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# Synthesis and antioxidant properties of pulvinic acids analogues

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ABSTRACT

The synthesis of three types of pulvinic acid analogues, using a diversity-oriented strategy starting from a single compound, dimethyl L-tartrate, is described. Lacey–Dieckmann condensation, alcohol dehydration and Suzuki–Miyaura cross-couplings were employed in the course of the analogues syntheses. The evaluation of the antioxidant properties of the 28 synthesized analogues was carried out using antioxidant capacity assays (protection of thymidine and β-carotene) and free radical scavenging assays (DPPH radical and ABTS radical cation). This allowed to assess the relative influence of the groups bonded to the tetronic ring and to the exocyclic double bond on the activity, as well as the importance of this exocyclic double bond. It was shown that the presence of an electron-donating group on the 3-position of the tetronic ring had a beneficial effect. It was shown in several assays that the presence of the exocyclic bond was not crucial to the activity.



## 1. Introduction

While oxygen is essential to life, its metabolism leads to the production of reactive oxygen species (ROS) which can be responsible for oxidative damages.[1] Under normal conditions, the concentrations of ROS are maintained at low levels by the action of the organism's defences, which may be enzymes such as superoxide dismutase or glutathione peroxidase, or small organic molecules such as ascorbic acid (vitamin C), tocopherols (vitamin E), and glutathione. These molecules, called antioxidants, are either produced by the organism or acquired through alimentation. The balance between the production and the elimination of ROS is thus normally controlled; however, when it is perturbed, oxidative stress is generated, leading to oxidative damages to biomolecules. Such damages have been associated with various pathologies, including Alzheimer's disease[2] and atherosclerosis,[3] and also, more generally, with ageing.[4] A treatment by antioxidants is potentially a way to overcome the oxidative stress, and then to improve the patient's condition.[5] Several studies have shown beneficial effects by antioxidant compounds such as ascorbic acid,[6] resveratrol,[7] and flavonoids.[8] Efforts have also been made to study synthetic antioxidants, which may be derived from naturally ones.[9]

In the course of a study aiming at evaluating the antioxidant activity of hundreds of compounds, a high-throughput screening test that made use of a competitive immunoassay technique was recently reported.[10] This test measured the protection of the DNA base thymidine submitted to three types of oxidative stresses. It allowed identifying norbadione A (**1**)[11,12] (Fig. 1), a pigment of the mushrooms *Xerocomus badius* (bay boletus) and *Pisolithus tinctorius*, as a potent antioxidant. It efficiently protected thymidine towards γ-irradiation, thus showing a potential activity as radioprotective agent, and also towards UV-exposure in the presence of $H_2O_2$. However, this product was found to be inefficient towards another oxidative stress, the Fenton-type oxidation, which was instead rather typical of a pro-oxidant activity.[13] Further studies showed that a structurally related pigment, di-*O*-methylatromentic acid (**2**), efficiently protected thymidine towards the three stress conditions, and did not show any pro-oxidant behavior. Compound **2** belongs to a larger pigment family typical of boletes, and also found in lichens, pulvinic acids, the simpler of which is pulvinic acid (**3**).[14] On the basis of these results, it was of interest to study the structure–activity relationship of various pulvinic acid analogues.

The pulvinic acid structure may be seen as a template to which many modifications can be made. Pulvinic acids are characterized by a tetronic acid moiety which is substituted by an aryl group and by a hydroxycarbonylalkylidene moiety. The natural pigments differ by the nature of the aryl groups, which are often hydroxylated.

We recently reported the antioxidant properties of analogues in which one of the aryl groups has been replaced by an alkyl group,[15] and now wish to disclose the synthesis and antioxidant activity evaluation of three types of pulvinic acid analogues (Fig. 2). The

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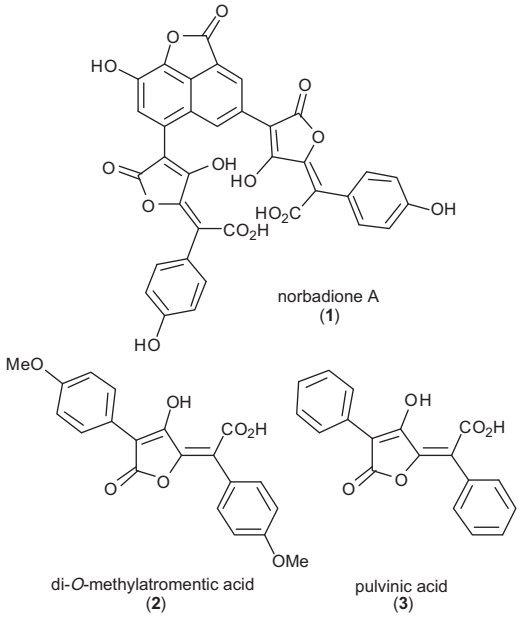



