Bioorganic & Medicinal Chemistry 16 (2008) 9056–9064

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Development of a second generation of inhibitors of microsomal prostaglandin E synthase 1 expression bearing the γ-hydroxybutenolide scaffold

Maurizio Aquino [a,†], Maria D. Guerrero [b,†], Ines Bruno [a,*], María C. Terencio [b], Miguel Paya [b], Raffaele Riccio [a]

[a] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano, SA, Italy
[b] Departamento de Farmacología, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andrés Estellés s/n, 46100 Burjasot, Valencia, Spain

ARTICLE INFO

Article history:
Received 6 February 2008
Revised 31 July 2008
Accepted 1 August 2008
Available online 6 August 2008

Dedicated to the memory of Professor Luigi Gomez-Paloma

Keywords:
γ-Hydroxybutenolide derivatives
$sPLA_2$
mPGES-1, COX-2

ABSTRACT

Petrosaspongiolide M (**PM**, **1**), a marine sesterterpene metabolite bearing the γ-hydroxybutenolide scaffold and displaying a potent inhibitory activity toward PLA_2 enzyme, was selected by us as an attractive target in order to explore its mechanism of action at molecular level. In the course of our investigations we decided to synthetically modify the parent compound to clarify the structural determinants responsible for the activity; in fact, very recently, our research group reported the synthesis and the pharmacological properties of a first collection of PM analogues generated by Ludi approach. The synthesized compounds showed a poor or moderate activity toward PLA_2 enzymes, nevertheless we discovered a potent and selective modulator of the expression of microsomal prostaglandin E synthase 1 (mPGES-1), an enzyme highly involved in the inflammatory response, which represents an interesting target for the development of a new class of anti-inflammatory agents. In this paper we report the synthesis of a further collection of nine analogues, having the same scaffold of PM, the γ-hydroxybutenolide, and bearing, as side chain, more complex aromatic portions, in substitution of the sesterterpene moiety. Their pharmacological behavior against PLA_2 enzymes as well as to modulate the expression of inducible cyclooxygenase 2 (COX-2) and mPGES-1 enzymes is also described.

1. Introduction

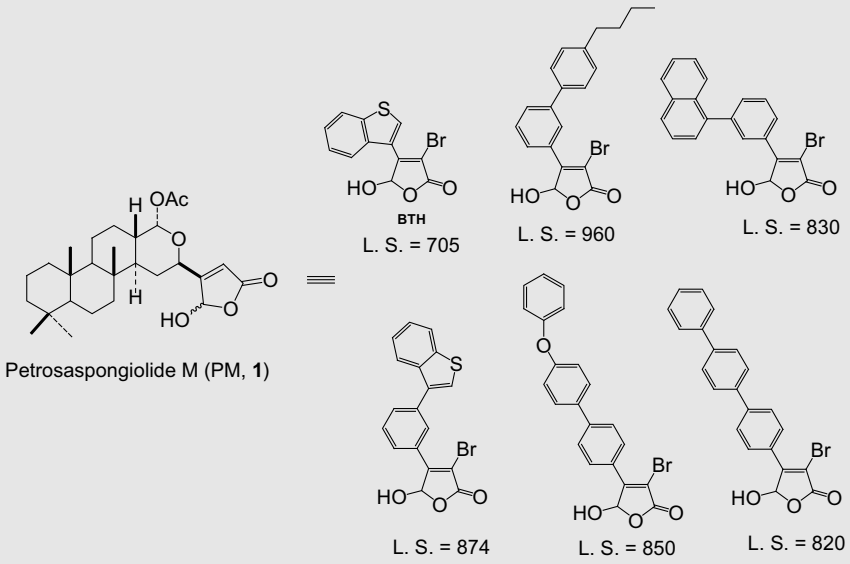
Continuing our studies on bioactive natural compounds, in the recent years we focused our attention on a marine metabolite, the PM (Table 1 and 1), possessing relevant anti-inflammatory properties due to its ability of irreversibly blocking PLA_2, the enzyme promoting the arachidonic acid cascade.[1] Its mechanism of action, in which the γ-hydroxybutenolide moiety is highly involved, was explored in great detail through Mass Spectrometry analysis and Molecular Modeling calculation and, finally, a 3D model of interaction of this natural ligand with its biological target was also proposed.[2] In consideration of the prominent role played by γ-hydroxybutenolide scaffold in the inhibition event, we decided to synthesize small collections of analogues having the same pharmacophore and different molecular fragments linked on it, in substitution of sesterterpene moiety, in order to explore some structure–activity relationships useful for the discovery of new binders with higher affinity for the target enzyme. In our recent paper, dealing with the synthesis of a first collection of γ-hydroxybutenolide analogues, we showed that the benzothiophene γ-hydroxybutenolide (Table 1, **BTH**) displayed an interesting selective modulation on the expression of microsomal prostaglandin E synthase 1 (mPGES-1), a key enzyme involved in the prostanoids production and located downstream along the arachidonic acid cascade.[3] This serendipitous discovery can be considered of great interest as mPGES-1 represents a potential target for the development of new therapeutics useful in the treatment of several diseases.[4] Encouraged by this finding we decided to undertake the synthesis of a further collection of unnatural variants of PM, bearing complex aromatic portions linked to the butenolide scaffold. As experienced for our previous compounds, also in this case to design the new analogues, we decided to take advantage from Ludi approach,[5] a computational method which has been proven to be fairly predictive of the affinity properties of potential ligands. Ludi was set in order to construct new promising binders, starting from the known parent compound PM (**1**) and replacing, the sesterterpene moiety of the natural product, with suitable molecular fragments; the 3-bromo γ-hydroxybutenolide, which resulted accepted by Ludi, was selected as scaffold, in order to simplify the synthetic approach. This choice was also made in consideration that in our parallel findings on PM analogues we found that the compounds lacking in the bromine atom on C-3 position of the scaffold displayed almost the same pharmacological behavior of the 3-bromo analogues.

In this paper, we describe in detail the synthesis and the pharmacological profile of the new collection of products.

* Corresponding author. Tel.: +39 089 969743; fax: +39 089 969702.
E-mail address: brunoin@unisa.it (I. Bruno).
† These authors contributed equally to the paper.

0968-0896/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2008.08.001

Table 1
Fragments suggested by Ludi and their calculated scores (L.S. = Ludi score)



2. Results and discussion

2.1. Chemistry

As mentioned before, to design our collection of compounds based on the suggestions coming from Ludi program which, starting from the 3D structure of the PM-bvPLA$_2$ complex,[2] generated a series of analogues of the parent compound (Table 1), according to our simplification plan.

In particular, our intention was to leave the pharmacophore γ-hydroxybutenolide scaffold quite unchanged, nevertheless, in order to simplify the synthetic strategy we preferred, at least, to use the 3-bromo substituted butenolide, which allowed us to use the commercially available mucobromic acid (Figs. 1 and 2) as starting material[6]; more incisive modifications were provided for the molecular recognition domain of PM, in fact the sesterterpene skeleton was decided to be replaced by several molecular fragments selected from the database connected to the software. From a structural point of view, the new generation of compounds, respect to the former ones recently reported,[3] possesses more complex aromatic fragments linked to the hydroxybutenolide scaffold, and that required a change of the synthetic approach adopted before. Based on the retro synthetic analysis (Fig. 1) and considering the two C–C disconnections indicated with broken lines, we decided to adopt a convergent synthetic approach (Fig. 2), in which, we first assembled separately the two aromatic building blocks **4** and **5**, through an organometallic catalyzed Suzuki cross coupling reactions,[7] and then we connected the generated

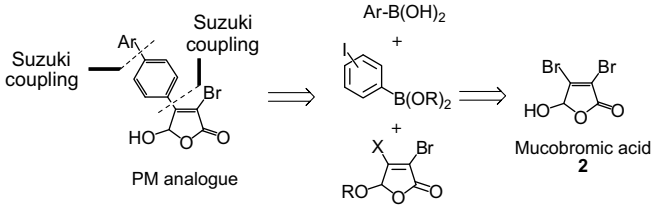


Figure 1. Retrosynthetic scheme.

