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Original article

Structure-based design, synthesis and preliminary anti-inflammatory activity of bolinaquinone analogues

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ABSTRACT

As a part of our drug discovery efforts we developed a series of simplified derivatives of bolinaquinone (BLQ), a hydroxyquinone marine metabolite, showing potent anti-inflammatory activity. Thirteen new hydroxyquinone derivatives closely related to BLQ were synthesized and tested on mouse macrophage-like RAW 264.7 cell line in order to investigate their ability to modulate the production of Prostaglandin E_2 (PGE_2). This optimization process led to the identification of three strictly correlated compounds with comparable and higher inhibitory potency than BLQ on PGE_2 production. To evaluate the affinity of BLQ and its analogues for *hs*PLA_2, surface plasmon resonance (SPR) experiments were performed.

1. Introduction

The search for biologically active natural products from marine sources continues to be an important scientific field that offers promising opportunities for the development of new compounds endowed with pharmacological properties.

Natural products with a quinoide system and a decalin-type or related aromatic side chain are characterized by pronounced and several biological properties (Chart 1).

For instance, the marine sesquiterpene quinones such as bolinaquinone (**1**) (**BLQ**) [1], nakijiquinone A–D [2] (**2a**–**d**) and nakijiquinone G-H [3] (**2e**–**f**), ilimaquinone [4] (**3**), avarol [5] (**4**), smenospongine (**5**), smenospongidine (**6**), smenospongiarine [6] (**7**) display antimicrobial, antiviral, anti-inflammatory and cytotoxic activities [7].

Among this class, the marine metabolite bolinaquinone expresses its anti-inflammatory activity by inhibition of secretory phospholipase A_2 (*s*PLA_2) [1].

Phospholipases A_2 (PLA_2) are a class of lipolytic enzymes that catalyze the hydrolysis of the *sn*-2 fatty acyl bond of phospholipids liberating free fatty acids and lysophospholipids [8]. Phospholipases A_2 include several unrelated protein families with common enzymatic activity but with different substrate specificities, cofactor requirements, subcellular localization and cellular functions.

Based on biophysical and biochemical properties more than 15 different forms of PLA_2 enzymes have been identified [8].

For their cellular location PLA_2 enzymes are classified as cytosolic PLA_2 (*c*PLA_2), intracellular PLA_2 (*i*PLA_2) and secretory PLA_2 (*s*PLA_2) [9].

Among the calcium dependent *s*PLA_2, type IIA (*s*PLA_2-IIA) is an isoform first isolated and purified from rheumatoid synovial fluid. Increased plasma levels of this enzyme were found in diseases that involve systemic inflammation such as sepsis, rheumatoid arthritis, and cardiovascular disease [10,11].

Therefore, compounds that can selectively block secretory PLA_2 activity and the assessment of their molecular mechanism of enzyme inactivation are of paramount importance in the field of anti-inflammatory drugs.

Among terpenoidic hydroquinone class, avarol was able to inhibit human recombinant synovial phospholipase A_2 activity with an $IC_{50} = 158$ μM, while quinone derivative avarone failed to show inhibitory activity [12]. BLQ, sharing a hydroxyl-*p*-quinone moiety connected to a trans-decalin terpene unit in a rearranged drimane skeleton is one of the most active metabolites with a selective profile against secretory PLA_2's [1].

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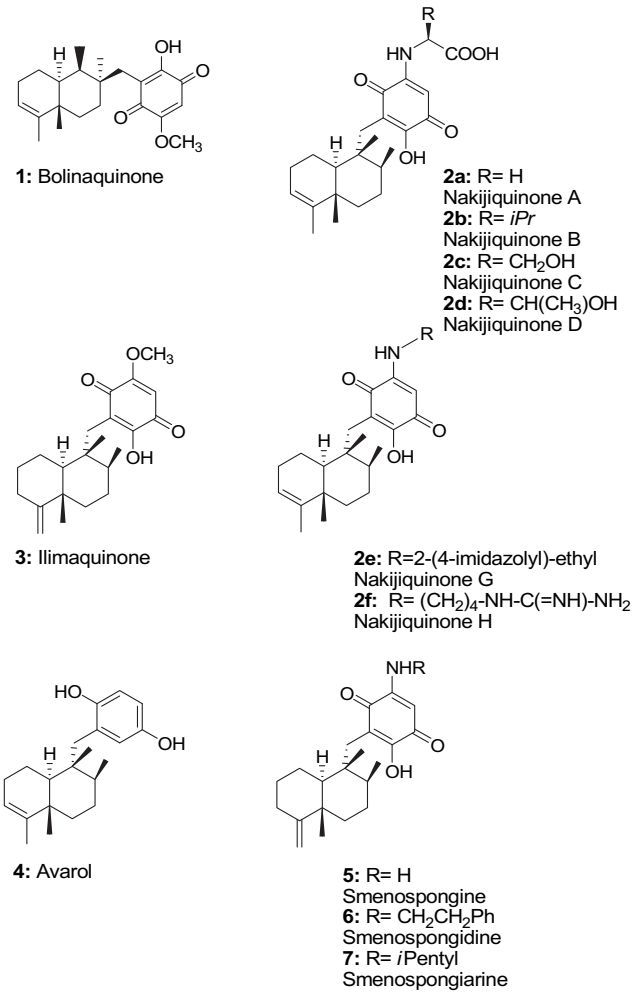


Chart 1. Formulas of natural sesquiterpene quinones and hydroquinones.

BLQ shows the ability to inhibit strongly *hs*PLA_2, bee venom PLA_2 (*bv*PLA_2), with IC_{50} values of 0.2 and 0.1 μM, respectively; remarkably, this product also exerts no effects on cytosolic PLA_2 (*c*PLA_2) [1].

Moreover, bolinaquinone is able to reduce the inflammatory response of adjuvant arthritis by inhibiting PGE_2, NO, and TNF production in paw homogenates without affecting PGE_2 levels in the stomach [13].

In a recent work [14] we have proposed, for the inactivation mechanism of *hs*PLA_2 [15] by BLQ, a key process in the inhibition consisting in the occupation of the active site's hydrophobic pocket by the sesquiterpene unit of the inhibitor.

The molecular basis of the human group IIA secretory phospolipase A_2 inactivation by BLQ is completely due to a non-covalent event involving the 2-hydroxy-1,4-benzoquinone responsible for the chelation with Ca^{2+} ions in the region of binding [16,17], orienting the inhibitor within the binding site.

Mainly, the oxygen atoms of hydroxyl-quinone system are of primary importance for the interaction, namely, both the C-1 carbonyl group and the deprotonated C-2 hydroxyl residue coordinate the calcium ion in a bidentate fashion.

Following our extensive investigation on the PLA_2 inactivation mechanism by BLQ we have developed a series of simplified derivatives related to the natural product.

The general design of novel inhibitors against inflammation-related diseases was guided by the modular composition of this metabolite.

Indeed, on the basis of these premises, we decided to rely on some well-reasoned structural changes of the basic molecule **1**, in the attempt to improve its pharmacological behaviour (Scheme 1).

To generate the chelation of calcium ion responsible of the competitive inhibition process of *hs*PLA_2, we generated focused collection of hydroxyl-benzoquinone analogues. Therefore, the quinone-type building block was not changed entirely but simplified by elimination of methoxy group.

In order to explore the non-covalent interactions with the large hydrophobic surface of the active site, thedrimane moiety of BLQ was replaced with different hydrophobic structures such as *n*-pentyl, benzyl, naphthyl and biphenyl units, also they were shifted from position 3 to 5.

To evaluate the impact of the single linker atom replacement on the overall profile of these molecules, we have substituted the carbon-linked of quinoide derivative with an NH or O bridge.

Optimization of the aromatic region was performed by introduction of different sterical hindrance structures as phenyl, naphthyl, biphenyl, thianthrenyl and dibenzofuranyl moiety directly attached to the quinoide core.

