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Synthesis of ferulic ester dimers, functionalisation and biological evaluation as potential antiatherogenic and antiplasmodial agents

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Abstract—Oxidative dimerization of ferulic acid methyl ester afforded dihydrobenzofuran derivative and new linear compound identified by X-ray crystallography. The gallate derivatized dihydrobenzofuran analogue was obtained and all compounds were evaluated for potential antiatherogenic, antiplasmodial (best IC_{50} = 0.8μM) and cytotoxic activities.

1. Introduction

Reactive oxygen species (ROS) are specific signalling molecules implicated in both physiological and pathophysiological conditions. Overproduction of ROS causes an imbalance between oxidants and antioxidants in favour of the former, leading to oxidative stress. This has been implicated in a variety of pathological and chronic degenerative processes including the development of cancer, atherosclerosis, inflammation, ageing and neurodegenerative disorders.[1] Concerning particularly atherosclerosis, low density lipoproteins (LDL) are involved in this pathogenesis after undergoing oxidative modifications in the arterial wall. Oxidized LDL (oxLDL) exhibit in vitro a variety of biological properties potentially involved in atherogenesis. In addition oxLDL are cytotoxic and induce apoptosis or necrosis on cultured vascular cells.

At the same time, it has long been recognized that *Plasmodium*-infected erythrocytes are under constant oxidative stress caused by exogenous ROS and reactive nitrogen oxide species (RNOS) produced by the immune system, and also by exogenous production of ROS generated during the digestion of host cell hemoglobin and all concomitant biochemical reactions.[2] Although ROS and RNOS are toxic to the parasites, they also induce oxidative and nitrosative stresses in the host and can damage host tissues.[3] In this way, it may be interesting to investigate compounds exhibiting both antiplasmodial and antioxidant activities that could be relevant as leads in the search for new antimalarials.

Phenolic compounds constitute an important class of natural substances. Lignins, the second most abundant biopolymer after cellulose, are complex aromatic polymers resulting from the oxidative polymerization of hydroxycinnamoyl alcohols, that is, *p*-coumaryl, coniferyl, sinapyl alcohols. It is well known that cinnamic acid derivatives, lignans and neolignans, can exhibit LDL-

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antioxidant activities.[4] On the other hand, dihydrobenzofuran lignans[5] and related *m*-hydroquinone[6] systems have been reported to possess marked antiplasmodial activities.

In the course of our ongoing research programme directed towards synthesis of phenolic compounds related to the lignification process, we previously described the synthesis of cinnamic compounds and phosphonocinnamic analogues with various biological activities.[7] In this report, we wish to describe the synthesis of some feruloyl dimeric systems, their functionalisation by the galloyl group and the evaluation of their antioxidant and antiplasmodial activities.

2. Chemistry

The synthesis of feruloyl dimers is shown in Figure 1. The oxidative coupling was carried out on the feruloyl methyl ester **1** obtained under refluxing methanol in the presence of a catalytic amount of sulfuric acid, in 86% yield after silica gel purification. The biomimetic oxidative coupling of ferulic acid methyl ester by using silver oxide Ag_2O was first carried out in anhydrous methylene chloride,[8] however no reaction occurred after two days under stirring in dark. Nevertheless the reaction proceeded smoothly until consumption of the starting material when using a toluene/acetone (2:1) mixture as solvent.[9a,b] Silica gel purification afforded a major fraction containing the known benzofuranic compound **2** (sole diastereoisomer) and a second one possessing different NMR pattern. After careful analytical chromatographic analysis and purification, dimeric ferulic ester **2′** was obtained. Compounds **2** and **2′** (45% and 5% yield, respectively) were fully characterized by spectroscopic analysis means, mono and bi dimensional NMR; compound **2′** was recrystallized and its X-ray spectra were obtained.

Bi dimensional NMR spectra of compound **2** (HSQC, HMBC) were recorded and unambigously confirmed the attribution of all atoms. It was noteworthy that the reaction generated preferentially only the dihydrobenzofuran skeleton **2** with a 7,8-*trans*-configuration. J_{7-8} value (8.4 Hz) for compound **2** was in the same order of magnitude as the J_{7-8} value (8.3 Hz) established by Pieters.[10] For compound **2′** the HMBC spectra induced an acyclic system. Moreover $H_{5'}/H_7$ observed NOESY correlation suggested a fold of compound **2′**. Phenoxy and allylester aromatic rings were not in the same plane; Allylester **2′** revealed a rotation about the C(4′)–O(5) axis (dihedral angle C(9)C(8)–O(5)C(4′) = 78.37°). The two aromatic rings adopt an