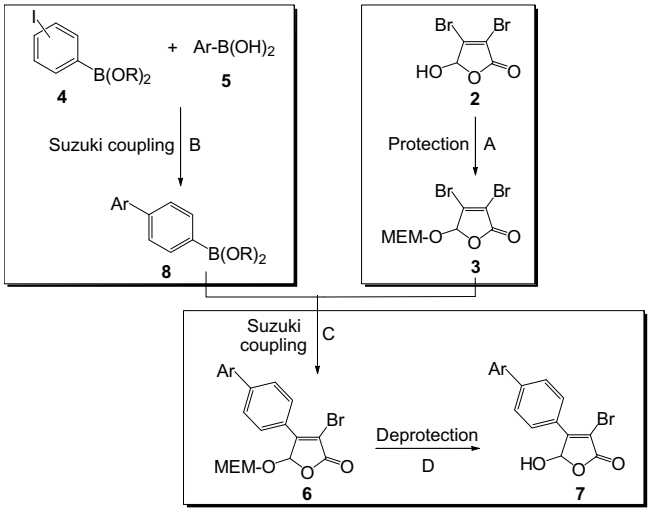
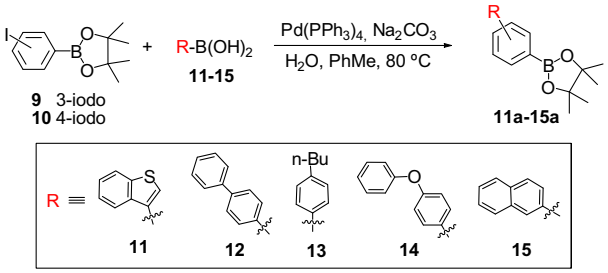


Figure 2. Synthetic strategy adopted for the synthesis of PM analogues.

aromatic portion **8** to the Mem protected[8] mucobromic acid **3**, through a second Suzuki reaction. Thus, a final deprotection step afforded our products.

According to this scheme, to search for the best experimental conditions, we utilized, as pilot reaction, the coupling of benzothy-



Scheme 1. Parallel synthesis of the aromatic building blocks.

ophene boronic acid **11** with the 3-iodophenyl boronic ester (**9**, Scheme 1), obtained from the related commercial acid,[9] in order to decrease its reactivity as nucleophile and to force the reaction toward the chemoselective formation of adduct **11a**.

From these preliminary trials, we had the opportunity to refine the conditions of the reaction (Table 2, entry 1) and, once we found the more efficient synthetic procedure, we applied it for the reaction between the selected boronic acids and the two aromatic pinacolic esters **9** and **10**; hence a parallel protocol was followed, by using the ASW1000 Chemspeed apparatus, to generate a small collection of organoboronic esters (Table 2).

The next stage consisted in the Suzuki coupling between these complex aromatic portions (**11a**–**15a**) and the Mem protected 3-bromo-γ-hydroxybutenolide scaffold (**3**), obtaining the coupling adducts **16a–24a**. Even in this case, we optimized the experimental conditions to improve the reaction yields, and, in order to speed up the reactions, we took advantage from microwave heating (Scheme 2).[10,11] The last step, consisting in the removal of the protecting Mem group, using $AlCl_3$ in CH_2Cl_2,[3] afforded our products in satisfactory yield (**16b**–**24b**, Scheme 2).

What we found, even in this case, was the formation, together with the desired monosubstituted γ-hydroxybutenolide analogues (**16b–20b**, Scheme 2), of lower amounts of bis-substituted adducts as major by-products. Differently from what we experienced in the synthesis of the first collection of compounds, in this case we obtained two types of bis-adducts: the symmetric ones (**21b** and **23b**, Scheme 2) and the mixed substituted ones (**22b** and **24b**, Scheme 2), these last due to the use, in the second Suzuki process, of the crude solution obtained from the previous step containing a mixture of the unreacted boronic acid and the bis aromatic adduct.

In any case we decided to select these by-products for pharmacological screening in order to gain more detail about structural requirements essential for the inhibition of the expression of mPGES-1.

2.2. Biology

Enzymatic inhibition of $sPLA_2$, mainly type IIA $sPLA_2$, is a pharmacological approach that can modulate the availability of arachidonic acid and consequently the production of PGE_2.[12] The enzyme concentration in serum tissues is correlated with diverse severity in several inflammatory pathologies such rheumatoid arthritis[13], septic shock[14], and psoriasis.[15]

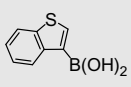
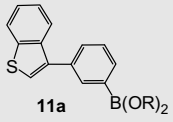
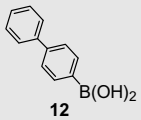
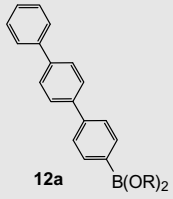
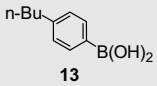
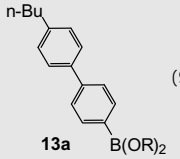
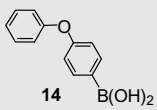
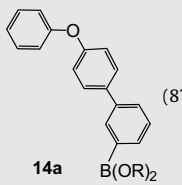
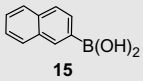
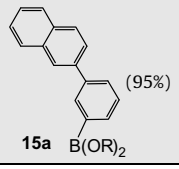
The new γ-hydroxybutenolide derivatives have been tested at 10 μM (Table 3), under the same experimental conditions, on four different $sPLA_2$ belonging to the groups IA (*Naja naja* venom), IB (porcine pancreatic enzyme), IIA (human synovial recombinant), and III (bee venom enzyme).

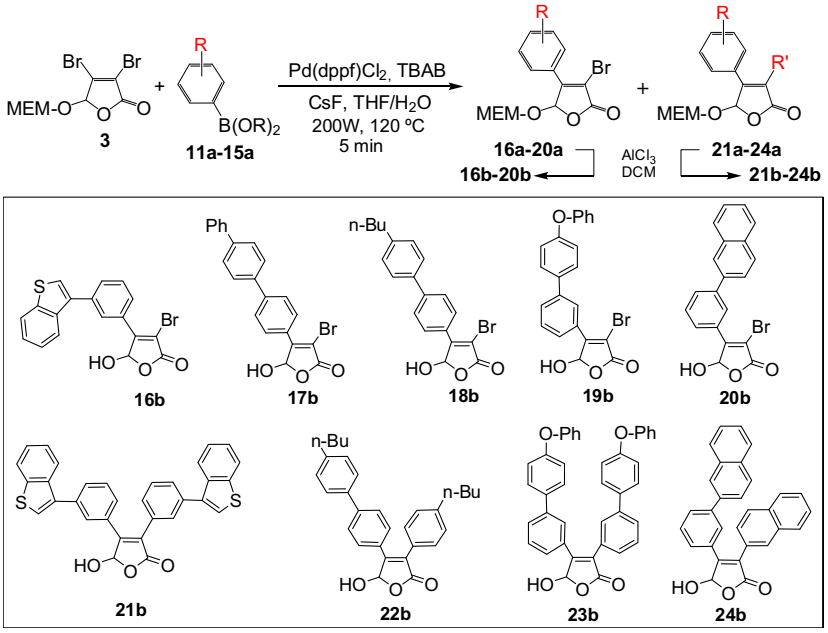
LY311727, a well-known inhibitor of group IIA $sPLA_2$, was used as a reference tool.[16]

None of the γ-hydroxybutenolide derivatives was able to inhibit preferentially human synovial $sPLA_2$, with inhibition percentages higher than 50%, as it did with the reference inhibitor LY311727.

The effect of the γ-hydroxybutenolide derivatives on PGE_2 production on mouse macrophage cell line RAW 264.7 stimulated with LPS was determined (Table 4).

Table 2
Experimental conditions for the synthesis of the aromatic building blocks

Entry	Boronic ester	Boronic acid	$Pd(PPh_3)_4$	Na_2CO_3 acq.	Time (h)	Product (yield, %)
1	9 (1 equiv)	 (1.2 equiv) **11**	1 mol%	2.4 equiv	6	 **11a** (89%)
2	9 (1 equiv)	 (1.2 equiv) **12**	1 mol%	2.4 equiv	6.5	 **12a** (92%)
3	9 (1 equiv)	 (1.2 equiv) **13**	1 mol%	2.4 equiv	6.2	 **13a** (93%)
4	10 (1 equiv)	 (1.2 equiv) **14**	1 mol%	2.4 equiv	7	 **14a** (87%)
5	10 (1 equiv)	 (1.2 equiv) **15**	1 mol%	2.4 equiv	6.5	 **15a** (95%)



Scheme 2. Microwave-assisted synthesis of PM analogues.