The purpose of the present study is to investigate the anti-inflammatory activity of a series of 2-hydroxy-1,4-benzoquinones, as well as to define the structure–activity relationships (SAR) of this new class. Moreover, preliminary investigations on the compounds synthesized in their potency against *hs*PLA_2 are evaluated.

This report describes the synthesis of new compounds that achieved good potency in the biochemical assay and cell-based system.

2. Chemistry

Synthesis of compounds **8a**–**d** (Scheme 2) was accomplished in three steps.

Commercially available 2,4-dimethoxyphenyl boronic acid (**10**) was subjected to Suzuki coupling with several benzylic halides (**11b**–**d**), to yield derivatives **12b**–**d**. After deprotection, compounds **13a**–**d** were oxidized with Fremy's salt yielding quinones **8a**–**d**. In the case of derivative **8a** we oxidized commercially available *n*-hexylresorcinol.

Compound **8e** was synthesized starting from 2,4,5-trimethoxybenzaldehyde (**14**) that was converted, via oxidation [18], to the phenol derivative **15** which reacted with 2-naphthyl boronic acid providing diaryl ether derivative **16** (Scheme 3).

Treatment of **16** with ammonium cerium nitrate in acetonitrile yielded desired compound.

For derivative **8f**, 2,4-dimethoxy aniline was submitted to Ullmann-type coupling with 2-bromonaphthalene to provide *N*-(2,4-dimethoxyphenyl)-naphthyl-2-amine (**18**). The resulting amine was deprotected and finally oxidized with Fremy's salt to give quinone **8f** (Scheme 4).

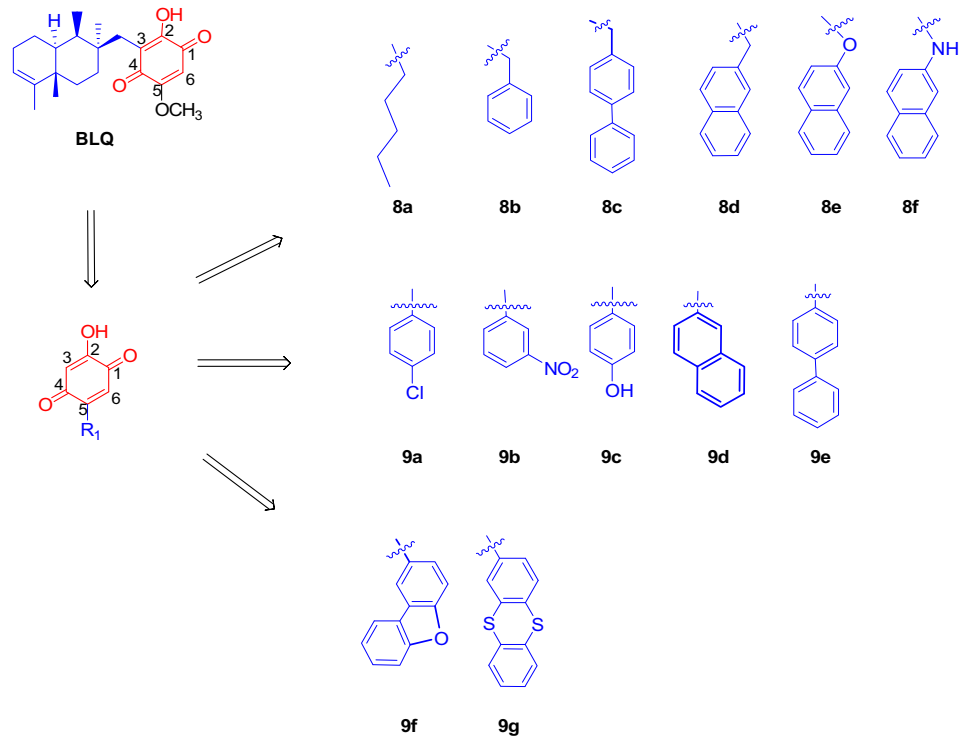
For the second set of analogues **9a**–**g**, a different procedure was followed (Scheme 5).

Commercially available 1-iodo-2,4-dimethoxybenzene (**20**) was subjected to Suzuki coupling, under microwave irradiation, with several boronic acids (**21a**–**g**), yielding the corresponding compounds, which were O-demethylated to provide resorcinol derivatives (**23a**–**g**) that were finally oxidized with Fremy's salt yielding quinones.

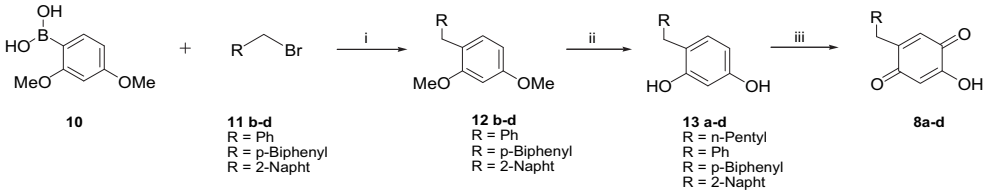
3. Biological results

3.1. Effects of BLQ and its analogues on LPS-induced PGE_2 production by LPS-stimulated RAW 264.7 cells

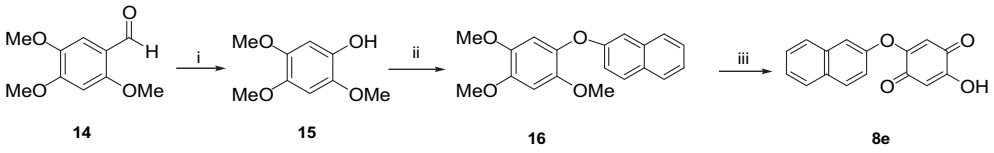
To assess the effect of BLQ and compounds **8a**–**f** and **9a**–**g** on LPS-induced PGE_2 production, RAW 264.7 macrophages cells were



Scheme 1. Modular composition of the bolinaquinone library.



Scheme 2. Reagents and conditions: (i) K_3PO_4, $Pd(OAc)_2$, PPh_3, toluene, 110 °C, 16 h; (ii) BBr_3, CH_2Cl_2, −15 °C to rt, 18 h; (iii) $NO(KSO_3)_2$, Na_2CO_3 THF−H_2O, rt, 10 h.



Scheme 3. Reagents and conditions: (i) H_2O_2, H_2SO_4, MeOH, rt, 2.5 h; (ii) 2-naphthyl boronic acid, $Cu(OAc)_2$, pyridine, CH_2Cl_2, rt, 48 h; (iii) CAN, CH_3CN, rt, 1 h.

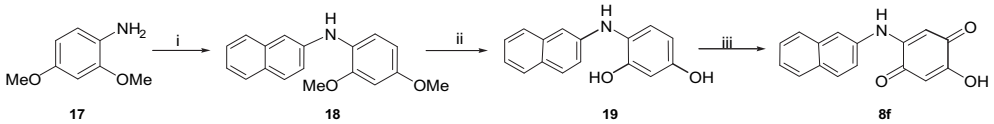
treated with various concentrations of analytes for 30 min and PGE_2 was induced by co-treatment with LPS (1 μg/mL) for another 24 h.

Neither LPS nor samples were added to the control group. Indomethacin (Indom.) was used as reference. Effects of BLQ and its analogues on LPS-induced PGE_2 production by LPS-stimulated RAW 264.7 cells are summarized in Table 1.

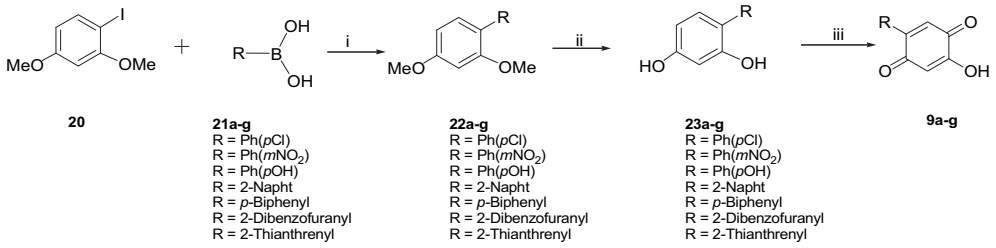
Analysis of the structure–activity relationships revealed the influence of the substituent at position 5 in the 2-hydroxy-1,4-benzoquinone ring.

