50 MHz) 80.8 (C_5), 114.9, 115.5, 115.7, 120.6, 122.6, 128.7 (C_4), 128.8, 129.8, 136.8 (C_3), 145.6 (C–OH), 146.4 (C–OH), 158.0 (C–OH), 169.5 (C_2); HRMS (nanoESI-FR-ICR) calcd for $C_{16}H_{12}O_6$ 300.06339, found 300.06386.

*4.3.5.3. 3-Hydroxy-4-(4-hydroxyphenyl)-5-(4-hydroxyphenyl)-2(5H)-furanone **4k***. Yield 75%; white solid; mp 236–238 °C; ^{1}H NMR (acetone-d_6, 200 MHz) 6.21 (s, 1H), 6.77 (m, 4H), 7.22 (dd, 2H, $^3J = 6.90$ Hz, $^4J = 2.05$ Hz), 7.53 (dd, 2H, $^3J = 6.90$ Hz, $^4J = 2.05$ Hz), 8.72 (br s, 2H); ^{13}C NMR (acetone-d_6, 50 MHz) 81.4 (C_5), 116.4, 116.8, 123.5, 129.0, 129.4, 130.68, 130.70 (C_4), 137.8 (C_3), 158.9 (C–OH), 159.4 (C–OH), 170.2 (C_2); MS (MALDI-TOF): *m/z* (%) = 285 ([MH^+], 100), 267 (18), 231 (10); HRMS (nanoESI-FR-ICR) calcd for $C_{16}H_{12}O_5$ 284.06847, found 284.06778.

*4.3.5.4. 4-(4-Fluorophenyl)-3-hydroxy-5-(4-hydroxyphenyl)-2(5H)-furanone **4l***. Yield 71%; white solid; mp 222–223 °C; ^{1}H NMR (acetone-d_6, 200 MHz) 6.32 (s, 1H), 6.81 (d, 2H, $^3J_{H-H} = 8.50$ Hz), 7.10 (t, 2H, $^3J_{H-H} = ^3J_{H-F} = 8.60$ Hz), 7.25 (d, 2H, $^3J_{H-H} = 8.50$ Hz), 7.70 (dd, 2H, $^3J_{H-H} = 8.40$ Hz, $^4J_{H-F} = 5.40$ Hz), 9.00 (s, 1H); ^{13}C NMR (acetone-d_6, 50 MHz) 81.6 (C_5), 116.5 ($^2J_{C-F} = 21.50$ Hz), 116.9, 127.8 (C_4), 128.4, 128.6 ($^4J_{C-F} = 3.40$ Hz), 130.8, 131.1 ($^3J_{C-F} = 8.20$ Hz), 139.7 (C–OH), 159.6 (C–OH), 163.4 ($^1J_{C-F} = 246.30$ Hz), 170.0 (C_2); MS (MALDI-TOF): *m/z* (%) = 287 ([MH^+], 100), 269 (10), 231 (18); HRMS (nanoESI-FR-ICR) calcd for $C_{16}H_{11}FO_4$ 286.06414, found 286.06430.

*4.3.5.5. 5-(3,5-Dihydroxyphenyl)-4-(4-fluorophenyl)-3-hydroxy-2(5H)-furanone **4m***. Yield 76%; brown solid; mp 92–94 °C; ^{1}H NMR (acetone-d_6, 200 MHz) 6.22 (s, 1H), 6.33 (t, 1H, $^4J_{H-H} = 2.05$ Hz), 6.40 (d, 2H, $^4J_{H-H} = 2.05$ Hz), 7.10 (dd, 2H, $^3J_{H-H} = ^3J_{H-F} = 9.10$ Hz), 7.73 (dd, 2H, $^3J_{H-H} = 9.10$ Hz, $^4J_{H-F} = 5.60$ Hz), 8.40 (br s, 2H), 9.52 (s, 1H); ^{13}C NMR (acetone-d_6, 50 MHz) 81.5 (C_5), 103.2, 104.9, 107.7, 116.7 ($^2J_{C-F} = 22.00$ Hz), 128.2 (C_4, $^5J_{C-F} = 0.80$ Hz), 128.7 ($^4J_{C-F} = 3.30$ Hz), 131.2 ($^3J_{C-F} = 8.20$ Hz), 139.8 (C_3, $^6J_{C-F} = 2.20$ Hz), 140.2, 160.6 (C–OH), 163.9 ($^1J_{C-F} = 248.20$ Hz), 170.2 (C_2); HRMS (nanoESI-FR-ICR) calcd for $C_{16}H_{11}FO_5$ 302.05905, found 302.05931.

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