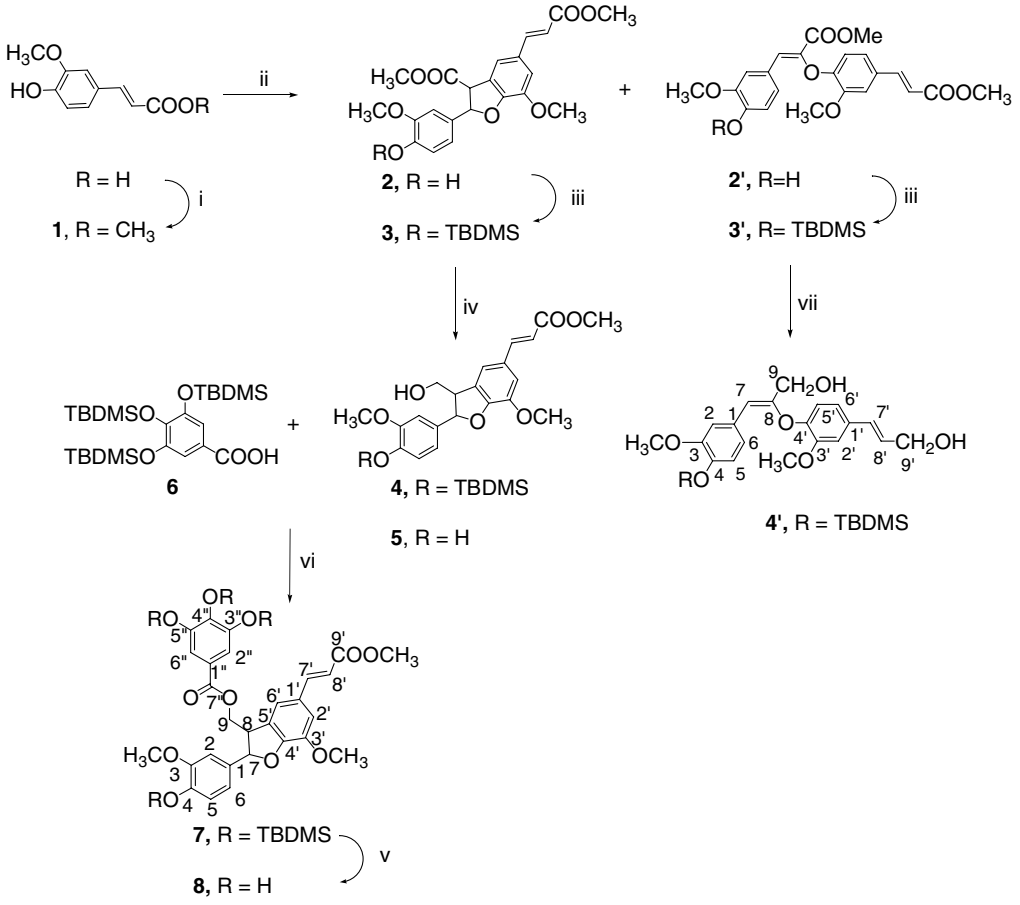

Figure 1. (i) MeOH, H_2SO_4, reflux; (ii) Ag_2O, toluene, acetone, rt, dark; (iii) TBDMSCl, $(i\text{-Pr})_2NEt$, DMF, rt; (iv) $LiBH_4$, THF, −78 °C–rt; (v) $Et_3N{*}HF$, THF, rt; (vi) 6, DCC, DMAP, 1,2-dichloroethane, reflux; (vii) DIBAL-H, $PhCH_3$, 0 °C.

almost orthogonal arrangement : the measured torsion angle between these two planes being 86.16°. Double bond C(7)–C(8) is not exactly in the phenoxy ring plane. Short torsion angle can be measured (dihedral angle C(8)C(7)–C(1)C(6) = −24.11°).

Moreover the measured distance $H_{5'}/H_7$ hydrogen atom distance (d = 13.645 Å) is in a range to confirm the NOESY correlation observed. Finally, the X-ray structure as shown in Figure 2, confirmed unambigously that the oxidative coupling leading to **2′** did not affect the geometry of the double bond (the allylic ester geometry is *Z*).

In order to obtain a final compound possesing the propenoyl ester functionality, selective reduction of the furanic ester group of compound **2** was undertaken. Gratifyingly we found that lithium tetrahydroborane could fulfil our objective. While no reaction occurs when operating in the presence of two equivalents of $LiBH_4$ in THF for several hours at room temperature, a sole product was obtained when using six equivalents under the same conditions.[11] The monoalcohol **5** was obtained in 65% of yield. Compound **5** failed to react in a suitable manner, under all examined conditions (detailed below) with gallic acid derivatives, presumably due to its free phenolic moiety. To circumvent the problem, compound **2** was first protected as a *tert*-butyldimethylsilyl ether. The reaction was carried out in DMF and in the presence of Hünig's base affording protected compound **3** in 90% yield after silica gel purification. Selective reduction of furanic ester group was as before led to monoalcohol **4** in 70% yield.

Next, we turned our attention to the introduction of the gallic acid to compounds **4** and **5**. Different experimental conditions were examined, that is, DCC activation as described by Kubo[12] by a Mitsunobu-like reaction as described by Appendino[13] by EDC/DMAP[14] or DCC/DMAP activation or simply by using pyridine.[15]

With trisilylated gallic acid[16] **6** and when operating with the phenolic derivative **5** in the presence of EDC/DMAP or DCC/DMAP the coupling reaction occurred in very low yield (less than 5%) along with many other by-products. With silylated compound **4** the same reactions afforded a 48% of yield of the coupled gallate ester **7** after silica gel purification.

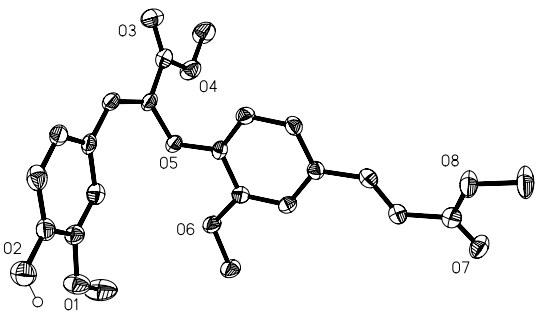

Figure 2. Crystallographic structure of compound **2′**.

Finally, silyl group elimination was performed by using a $Et_3N{\cdot}3HF$ complex in the presence of Et_3N in THF. After 50 min, the reaction mixture was directly applied on a silica column and eluted affording gallate ester **8** in 80% yield. It is noteworthy that aqueous treatment before the purification step decreased the yield drastically (less than 50%).

Compound **2′** possessing two allylic esters could not be reduced by lithium tetrahydroborane. This can be achieved by using DIBAL-H. While the reaction is quite sluggish when compound **2′** is used, silyl protection of the phenolic group, achieved as before, followed by DIBAL-H reduction of **3′** in toluene at 0 °C, afforded after purification the diol **4′** in 25% yield.[17] This compound can be a valuable synthon for further (alcohol or aldehyde) functionalisation.

3. Biological results and discussion

3.1. Antioxidant and cytoprotective effects

Compounds **2**, **2′**, **5** and **8** were studied on cell-mediated LDL oxidation to determine their antioxidant properties. All compounds were evaluated for their ability to protect native human LDL from HMEC-1-induced oxidation. HMEC-1 are human microvascular endothelial cells able to oxidize LDL and thus are suitable to modellize the pathophysiological events (LDL oxidation) that occur within the vascular wall, and the protective effect of antioxidants.[18] LDL oxidation was determined by using the thiobarbituric acid reactive substance assay (TBARS) and expressed as a percentage of TBARS formed in LDL in contact with HMEC-1 in the absence of antioxidant. Since LDL oxidation by cultured cells renders them cytotoxic[19] and since antioxidants are able to block the LDL oxidation process by cells,[20] we evaluated the residual cytotoxicity of LDL previously oxidized by HMEC-1 in presence or absence of the newly synthesized derivatives, by the MTT assay. The data were expressed as a percentage of the unstimulated control (cell incubated without LDL).