Table 3
Inhibitory activity of the γ-hydroxybutenolide derivatives at 10 μM on different $sPLA_2$ belonging to the groups IIA, IA (*Naja naja* venom), IB (porcine pancreatic enzyme), and III (bee venom enzyme)

Compound (10 μM)	GroupIIA-$sPLA_2$ % I	GroupIA-$sPLA_2$ % I	GroupIB-$sPLA_2$ % I	GroupIII-$sPLA_2$ % I
16b	30.0 ± 5.2**	11.7 ± 3.4	28.6 ± 4.3**	13.9 ± 4.2
17b	12.1 ± 2.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18b	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.5 ± 1.8
19b	2.4 ± 1.5	5.8 ± 5.3	0.0 ± 0.0	5.3 ± 3.0
20b	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
21b	2.4 ± 1.1	3.6 ± 2.2	8.3 ± 0.9	4.0 ± 1.8
22b	4.4 ± 2.6	2.6 ± 1.8	0.0 ± 0.0	2.8 ± 1.3
23b	10.6 ± 2.2	16.6 ± 2.9	10.2 ± 7.9	6.0 ± 4.5
24b	14.8 ± 1.0	2.0 ± 1.3	0.0 ± 0.0	2.8 ± 1.9
LY	96.3 ± 1.7**	7.9 ± 5.6	36.9 ± 11.0**	2.4 ± 1.8

Results show means ± SEM ($n = 6$). Statistical significances: $^{**}p < 0.01$, with respect to the corresponding enzyme control group (IIA $sPLA_2$ = 12129 ± 384 cpm; IA $sPLA_2$ = 10973 ± 350 cpm; IB $sPLA_2$ = 8008 ± 47 cpm; III $sPLA_2$ = 14854 ± 1054 cpm). Enzyme control group contains the vehicle (ethanol 1%).

Table 4
Inhibitory activity and cytotoxic effect of the γ-hydroxybutenolide derivatives at 10 μM on the production of PGE_2 in LPS-stimulated RAW 264.7 cells

Compound (10 μM)	% Inhibition	IC_{50} (μM)	% Toxicity
16b	33.6 ± 13.7	n.d.	0.0
17b	73.1 ± 4.1**	3.61 (2.3–6.8)	1.2
18b	62.2 ± 4.3**	2.24 (1.2–3.4)	5.5
19b	51.0 ± 14.0**	n.d.	0.0
20b	66.8 ± 4.7**	n.d.	18.6**
21b	69.2 ± 8.4**	3.23 (1.0–6.7)	4.1
22b	58.7 ± 6.9**	4.59 (3.5–6.4)	0.0
23b	22.2 ± 10.3	n.d.	0.0
24b	79.6 ± 4.7**	0.84 (0.4–1.4)	8.6
BTH	72.2 ± 5.7**	1.80 (0.5–3.8)	0.0

Results show means ± SEM ($n = 6$). Statistical significances: $^{**}p < 0.01$, with respect to the LPS-stimulated control group (contains the vehicle ethanol 1%). PGE_2 (non-stimulated cells = 0.6 ± 0.2 ng/ml; LPS-stimulated cells = 16.0 ± 1.6 ng/ml). n.d., not determined.

After 18 h stimulation, compounds **17b**, **18b**, **20b**, **21b**, **22b**, and **24b** were able to inhibit PGE_2 production with a percentage of inhibition higher than 50% at 10 μM, showing IC_{50} values in the micromolar range. On the other hand, all the derivatives except **20b,** which was discarded, were devoid of significant cytotoxic effects on RAW 264.7 at concentrations up to 10 μM, as assessed by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan (Table 4).

Western blot analysis for COX-2 and mPGES-1 proteins using 18 h LPS-stimulated RAW 264.7 cells (Fig. 3) shows clearly that compounds **17b**, **18b**, **21b**, **22b**, and **24b** inhibit dose-dependently mPGES-1 expression, without any effect on COX-2 expression, whereas dexamethasone, as expected, reduced the expression of both inducible proteins.

The three enzymes mainly involved in the biosynthesis of PGE_2 are PLA_2, COX-2, and mPGES-1.[17] Due to the great interest evoked by mPGES-1 enzyme, as an attractive target for the development of a new class of anti-inflammatory drugs, these γ-hydroxybutenolide derivatives by inhibiting PGE_2 production through the selective inhibition of mPGES-1 expression, can be considered interesting candidates. The obtained biological results, however, do not allow us yet to draw a clear structure–activity relationship, in any case we can make few observations, waiting to gather more data which can illuminate the structural aspects involved in the biological behavior. For example, altogether, the present compounds, except the case of compound **16b**, show an increased activity compared to the former collection of analogues,[3] probably depending on the presence of more bulky aromatic substituents on the C-4 position of the scaffold; this hypothesis seems to be strengthened by the unexpected observation that the reaction by-products bis-substituted hydroxybutenolides (**21b** and **24b**), possessing a further bulky appendage on the scaffold, displayed an interesting activity. Notably the **23b** analogue, belonging to the same series of products, is completely inactive; probably the presence of an oxygen atom in the side chain is detrimental for the activity, as, also the related monosubstituted compound, **19b**, proved to be totally ineffective. In any case, to put these results under the right perspective, we have to consider them as a useful basis for the structural optimization process and, furthermore, this selective pharmacological profile could be of great interest to discover new promising anti-inflammatory agents, as well as to discern the role of mPGES-1 in a great variety of inflammatory pathologies.

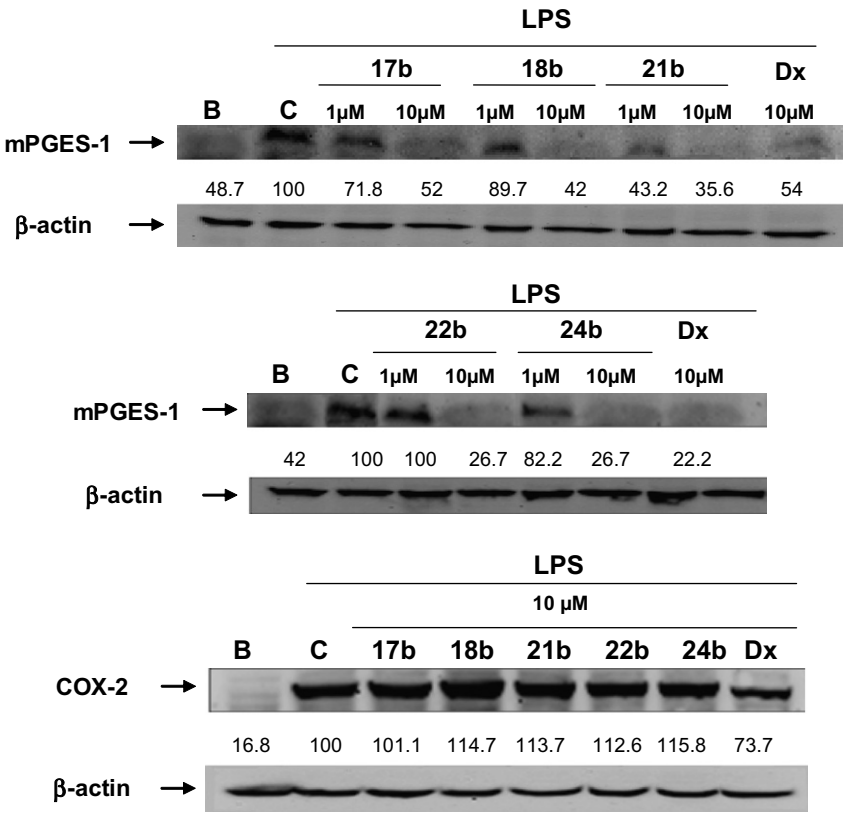


Figure 3. Effect of γ-hydroxybutenolides on COX-2 and mPGES-1 expression in LPS-stimulated RAW 264.7 cells. The figure is representative of two similar experiments. B: normal cells. C: LPS-stimulated cells. Dx, dexamethasone.