The introduction of an aryl group has been shown to significantly affect the potency of such derivatives. As shown in Table 1 the benzyl ring was well tolerated, while replacement with the biphenyl moiety was found to be deleterious (IC_{50} values 26.0 for **8b** vs 56.7 for **8c**). Likewise, the *n*-hexyl analogue **8a** showed a significant reduction in potency.

In contrast, 5-naphthyl derivatives **8d**, **8e** and **8f** displayed an IC_{50} values in the micromolar range which is similar to the value obtained with **BLQ**. Besides compound **8d** exerted an inhibitory



Scheme 4. Reagents and conditions: (i) 2-Bromonaphthalene, $Pd(OAc)_2$, PPh_3, *t*-BuONa, toluene, 110 °C, 40 h; (ii) BBr_3, CH_2Cl_2, −15 °C to rt, 19 h; (iii) $NO(KSO_3)_2$, Na_2CO_3, THF−H_2O, rt, 2.5 h.



Scheme 5. Reagents and conditions: (i) K_2CO_3, $PdCl_2$(dppf), Ethanol, MW, 25 min; (ii) BBr_3, CH_2Cl_2, −15 °C to rt, 18 h; (iii) $NO(KSO_3)_2$, EtOAc–H_2O, Na_2HPO_4, 0 °C to rt, 20 h.

potency higher than the lead compound **1** with an observed IC_{50} of 4.0 μM.

Remarkably **8d** inhibitor, as shown in Fig. 1, showed good ability in reducing LPS-induced PGE_2 release with different degrees of potency. In particular, compound **8d** showed a concentration dependent inhibition of PGE_2 release (−66% at 10 μM and −90% at 100 μM, panel b).

Furthermore a few compounds were synthesized with variations in the aromatic system directly attached to quinone ring. The analogues lacking the methyl spacer were inactive (IC_{50} values from 41.0 to 65.9 for compounds **9a**–**g**) suggesting that the linker between the hydrophobic pocket and quinone ring is essential for the activity.

Next, to determine the affinity of **BLQ** and its analogues for *hs*PLA_2, surface plasmon resonance (SPR) experiments were performed.

3.2. Surface plasmon resonance experiments

SPR is an optical technique suitable for characterizing macromolecular interactions [19,20] based on the evanescent wave phenomenon to measure changes in refractive index very close to a sensor surface. The binding between a compound in solution and its ligand immobilized on the sensor surface results in a change in the refractive index.

The interaction is monitored in real time and the amount of bound ligand and rates of association and dissociation can be measured. First, *hs*PLA_2 was immobilized on a CM-5 sensor chip and, then, tested compounds were singly injected over the enzyme-modified sensor chip at different concentrations from 50 nM to 1000 nM, in presence of Ca^{2+} ion ($CaCl_2$, 500 μM).

In Fig. 2, the sensograms obtained from the binding of *hs*PLA_2-IIA to **8d** at four different concentrations in presence of $CaCl_2$ are shown.

Table 1
Evaluation of the affinity for *hs*PLA_2 and inhibition of PGE_2 production from LPS-treated RAW 264.7 cells by BLQ and the synthetic quinones.

	K_{DSPR}[a]	Sd (K_{DSPR})	IC_{50}(μM)[b]
BLQ	4.88×10^{-7}	2.93×10^{-7}	5.2 ± 0.5
8a	4.28×10^{-5}	3.93×10^{-5}	87.1 ± 10.5
8b	4.58×10^{-7}	2.73×10^{-7}	26.0 ± 3.6
8c	4.55×10^{-7}	3.27×10^{-7}	56.7 ± 3.4
8d	3.88×10^{-7}	3.14×10^{-7}	4.0 ± 0.9
8e	5.01×10^{-7}	3.49×10^{-7}	13.0 ± 0.2
8f	9.59×10^{-7}	8.27×10^{-7}	18.0 ± 0.6
9a	7.01×10^{-7}	4.05×10^{-7}	41.0 ± 3.4
9b	7.49×10^{-7}	4.49×10^{-7}	40.5 ± 5.6
9c	3.33×10^{-7}	2.49×10^{-7}	52.1 ± 2.8
9d	5.03×10^{-5}	3.63×10^{-5}	65.9 ± 9.3
9e	1.03×10^{-6}	9.11×10^{-7}	61.7 ± 7.2
9f	2.87×10^{-7}	2.40×10^{-7}	55.1 ± 3.3
9g	4.67×10^{-6}	2.01×10^{-6}	60.5 ± 9.3

[a] Experimental dissociation constants of BLQ and ligands.
[b] IC_{50}(μM) on PGE_2 release by LPS-stimulated mouse macrophage-like cells. Results are mean ± s.e.m. of 3 experiments performed in triplicate.

As expected, the increase of response units (RU) in the association phase and the slope of the dissociation phase of the complex are clearly dependent on the analyte concentration.

The analysis of the ascendant region of the sensograms allowed to extrapolate the k_a values, whereas the time required to completely dissociate the complex and the slope of the curves in their descending region directly depends on k_d of the complex.

On the basis of these experiments, the dissociation constants K_{DSPR} of the ligand-*hs*PLA_2 complexes were calculated using a 1:1 Langmuir algorithm and summarized in Table 1.

Even if the use of Biacore 2000 was not completely suitable in the study of small molecules–protein interaction, the obtained data were enough accurate to get preliminary SAR analysis.

The analysis with SPR elucidated that for *hs*PLA_2's interaction the aromatic groups instead of *n*-hexyl chain must be present (**1** vs **8a**). The *hs*PLA_2 interaction would appear to require a bicycle ring in

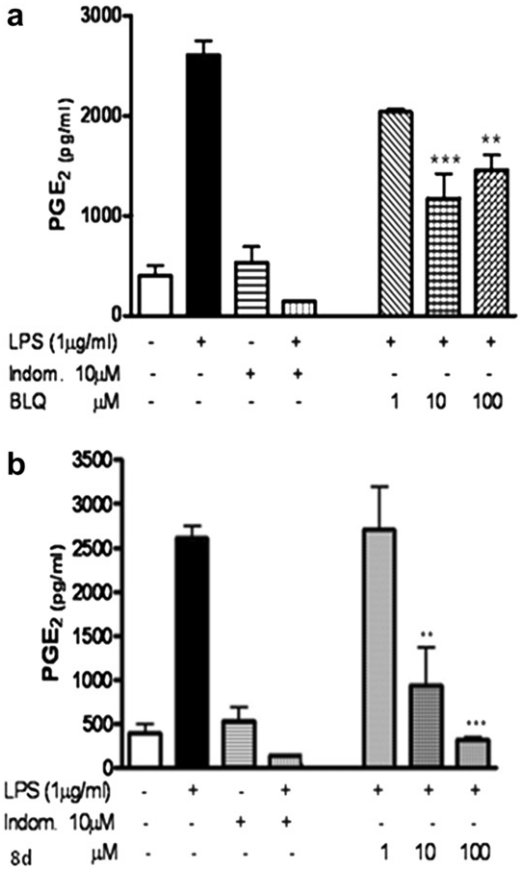


Fig. 1. Effect of BLQ and analogue **8d** on PGE_2 release by LPS-stimulated mouse macrophage-like cells. Compounds were added to the cells 30 min before LPS (1 μg/mL). Supernatants were collected after 24 h for PGE_2 assay. Results are mean ± s.e.m. of 3 experiments performed in triplicate. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs LPS (Student's *t* test).