**Figure 1.** Norbadione A and pulvinic acids.

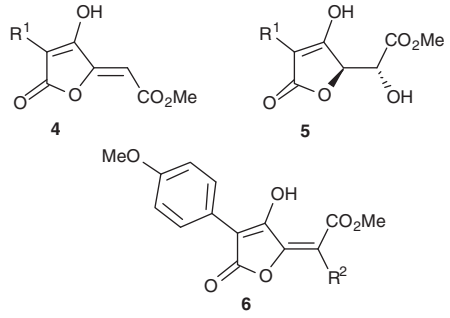



**Figure 2.** Structures of targeted pulvinic acids analogues **4**, **5** and **6**.

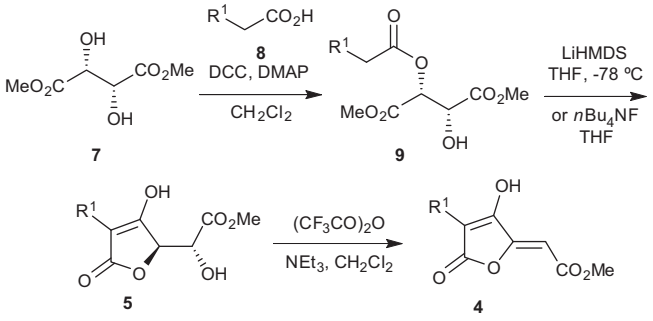
aim was in the first place to assess the relative effect on the activity of the group $R^1$, which is bound to the lactone ring, and the group $R^2$, which is placed on the exocyclic double bond; variously substituted compounds **4** and **6** would inform us on that matter. We also wished to evaluate the importance of the double bond by comparing the activities of compounds **4** versus **5**. Compounds **5** also present some analogies with ascorbic acid, which is a tetronic acid substituted by a 1,2-dihydroxyethyl group.

In this paper, we give a full account on the synthesis of nine alkenes **4**, ten tetronic acid derivatives **5**, and nine methyl pulvinates **6**, which were all obtained from a single precursor, dimethyl L-tartrate, using a strategy that we previously reported in a preliminary communication.[16] The antioxidant properties of these compounds were also evaluated, using two kinds of systems: the thymidine protection immuno-enzymatic assay and the β-carotene bleaching assay may be viewed as antioxidant capacity assays, while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method are free radical scavenging assays.

## 2. Results and discussion

### 2.1. Synthesis of analogues

Compounds **4** and **5** were prepared from dimethyl L-tartrate **7** as summarized in Scheme 1. In the first step, esterification of the




**Scheme 1.** Synthesis of tetronic acids **5** and alkenes **4**.

diol with acids **8** in the presence of dicyclohexylcarbodiimide (DCC) and catalytic 4-(dimethylamino)pyridine (DMAP) afforded the corresponding esters **9a–i** (Table 1).

Several conditions were tested in order to minimize the formation of diesters. The formation of diesters was limited to less than 20% by using 1 equiv of acid and 1 equiv of DCC; the reagents were added at 0 °C, and then the reaction mixture was stirred at room temperature. The diesters formed were readily removed by silica gel chromatography, and were not usually isolated. Esters **9a–i** were obtained in 38–67% yield.

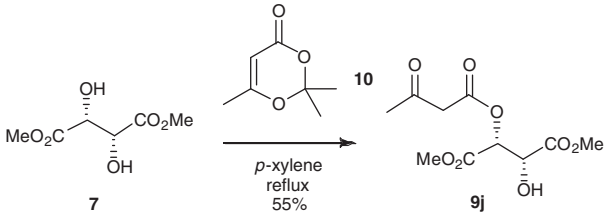
The acetoacetyl derivative of dimethyl L-tartrate **9j** was prepared using another method (Scheme 2). Thus, treatment of dimethyl L-tartrate with 2,2,6-trimethyl-1,3-dioxin-4-one **10**[17] in *p*-xylene at reflux afforded compound **9j** in 55% yield.