Preliminary studies were undertaken on all synthesized compounds in order to determine the dose effect on LDL oxidation as measured by the TBARS formation and the oxLDL cytotoxicity as measured by the MTT assay. Figure 3a and b represent the results for compounds **2**, **2′**, **5** and **8**. Compounds **2′** and **8** present good antioxidant and cytoprotective properties starting from 1 to 100 μM. Figure 3a indicates that the **2**, **2′** and **8** derivatives exert a strong antioxidant effect at 10 μM, whereas the compound **5** is efficient only at 100 μM. Conversely, the compound **2′** (and to a lesser extent **2** and **8**) by protecting efficiently LDL against cell-induced oxidation prevented their cytotoxicity by comparison with LDL oxidized by cells in the absence of antioxidant (Fig. 3b).

All the synthetized compounds exhibited antioxidant and cytoprotective effects. Dihydrobenzofuran **8** pre-

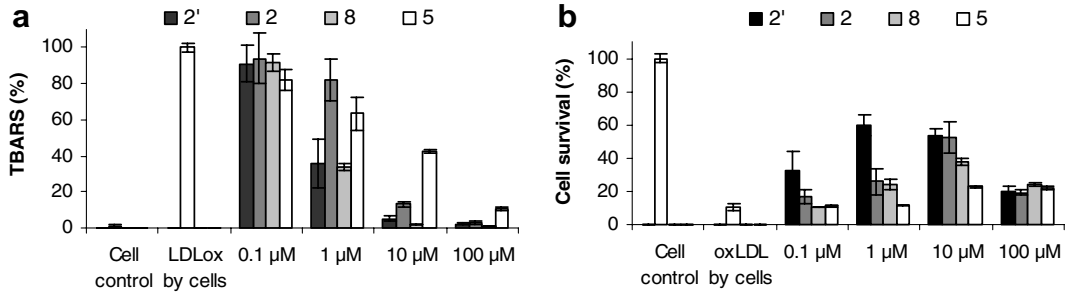

Figure 3. Antioxidant effect: (a) effect of didydrobenzofuran derivatives on HMEC-1-mediated LDL oxidation, evaluated by TBARS formation. Native LDL (100 μg/mL) were incubated with subconfluent HMEC-1 in RPMI 1640, in presence of 1 μM Cu^{2+} and in presence (or absence) of didydrobenzofuran compounds. After 6 h contact, the TBARS content was determined in the culture medium as reported in Section 5. Results are expressed as % of TBARS formed in LDL oxidized by HMEC-1 in absence of antioxidant (i.e., 20 ± 5 nmol TBARS/mg apoB protein). The TBARS content of control native LDL (i.e., native LDL in presence of 1 μM Cu^{2+} without HMEC-1 cells) was evaluated to verify the lack of endogenous oxidation in LDL and was around 0.5 ± 0.15 nmol TBARS/mg apoB protein. Each bar represents the mean for three separate determinations. (b) The different compounds were tested on LDL oxidation (TBARS) and subsequent cytotoxicity (MTT) elicited by HMEC-1. The results are expressed as % of viability in the unstimulated control (MTT done on control cells incubated in fresh RPMI culture medium in the absence of LDL and antioxidant).

sents the best results in this series at 10 μM, that is, 2.2% of residual TBARS in LDL and 59.9% of viable cells evaluated by the MTT test. The antioxidant capacity of phenols is generally ascribed to the reaction with oxidants to form resonance-stabilized phenoxyl radicals. This activity is strengthened by the presence of other hydroxyl group, as in caffeic or gallic acids, through the formation of intramolecular hydrogen bond. Moreover, the phenolic adjacent methoxy group may provide stability to the phenoxy radical.[21] Compound **8** by its structure sums these two effects. This can explain its good antioxidant and cytoprotective capacity.

All synthesized derivatives possess 3-OMe group surrounding 4-hydroxy function on the aromatic ring in order to stabilize radical generated on hydroxyl position as mentioned above and exhibit an antioxidant and cytoprotective effect in the same order of probucol (TBARS: 10% and MTT : 92% (at 10μM)) and vitamin E (TBARS: 55% and MTT 29% (at 10 μM)) under the same experimental conditions.

In another set of experiments, we tested the direct cytoprotective effect of the synthesized molecules against the cytotoxicity of oxidized LDL. This was studied by incorporating various concentrations of the tested drugs in the culture medium of HMEC-1 simultaneously with previously oxidized LDL (i.e., 200 μg/mL of LDL oxidized by UV irradiation[22] in the absence of any additive). As shown in Figure 4, all the tested compounds increased significantly the resistance of cells against the cytotoxic effect of oxidized LDL (at the tested concentration of 10 μM, 40–60% living cells versus less than 20% in presence of oxidized LDL alone), and were found as efficient as Probucol and Vitamin E (up to 55% and 40% of living cells, respectively). By comparison to the other compounds, the derivative **2′** was the most efficient by exhibiting two kind of protective effects: 1/an antioxidant activity allowing to inhibit LDL oxidation occurring in the culture medium (thus outside the cell), and 2/a direct cytoprotective effect which prevented the cytotoxicity of oxidized LDL (inside the cell).

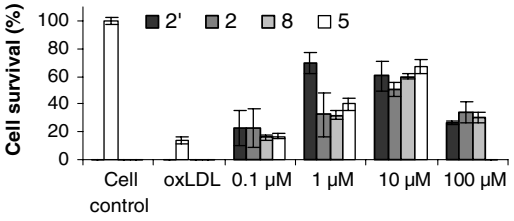

Figure 4. Direct cytoprotective effect of some dihydrobenzofuran derivatives against the cytotoxicity of (previously) oxidized LDL. A fixed concentration of oxidized LDL (200 μg apoB/ml of LDL oxidized by UV irradiation as described in Section 5) was added simultaneously to the culture medium with variable concentrations of the different antioxidants. The cell viability was determined after 24 h by the MTT test. The results are means (±SEM) of three separate experiments.