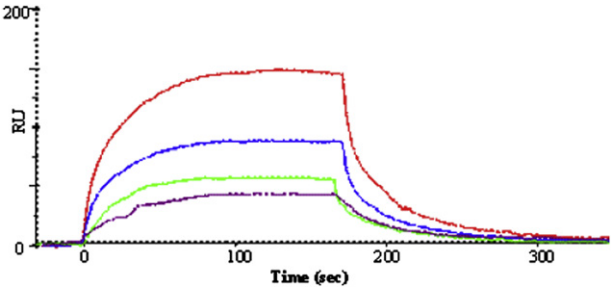


Fig. 2. Sensograms obtained from the binding of hsPLA$_2$ to **8d** in presence of $CaCl_2$ at four different concentrations (50 nM grey curve; 300 nM green curve, 700 nM blue curve, 1000 nM red curve). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

the hydrophobic pocket and to tolerate various building blocks including large and hydrophobic aryl ring like dibenzofuran instead of no-planar tricyclic derivative as thianthrene (**9f** vs **9g**). The presence of a condensate bicycle group should be favored and the introduction of a methylene bridge increases binding affinity probably because the compounds should fit better with the enzyme binding domain (**8c**–**d** vs **9d**–**e**).

4. Results and discussion

Prostaglandins and leukotrienes are the products of arachidonate cascade and mediator systems exerting numerous vascular and inflammatory effects.

Group II secretory PLA$_2$ can act as a signalling agent contributing to the inflammatory response. Group IIA *hs*PLA$_2$ has been reported to release arachidonic acid in some systems and may provide the substrate for both cyclooxygenase (COX) and 5-lipoxigenase (5-LO) products formation in mast cells.

The purpose of the present study was to investigate the modulatory effect of the natural compound bolinaquinone and its analogues on PGE$_2$ production on mouse macrophage cell line RAW 264.7.

Our in vitro test indicated that almost all compounds inhibited PGE$_2$ production with different potency.

In particular, this paper addresses the influence of substituents on the 2-hydroxyl-1,4-benzoquinone series in the structure-based design of anti-inflammatory compounds.

The data we gained on the binding with *hs*PLA$_2$, except for derivatives **9a**–**c** and **9f** was in good agreement with cellular analysis. In conclusion, we have described the discovery and optimization of a novel series of hydroxyquinones with potent anti-inflammatory activity. Further explorations to clarify potential and selectivity modulation on PGE$_2$ production are ongoing in our group.

5. Experimental

5.1. General

Microwaves experiments were performed in a CEM Discover monomode reactor (CEM Corp., Matthews, NC). All reactions were conducted in a specially adapted cylindrical Pyrex vessel. All reagents were analytical grade and purchased from Sigma–Aldrich (Milano-Italy). Flash chromatography was performed on Carlo Erba silica gel 60 (230 ÷ 400 mesh; Carlo Erba, Milan, Italy). TLC was carried out using plates coated with silica gel 60F 254 nm purchased from Merck (Darmstadt, Germany). Melting points were determined in open capillary tubes on a Electrothermal 9100 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were registered on a Bruker AC 300. Chemical shifts are reported in ppm. All target compounds were assessed for purity by analytical high performance liquid chromatography (HPLC), using an Agilent 1100 series, UV detector monitoring at 254 nm, HP Chem Station software, and a Waters C18 RP-column (5 μm, 300 mm × 3.9 mm). All target compounds were found to be >95% purity. MS spectrometry analysis ESI-MS was carried out on a Finnigan LCQ Deca *ion trap* instrument. Microanalyses were carried out on Carlo Erba 1106 elemental analyzer.

5.2. General procedure for the synthesis of 1-substituted-2,4-dimethoxybenzene derivatives **12b**–**d**

2,4-Dimethoxyphenyl boronic acid (0.205 g, 1.5 equiv.) in dry toluene (4 mL) was reacted at room temperature for 10 min with benzyl halide (1.0 equiv.) in the presence of K_3PO_4 (2.0 equiv.), PPh_3 (0.02 equiv.) and $Pd(OAc)_2$ (0.01 equiv.) under argon atmosphere. Bromine derivative (1.0 equiv.) was added and the reaction was heated at 100 °C for 16 h. A solution of NaOH (1 M) was added and the crude product was extracted with diethyl ether. Final compounds were purified by column chromatography over silica gel.

5.2.1. 1-Benzyl-2,4-dimethoxybenzene (**12b**)

Elution with hexane/EtOAc (90:10) afforded **12b** (97%) as pale oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.92 (s, 6H), 4.03 (s, 2H), 6.55 (dd, J = 1.1 Hz, 1H), 6.62 (s, 1H), 7.11 (d, J = 7.0 Hz, 1H), 7.32–7.44 (m, 5H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 35.9, 55.9, 100.9, 107.1, 120.3, 126.3, 128.3, 129.3, 130.3, 141.6, 158.9, 159.2.

5.2.2. 1-[(1,1′-Biphenyl)-4-yl]-2,4-dimethoxybenzene (**12c**)

Elution with light petroleum/EtOAc (90:10) afforded **12c** (91%) as yellow oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.92 (s, 6H), 4.13 (s, 2H), 6.42 (d, J = 8.1 Hz, 1H), 6.56 (s, 1H), 7.05 (s, 1H), 7.31(s, 2H), 7.40 (d, J = 7.1 Hz, 1H), 7.47 (t, J = 7.7 Hz, 2H), 7.55 (m, 2H), 7.62 (q, J = 8.1 Hz, 2H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 35.0, 56.2, 102.0, 106.1, 120.3, 127.7, 128.0, 129.5, 130.3, 131.0, 134.0, 136.5, 140.5, 159.0.

5.2.3. 1-[(2-Naphthyl)methyl]-2,4-dimethoxybenzene (**12d**)

Elution with hexane afforded **12d** (70%) as light-yellow oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.85 (s, 6H), 4.16 (s, 2H), 6.42 (dd, J = 4.0 Hz, 1H), 6.55 (s, 1H), 7.08 (s, 1H), 7.37–7.47 (m, 3H), 7.63 (s, 1H), 7.86 (t, J = 8.6 Hz, 3H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 36.7, 56.5, 101.0, 107.7, 122.3, 125.1, 126.0, 127.0, 128.0, 131.3, 133.5, 134.7, 137.2, 160.0.

5.3. General procedure for the synthesis of 1-substituted-2,4-benzenediol derivatives (**13b**–**d**, **23a**–**g**) and 1-(2-Naphthylamino)-2,4-benzenediol (**19**)

The 2,4-dimethoxybenzene aryl derivatives (1.0 equiv.) in dry dichloromethane (12 mL), cooled to −15 °C, were reacted in the presence of boron tribromide (8.8 equiv.). The mixture was stirred for 18 h then deionised water was added, followed by dichloromethane. The combined organic layer was dried over Na_2SO_4, filtered, and purified to obtain desired compounds.

5.3.1. 1-Benzyl-2,4-benzenediol (**13b**)

Elution with hexane/EtOAc (70:30) afforded **13b** (95%) as white powder.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.10 (s, 2H), 4.75 (br, 2H), 6.35 (s, 1H), 6.38 (d, 1H, J = 7.1 Hz), 6.93 (d, 1H, J = 8.1 Hz), 7.22–7.30 (m, 3H), 7.31 (d, 2H, J = 7.4 Hz).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 35.3, 104.1, 109.0, 122.2, 126.3, 128.5, 131.0, 141.7, 156.8, 157.7. Mp: 110 °C.

*5.3.2. 1-[(1,1′-Biphenyl)-4-yl]-2,4-benzenediol (**13c**)*

Elution with $CHCl_3$/MeOH (95:5) afforded **13c** (80%) as pale powder.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.93 (s, 2H), 4.85 (br, 2H), 6.46 (s, 1H), 6.53 (s, 1H), 7.02 (d, 1H, $J = 8.1$ Hz), 7.33 (s, 1H), 7.45 (d, 1H, $J = 7.8$ Hz), 7.53 (t, $J = 7.6$ Hz, 2H), 7.61–7.73 (m, 4H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 35.6, 104.1, 109.0, 122.2, 127.7, 128.8, 129.3, 131.1, 134.0, 136.5, 140.5, 156.8. Mp: 117.6 °C.