The preparation of tetronic acids via a Dieckmann condensation was first described by Lacey[18] and has since been frequently employed, especially in the course of syntheses of natural products containing a tetronic acid moiety.[19] Brandänge et al. have reported the Dieckmann condensation of dimethyl *O*-acetyltartrate using lithium hexamethyldisilazide as base.[20] Two conditions were employed for the cyclization of esters **9** to the corresponding tetronic acids **5**, depending on the nature of the substituent $R^1$ (Table 1). Compounds **5a–g** were obtained, in 42–79% yield, by treatment of the esters with 3 equiv of lithium hexamethyldisilazide

**Table 1**
Synthesis of esters **9** and tetronic acids **5**

| Entry | $R^1$ | Acid | **9** (% yield) | Base[a] | **5** (% yield) |
|---|---|---|---|---|---|
| 1 | 4-(MeO)$C_6H_4$ | **8a** | **9a** (65) | LiHMDS | **5a** (64) |
| 2 | Ph | **8b** | **9b** (67) | LiHMDS | **5b** (59) |
| 3 | 4-Me$C_6H_4$ | **8c** | **9c** (57) | LiHMDS | **5c** (70) |
| 4 | 4-Br$C_6H_4$ | **8d** | **9d** (59) | LiHMDS | **5d** (79) |
| 5 | 4-F$C_6H_4$ | **8e** | **9e** (52) | LiHMDS | **5e** (55) |
| 6 | 4-(BnO)$C_6H_4$ | **8f** | **9f** (62) | LiHMDS | **5f** (42) |
| 7 | 2-Thienyl | **8g** | **9g** (55) | LiHMDS | **5g** (54) |
| 8 | 4-($NO_2$)$C_6H_4$ | **8h** | **9h** (42) | $Bu_4NF$ | **5h** (60) |
| 9 | BnOC(O) | **8i** | **9i** (40) | $Bu_4NF$ | **5i** (58) |
| 10 | $CH_3C(O)$ | — | **9j**[b] | $Bu_4NF$ | **5j** (50) |

[a] Base employed for the conversion of compound **9** to tetronic acid **5**.
[b] Compound **9j** was prepared as described in Scheme 2.




**Scheme 2.** Synthesis of the acetoacetic ester **9j**.

(LiHMDS) in THF at −78 °C, according to the method described by Brandänge.

However, these conditions were not satisfactory for the preparation of tetronic acid **5h**, leading to degradation products. We then found that a mere treatment of esters **9h–j** with tetrabutylammonium fluoride[21] in THF at room temperature allowed the clean formation of the corresponding tetronic acids **5h–j**, obtained in 50–60% yield. In compounds **9h–j**, $R^1$ is an electron-withdrawing group (4-nitrophenyl, benzyl ester and acetyl); hence the adjacent methylene protons are more acidic and the use of a weaker base was possible. It is noteworthy that all the tetronic acids prepared were isolated as stereochemically pure isomers, indicating that no epimerization had occurred under the cyclization conditions. Hydrogenolysis of benzyl ether **5f** under usual conditions ($H_2$, 10% Pd/C, MeOH–DMF) afforded quantitatively the corresponding phenol **5k**.

Tetronic acids **5a–k** were notably characterized by the presence, in the $^1$H NMR spectra, of a doublet at 5.04–5.42 ppm, corresponding to the proton on the lactone ring, and a doublet at 4.78–5.00 ppm corresponding to the α-hydroxyl proton. Interestingly, in $^1$H NMR, compound **5j** appears as a mixture of two stereoisomers, due to an enolization of the acetyl carbonyl function (Fig. 3). For example the protons of the two methyl groups appear as singlets at $\delta = 2.57$ and 2.59 ppm.

Alcohols **5a–e**, **5g–k** were then converted to the corresponding alkenes in dehydrating conditions, using trifluoroacetic anhydride (3 equiv) and triethylamine (6 equiv) in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP), according to a previously reported method (Table 2).[22] However, the dehydration of ketone **5j** could not be obtained under these conditions and other methods tested also failed. For compound **5k**, which contains a phenol function, higher amounts of reagents have been employed.