3.2. Antiplasmodial activity and cytotoxicity

Compounds **2**, **2′**, **5** and **8** were evaluated for their ability to inhibit in vitro *Plasmodium falciparum* grown in asynchronous culture conditions.[23] To this end, the chloroquine-resistant *Plasmodium* strain FCM29 was incubated first with a 10 μg/mL single concentration of test compounds in triplicate. Inhibition of parasite growth was monitored by measuring the specific incorporation of tritiated hypoxanthine into the parasites, and the percentage inhibition was calculated relative to the control. Chloroquine was used as reference compound. Compounds having more than 80% inhibition were further tested for the determination of their median inhibitory concentration (IC_{50}) using serial concentrations of the test compound ranging from 0.01 to 10 μg/mL. Results are summarized in Table 1. In this study, compound **8** exhibited antiplasmodial activities with an IC_{50} value of 798 ± 12 nM. The standard reference drug chloroquine had an IC_{50} value of 264 ± 11 nM. The remaining compounds **2**, **2′** and **5** did not show significant

Table 1. Biological activities of compounds **2**, **2′**, **5** and **8** on the FCM29 strain of *P. falciparum* and the P388 cancerous cell lines

Compound	Antiplasmodial activity		Cytotoxicity activity	
	% of inhibition (10 μg/mL)	IC_{50} (nM)	% of inhibition (10 μg/mL)	IC_{50} (nM)
2	0		85.2	772 ± 21
2′	23.8		0	
5	25.7		8.63	
8	93.7	798 ± 12	16.4	
Chloroquine		264 ± 11		—
Camptothecin		—		32.1 ± 0.2

antiplasmodial activities. Interestingly, the presence of the galloyl moiety in compound **8** led to the appearance of antiplasmodial effects with respect to the parent compound **5**. A decrease in cytotoxicity from compound **2** to compound **8** was noticed. This should be explained by the presence of galloyl moiety on dibenzofuran **8.** Gallate ester might be a good substitute to enhance the antimalarial activities of existing or potential drugs.

Cytotoxicity activities of these compounds were also evaluated against the murine P388 leukaemia cells. To this purpose, cells were incubated with a 10 μg/mL single concentration of test compounds in triplicate. Inhibition of cell growth was monitored by colorimetric method using neutral red, and the % inhibition was calculated with respect to the blank control. Camptothecin was used as reference drug. Compounds having more than 80% inhibition were further tested for the determination of their IC_{50} (Table 1) using serial concentrations ranging from 0.01 to 10 μg/mL. Compound **2** showed cytotoxic activities against P388 cell lines with IC_{50} of 772 ± 21 nM, whereas the remaining compounds were devoid of such activities. Camptothecin had 32.1 ± 0.2 nM as IC_{50} value. It is noteworthy that compound **8** had selective antiplasmodial activities.

4. Conclusion

In conclusion, based on the oxidative coupling of feruloyl ester, we have synthetized a series of compounds possessing strong and different biological properties. Thus, compound **2′**, which its X-ray data analysis established its structure unambigously, presented the best antiatherogenic effect (antioxidant activity and cytoprotective effect). Compound **2** presented the best activity against the murine P388 leukaemia cells, while its derivatized compound **8** showed a very good antiplasmodial potency coupled also with an antioxidant one.

Our efforts, focusing on a more efficient synthesis of compound **2′**, its selective reduction and functionalisation of both feruloyl dimers with gallate and other interesting frames, along with their activities, will be reported in due course. Moreover, the role of gallate group in enhancing the antiplasmodial activities of compounds deserves further investigation.

5. Experimental

5.1. Chemistry

Reagents and solvents were purchased from usual commercial sources. Silica gel (Kieselgel 60, Merck) was used for column chromatography. Compounds were detected on TLC plates by exposure to UV light (254 nm). Melting points were measured on a Buchi melting point apparatus without correction. Mass spectra (MS) were obtained on a triple quadrupole spectrometer, Thermo Finnigan TSQ 7000. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-300, AC-400 or AC-500 spectrometer, and chemical shifts are expressed in ppm downfield of tetramethylsilane (TMS), which was used as an internal reference. All atoms were numbered as below to facilitate the NMR signals attribution.

Compound **1** was synthesized by the reported procedure.[24]

5.1.1. Methyl (*E*)-3-[2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]-prop-2-enoate (2). A solution of (**1**) (4.13 g, 19.8 mmol) in a mixture of dry toluene/acetone (80:40 mL) was added with silver(I) oxide (3.67 g, 15.8 mmol) under nitrogen atmosphere at room temperature. After stirring for 8 h under darkness conditions, the mixture was filtered through a silica pad and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography with petroleum ether/AcOEt (5:2) yielding a mixture of diastereoisomer (**2**) and compound (**2′**) (2.05 g, 50%). The major diastereoisomer (**2**) and the minor one (**2′**) were separated by preparative HPLC (isocratic mode, CH_3CN/H_2O 35:65) yielding pur major diastereoisomer (**2**) (1.84 g, 45%), pure compound (**2′**) (0.21 g, 5%). The minor diastereoisomer (**2**) was not characterized. Mp 153–155 °C; DCI-MS (*m*/*z*) 432 $(M+NH_4)^+$; all NMR data were according to previous results.[9,10]

5.1.2. Methyl (*Z*)-3-(4-hydroxy-3-methoxyphenyl)-2-{2-methoxy-4[(*E*)-3-methoxy-3-oxoprop-1-enyl]phenoxy}-prop-2-enoate (2′). The title compound was obtained during the synthesis of compound (**2**) as yellow crystals (0.72 g, 17.5%). Mp 186–188 °C DCI-MS (*m*/*z*) 432 $(M+NH_4)^+$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.63 (d, J = 16 Hz, 1H, H-7′), 7.42 (d, J = 2 Hz, 1H, H-2), 7.40 (s, 1H, C-7), 7.16 (dd, J = 8.5 Hz, J = 2 Hz, 1H, H-6), 7.15 (s, 1H, H-2′), 7.01 (dd, J = 8.5 Hz, J = 2 Hz, 1H, H-6′), 6.88 (d, J = 8.5 Hz, 1H, H-5), 6.78 (d, J = 8.5 Hz, 1H, H-5′), 6.34 (d, J = 16 Hz, 1H, H-8′), 5.85 (s, 1H, OH), 4.00 (s, 3H, 3′-OCH_3), 3.82 (s, 3H, 9′-OCH_3), 3.80 (s, 3H, 9-OCH_3), 3.78 (s, 3H, 3-OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.53 (C-9′), 164.01 (C-9), 149.06 (C-3′), 147.64 (C-4′), 147.61 (C-4), 146.53 (C-3), 144.43 (C-7′), 137.20 (C-8), 129.31 (C-1′), 128.4 (C-7), 125.66 (C-6), 124.57 (C-1), 122.17 (C-6′), 116.33 (C-8′), 114.55 (C-5), 113.82 (C-5′), 112.06 (C-2),

111.20 (C-2′), 56.19 (3′-OCH_3), 55.56 (3-OCH_3), 52.46 (9-OCH_3), 51.68 (9′-OCH_3).