3. Experimental

3.1. Ludi design

The Ludi module of Insight II (Accelrys, San Diego, CA, USA) was used for the in silico design. Computation was performed on a Silicon Graphics Indigo 2 workstation equipped with a R10000 processor. The 3D complex structure bvPLA$_2$ (1POC)-PM[4] was imported into the graphic modeling program Insight II; the tetracyclic portion of PM was deleted from the active site of the above-mentioned adduct, whereas the tridimensional coordinates of the γ-hydroxybutenolide ring were kept unaltered. On this dataset, Ludi performed a database screening on the User_link_library (provided by Accelerys version 1998 and 2000.2) to select appropriate aromatic and heteroaromatic fragments to replace the sesterterpene skeleton of PM by linking the γ-hydroxybutenolide ring. The value of the maximum RMS deviation was fixed at 0.4 Å, the lipo weight was set at 10, the H bond weight was set at 1, and the value of the minimum separation was definitively 3.00. The other parameters were used as standard default. For each fragment, the Ludi score was calculated by means of the scoring function mentioned as *energy estimate_3*.

3.2. General methods

All water and air sensitive reactions were carried out under an inert atmosphere (N_2) in oven- or flame-dried glassware. Ethyl acetate, methylene chloride, and toluene were distilled from CaH_2 immediately prior to use. Water was degassed under vacuum (10 mbar). All reagents were used from commercial sources without any further purification. Organic extracts were dried over anhydrous Na_2SO_4. Reactions were monitored on silica gel 60 F254 (Merck) plates and visualized with potassium permanganate or cerium sulfate and under UV (λ = 254, 365 nm). Flash column chromatography was performed using Merck 60/230-400 mesh silica gel. Analytical and semipreparative reverse phase HPLC purifications were performed on a Waters instrument using Jupiter C-18 column (250 × 4.60 mm, 5 μm, 300 Å; 250 × 10.00 mm, 10 μm, 300 Å, respectively). Purity grade of final products was determined on a Agilent 1100 HPLC using two different analytical reverse phase columns (Method A: Jupiter C-18, 250 × 4.60 mm, 5 μm, 300 Å; Method B: Jupiter C-4, 250 × 4.60 mm, 5 μm, 300 Å). Parallel reactions were performed on Chemspeed Automated Synthesis Workstation ASW1000. Reaction yields refer to chromatographically and spectroscopically pure products. Proton detected (^{1}H, HMBC, HSQC) and carbon detected NMR spectra were recorded on Bruker instruments of Avance series operating at 300, 400, and 600 MHz and 75, 100, and 150 MHz, respectively. Chemical shifts are expressed in parts per million (ppm) on the delta (δ) scale. In ^{1}H NMR chemical shift description, Roman numbers in superscript (I, II, and III) are referred to the aromatic rings (first, second, third) linked in sequence to the scaffold; when letters are present, A protons belong to aromatic rings in C-4 position and B protons in C3 position. The solvent peak was used as internal reference: for ^{1}H NMR $CDCl_3$ = 7.26 ppm; for ^{13}C NMR: $CDCl_3$ = 77.0 ppm. Multiplicities are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets; b, broad. High resolution mass spectra (HRMS) were recorded on a Q/TOF Premier WATERS (Milford, MA, USA) mass spectrometer using an electrospray ion source (ESI-MS).

3.2.1. 3,4-Dibromo-5-(2-methoxy-ethoxymethoxy)-5*H*-furan-2-one (3)

To a solution of mucobromic acid (**2**) (100 mg, 0.387 mmol) in 10 mL of dry dichloromethane, MEM-Cl (66 μl, 0.581 mmol) was

added. Diisopropylethylamine (101 μl, 0.581 mmol) was added dropwise over a period of 15 min. After 4 h, the reaction mixture was quenched with 20 mL of HCl 1 M. The aqueous layer was extracted with dichloromethane (2 × 30 mL) and the organics were dried, filtered, and concentrated in vacuo to leave dark oil. The crude oil was purified by flash chromatography (10% diethyl ether/hexane) to give **3** (115 mg, 86% yield): ^{1}H NMR δ (300 MHz; $CDCl_3$): δ 6.10 (1H, s, *H*-5), 5.20 (1H, d, *J* = 7 Hz, OC*H*HO), 4.87 (1H, d, *J* = 7 Hz, OCH*H*O), 3.79 (1H, m, OC*H*HCH$_2$O), 3.40 (3H, s, OCH_3), 3.60 (1H, m, OCH*H*CH$_2$O), 3.54 (2H, dd, OCH$_2$CH_2O); ^{13}C NMR δ (75 MHz; $CDCl_3$): δ 167.4, 144.2, 119.1, 100.4, 95.2, 72.1, 69.0, 59.8; HRMS calcd for $C_8H_{11}Br_2O_5$: $[M+H]^+$ 344.8973, 346.8953, 348.8932 (ratio 1:2:1); found $[M+H]^+$ 344.8942, 346.8925, 348.8918 (ratio 1:2:1).

3.2.2. General procedure for the esterification of the boronic acids

To a solution of 3- or 4-iodophenylboronic acid (100 mg, 0.403 mmol) in 6 mL of dry ethyl acetate, 2,3-dimethylbutane-2,3-diol (47.5 mg, 0.403 mmol) was added, upon stirring and under argon. The mixture was then stirred at rt for 4 h. After dilution with ethyl acetate were added 1 g of Na_2SO_4 and 1 g of $CaCl_2$ in order to remove water. The mixture was finally filtered and concentrated on the rotavapor. The crude did not require any further purification.

3.2.3. 2-(4-Iodo-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane (9)

Yield: 90%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 7.72 (2H, d, *J* = 8.2 Hz, H_2-3), 7.51 (2H, d, *J* = 8.2 Hz, H_2-2), 1.33 (12H, s, (CH_3)$_4$); ^{13}C NMR δ (75 MHz; $CDCl_3$): 137.4, 130.2, 127.1, 97.0, 88.4, 21.6; HRMS calcd for $C_{12}H_{17}BIO_2$: $[M+H]^+$ 331.0361; found 331.0355.

3.2.4. 2-(3-Iodo-phenyl)-4,4,5,5-tetramethyl-[1,3,2]dioxaborolane (10)

Yield: 97%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 8.16 (1H, s, *H*-2), 7.76 (2H, d, *J* = 6.3 Hz, *H*-4, *H*-6), 7.10 (1H, t, *J* = 7.6 Hz, *H*-5); ^{13}C NMR δ (75 MHz; $CDCl_3$): 137.4, 130.2, 127.1, 97.0, 88.4, 21.6; HRMS calcd for $C_{12}H_{17}BIO_2$: $[M+H]^+$ 331.0361; found 331.0358.

3.2.5. General procedure for the Chemspeed® assisted coupling of the aromatic building blocks

All the automatic sequences were programmed through the Chemspeed® software. The boronic acids (1.2 equiv, see Table 2) were manually introduced, under argon, in five reactors (12 mL vol). The *stock* solutions were then prepared : 600 mg (1.82 mmol) of 4-iodophenylboronic ester in 6 mL of toluene; 400 mg (1.21 mmol) of 3-iodophenylboronic ester in 4 mL of toluene; 35 mg (0.052 mmol) of $Pd(PPh_3)_4$ in 5 mL of toluene; 10 mL of an aqueous solution 0.73 M of Na_2CO_3 (Table 2). All the stock solutions were then sequentially added, in equal proportions, to the six reactors respecting the order presented in Table. 2. The reactors were then stirred at 900 rpm setting the temperature to 80 °C. Every 2 h TLC controls collecting small aliquots of the mixtures were programmed. Time reactions varied in function of the substrates (Table. 2). The workup was automatically performed adding 4 mL of an aqueous solution of HCl 1 N then extracting with toluene (2× 4 mL). The organics were then dried over Na_2SO_4, filtered and concentrated. The crudes were directly used in the next step.