While alkene **4i** was obtained as a pure *Z*-isomer, the other alkenes **4a–h** were obtained as mixtures of stereoisomers, in which the *E*-isomer accounted usually for 10–20%. The isomers were distinguished on the basis of the chemical shift of the ethylenic proton, which appears at upper field in the *E*-isomer (for compounds **4a**, $\delta_E = 6.15$ ppm, $\delta_Z = 5.91$ ppm). In each case, the two isomers were easily separated, and only the major, more polar *Z*-isomer was usually recovered. The yields of the reactions from compounds **3**, in which the aryl group is substituted either by an electron-withdrawing or electron-donating function, were generally good. However, lower yields were obtained from 2-thienyl derivative **5g** (37%) and from phenol **5k** (37%).

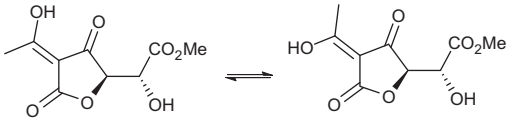



**Figure 3.** Structures of compound **5j**.

**Table 2**
Synthesis of alkenes **4a–h**

| Entry | $R^1$ | Tetronic acid | Alkene | Yield[a] (%) |
|---|---|---|---|---|
| 1 | 4-(MeO)$C_6H_4$ | **5a** | **4a** | 83 |
| 2 | Ph | **5b** | **4b** | 62 |
| 3 | 4-Me$C_6H_4$ | **5c** | **4c** | 83 |
| 4 | 4-Br$C_6H_4$ | **5d** | **4d** | 63 |
| 5 | 4-F$C_6H_4$ | **5e** | **4e** | 74 |
| 6 | 4-(HO)$C_6H_4$ | **5k** | **4f** | 44[b] |
| 7 | 2-Thienyl | **5g** | **4g** | 37 |
| 8 | 4-($NO_2$)$C_6H_4$ | **5h** | **4h** | 73 |
| 9 | BnOC(O) | **5i** | **4i** | 83 |

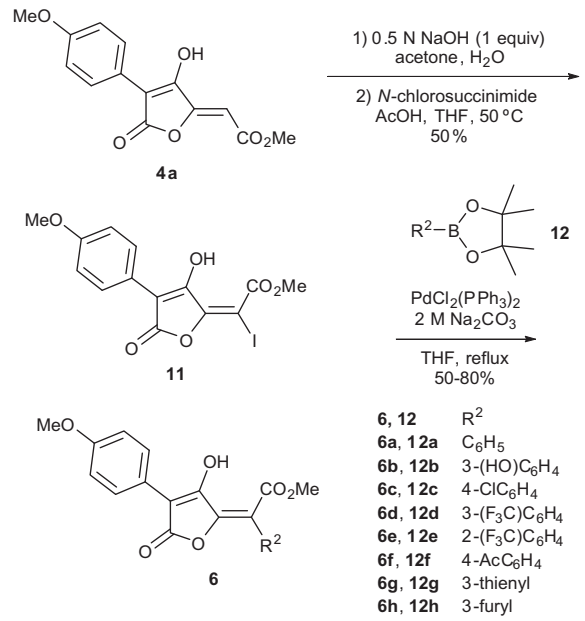
[a] Yield of isolated *Z*-isomer.
[b] 9 Equiv of $Et_3N$ and 4.5 equiv of $(CF_3)_2CO$ were employed.

In the case of alkene **4a**, the two isomers were fully characterized.[16] In infrared, a strong band at 2594 $cm^{-1}$, corresponding to a chelated hydroxyl, was observed for the *E*-alkene, while a strong band at 3524 $cm^{-1}$, due to a nonchelated hydroxyl, was observed for the *Z*-alkene.[23] It is then not surprising that the *E*-isomers, in which the hydroxyl group is chelated by the ester function, are distinctly less polar than the Z-isomers. Hence the synthetic sequence described allowed to prepare various compounds **4**, in which the tetronic acid moiety is substituted by a methoxycarbonylmethylene in position 5 and by various aryl and heteroaryl groups in position 3. The synthesis of the alkenes **4** was easily performed on a multi-gram scale.