*5.3.3. 1-[(2-Naphthyl)methyl]-2,4-benzenediol (**13d**)*

Elution with $CHCl_3$/MeOH (95:5) afforded **13d** (70%) as pale solid.

^{1}H NMR (CD_3OD, 300 MHz) δ 4.08 (s, 2H), 6.35 (d, 1H, $J = 8.1$ Hz), 6.42 (s, 1H), 6.93 (d, 1H, $J = 8.1$ Hz), 7.42 (m, 3H), 7.62 (s, 1H), 7.83–7.92 (m, 3H).

^{13}C NMR (CD_3OD, 300 MHz) δ 36.0, 105.0, 110.0, 122.5, 125.1, 126.0, 127.6, 128.0, 131.3, 133.7, 135.2, 157.0, 158.0. Mp: 128 °C.

*5.3.4. 1-(2-Naphthylamino)-2,4-benzenediol (**19**)*

Elution with light petroleum/EtOAc (50:50), afforded **19** as a dark brown solid (85%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.92 (br, 1H), 5.15 (br, 1H), 6.13 (s, 1H), 6.47 (m, 1H), 6.63 (s, 1H), 6.92 (s, 1H), 7.06 (d, 1H, $J = 8.5$ Hz), 7.15 (d, 1H, $J = 8.9$ Hz), 7.42 (m, 2H), 7.6 1 (d, 1H, $J = 7.6$ Hz), 7.92 (d, 2H, $J = 9.2$ Hz).

^{13}CNMR ($CDCl_3$, 300 MHz) δ 103, 108, 110, 119,122, 124, 127, 129, 134, 142, 149. MS (ESI) *m*/*z* 253.29. Mp: 238.3 °C.

*5.3.5. 1-(4-Chlorophenyl)-2,4-benzenediol (**23a**)*

Elution with hexane/EtOAc (90:10 to 80:20) afforded **23a** (76%) as pale solid.

^{1}H NMR (CD_3OD, 300 MHz) δ 6.37 (m, 2H), 7.10 (d, $J = 8.5$ Hz, 1H), 7.35 (d, $J = 9.4$ Hz, 2H), 7.52 (d, $J = 8.1$ Hz, 2H).

^{13}C NMR (CD_3OD, 300 MHz) δ 104.1, 109.0, 119.8, 128.3, 129.4, 130.7, 133.2, 134.6, 157.1, 158.8. Mp: 68.9 °C.

*5.3.6. 1-(3-Nitrophenyl)-2,4-benzenediol (**23b**)*

Elution with hexane/EtOAc (80:20) afforded **23b** (86%) as yellow oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.50 (s, 1H), 6.60 (d, $J = 8.1$ Hz, 1H), 7.23 (d, $J = 8.3$ Hz, 1H), 7.65 (t, $J = 7.6$ Hz, 1H), 7.88 (d, $J = 7.4$ Hz, 1H), 8.23 (d, $J = 8.3$ Hz, 1H), 8.41 (s, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 104.1, 109.0, 119.8, 120.0, 122.5, 130.2, 131.2, 134.0, 137.4, 148.9, 157.1, 158.8. Mp: 138.2 °C.

*5.3.7. 1-(4-Hydroxyphenyl)-2,4-benzenediol (**23c**)*

Elution with light petroleum/EtOAc (85:15 to 80:20) afforded **23c** (80%) as white solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.26 (dd, 1H, $J = 8.1$ Hz), 6.36 (s, 1H), 6.71 (d, 2H, $J = 8.4$ Hz), 6.94 (d, 1H, $J = 7.8$ Hz), 7.24 (d, 2H, $J = 8.2$ Hz), 9.16 (s, 3H).

^{13}C NMR (DMSO-d_6, 300 MHz) δ 104.1, 109.0, 116.4, 119.8, 128.1, 129.3, 130.7, 158.1, 159.8. Mp: 118.6 °C.

*5.3.8. 1-(2-Naphthyl)-2,4-benzenediol (**23d**)*

Elution with hexane/EtOAc (70:30) afforded **23d** (70%) as pale-yellow solid

^{1}H NMR (CD_3OD, 300 MHz) δ 6.45 (s, 1H), 6.48 (m, 1H), 6.98 (d, $J = 7.1$ Hz, 1H), 7.30–7.53 (m, 4H), 7.67 (d, $J = 8.1$ Hz, 1H), 7.82–7.90 (m, 2H).

^{13}C NMR (CD_3OD, 300 MHz) δ 104.1, 109.0, 119.8, 124.9, 126.2, 128.1, 129.2, 130.7, 133.1, 134.2, 157.1, 158.8. Mp: 125.7 °C.

*5.3.9. 1-[4-(1,1′-Biphenyl)]-2,4-benzenediol (**23e**)*

Elution with hexane/EtOAc (90:10 to 70:30) afforded **23e** (82%) as white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.48 (s, 1H), 6.50 (m, 1H), 7.20 (d, $J = 7.4$ Hz, 1H), 7.38 (d, $J = 7.2$ Hz, 1H), 7.49 (t, $J = 8.3$ Hz, 2H), 7.46 (m, 2H), 7.68 (t, $J = 8.3$ Hz, 4H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 104.1, 109.0, 119.8, 127.7, 127.9, 128.4, 129.3, 130.7, 135.4, 136.5, 157.1, 158.8. Mp: 400 °C dec.

*5.3.10. 1-(2-Dibenzofuranyl)-2,4-benzendiol (**23f**)*

Elution with hexane/EtOAc (90:10 to 60:40) afforded **23f** (83%) as white solid.

^{1}H NMR (CD_3OD, 300 MHz) δ 6.85 (s, 1H), 6.87 (s, 1H), 7.48 (t, $J = 6.5$ Hz, 1H), 7.68 (t, $J = 7.2$ Hz, 1H), 7.79 (d, $J = 8.5$ Hz, 1H), 7.96 (q, $J = 7.2$ Hz, 2H), 8.13 (d, $J = 7.2$ Hz, 1H), 9.0 (s, 1H).

^{13}C NMR (CD_3OD, 300 MHz) δ 104.1, 109.0, 111.6, 112.3, 113.0, 119.8, 121.0, 121.5, 123.3, 124.7, 130.7, 132.9, 137.8, 145.8, 156.8, 157.1, 158.8. Mp: 400 °C dec.

*5.3.11. 1-(2-Thianthrenyl)-2,4-benzenediol (**23g**)*

Elution with hexane/EtOAc (95:5) afforded **23g** (77%) as pale oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.56 (s, 1H), 6.75 (s, 1H), 7.09 (d, $J = 8.5$ Hz, 1H), 7.22–7.30 (m, 2H), 7.43 (d, $J = 8.1$ Hz, 1H), 7.51–7.57 (m, 3H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 104.1, 109.0, 119.8, 127.0, 127.9, 130.5, 130.7, 131.8, 132.3, 135.6, 136.6, 137.7, 138.2, 157.1, 158.8. Mp: 310.2 °C.

*5.4. General procedures for the synthesis of 2-hydroxy-2,5-cyclohexadiene-1,4-dione-5-substituted derivatives (**8a–d**, **8f**)*

To a solution of the appropriate dihydroxyl derivatives (1.0 equiv.) in tetrahydrofuran (6 mL) at room temperature, a solution of Fremy's salt (2.5 equiv.) in 0.7 mL of 15% Na_2CO_3, (1.6 equiv.) was added. The solution was stirred vigorously until the reaction was completed (10 h for **8a–d**, 19 h for **8f**). Desired compounds were obtained after purification by flash chromatography.