3.2.6. Microwave-assisted Suzuki coupling: general procedure

In a CEM Discover vial were placed 3,4-dibromo-5-(2-methoxy-ethoxymethoxy)-5*H*-furan-2-one (1 equiv), the boronic ester (1.5 equiv), $Pd(dppf)Cl_2$ (0.03 equiv), TBAB (1 equiv), and CsF (4 equiv). All the reactions were performed on 100 mg scale of furan-2-one. Under Ar were added water (500 μl) and THF (500 μl). The mixture was irradiated for 5 min, setting the power to 200 W, the temperature to 120 °C, the pressure to 250 psi and the Power Max ON. After diluting (10 mL) with DCM, 10 mL of an aqueous solution of HCl 1 N was added. The aqueous layer was extracted with DCM (3× 15 mL). The organics were then dried over Na_2SO_4, filtered, and concentrated. The crude was purified by flash chromatography (9:1 hexane/diethyl ether to 6:4 hexane/diethyl ether).

3.2.7. 4-(3-Benzo[b]thiophen-3-yl-phenyl)-3-bromo-5-(2-methoxy-ethoxymethoxy)-5H-furan-2-one (16a)

Yield: 68%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 8.07 (1H, s, *H*-2^{I}), 7.93 (2H, d, *J* = 6.9 Hz, *H*-8^{II}, *H*-4^{I}), 7.87 (1H, m, *H*-5^{II}), 7.75 (1H, d, *H*-6^{I}), 7.64 (1H, t, *H*-5^{I}), 7.48 (1H, s, *H*-2^{II}), 7.41 (2H, m, *H*-6^{II}, *H*-7^{II}), 6.64 (1H, s, *H*-5), 5.17 (1H, d, *J* = 7 Hz, OC*H*HO), 4.85 (1H, d, *J* = 7 Hz, OCH*H*O), 3.73–3.64 (2H, m, OCH_2CH$_2$O), 3.60–3.52 (2H, m, OCH$_2$CH_2O), 3.23 (3H, s, OCH_3); HRMS calcd for $C_{22}H_{20}BrO_5S$: $[M+H]^+$ 475.0215, 477.0194 (ratio 1:1); found 475.0208, 477.0190 (ratio 1:1).

3.2.8. 3-Bromo-5-(2-methoxy-ethoxymethoxy)-4-[1,1′;4′,1″]terphenyl-4-yl-5H-furan-2-one (17a)

Yield: 81%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 8.03 (2H, d, *J* = 8.4 Hz, *H*-2^{I}, *H*-6^{I}), 7.79 (2H, d, *J* = 8.4 Hz, *H*-3^{I}, *H*-5^{I}), 7.70–7.64 (4H, m, *H*-2^{II}, *H*-3^{II}, *H*-5^{II} *H*-6^{II}), 7.51–7.46 (4H, m, *H*-2^{III}, *H*-3^{III}, *H*-5^{III} *H*-6^{III}), 7.39 (1H, t, *H*-4^{III}), 6.63 (1H, s, *H*-5), 5.21 (1H, d, *J* = 7 Hz, OC*H*HO), 4.91 (1H, d, *J* = 7 Hz, OCH*H*O), 3.89–3.84 (1H, m, OC*H*HCH$_2$O), 3.72–3.66 (1H, m, OCH*H*CH$_2$O), 3.59 (2H, t, *J* = 8.4 Hz, OCH$_2$CH_2O), 3.43 (3H, s, OCH_3); HRMS calcd for $C_{26}H_{24}BrO_5$: $[M+H]^+$ 495.0807, 497.0787 (ratio 1:1); found 495.0817, 497.0793 (ratio 1:1).

3.2.9. 3-Bromo-4-(4′-butyl-biphenyl-4-yl)-5-(2-methoxy-ethoxymethoxy)-5H-furan-2-one (18a)

Yield: 72%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 7.95 (2H, d, *J* = 8.7 Hz, *H*-2^{I}, *H*-6^{I}), 7.70 (2H, d, *J* = 8.7 Hz, *H*-3^{I}, *H*-5^{I}), 7.48 (2H, d, *J* = 8.4 Hz, *H*-2^{II}, *H*-6^{II}), 7.26 (2H, d, *J* = 8.4 Hz, *H*-3^{II}, *H*-5^{II}), 6.61 (1H, s, *H*-5), 5.19 (1H, d, *J* = 7 Hz, OC*H*HO), 4.90 (1H, d, *J* = 7 Hz, OCH*H*O), 3.86–3.78 (1H, m, OC*H*HCH$_2$O), 3.70–3.62 (1H, m, OCH*H*CH$_2$O), 3.57 (2H, t, OCH$_2$CH_2O), 3.41 (3H, s, OCH_3), 2.64 (2H, t, *J* = 7.8 Hz, PhCH_2), 1.58 (2H, quint., J_1 = 7.5 Hz, J_3 = 15.4 Hz, PhCH$_2$CH_2), 1.31 (2H, sest., J_1 = 7.14 Hz and J_3 = 14.5 Hz, CH_2CH$_3$), 0.92 (3H, t, *J* = 7.6 Hz, CH_3); HRMS calcd for $C_{24}H_{28}BrO_5$: $[M+H]^+$ 475.1120, 477.1100 (ratio 1:1); found 475.1114, 477.1108 (ratio 1:1).

3.2.10. 3-Bromo-5-(2-methoxy-ethoxymethoxy)-4-(4′-phenoxy-biphenyl-3-yl)-5H-furan-2-one (19a)

Yield: 74%; ^{1}H NMR δ (600 MHz; $CDCl_3$): 8.14 (1H, s, *H*-2^{I}), 7.82 (1H, d, *J* = 7.8 Hz, *H*-4^{I}), 7.69 (1H, d, *J* = 7.8 Hz, *H*-6^{I}), 7.55 (1H, t, *J* = 7.8 Hz, *H*-5^{I}), 7.53 (2H, d, *J* = 8.4 Hz, *H*-2^{II}, *H*-6^{II}), 7.34 (2H, t, *J* = 7.8 Hz, *H*-3^{III}, *H*-5 ^{III}), 7.11 (1H, t, *J* = 7.8 Hz, *H*-4^{III}), 7.08 (2H, d, *J* = 8.4 Hz, *H*-3^{II}, *H*-5^{II}), 7.06 (2H, d, *J* = 7.8 Hz, *H*-2 ^{III}, *H*-6^{III}), 6.58 (1H, s, *H*-5), 5.15 (1H, d, *J* = 7 Hz, OC*H*HO), 4.86 (1H, d, *J* = 7 Hz, OCH*H*O), 3.70–3.62 (2H, m, OCH_2CH$_2$O), 3.56–3.48 (2H, t, OCH$_2$CH_2O), 3.25 (3H, s, OCH_3); HRMS calcd for $C_{26}H_{24}BrO_6$: $[M+H]^+$ 511.0756, 513.0736 (ratio 1:1); found 511.0746, 513.0730 (ratio 1:1).

3.2.11. 3-Bromo-5-(2-methoxy-ethoxymethoxy)-4-(3-naphthalen-1-yl-phenyl)-5H-furan-2-one (20a)

Yield: 66%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 8.09 (1H, s, *H*-2^{I}), 8.05 (1H, s, *H*-1^{II}), 7.78 (1H, d, *H*-4^{I}), 7.75 (1H, d, *H*-3^{II}), 7.69 (1H, d, *H*-6^{I}), 7.50 (1H, d, *H*-4^{II}), 7.49–7.46 (2H, m, *H*-7^{II}, *H*-8^{II}), 7.44 (1H, t, *H*-5^{I}), 7.41–7.40 (1H, m, *H*-6^{II}), 7.38–7.36 (1H, m, *H*-9^{II}), 6.75 (1H, s, *H*-5), 5.29 (1H, d, *J* = 7 Hz, OC*H*HO), 4.94 (1H, d, *J* = 7 Hz, OCH*H*O), 3.83–3.71 (1H, m, OC*H*HCH$_2$O), 3.67–3.55 (1H, m, OCH*H*CH$_2$O), 3.41 (2H, m, OCH$_2$CH_2O), 3.25 (3H, s, OCH_3); HRMS calcd for $C_{24}H_{22}BrO_5$: $[M+H]^+$ 469.0651, 471.0630 (ratio 1:1); found 469.0645, 471.0641 (ratio 1:1).