5.1.3. Methyl (*E*)-3-[2-(4-tertiobutyldimethylsilyloxy-3-methoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]-prop-2-enoate (3). Diisopropylethylamine (1.23 mL, 7.43 mmol) was syringed into a solution of (**2**) (0.86 g, 2.08 mmol) and *tert*-butyldimethylsilyl chloride (0.94 g, 6.26 mmol) in 26 mL of dry DMF under Ar. The reaction mixture was stirred for 2 h 30 min at room temperature and was poured into ice-cold water. The product was extracted into Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to provide an oil (1.08 g, 97%). DCI-MS (*m*/*z*) 546 $(M+NH_4)^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.51 (d, J = 16 Hz, 1H, H-7′), 7.05 (s, 1H, H-6′), 6.88 (s, 1H, H2′), 6.74 (d, J = 1.8 Hz, 1H, H-2), 6.72 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H, H-6), 6.67 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H, H-5), 6.17 (d, J = 15.9 Hz, 1H, H-8′), 5.98 (d, J = 8.4 Hz, 1H, H-7), 4.23 (d, J = 8.0 Hz, 1H, H-8), 3.78 (s, 3H, 3′-OCH_3), 3.70 (s, 3H, 3-OCH_3), 3.67 (s, 3H, 9-OCH_3), 3.65 (s, 3H, 9′-OCH_3), 0.84 (s, 9H, *t*-Bu), 0.00 (s, 6H, Si–CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.72 (C-9), 167.55 (C-9′), 151.11 (C-4′), 149.96 (C-3), 145.48 (C-4), 144.70 (C-7′), 144.66 (C-3′), 132.81 (C-1), 128.49 (C-1′), 125.69 (C-5′), 120.95 (C-6), 118.73 (C-6′), 117.88 (C-8′), 115.47 (C-5), 112.02 (C-2′), 110.10 (C-2), 87.42 (C-7), 56.07 (3′-OCH_3), 55.51 (3-OCH_3), 55.39 (C-8), 52.82 (9-OCH_3), 51.58 (9′-OCH_3), 25.65 (CH_3, *t*-Bu), 18.39 (C, *t*-Bu), −4.68 (Si–CH_3).

5.1.4. Methyl (*Z*)-3-(4-tertiobutyldimethylsilyloxy-3-methoxyphenyl)-2-{2-methoxy-4-[(*E*)-3-methoxy-3-oxo-prop-1-enyl]phenoxy}-prop-2-enoate (3′). It was synthetized by the same procedure as for compound (**3**) (yield 71%). DCI-MS (*m*/*z*) 546 $(M+NH_4)^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.48 (d, J = 16 Hz, 1H, H-7′), 7.20 (d, J = 1.8 Hz, 1H, H-2), 7.14 (s, 1H, H-7), 7.00 (d, J = 1.8 Hz, 1H, H-2′), 6.97 (dd, J = 8.5 Hz, J = 2 Hz, 1H, H-6), 6.86 (dd, J = 8.5 Hz, J = 2 Hz, 1H, H-6′), 6.66 (d, J = 8.5 Hz, 1H, H-5), 6.63 (d, J = 8.5 Hz, 1H, H-5′), 6.18 (d, J = 16 Hz, 1H, H-8′), 3.85 (s, 3H, 3′-OCH_3), 3.66 (s, 3H, 9′-OCH_3), 3.64 (s, 3H, 9-OCH_3), 3.54 (s, 3H, 3-OCH_3), 0.83 (s, 9H, $C(CH_3)_3$), 0.00 (s, 6H, CH_3).

5.1.5. Methyl (*E*)-3-[2-(4-tertiobutyldimethylsilyloxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]-prop-2-enoate (4). $LiBH_4$ (33 mg; 1.5 mmol) was suspended in dry THF (3 mL) and added to a solution of compound (**3**) (123 mg; 0.23 mmol) dissolved in dry THF (5 mL) under argon at −78 °C. The reaction mixture was stirred for 30 min h at −78 °C then 2 h at room temperature. Aqueous THF (80%, 10 mL) was added slowly and aqueous 0.1 M ammonium chloride (5 mL) was then added. The mixture was extracted with EtOAc (3× 10 mL), and the combined organic extracts were washed with water, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel chromatography with petroleum ether/EtOAc (45:55) yielding pure monoalcohol (**4**) (98 mg, 84%). DCI-MS (*m*/*z*) 518 $(M+NH_4)^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.49 (d, J = 16 Hz, 1H, H-7′), 6.93 (s, 1H, H-6′), 6.87 (s, 1H, H-2′), 6.75 (d, J = 1.2 Hz, 1H, H-2), 6.70 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H, H-6), 6.67 (d, J = 8.1 Hz, 1H, H-5), 6.15 (d, J = 15.9 Hz, 1H, H-8′), 5.48 (d, J = 6.9 Hz, 1H, H-7), 3.80 (m, 2H, C-9), 3.77 (s, 3H, 3′-OCH_3), 3.65 (s, 3H, 3-OCH_3), 3.63 (s, 3H, 9′-OCH_3), 3.50 (m, 1H, C-8), 0.85 (s, 9H, *t*-Bu), 0.00 (s, 6H, Si–CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.73 (C-9′), 151.07 (C-4′), 150.64 (C-3), 145.21 (C-4), 144.96 (C-7′), 144.53 (C-3′), 133.91 (C-1), 128.77 (C-1′), 128.32 (C-5′), 120.83 (C-6), 118.69 (C-6′), 117.33 (C-8′), 115.02 (C-5), 111.76 (C-2′), 110.12 (C-2), 88.62 (C-7), 63.86 (C-9), 56.01 (3′-OCH_3), 55.95 (3-OCH_3), 55.49 (C-8), 53.10 (9-OCH_3), 51.57 (9′-OCH_3), 25.65 (CH_3, *t*-Bu), 18.38 (C, *t*-Bu), −4.69 (Si–CH_3).

5.1.6. (*Z*)-3-(4-tertiobutyldimethylsilyloxy-3-methoxyphenyl)-2-{2-methoxy-4-[(*E*)-3-hydroxy prop-1-enyl]phenoxy}-prop-2-enol (4′). Compound (**3′**) (11.4 mg, 21.6 μmol) dissolved in 1 mL of toluene freshly distilled, under nitrogen, was cooled in an ice-water bath, and diisobutylaluminium hydride (30 μL of 1.5 M solution, 181 μmo1, 8.4 equiv) in toluene was slowly added. After addition was complete, stirring was performed for one night. The reaction mixture was then carefully quenched with ethanol (5 mL). The solvents were partially removed under vacuo at 40 °C. Water (10 mL) was added, and the aqueous layer was extensively extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography with petroleum ether/EtOAc (5:5) yielding pur diol (**4′**) (6.7 mg, 66%).