*5.4.1. 5-Hexyl-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**8a**)*

Elution with hexane/EtOAc (50:50) afforded **8a** (60%) as yellow oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.05 (m, 3H), 1.23 (m, 5H), 1.31 (m, 3H), 2.5 (t, 2H, $J = 8.1$ Hz), 6.15 (s, 1H), 6.37 (s, 1H), 8.60 (br, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 40.5, 110.1, 125.8, 128.7, 129.1, 133.0, 135.3, 137.5, 169.0, 181.3, 187.2. MS (ESI) *m*/*z*: 213.01. Anal. Calcd for $C_{13}H_{10}O_3$: C 72.89, H 4.71, O 22.41. Found: C 72.40, H 4.11, O 21.99.

*5.4.2. 5-Benzyl-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**8b**)*

Elution with hexane/EtOAc (50:50) afforded **8b** (60%) as yellow oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.83 (s, 2H), 6.12 (s, 1H), 6.37 (s, 1H), 7.20 (d, 2H, $J = 8.2$ Hz), 7.39 (m, 3H), 8.60 (br, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 40.5, 110.1, 125.8, 128.7, 129.1, 133.0, 135.3, 137.5, 169.0, 181.3, 187.2. MS (ESI) *m*/*z*: 213.01. Anal. Calcd for $C_{13}H_{10}O_3$: C 72.89, H 4.71, O 22.41. Found: C 72.40, H 4.11, O 21.99.

*5.4.3. 5-[(1,1′-Biphenyl)-4-yl]-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**8c**)*

Elution with EtOAc afforded **8c** (55%) as brown oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 2H), 6.15 (s, 1H), 6.44 (s, 1H), 7.26 (s, 1H), 7.46 (d, 2H, $J = 7.1$ Hz) 7.56 (t, 2H, $J = 7.7$ Hz), 7.60 (t, 4H, $J = 5.5$ Hz).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 40.5, 111.0, 127.6, 128.0, 129.3, 130.0, 133.0, 135.3, 136.7, 169.0, 180.3, 188.5. MS (ESI) *m*/*z*: 289.09. Anal. Calcd. for $C_{19}H_{14}O_3$: C 78.61, H 4.86, O 16.53. Found: C 78.43, H 4.65, O 16.02.

*5.4.4. 5-[(2-Naphthyl)methyl]-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**8d**)*

Elution with hexane/EtOAc (50:50) afforded **8d** (50%) as orange oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.93 (s, 2H), 6.14 (s, 1H), 6.31 (s, 1H), 7.26 (s, 1H), 7.57 (d, 2H, J = 6.9 Hz), 7.64 (s, 1H), 7.82–7.84 (m, 3H), 8.64 (br, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 41.0, 110.1, 125.0, 126.0, 127.0, 127.6, 128.0, 131.8, 133.0, 135.2, 135.7, 169.0, 180.3, 188.5. MS (ESI) m/z: 263.0. Anal. Calcd for $C_{17}H_{12}O_3$: C 77.26, H 4.58, O 18.16. Found: C 76.90, H 4.20, O 18.07.

5.4.5. 5-(2-Naphthylamino)-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**8f**)

Elution with EtOAc/hexane (90:10) afforded **8f** as a violet solid (50%).

^{1}H NMR (CD_3OD, 300 MHz) δ 2.2 (s, 1H), 5.5 (s, 1H), 7.00 (m, 2H), 7.20 (t, 1H, J = 7.6 Hz), 7.32 (t, 1H, J = 8.1 Hz), 7.52 (d, 1H, J = 8.8 Hz), 7.66–7.80 (m, 3H).

^{13}C NMR (CD_3OD, 300 MHz) δ 103.2, 108.5, 110.0, 116.1, 122.3, 124.0, 126.4, 128.3, 132.6, 135.0, 148.0, 180.5, 181.2. MS (ESI) m/z: 280.4. Anal. Calcd for $C_{16}H_{11}NO_3$: C 72.45, H 4.18, N 5.28, O 18.09. Found: C 73.05, H 3.99, N 5.67, O 17.97. Mp: 389 °C.

5.5. 2-(2,4,5-Trimethoxyphenoxy)naphthalene (**16**)

2,4,5-Trimethoxyphenol **15** (0.280 g, 1 equiv.) in dry dichloromethane (10 mL) was reacted with 2-naphthalenboronic acid (2.2 equiv.), $Cu(OAc)_2$ (1.5 equiv.) and pyridine (2.5 equiv.) at room temperature for 48 h. The reaction mixture was diluted with water and extracted with dichloromethane. Final compound was purified by column chromatography over silica gel on silica gel with a mixture of hexane/EtOAc (50:50) as eluent to give **16** (0.450 g, 97%) as a light-yellow oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 6H), 3.93 (s, 3H), 6.76 (s, 2H), 7.15 (s, 1H), 7.31 (s, 1H), 7.43 (d, 2H, J = 8.1 Hz), 7.56 (d, 1H, J = 6.4 Hz), 7.75 (d, 1H, J = 8.5 Hz), 7.90 (d, 2H, J = 8.1 Hz).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 60.0, 100.2, 105.3, 110.7, 123.6, 126.5, 127.2, 129.1, 135.6, 140.3, 143.0, 145.2, 153.6.

5.6. 5-(2-Naphthyloxy)2-hydroxy-2,5-cyclohexadiene-1,4-dione (**8e**)

A water solution of ammonium cerium(IV) nitrate (CAN) (2.5 equiv.) was added rapidly to 2-(2,4,5-trimethoxyphenoxy) naphthalene (**16**) (0.199 g, 1 equiv.) in acetonitrile (16 mL) at room temperature. After 1 h the solvent was evaporated. The mixture was diluted with water, extracted with ethyl acetate. Column chromatography, with a mixture of hexane/EtOAc (40:60) as eluent, provided final compound **8e** (0.085 g; 53%) as yellow solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.10 (br, 1H), 5.79 (s, 1H), 6.10 (s, 1H), 7.00 (d, 1H, J = 8.3 Hz), 7.30 (s, 1H), 7.53 (m, 2H), 7.60 (d, 1H, J = 7.6 Hz), 7.70 (d, 1H, J = 6.9 Hz), 8.00 (d, 1H, J = 7.6 Hz).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 106.1, 110.5, 126.2, 127.5, 128.3, 129.0, 131.1, 132.7, 135.6, 150.5, 169.6, 183.0, 185.4. MS (ESI) m/z: 288.41. Anal. Calcd for $C_{16}H_{10}O_4$: C 72.18, H 3.79, O 24.04. Found: C 73.07, H 4.01, O 24.78. Mp: 104.5 °C.

5.7. N-(2,4-Dimethoxyphenyl)naphthyl-2-amine (**18**)

2-4-Dimethoxyaniline **17** (0.377 g, 5 equiv.) in dry toluene was reacted with 2-bromonaphthalene (1.0 equiv.), $Pd(OAc)_2$ (0.04 equiv.), PPh_3 (0.16 equiv.), sodium *tert*-butoxide (1.2 equiv.). The mixture was heated at 100 °C for 40 h and after neutralized with a solution of NH_4Cl. The crude product was extracted with diethyl ether and the final compound was purified by column chromatography over silica gel, using light petroleum/EtOAc (90:10) as eluent, to give **18** as a dark orange oil (0.137 g, 77%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.71 (s, 3 H), 3.84 (s, 3H), 5.81 (br, 1H), 6.22 (d, 1H, J = 8.9 Hz), 6.53 (s, 1H), 7.25 (d, 1H, J = 8.9 Hz), 7.33 (t, 1H, J = 7.5 Hz), 7.45 (m, 3H), 7.67 (d, 1H, J = 7.6 Hz), 7.91 (d, 2H, J = 9.2 Hz).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 58.2, 102.8, 108.0, 110.3, 119.1, 120.2, 124.5, 126.6, 128.1, 134.0, 144.1, 150.3. MS (ESI) m/z: 280.46.