3.2.12. 3,4-Bis-(3-benzo[b]thiophen-3-yl-phenyl)-5-(2-methoxy-ethoxymethoxy)-5H-furan-2-one (21a)

Yield: 20%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 7.87 (2H, dd, *H*-5^{II}A, *H*-5^{II}B), 7.68–7.64 (6H, m, *H*-4^{I}A, *H*-5^{I}A, *H*-2^{II}A, *H*-6^{II}A, *H*-7^{II}A, *H*-8^{II}A), 7.62–7.53 (6H, m, *H*-4^{I}B, *H*-5^{I}B, *H*-2^{II}B, *H*-6^{II}B, *H*-7^{II}B, *H*-8^{II}B), 7.37–7.29 (4H, m, *H*-6^{I}A, 6^{I}B, *H*-2^{I}A, *H*-2^{I}B), 6.64 (1H, s, *H*-5), 5.24 (1H, d, *J* = 7 Hz, OC*H*HO), 4.84 (1H, d, *J* = 7 Hz, OCH*H*O), 3.73–3.50 (2H, m, OCH_2CH_2O), 3.33 (2H, t, OCH_2CH_2O), 3.25 (3H, s, OCH_3); HRMS calcd for $C_{36}H_{29}O_5S_2$: $[M+H]^+$ 605.1456; found 605.1464.

3.2.13. 4-(4′-Butyl-biphenyl-4-yl)-3-(4-butyl-phenyl)-5-(2-methoxy-ethoxymethoxy)-5H-furan-2-one (22a)

Yield: 16%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 7.59–7.56 (4H, m, *H*-2^{I}A, *H*-3^{I}A, *H*-5^{I}A, *H*-6^{I}A), 7.54–7.50 (4H, m, *H*-2^{II}A, *H*-3^{II}A, *H*-5^{II}A, *H*-6^{II}A), 7.27–7.25 (4H, dd, *H*-2^{I}B, *H*-6^{I}B), 6.60 (1H, s, *H*-5), 5.25 (1H, d, *J* = 7 Hz, OC*H*HO), 4.93 (1H, d, *J* = 7 Hz, OCH*H*O), 3.85–3.78 (1H, m, OC*H*HCH_2O), 3.72–3.65 (1H, m, OCH*H*CH_2O), 3.58 (2H, t, OCH_2CH_2O), 3.42 (3H, s, OCH_3), 2.65 (4H, m, $(PhCH_2)_2$), 1.63 (4H, m, $(PhCH_2CH_2)_2$), 1.38 (4H, m, $(CH_2CH_3)_2$), 0.94 (6H, m, $(CH_2CH_3)_2$); HRMS calcd for $C_{30}H_{41}O_5$: $[M+H]^+$ 529.2954; found 529.2948.

3.2.14. 5-(2-Methoxy-ethoxymethoxy)-3,4-bis-(4′-phenoxy-biphenyl-3-yl)-5H-furan-2-one (23a)

Yield: 10%; ^{1}H NMR δ (600 MHz; $CDCl_3$): 7.69 (2H, d, *J* = 7.8 Hz, *H*-4^{I}A, *H*-4^{I}B), 7.58 (2H, d, *J* = 7.8 Hz, *H*-6^{I}A, *H*-6^{I}B), 7.44–7.42 (3H, d, *J* = 8.4 Hz, *H*-2^{II}B, *H*-5^{I}B, *H*-6^{II}B), 7.33–7.30 (6H, m, *H*-2^{I}A, *H*-3^{III}A, *H*-5^{III}A, *H*-2^{I}B, *H*-3^{III}B, *H*-5^{III}B), 7.12 (2H, dt, *H*-4^{III}A, *H*-4^{III}B), 6.98 (4H, d, *H*-3^{II}A, *H*-5^{II}A, *H*-3^{II}B, *H*-5^{II}B), 6.86 (4H, d, *H*-2^{III}A, *H*-6^{III}A, *H*-2^{III}B, *H*-6^{III}B), 6.65 (1H, s, *H*-5), 5.12 (1H, d, *J* = 7 Hz, OC*H*HO), 4.80 (1H, d, *J* = 7 Hz, OCH*H*O), 3.80–3.70 (2H, m, OCH_2CH_2O), 3.50–3.42 (2H, t, OCH_2CH_2O), 3.25 (3H, s, OCH_3); HRMS calcd for $C_{44}H_{37}O_7$: $[M+H]^+$ 677.2534; found 677.2528.

3.2.15. 5-(2-Methoxy-ethoxymethoxy)-3-naphthalen-2-yl-4-(3-naphthalen-2-yl-phenyl)-5H-furan-2-one (24a)

Yield: 14%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 8.18 (1H, s, *H*-1^{II}A), 8.17 (1H, s, *H*-1^{I}B), 7.90 (2H, m, *H*-9^{II}A, *H*-9^{I}B), 7.87–7.83 (4H, m, *H*-3^{II}A, *H*-4^{II}A, *H*-3^{I}B *H*-4^{I}B), 7.81 (1H, d, *H*-4^{I}A), 7.79 (1H, d, *H*-6^{I}A), 7.72 (2H, m, *H*-6^{II}A, *H*-6^{I}B), 7.67 (1H, s, *H*-2^{I}A), 7.53–7.51 (4H, m, *H*-7^{II}A, *H*-8^{II}A, *H*-7^{I}B *H*-8^{I}B), 7.48 (1H, t, *H*-5^{I}A), 6.75 (1H, s, *H*-5), 5.29 (1H, d, *J* = 7 Hz, OC*H*HO), 4.94 (1H, d, *J* = 7 Hz, OCH*H*O), 3.83–3.72 (1H, m, OC*H*HCH_2O), 3.67–3.58 (1H, m, OCH*H*CH_2O), 3.41 (2H, t, *J* = 8.4 Hz, OCH_2CH_2O), 3.28 (3H, s, OCH_3); HRMS calcd for $C_{34}H_{28}O_5$: $[M+H]^+$ 516.1937; found 516.1921.

3.2.16. General procedure for MEM cleavage

To a suspension of $AlCl_3$ (5 equiv) in dry DCM (3 mL), at 0 °C and upon stirring, the protected hydroxybutenolide in 2 mL of dry DCM was added dropwise. The mixture was stirred at 0 °C for 5 h. The mixture was then washed with aqueous $NaHCO_3$ sat. (5 mL) and then with brine (5 mL). The aqueous layers were extracted with DCM (3× 10 mL). The organics were then dried over Na_2SO_4, filtered, and concentrated.

3.2.17. 4-(3-Benzo[*b*]thiophen-3-yl-phenyl)-3-bromo-5-hydroxy-5H-furan-2-one (16b)

Yield: 74%; ^{1}H NMR δ (600 MHz; $CDCl_3$): 8.23 (1H, s, *H*-2^I), 8.03 (1H, d, *H*-4^I), 7.96 (2H, dd, *H*-8^{II}), 7.93 (1H, dd, *H*-5^{II}), 7.78 (1H, d, *H*-6^I), 7.66 (1H, t, *H*-5^I), 7.50 (1H, s, *H*-2^{II}), 7.45–7.43 (2H, m, *H*-6^{II}, *H*-7^{II}), 6.58 (1H, s, *H*-5); ^{13}C NMR δ (150 MHz; $CDCl_3$): 166.8, 154.7, 141.1, 140.4, 138.7, 136.9, 135.7, 131.8, 129.7, 128.7, 127.6, 125.0, 124.8, 124.6, 123.2, 122.5, 111.3, 96.5; HRMS calcd for $C_{18}H_{12}BrO_3S$: $[M+H]^+$ 386.9691, 388.9670 (ratio 1:1); found 386.9684, 388.9662 (ratio 1:1).

3.2.18. 3-Bromo-5-hydroxy-4-[1,1′;4′,1″]terphenyl-4-yl-5H-furan-2-one (17b)

Yield: 77%; ^{1}H NMR δ (600 MHz; $CDCl_3$): 8.14 (2H, d, *J* = 8.4 Hz, *H*-2^I, *H*-6^I), 7.82 (2H, d, *J* = 8.4 Hz, *H*-3^I, *H*-5^I), 7.67–7.64 (4H, m, *H*-2^{II}, *H*-3^{II}, *H*-5^{II} *H*-6^{II}), 7.50–7.48 (4H, m, *H*-2^{III}, *H*-3^{III}, *H*-5^{III} *H*-6^{III}), 7.40 (1H, t, *H*-4^{III}), 6.59 (1H, s, *H*-5); ^{13}C NMR δ (150 MHz; $CDCl_3$): 167.5, 155.2, 145.3, 145.0, 142.5, 141.8, 139.7, 130.6, 130.5, 130.2, 130.0, 129.7, 129.4, 129.3, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 111.6, 98.9; HRMS calcd for $C_{22}H_{15}BrO_3$: $[M+H]^+$ 407.0283, 409.0262 (ratio 1:1); found 407.0266, 409.0250 (ratio 1:1).