DCI-MS (*m*/*z*) 490 $(M+NH_4)^+$; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.14 (s, 2H, 9-OH, 9′-OH), 7.11 (d, J = 1.8 Hz, 1H, H-2), 6.88 (d, J = 1.8 Hz, 1H, H-2′), 6.86 (dd, J = 8.1 Hz, J = 2.1 Hz, 1H, H-6′), 6.80 (d, , J = 8.1 Hz, 1H, H-5), 6.73 (dd, J = 8.1 Hz, J = 1.8Hz, 1H, H-6), 6.63 (d, , J = 8.4 Hz, 1H, H-5′), 6.43 (d, J = 15.9 Hz, 1H, H-7′), 6.18 (dt, J = 15.9 Hz, J = 5.7 Hz, 1H, H-8′), 5.96 (s, 1H, H-7), 4.19 (dd, J = 5.7 Hz, J = 1.2 Hz, 2H, H-9′), 4.04 (s, 2H,C-9), 3.80 (s, 3H, 3′-OCH_3), 3.55 (s, 3H, 3-OCH_3), 0.86 (s, 9H, $C(CH_3)_3$), 0.00 (s, 6H, CH_3).

5.1.7. Methyl (*E*)-3-[2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]-prop-2-enoate (5). The title compound was synthetized from compound (**2**) by the same procedure as for compound (**4**) synthesis (yield 87%). DCI-MS (*m*/*z*) 404 $(M+NH_4)^+$; ^1H NMR (DMSO-d_6, 500 MHz) δ 9.09(s, H, 4-OH), 7.62 (d, J = 16.0 Hz, 1H, H-7′), 7.29(s, 1H, H-2′), 7.28 (s, 1H, H-6′), 6.93 (s, 1H, H-2), 6.78 (s, 2H, H-5, H-6), 6.53 (d, J = 16.0 Hz, 1H, H-8′), 5.56 (d, J = 6.5 Hz, , 1H, H-7), 5.09 (m, 1H, OH-9), 3.84 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.74 (m, 1H, H-9b), 3.72 (s, 3H, OCH_3), 3.67 (m, 1H, H-9a), 3.51 (td, J = 6.0 Hz, J = 6.0 Hz, 1H, H-8);^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.53 (C-9′), 145.59 (C-7′), 150.44 (C-4′), 148.05 (C-3), 147.01 (C-4), 144.44 (C-3′),

132.35 (C-1), 130.35 (C-2), 128.02 (C-1′), 112.88 (C-5′), 119.13 (C-6), 118.53 (C-6′), 115.11 (C-8′), 115.82 (C-5), 110.84 (C-2′), 88.45 (C-7), 63.15 (C-9), 56.27 (3′-OCH_3), 56.08 (3-OCH_3), 53.07 (C-8), 51.75 (9-OCH_3).

5.1.8. 3,4,5-Tri(tertiobutyldimethylsilyloxy)benzoic acid (6). The title compound was synthetized by the reported procedure [17] without further modifications (yield 99%).

5.1.9. Methyl (*E*)-3-[2-(4-tertiobutyldimethylsilyloxy-3-methoxyphenyl)-3-(3,4,5-tri(tertiobutyldimethylsilyloxy)phenylcarbonyloxymethyl)-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]-prop-2-enoate (7). To a solution of (**4**) (0.2 mg, 0.41 mmol) and 2,4,5-tri(*tertio*butyldimethylsilyloxy)benzoic acid (**6**) (0.42, 0.81 mmol) in 1,2-dichloroethane (28 mL) were added DCC (0.084 g, 0.81 mmol) and DMAP (0.17 g, 0.81 mmol). After stirring for 6 h under reflux, the reaction mixture was concentrated and purified by silica gel chromatography (petroleum ether/EtOAc, 9:1) to afford compound (**7**) (0.19 g, 47%) as an oil and 88 mg (44 %) of the starting material (**4**) was recovered; DCI-MS (*m*/*z*) 1012.7 $(M+NH_4)^+$. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50 (d, J = 15.9 Hz, 1H, H-7′), 7.02 (s, 2H, H-2″, H-6″), 6.95 (d, J = 0.9 Hz, 1H, H-6′), 6.88 (d, J = 0.9 Hz, 1H, H-2′), 6.72 (d, J = 1.2 Hz, 1H, H-2), 6.70 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H, H-6), 6.65 (d, J = 8.1 Hz, 1H, H-5), 6.15 (d, J = 15.6 Hz, 1H, H-8′), 5.47 (d, J = 7.5 Hz, 1H, H-7), 4.46 (dd, J = 11.4 Hz, J = 5.1 Hz, 1H, H-9b), 4.40 (dd, J = 11.4 Hz, J = 6.0 Hz, 1H, H-9a), 3.79 (s, 3H, 3′-OCH_3), 3.77 (m, 1H, H-8), 3.65 (s, 3H, 3-OCH_3), 3.59 (s, 3H, 9′-OCH_3), 0.85 (s, 9H, *t*-Bu), 0.84 (s, 9H, *t*-Bu), 0.78 (s, 18H, *t*-Bu), 0.06 (s, 6H, Si–CH_3); 0.05 (s, 6H, Si–CH_3); 0.00 (s, 6H, Si–CH_3); −0.01 (s, 6H, Si–CH_3); ^{13}C NMR ($CDCl_3$+CD_3OD, 75 MHz) δ 167.59 (C-9′), 166.20 (C-7″), 151.18 (C-4′), 150.40 (C-3), 148.51 (C-3″, C-5″), 145.40 (C-4), 144.84 (C-7′), 144.64 (C-3′), 143.56 (C-4″), 133.19 (C-1), 128.61 (C-5′), 128.30 (C-1′), 121.20 (C-1″), 120.93 (C-6), 118.73 (C-6′), 117.38 (C-5), 115.43 (C-8′), 115.34 (C-2″, C-6″), 111.65 (C-2′), 109.94 (C-2), 88.61 (C-7), 64.47 (C-9), 55.94 (3′-OCH_3), 55.46 (3-OCH_3), 51.56 (C-8), 50.47 (9′-OCH_3), 26.12 (CH_3, *t*-Bu), 26.07 (CH_3, *t*-Bu), 25.68 (CH_3, *t*-Bu), 18.76(C3″ − 5″, *t*-Bu), 18.47 (C, *t*-Bu), 18.43 (C, *t*-Bu), −3.70 (Si–CH_3), −3.72 (Si–CH_3), −4.64 (Si–CH_3).