5.8. General procedures for the synthesis of compounds (**22a–g**)

1-Iodo-2,4-dimethoxybenzene **20** (0.200 g, 1.0 equiv.) in ethanol (4 mL) was reacted with arylboronic acid (1.2 equiv.), K_2CO_3 (2.0 equiv.), $PdCl_2$(dppf) (5% mol). MW irradiation of 60 W, for 25 min, was used, the temperature being ramped from room temperature to 110 °C. To the resulting mixture water and ethyl acetate (20 mL of each) were added. Final compounds were purified by flash chromatography over silica gel.

5.8.1. 1-(4-Chlorophenyl)-2,4-dimethoxybenzene (**22a**)

Elution with hexane/EtOAc (95:5) afforded **22a** (70%) as white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.80 (s, 6H), 6.64 (m, 2H), 7.25 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 7.2 Hz, 2H), 7.47 (d, J = 8.5 Hz, 2H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 56.2, 55.9, 100.9, 107.1, 117.9, 129.3, 129.4, 129.9, 133.2, 134.6, 158.8, 160.6. Mp: 48.2 °C.

5.8.2. 1-(3-Nitrophenyl)-2,4-dimethoxybenzene (**22b**)

Elution with hexane/EtOAc (95:5) afforded **22b** (80%) as yellow solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.93 (s, 6H), 6.64 (m, 2H), 7.25 (d, J = 8.1 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.85 (d, J = 7.0 Hz, 1H), 8.15 (d, J = 8.3 Hz, 1H), 8.42 (s, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 56.2, 55.9, 100.9, 107.1, 117.9, 120.0, 122.5, 129.9, 130.2, 134.0, 137.4, 148.9, 158.8, 160.6. Mp: 85.9 °C.

5.8.3. 2′,4′-Dimethoxy-[1,1′-biphenyl]-4-ol (**22c**)

Elution with CH_2Cl_2/MeOH (95:5 to 50:50) afforded **22c** (74%) as white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.85 (s, 3H), 3.93 (s, 3H), 5.02 (br, 1H), 6.64 (m, 2H), 6.90 (d, 2H, J = 6.8 Hz), 7.10 (d, 1H, J = 8.1 Hz), 7.40 (d, 2H, J = 8.5 Hz).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 56.2, 55.9, 100.9, 107.1, 116.4, 117.9, 129.3, 129.9, 157.4, 158.8, 160.6. Mp: 48.2 °C.

5.8.4. 1-(2-Naphthyl)-2,4-dimethoxybenzene (**22d**)

Elution with hexane/EtOAc (95:5 to 90:10) afforded **22d** (70%) as pale-yellow solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.55 (s, 3H), 3.82 (s, 3H), 6.68 (m, 2H), 7.25 (d, 1H, J = 8.1 Hz), 7.40–7.60 (m, 4H), 7.70 (d, 2H, J = 8.5 Hz), 8.00 (t, 1H, J = 9.2 Hz).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 56.2, 55.9, 100.9, 107.1, 117.9, 125.1, 126.3, 126.8, 127.2, 128.3, 129.9, 133.1, 134.2, 136.7, 158.8, 160.6. Mp: 118.9 °C.

5.8.5. 1-[4-(1,1′-Biphenyl)]-2,4-dimethoxybenzene (**22e**)

Elution with hexane/EtOAc (95:5 to 90:10) afforded **22e** (75%) as white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.81 (s, 3H), 3.94 (s, 3H), 6.68 (m, 2H), 7.20–7.40 (m, 3H), 7.55 (t, 3H, J = 6.2 Hz), 7.70–7.80 (m, 4H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 55.9, 56.2, 100.9, 107.1, 117.9, 127.7, 127.9, 128.4, 129.3, 129.9, 135.4, 136.5, 158.8, 160.6. Mp: 115.3 °C.

5.8.6. 1-(2-Dibenzofuranyl)-2,4-dimethoxybenzene (**22f**)

Elution with hexane/EtOAc (95:5) afforded **22f** (80%) as white solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.83 (s, 3H), 3.91 (s, 3H), 6.68–6.69 (m, 2H), 7.29–7.36 (m, 6H), 7.96–7.99 (m, 2H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 56.2, 55.9, 100.9, 107.1, 111.6, 112.1, 113.0, 117.9, 119.4, 121.0, 122.5, 123.3, 124.7, 129.9, 134.0, 136.4, 145.8, 156.3, 158.8. Mp: 116.8 °C.

*5.8.7. 1-(2-Thianthrenyl)-2,4-dimethoxybenzene (**22g**)*

Elution with hexane/EtOAc (95:5) afforded **22g** (77%) as yellow solid.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.83 (s, 3H), 3.93 (s, 3H), 6.63 (m, 2H), 7.25–7.27 (m, 4H), 7.29 (d, 2H, J = 7.8 Hz), 7.51 (d, 2H, J = 8.11 Hz).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 55.9, 56.2, 100.9, 107.1, 117.9, 127.0, 127.9, 129.9, 130.5, 131.8, 132.3, 135.6, 136.6, 137.7, 138.2, 158.8, 160.6. Mp: 140.4 °C.

*5.9. General procedure for the synthesis of 2-hydroxy-2,5-cyclohexadiene-1,4-dione 5-substituted derivatives (**9a**–**g**)*

Dihydroxyl derivatives **23a**–**g** (0.064 g, 1.0 equiv.) in ethyl acetate (15 mL) cooled to 0 °C were reacted, under argon with Fremy's salt (2.5 equiv.) and $Na_2HPO_4(H_2O)_2$ (1.6 equiv.) at room temperature for 20 h. The aqueous layer was back-extracted with ethyl acetate. Final compounds were purified by flash chromatography.

*5.9.1. 5-(4-Chlorophenyl)-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**9a**)*

Elution with hexane/EtOAc (80:20) afforded **9a** (55%) as purple solid.

^{1}H NMR (CD_3OD, 300 MHz) δ 5.66 (s, 1H), 6.50 (s, 1H), 7.28 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 7.2 Hz, 2H), 8.51 (s, 1H). ^{13}C NMR (CD_3OD, 300 MHz) δ 110.1, 127.8, 128.8, 130.7, 132.7, 133.5, 145.7, 169.0, 181.3, 187.0. MS (ESI) m/z: 235.01. Anal. Calcd for $C_{12}H_7ClO_3$: C 61.43, H 3.01, Cl 15.11, O 20.46. Found: C 61.08, H 2.90, Cl 15.01, O 20.10. Mp: 400 °C dec.

*5.9.2. 5-(3-Nitrophenyl)-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**9b**)*

Elution with EtOAc afforded **9b** (60%) as purple solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.30 (s, 1H), 6.90 (s, 1H), 7.64 (t, J = 8.4 Hz, 2H), 7.83 (d, J = 8.1 Hz, 1H), 8.36 (s, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 110.1, 120.3, 121.3, 129.6, 132.5, 133.5, 132.7, 145.7, 148.3, 169.0, 181.3, 187.0. MS (ESI) m/z: 246.05. Anal. Calcd for $C_{12}H_7NO_5$: C 58.78, H 2.88, N 5.71, O 32.63. Found: C 58.10, H 2.44, N 5.10, O 32.08. Mp: 400 °C dec.

*5.9.3. 5-(4-Hydroxyphenyl)-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**9c**)*

Elution with EtOAc/MeOH (80:20) afforded **9c** (50%) as brown solid.

^{1}H NMR (CD_3OD, 300 MHz) δ 6.62 (s, 1H), 6.70 (s, 1H), 6.94 (d, J = 9.3 Hz, 2H), 7.53 (d, J = 8.7 Hz, 2H), 8.05 (br, 1H).