3.2.19. 3-Bromo-4-(4′-butyl-biphenyl-4-yl)-5-hydroxy-5H-furan-2-one (18b)

Yield: 70%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 8.09 (2H, d, *J* = 8.7 Hz, *H*-2^I, *H*-6^I), 7.74 (2H, d, *J* = 8.7 Hz, *H*-3^I, *H*-5^I), 7.56 (2H, d, *J* = 8.4 Hz, *H*-2^{II}, *H*-6^{II}), 7.30 (2H, d, *J* = 8.4 Hz, *H*-3^{II}, *H*-5^{II}), 6.54 (1H, s, *H*-5), 2.64 (2H, t, *J* = 7.8 Hz, PhCH_2), 1.58 (2H, quint., J_1 = 7.5 Hz, J_3 = 15.4 Hz, $PhCH_2CH_2$), 1.31 (2H, sest., J_1 = 7.14 Hz and J_3 = 14.5 Hz, CH_2CH_3), 0.92 (3H, t, *J* = 7.6 Hz, CH_2CH_3); ^{13}C NMR δ (75 MHz; $CDCl_3$): 167.5, 154.7, 143.3, 143.1, 136.6, 136.4, 129.2, 129.0, 128.3, 128.0, 127.3, 127.1, 126.9, 126.2, 109.7, 98.1, 37.6, 33.4, 22.5, 14.0; HRMS calcd for $C_{20}H_{19}BrO_3$: $[M+H]^+$ 387.0518, 389.0497 (ratio 1:1); found 387.0528, 387.0485 (ratio 1:1).

3.2.20. 3-Bromo-5-hydroxy-4-(4′-phenoxy-biphenyl-3-yl)-5H-furan-2-one (19b)

Yield: 93%; ^{1}H NMR δ (600 MHz; $CDCl_3$): 8.21 (1H, s, *H*-2^I), 7.93 (1H, d, *J* = 7.8 Hz, *H*-4^I), 7.75 (1H, d, *J* = 7.8 Hz, *H*-6^I), 7.60 (1H, t, *J* = 7.8 Hz, *H*-5^I), 7.57 (2H, d, *J* = 8.4 Hz, *H*-2^{II}, *H*-6^{II}), 7.39 (2H, t, *J* = 7.8 Hz, *H*-3^{III}, *H*-5^{III}), 7.16 (1H, t, *J* = 7.8 Hz, *H*-4^{III}), 7.12 (2H, d, *J* = 8.4 Hz, *H*-3^{II}, *H*-5^{II}), 7.09 (2H, d, *J* = 7.8 Hz, *H*-2^{III}, *H*-6^{III}), 6.58 (1H, s, *H*-5) ^{13}C NMR δ (150 MHz; $CDCl_3$): 166.6, 157.8, 157.0, 155.2, 141.8, 135.2, 134.6, 134.3, 130.4, 130.2, 129.7, 129.6, 129.4, 129.3, 128.7, 127.8, 127.3, 123.9, 119.5, 119.3, 111.3, 98.1; HRMS calcd for $C_{22}H_{16}BrO_4$: $[M+H]^+$ 423.0232, 425.0211 (ratio 1:1); found 423.0224, 425.0204 (ratio 1:1).

3.2.21. 3-Bromo-5-(2-methoxy-ethoxymethoxy)-4-(3-naphthalen-1-yl-phenyl)-5H-furan-2-one (20b)

Yield: 80%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 8.16 (1H, s, *H*-2^I), 8.12 (1H, s, *H*-1^{II}), 7.82 (1H, d, *H*-4^I), 7.80 (1H, d, *H*-3^{II}), 7.70 (1H, d, *H*-6^I), 7.54 (1H, d, *H*-4^{II}), 7.53–7.50 (2H, m, *H*-7^{II}, *H*-8^{II}), 7.51 (1H, t, *H*-5^I), 7.43–7.42 (1H, m, *H*-6^{II}), 7.41–7.39 (1H, m, *H*-9^{II}), 6.68 (1H, s, *H*-5); ^{13}C NMR δ (75 MHz; $CDCl_3$): 170.1, 154.7, 140.4, 137.1, 135.0, 133.5, 130.0, 129.6, 129.4, 129.3, 129.0, 128.5, 127.8, 127.1, 126.0, 125.8, 125.7, 124.9, 110.4, 97.0; HRMS calcd for $C_{20}H_{14}BrO_3$: $[M+H]^+$ 381.0126, 383.0106 (ratio 1:1); found 381.0112, 383.0120 (ratio 1:1).

3.2.22. 3,4-Bis-(3-benzo[b]thiophen-3-yl-phenyl)-5-hydroxy-5H-furan-2-one (21b)

Yield: 88%; ^{1}H NMR δ (300 MHz; $CDCl_3$): 7.88 (2H, dd, *H*-5^{II}A, *H*-5^{II}B), 7.71–7.66 (6H, m, *H*-4^{I}A, *H*-5^{I}A, *H*-2^{II}A, *H*-6^{II}A, *H*-7^{II}A, *H*-8^{II}A), 7.54–7.61 (6H, m, *H*-4^{I}B, *H*-5^{I}B, *H*-2^{II}B, *H*-6^{II}B, *H*-7^{II}B, *H*-8^{II}B), 7.35 (1H, d, *H*-6^{I}A), 7.32 (1H, d, *H*-6^{I}B), 7.31 (1H, s, *H*-2^{I}A), 7.28 (1H, s, *H*-2^{I}B), 6.60 (1H, s, *H*-5); ^{13}C NMR δ (150 MHz; $CDCl_3$): 170.1, 156.1, 141.3, 141.0, 140.6, 140.4, 138.5, 138.4, 137.0 136.9, 135.8, 135.7, 131.8, 131.6, 129.5, 129.4, 128.8, 128.7, 127.6, 127.3, 125.4, 125.0, 124.8, 124.7, 124.6, 124.5, 123.0, 122.8, 122.7, 122.2, 122.0, 96.3; HRMS calcd for $C_{32}H_{22}O_3S_2$: $[M+H]^+$ 517.0932; found 517.0922.

3.2.23. 4-(4′-Butyl-biphenyl-4-yl)-3-(4-butyl-phenyl)-5-hydroxy-5H-furan-2-one (22b)

Yield: 80%. ^{1}H NMR δ (300 MHz; $CDCl_3$): 7.59–7.56 (4H, m, *H*-2^{I}A, *H*-3^{I}A, *H*-5^{I}A, *H*-6^{I}A), 7.54–7.50 (4H, m, *H*-2^{II}A, *H*-3^{II}A, *H*-5^{II}A,

H-6^{II}A), 7.27–7.25 (4H, dd, *H*-2^{I}B, *H*-6^{I}B), 6.60 (1H, s, *H*-5), 2.65 (4H, m, (PhCH_2)$_2$), 1.63 (4H, m, (PhCH_2CH_2)$_2$), 1.38 (4H, m, CH_2CH_3)$_2$), 0.94 (6H, m, CH$_2$CH_3)$_2$); ^{13}C NMR δ (75 MHz; CDCl$_3$): 169.9, 154.5, 140.4, 137.6, 137.1, 134.5, 134.1, 133.9, 133.0, 132.7, 130.6, 129.5, 129.2, 128.6, 128.3, 128.1, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 126.5, 126.2, 126.0, 125.3, 124.8, 96.6; HRMS calcd for $C_{30}H_{32}O_3$: $[M+H]^+$ 440.2351; found 440.2342.