5.1.10. Methyl (*E*)-3-[2-(4-hydroxy-3-methoxyphenyl)-3-(3,4,5-trihydroxyphenylcarbonyloxymethyl)-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]-prop-2-enoate (8). was synthetized by the same procedure as for compound (**5**) synthesis (yield 85%). DCI-MS (*m*/*z*) 556 $(M+NH_4)^+$; ^{1}H NMR (CD_3COCD_3, 300 MHz) δ 8.36 (s, 2H, OH-3″, OH-5″), 8.20 (s, 1H, OH-4″), 7.76 (s, 1H, OH-4), 7.64(d, J = 16.0 Hz, 1H, H-7′), 7.35 (s, 1H, H-6′), 7.20 (d, J = 1.5 Hz H, H-2′), 7.15 (s, 2H, H-2″, H-6″), 7.08 (d, J = 1.9 Hz, 1H, H-2), 6.94 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H, H-6), 6.85 (d, J = 8.1 Hz, 1H, H-5), 6.44 (d, J = 16.0 Hz, 1H, H-8′), 5.75 (d, J = 6.5 Hz, 1H, H-7), 4.65 (dd, J = 11.1 Hz, J = 5.4 Hz, 1H, H-9b), 4.58(dd, J = 11.1 Hz, J = 7.2 Hz, 1H, H-9a), 3.95 (s, 3H, 3′-OCH_3), 3.94 (m, 1H, H-8), 3.79 (s, 3H, 3-OCH_3), 3.73 (s, 3H, 9′-OCH_3); ^{13}C NMR (CD_3COCD_3, 75 MHz) δ 168.78(C-9′), 167.50(C-7″), 152.42 (C-4′), 148.72 (C-3), 147.48 (C-3′), 147.00 (C-3″, C-5″), 146.52 (C-7′), 146.48 (C-4), 139.92 (C-4″), 134.05 (C-1), 130.40 (C-1′), 130.20 (C-5′), 122.24 (C-1″), 120.50 (C-6), 119.61 (C-6′), 116.82 (C-5), 116.76 (C-8′), 114.41 (C-2′), 111.30 (C-2), 110.84 (C-2″ ,C-6″), 90.33 (C-7), 67.02 (C-9), 57.34 (3′-OCH_3), 57.08 (3-OCH_3), 52.49(9′-OCH_3), 52.16 (C-8).

5.1.11. X-ray crystallographic data. Crystal data for (**2′**): $C_{22}H_{22}O_8$, M = 414.40, orthorhombic, $P2_12_12_1$, a = 7.567(3) Å, b = 16.118(6) Å, c = 16.383(6) Å, V = 1998.3(14) Å^3, Z = 4, T = 173(2) K. 10059 reflections (3369 independent, R_{int} = 0.0435) were collected at low temperatures using an oil-coated shock-cooled crystal on a Bruker-AXS CCD 1000 diffractometer with MoKα radiation (λ = 0.71073 Å). The structure was solved by direct methods (SHELXS-97, G. M. Sheldrick, *Acta Crystallogr. A* **1990**, *46*, 467–473) and all nonhydrogen atoms were refined anisotropically using the least-squares method on F^2 (SHELXL-97, Program for Crystal Structure Refinement, G. M. Sheldrick, University of Göttingen 1997). Largest electron density residue: 0.146 e Å^{-3}, R_1 (for $I > 2\sigma(I)$) = 0.0365 and wR_2 = 0.0748 (all data) with $R_1 = \sum \|F_o\| - |F_c\| / \sum |F_c|$ and $wR_2 = (\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2)^{0.5}$. CCDC 642002 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

5.2. Pharmacological methods

5.2.1. Cell culture. HMEC-1 line was obtained from CDC (Atlanta, US)[18] and was a generous gift from Dr. F. Trottein (Institut Pasteur, Lille). Cells were grown in MCDB-131 supplemented with 10% heat inactivated foetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Twenty-four hours before LDL incorporation, cells were starved in serum-free RPMI medium.

5.2.2. LDL isolation. LDL were isolated by ultracentrifugation from the pooled plasma of healthy normolipidemic human subjects and dialysed against PBS containing 100 μmol/L EDTA, as previously indicated.[25]

5.2.3. Antioxidant effect. To evaluate cell-mediated LDL oxidation, HMEC-1 were seeded in 24-multiwell plates. The standard culture medium (on sparse proliferative cells) was removed and replaced by serum-free RPMI-1640 containing native LDL (100 μg apoB/mL) and the compounds diluted in DMSO were added to the culture medium at variable concentrations. Cells were incubated overnight at 37 °C. At the end of the incubation, LDL-containing medium was immediately used for determining thiobarbituric acid reactive substances (TBARS) formation using the fluorimetric procedures of Yagi.[26]

5.2.4. Cell viability. Cells were incubated in presence of LDL (previously oxidized by cells overnight in presence of variable concentrations of antioxidants) until the cell viability was evaluated at the indicated time (24 h pulse period, under standard conditions). To test the direct cytoprotective effect of antioxidants, cells were incubated for 24 h with UV/copper oxidized LDL (200 μg/mL) in presence of variable concentrations of dihydrobenzofuran derivatives. The cell viability was determined by using the MTT test (based on the reduction of diphenyltetrazolium bromide salt by mitochondrial enzymes, according to Price and McMillan).[27]

5.2.5. In vitro antiplasmodial test. The in vitro antiplasmodial test was based on the inhibition of [G-3H]hypoxanthine uptake by *P. falciparum* cultured in human blood. Briefly, *P. falciparum* parasites were maintained in culture in a specific medium composed of RPMI 1640, 25 mM Hepes, 25 mM $NaHCO_3$, and 10% pooled human serum, with uninfected human red blood cells at 2.5% haematocrit. The cell suspension (1% parasitaemia), was distributed at 0.2 mL per well into flat-bottomed 96-well plates containing a single concentration (10 μg/mL) of test compounds in triplicate, alongside untreated controls. Chloroquine was used as a standard reference drug. The culture was then incubated at 37 °C during 18 h under microaerophilic conditions obtained with the candle jar method. Tritiated hypoxanthine (0.5 μCi per well) was then added to each well and incubation continued at 37 °C in the required atmosphere for further 24 h. The contents of the well were then frozen at −30 °C and unfrozen at 50 °C to lyse the cells, harvested by filtration on glass filter papers using a Skatron apparatus and finally washed several times with water. Thereafter, the discs were dried, added to toluene scintillator in vials and the radioactivity incorporated into parasites was estimated in a Wallac MicroBeta TriLux microplate liquid scintillation counter. The inhibition percentage of parasite growth was calculated with respect to the controls.