^{13}C NMR (CD_3OD, 300 MHz) δ 110.1, 115.8, 125.2, 127.8, 132.7, 157.7, 169.0, 181.3, 187.0. MS (ESI) m/z: 214.2. Anal. Calcd for $C_{12}H_8O_4$: C 66.67, H 3.73, O 29.60. Found: C 66.01, H 3.60, O 29.15. Mp: 400 °C dec.

*5.9.4. 5-(2-Naphthyl)-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**9d**)*

Elution with hexane/EtOAc (50:50) afforded **9d** (53%) as light-yellow oil.

^{1}H NMR (CD_3OD, 300 MHz) δ 6.74 (s, 1H), 7.37 (s, 1H), 7.42 (m, 1H), 7.55–7.62 (m, 2H), 7.74 (d, 1H, J = 8.1 Hz), 7.93 (t, 3H, J = 7.8 Hz).

^{13}C NMR (CD_3OD, 300 MHz) δ 110.1, 123.5, 125.1, 126.0, 126.4, 127.7, 128.1, 128.2, 132.7, 133.2, 133.6, 134.8, 145.7, 169.0, 181.3, 187.0. MS (ESI) m/z: 249.2. Anal. Calcd for $C_{16}H_{10}O_3$: C 76.79, H 4.03 O 19.18. Found: C 76.70, H 3.99, O 19.07.

*5.9.5. 5-[4-(1,1′-Biphenyl)]-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**9e**)*

Elution with hexane/EtOAc (50:50) afforded **9e** (48%) as orange oil.

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.25 (s, 1H), 7.00 (s, 1H), 7.20–7.32 (m, 3H), 7.61–7.68 (m, 6H), 8.74 (br, 1H).

^{13}C NMR ($CDCl_3$, 300 MHz) δ 110.1, 126.9, 127.7, 127.9, 127.8, 129.3, 131.5, 132.7, 135.7, 136.5, 145.7, 169.0, 181.3, 187.0. MS (ESI) m/z: 275.09. Anal. Calcd for $C_{18}H_{12}O_3$: C 78.25, H 4.38, O 17.37. Found: C 78.10, H 4.08, O 17.15.

*5.9.6. 5-(2-Dibenzofuranyl)-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**9f**)*

Elution with hexane/EtOAc (60:40) afforded **9f** (56%) as brown solid.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.11 (s, 1H), 6.99 (s, 1H), 7.30–7.36 (m, 3H), 7.46 (d, J = 7.4 Hz, 1H), 7.8–8.0 (m, 3H), 11.0 (br, 1H).

^{13}C NMR (DMSO-d_6, 300 MHz) δ 110.1, 111.5, 111.6, 113.0, 119.1, 121.0, 121.7, 123.3, 124.7, 129.6, 132.7, 134.0, 145.7, 145.8, 156.3, 169.0, 181.3, 187.0. MS (ESI) m/z: 290.0. Anal. Calcd for $C_{18}H_{10}O_4$: C 74.48, H 3.47, O 22.05. Found: C 74.13, H 3.40, O 21.98. Mp: 400 °C dec.

*5.9.7. 5-(2-Thianthrenyl)-2-hydroxy-2,5-cyclohexadiene-1,4-dione (**9g**)*

Elution with $CHCl_3$/MeOH (90:10) afforded **9g** (44%) as yellow oil.

^{1}H NMR (CD_3OD, 300 MHz) δ 6.42 (d, 1H, J = 7.6 Hz), 6.45 (s, 1H), 6.99 (d, 1H, J = 8.1 Hz), 7.58–7.61 (m, 2H), 7.62–7.67 (m, 2H), 7.82 (t, 2H, J = 7.4 Hz), 8.52 (br, 1H).

^{13}C NMR (CD_3OD, 300 MHz) δ 111.1, 125.5, 126.6, 127.9, 130.7, 131.8, 132.7, 136.9, 137.6, 138.2, 145.7, 169.0, 182.3, 188.0. MS (ESI) m/z: 338.99. Anal. Calcd for $C_{18}H_{10}O_3S_2$: C 63.89, H 2.98, O 14.18, S 18.95. Found: C 63.70, H 2.50, O 14.04, S 18.46.

6. Biological protocols

6.1. Cell culture

The mouse macrophage-like cell line RAW 264.7, was purchased from American Type Culture Collection (ATCC, LGC Promochem, Sesto San Giovanni, Milan, Italy). RAW 264.7 macrophages were grown in DMEM supplemented with 10% heat-inactivated FBS, 4 mM L-Glutamine, 1000 units/mL penicillin and 10000 μg/mL streptomycin (all from Cambrex Bioscience, Verviers, Belgium) at 37 °C in an atmosphere of 95% O_2 and 5% CO_2. RAW 264.7 cells were plated at a density of 5×10^5cells/well in 6-well culture plates. Cells were allowed to adhere overnight, rinsed twice and cultured for 16 h in serum-free DMEM. After pre-incubation for 16 h, the test compounds were added 30 min before co-treatment with LPS (1 μg/mL) for another 24 h. Test compounds were dissolved in DMSO on the day of the experiment and diluted with serum-free DMEM at appropriate concentrations (1–100 μM). The final concentration of DMSO was adjusted to 0.1% (v/v). Control groups also received the same amount of DMSO.

6.2. PGE_2 enzyme immunoassay

RAW 264.7 supernatants were analyzed for PGE_2 using a commercially available kit (Cayman Chem, Ann Arbor, MI, USA). Due to the rapid metabolism to an inactive metabolite of PGE_2 in vivo, concentrations detected in samples are very low: a more accurate index of PGE_2 biosynthesis and excretion is obtained by converting PGE_2 to a single, stable derivative (13,14-dihydro-15-keto-prostaglandin A_2) easily quantifiable by an enzyme immunoassay (EIA) procedure. Briefly, the assay is based on the

competition between free PGE_2 and a PGE_2 acetylcholinesterase conjugate (PGE_2 tracer) for a limited amount of PGE_2-specific monoclonal antibody; the amount of PGE_2 tracer binding to monoclonal antibody will be inversely proportional to the concentration of PGE_2 in the well. The results are shown as mean ± SEM of three separate experiments made in triplicate.

7. Biacore assays - surface plasmon resonance experiments

7.1. Materials

Biacore 2000, CM5 sensor chip, and coupling reagents (*N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxy-succinimmide (NHS), and ethanolamine–HCl) were purchased from GE Healthcare (United Kingdom). Human synovial phospholipase A_2 was expressed and purified as reported by Othman et al. [21]. Human synovial PLA_2 was immobilized onto CM5 sensor chip using standard amine coupling [22] Phosphate–buffered saline, which consisted of 20 mM Na_2HPO_4 and 150 nM NaCl, pH 7.4, was used as running buffer. The carboxymethyl dextran surface was activated with a 5-min injection of a 1:1 ratio of 0.4 M EDC and 0.1 M NHS at 5 μl/min human synovial PLA_2 was coupled to the surface with a 7-min injection of a protein diluted in 10 mM sodium acetate, pH 6; protein concentration was selected to obtain an optimal response (around 2000 RU).

Remaining active groups were blocked with a 7-min injection of 1.0 M ethanolamine-HCl, pH 8.5, at 5 μl/min. Biosensor experiments were carried out on different compounds: **8a**–**f** and **9a**–**g**, and **BLQ**. All compounds were diluted in 10 mM phosphate saline buffer (pH 7.4) in presence and in absence of 200 μM $CaCl_2$ at 3% final concentration of methanol and analyzed at concentrations of 50 nM, 300 nM, 700 nM and 1 μM. Each concentration was tested at least three times. All the obtained complexes dissociated back to baseline within a reasonable time frame, therefore, no regeneration was required. The interaction experiments were carried out at a flow rate of 10 μl/min, employing a 3 min injection time. The dissociation time was set at 300 s.

Rate constants for association (k_a) dissociation (k_d) and the dissociation constant (K_D) were obtained by globally fitting data from all the injection of different concentration of each compound, using the BIAevaluation software, using the simple 1:1 Langmuir binding model.

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