3.2.24. 5-Hydroxy-3,4-bis-(4′-phenoxy-biphenyl-3-yl)-5H-furan-2-one (23b)

Yield: 86%; ^{1}H NMR δ (300 MHz; CDCl$_3$): 7.71 (2H, d, *J* = 7.8 Hz, *H*-4^{I}A, *H*-4^{I}B), 7.62 (2H, d, *J* = 7.8 Hz, *H*-6^{I}A, *H*-6^{I}B), 7.43–7.45 (3H, d, *J* = 8.4 Hz, *H*-2^{II}B, *H*-5^{I}B, *H*-6^{II}B), 7.34–7.36 (6H, m, *H*-2^{I}A, *H*-3^{III}A, *H*-5^{III}A, *H*-2^{I}B, *H*-3^{III}B, *H*-5^{III}B), 7.17 (2H, dt, *H*-4^{III}A, *H*-4^{III}B), 7.00 (4H, d, *H*-3^{II}A, *H*-5^{II}A, *H*-3^{II}B, *H*-5^{II}B), 6.92 (4H, d, *H*-2^{III}A, *H*-6^{III}A, *H*-2^{III}B, *H*-6^{III}B), 6.60 (1H, s, *H*-5); ^{13}C NMR δ (75 MHz; CDCl$_3$): 170.2, 157.0, 156.9, 156.4, 156.3, 141.6, 141.5, 135.9, 135.8, 134.8, 134.7, 134.3, 134.2, 133.7, 133.6, 132.6, 132.2, 130.8, 130.5, 129.6, 129.5, 129.3, 129.2, 128.9, 128.7, 127.8, 127.7, 127.5, 127.4, 126.9, 126.7, 126.3, 125.9, 125.6, 125.5, 124.7, 124.5, 119.6, 119.3, 96.2; HRMS calcd for $C_{40}H_{28}O_5$: $[M+H]^+$ 588.1937; found 588.1921.

3.2.25. 5-Hydroxy-3-naphthalen-2-yl-4-(3-naphthalen-2-yl-phenyl)-5H-furan-2-one (24b)

Yield: 81%; ^{1}H NMR δ (300 MHz; CDCl$_3$): 8.18 (1H, s, *H*-1^{II}A), 8.17 (1H, s, *H*-1^{I}B), 7.90 (2H, m, *H*-9^{II}A, *H*-9^{I}B), 7.87–7.83 (4H, m, *H*-3^{II}A, *H*-4^{II}A, *H*-3^{I}B *H*-4^{I}B), 7.81 (1H, d, *H*-4^{I}A), 7.79 (1H, d, *H*-6^{I}A), 7.72 (2H, m, *H*-6^{II}A, *H*-6^{I}B), 7.67 (1H, s, *H*-2^{I}A), 7.53–7.51 (4H, m, *H*-7^{II}A, *H*-8^{II}A, *H*-7^{I}B *H*-8^{I}B), 7.48 (1H, t, *H*-5^{I}A), 6.69 (1H, s, *H*-5); ^{13}C NMR δ (75 MHz; CDCl$_3$): 169.9, 154.5, 140.4, 137.6, 137.1, 134.5, 134.1, 133.9, 133.0, 132.7, 130.6, 129.5, 129.2, 128.6, 128.3, 128.1, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 126.5, 126.2, 126.0, 125.3, 124.8, 96.6; HRMS calcd for $C_{30}H_{20}O_3$: $[M+H]^+$ 428.1412; found 428.1402.

3.3. Materials

[5,6,8,11,12,14,15(*n*)-^{3}H] PGE_2 and [9,10-^{3}H]oleic acid were purchased from Amersham Biosciences (Barcelona, Spain). LY311727 was a gift from Lilly Corporate Center (Indianapolis, IN). The rest of reagents were from Sigma (St. Louis, MO). *Escherichia coli* strain CECT 101 was a gift from Professor Uruburu, Department of Microbiology, University of Valencia, Spain.

3.4. Assay of $sPLA_2$

$sPLA_2$ activity was assayed using [^{3}H]oleate labeled membranes of *E. coli*, following a modification of the method of Franson and co-workers.[18,19] *E. coli* strain CECT 101 was grown for 6–8 h at 37 °C in the presence of 5 μCi/mL [^{3}H]oleic acid (specific activity 10 Ci/mmol) until the end of the logarithmic phase. After centrifugation at 1800*g* for 10 min at 4 °C, the membranes were washed, resuspended in phosphate-buffered saline (PBS), and autocleaved for 30–45 min. At least 95% of the radioactivity was incorporated into the phospholipid fraction. *N. naja* venom (Group IA $sPLA_2$), porcine pancreatic (Group IB $sPLA_2$), human recombinant synovial (Group IIA $sPLA_2$), and bee venom (Group III $sPLA_2$) enzymes were used as sources of $sPLA_2$. Enzymes were diluted in 10 μl of 100 mM Tris–HCl, 1 mM $CaCl_2$ buffer, pH 7.5, and preincubated at 37 °C for 5 min with 2.5 μl of test compound dissolved in ethanol or 2.5 μl of ethanol (control group) to get a final volume of 250 μl. Incubation proceeded for 15 min in the presence of 20 μl of [^{3}H]oleic-*E. coli* membranes and was terminated by addition of 100 μl ice-cold solution of 0.25% bovine serum albumin (BSA) solution in saline to a final concentration of 0.07% (w/v). After centrifugation at 2500*g* for 10 min at 4 °C, the radioactivity (cpm) in the supernatants was determined by liquid scintillation counting.

3.5. Western blot assay of COX-2 and mPGES-1

Cellular lysates from RAW 264.7 (murine macrophages, 1.5×10^6 cells/mL) incubated for 18 h with LPS (1 μg/mL) were obtained with lysis buffer A [10 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES), pH 8.0, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 10 mM KCl, 1 mM dithiothreitol, 5 mM NaF, 1 mM Na_3VO_4, 10 mM Na_2MoO_4, 1 μg/mL leupeptin, 0.1 μg/mL aprotinin, and 0.5 mM phenylmethanesulfonyl fluoride). Following centrifugation (10,000*g*, 15 min, 4 °C), supernatant protein was determined by the Bradford method with BSA as standard. COX-2 or mPGES-1 protein expression was studied in the total fraction or microsomal fractions, respectively. Equal amounts of protein (50 μg for both COX-2 and mPGES-1) were loaded on SDS–15% PAGE and transferred onto poly(vinylidene difluoride) membranes for 90 min at 125 mA. Membranes were blocked in PBS (0.02 M, pH 7.0)-Tween 20 (0.1%), containing 3% (w/v) nonfat milk, and incubated with specific polyclonal antibody against COX-2 (1/1000) or mPGES-1 (1/200). Finally, membranes were incubated with peroxidase-conjugated goat anti-rabbit IgG (1/10,000). The immunoreactive bands were visualized using an enhanced chemiluminescence system (Amersham Biosciences, Barcelona, Spain).

3.6. Culture of murine macrophage RAW 264.7 cell line

The mouse macrophage cell line RAW 264.7 (Cell Collection, Department of Animal Cell Culture, C.S.I.C., Madrid, Spain) was cultured in Dulbecco's modified Eagle's medium (DMEM) containing 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum. Cultures were maintained at 37 °C in 5% CO2 (air/CO_2 95:5) humidified incubator. Cells were resuspended at a concentration of 1.5×10^6 cells/mL.

3.7. PGE_2 production in RAW 264.7 macrophages

RAW 264.7 macrophages (1.5×10^6 cells/mL) were co-incubated in 96-well culture plate (200 μl) with 1 μg/mL of *E. coli* [serotype 0111:B4] lipopolysaccharide (LPS) at 37 °C for 20 h in the presence of 2.0 μl of test compound dissolved in ethanol or 2.0 μl of ethanol (control group). PGE_2 levels were determined in culture supernatants by radioimmunoassay.[20] The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan[21] was used to assess the possible cytotoxic effects of compounds.

3.8. Statistical analysis

The results are presented as means ± SEM; *n* represents the number of experiments. Inhibitory concentration 50% (IC_{50}) values were calculated from at least four significant concentrations (*n* = 6). The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons. Significance was assumed at a *p* value of 0.05 or less.

Acknowledgments

Financial support by the University of Salerno and MIUR (Rome) is gratefully acknowledged. M.D.G. was the recipient of a research fellowship from FPU program (AP 20041633) of Spanish Ministerio de Educación y Ciencia. This work was supported in part by Grant FIS PI051659 from Spanish Instituto de Salud Carlos III.

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