For test compounds having inhibition percentage higher than 80, serial concentrations ranging from 0.01 to 10 μg/mL were tested using the same method. The inhibitory concentrations 50 (IC_{50}) were determined by linear regression method. Three separate experiments were made for each test.

5.2.6. Cytotoxicity test. Murine P388 leukaemia cells were grown in RPMI 1640 medium containing 0.001 mM β-mercaptoethanol, 10 mM L-glutamine, 100 IU/ml G-penicillin, 100 μg/mL streptomycin, supplemented with 10% foetal calf serum. Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The inoculum seeded at 2×10^4 cells/ml at an optimal volume of 0.2 mL per well was distributed into 96-well microculture plates containing a single concentration (10 μg/mL) of test compounds, alongside untreated controls. Camptothecin was used as standard reference drug. Culture was then incubated at 37 °C for 72 h in the required atmosphere. Thereafter, cells were incubated at 37 °C with 0.01% neutral red (0.2 mL per well) for 3 h and then washed with 1 N PBS and finally lysed with SDS. After 10 min agitation on a microculture plate shaker, the plates were transferred to a Titertek Twinreader equipped with a 540 nm filter to measure absorbance of the extracted dye. Cell viability was expressed as the percentage of cells incorporating dye relative to the untreated controls. IC_{50} values were determined as described for the antiplasmodial test.

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References and notes

1. Zhou, Y. C.; Zheng, R. L. *Biochem. Pharmacol.* **1991**, *42*, 1177–1179.
2. Becker, K.; Tilley, L.; Vennerstrom, J. L.; Roberts, D.; Rogerson, S.; Ginsburg, H. *Int. J. Parasitol.* **2004**, *34*, 163–189.
3. Fang, F. C. In *Nitric Oxide and Infection*; Kluwer Academic/Plenum: New York, 1999.
4. Andreasen, M. F.; Lanbdo, A.-K.; Christensen, L. P.; Hansen, A.; Meyer, A. S. *J. Agri. Food Chem.* **2001**, *49*, 4090–4096.
5. Van Miert, S.; Van Dyck, S.; Schmidt, T. J.; Brun, R.; Vlietinck, A.; Lemière, G.; Pieters, L. *Bioorg. Med. Chem.* **2005**, *13*, 661–669.
6. Go, M.-L. *Med. Res. Rev.* **2003**, *23*, 456–487.
7. Lapeyre, C.; Delomenède, M.; Bedos-Belval, F.; Duran, H.; Negre-Salvayre, A.; Baltas, M. *J. Med. Chem.* **2005**, *48*, 8115–8124.
8. Quideau, S.; Ralph, J. *Holzforschung* **1994**, *48*, 12–22.
9. (a) Lemiere, G.; Gao, M.; De Groot, A.; Dommisse, R.; Lepoivre, J.; Pieters, L.; Buss, V. *J. Chem. Soc., Perkin Trans 1* **1995**, 1775–1779; (b) Apers, S.; Papers, D.; Bürgermeister, J.; Baronikova, S.; Van Dyck, S.; Lemiere, G.; Vlietinck, A.; Pieters, L. *J. Nat. Prod.* **2002**, *65*, 718–720.
10. Pieters, L.; Van Dyck, S.; Gao, M.; Bai, R.; Hamel, E.; Vlietinck, A.; Lemiere, G. *J. Med. Chem.* **1999**, *42*, 5475–5481.
11. Orlandi, M.; Rindone, B.; Molteni, G.; Rummakko, P.; Brunow, G. *Tetrahedron* **2001**, *57*, 371–378.
12. Kubo, I.; Fujita, K.-I.; Nihei, K.-I. *J. Agri. Food Chem.* **2002**, *50*, 6692–6693.
13. Appendino, G.; Minassi, A.; Dadario, N.; Bianchi, F.; Cesare Tron, G. *Org. Lett.* **2002**, *4*, 3839–3841.
14. Lapeyre, C.; Bedos-Belval, F.; Duran, H.; Baltas, M.; Gorrichon, L. *Tetrahedron Lett.* **2003**, *44*, 2445–2447.
15. He, Q.; Shi, B.; Yao, K.; Luo, Y.; Ma, Z. *Carbohydr. Res.* **2001**, *335*, 245–250.
16. Feldman, K. S.; Sambandam, A.; Bowers, K. E.; Appel, H. M. *J. Org. Chem.* **1999**, *64*, 5794–5803.
17. Quideau, S.; Ralph, J. *J. Agri. Food. Chem.* **1992**, *40*, 1108–1110.
18. Ades, E. W.; Candal, F. J.; Swerlick, R. A.; George, V. G.; Summers, S.; Bosse, D. C.; Lawley, T. J. *J. Invest. Dermatol.* **1992**, *99*, 683–690.

19. Mayer, D.; Muhlhofer, A.; Biesalski, H. K. *Eur. J. Med. Res.* **2001**, *296*, 201–208.
20. Mashima, R.; Witting, P. K.; Stocker, R. *Curr. Opin. Lipidol.* **2001**, *12*, 411–418.
21. Rajan, P.; Vedernikova, I.; Cos, P.; Vanden Berghe, D.; Augustyns, K.; Haemers, A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 215–217.
22. (a) Wright, J. S.; Johnson, E. R. *J. Am. Chem. Soc.* **2001**, *123*, 1173–1183; (b) Escargueil-Blanc, I.; Salvayre, R.; Nègre-Salvayre, A. *FASEB J.* **1994**, *8*, 1075–1080.
23. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
24. Tanaka, H.; Kato, I.; Ito, K. *Chem. Pharm. Bull.* **1987**, *35*, 3603.
25. Mabile, L.; Meilhac, O.; Escargueil-Blanc, I.; Troly, M.; Pieraggi, M. T.; Salvayre, R.; Negre-Salvayre, A. *Arterioscler. Thromb. Vasc. Biol.* **1997**, *17*, 1575–1582.
26. Yagi, K. *Chem. Phys. Lipids* **1987**, *45*, 337–351.
27. Price, P.; McMillan, T. J. *Cancer Res.* **1989**, *50*, 1392–1396.