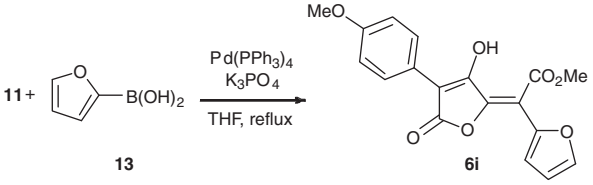
Several methyl pulvinates **6a–h**, in which a 4-methoxyphenyl group is attached to the tetronic acid moiety, and which contain various $R^2$ aryl or heteroaryl substituents were prepared from alkene **4a** as reported previously (Scheme 3).[16] The main features of their preparation were the iodination of alkene **4a** leading to iodide **11**, which was accompanied by an inversion of the double bond configuration, and the Suzuki–Miyaura cross-couplings carried out from iodide **11** and various boronates **12**, using the conditions reported by Occhiato et al.[24] It is worthy of note that these cross-couplings were carried out using iodide **11**, in which the enol function was left unprotected. The physical and spectroscopic characteristics of the known compound **6a** were in agreement with the reported values.[25]

Methyl pulvinate **6i**, which contains a 2-furyl group, was prepared according to a different procedure, using 2-furylboronic acid instead of the corresponding boronate.[26] The expected compound was isolated in 35% yield (Scheme 4).

Methyl pulvinates **6a–i** were thus conveniently prepared from readily available dimethyl L-tartrate, using a four-step flexible




**Scheme 3.** Synthesis of methyl pulvinates **6**.




**Scheme 4.** Synthesis of methyl pulvinate **6i**.

approach, characterized by the fact that no functional group protection was needed. The antioxidant properties of methyl pulvinates **6a–i**, tetronic acids **5a–e** and **5g–j** and alkenes **4a–h** were then evaluated as described below.

### 2.2. Antioxidant assays

A series of assays were then performed in order to evaluate the antioxidant properties of the synthesized compounds.[27] Firstly, the protection of thymidine towards three types of oxidative conditions (γ-rays, UV/$H_2O_2$, and Fenton oxidation) was determined using the previously described immuno-enzymatic assay.[10,13] Then, the radical scavenging properties of the compounds were studied using assays based on two oxidants, the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH·) and the radical cation of (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$). Finally the evaluation of β-carotene protection in a lipidic emulsion under oxidative conditions by the compounds was realized. In each case, reference compounds, that is, known antioxidant compounds were also tested.

#### 2.2.1. Protection of thymidine

The degradation of the DNA base thymidine under oxidative treatment is well known to lead to numerous compounds.[28] In this method, thymidine was oxidized under a variety of conditions, and the amount of remaining intact thymidine was determined owing to a competitive immuno-enzymatic assay, which makes use of a specific anti-thymidine monoclonal antibody.[10] The protection of thymidine by the synthesized compounds under three types of oxidative conditions are summarized in Table 3. The first oxidative condition involved an irradiation at 340 Gy, using a cesium-137 source; the second one consisted in a UV irradiation of a hydrogen peroxide solution $\lambda = 254$ nm, 1.75 J $cm^{-2}$); finally, Fenton conditions, a treatment with $Fe^{2+}$/EDTA and hydrogen peroxide, were also employed.

As previously stated, differences in the protection effect are expected to be observed, depending on the oxidation conditions.[13] Both the radiolysis of water and the homolytic cleavage of the oxygen-oxygen bond of hydrogen peroxide under UV irradiation lead to the formation of hydroxyl radicals, which would then react with thymidine. In these cases, compounds would efficiently protect thymidine by trapping first the hydroxyl radical or by breaking peroxidation chains. Regarding the Fenton system, it is supposed to generate, along with hydroxyl radicals, iron-containing oxidants.[29] In previous work, it was shown that under the Fenton conditions, the protective effects observed were significantly different from those obtained using the two other conditions.[13]

Most of the hydroxy esters **5** led to a good protection of thymidine in the test using γ-rays. Compound **5b** in which $R^1$ is a phenyl group gave a protection of 62%. Compounds **5d**, **5h**, and **5k** in which the aromatic ring is 4-substituted by a bromide, a nitro group and a hydroxyl function, respectively, led to similar results. In this series, compound **5j**, which is substituted by an acetyl group, was inefficient, while **5a**, which contains a 4-methoxyphenyl group, gave the best result. Alkenes **4** were also generally good protectors (over 50%, except for 4-tolyl derivative **4c** and benzyl ester **4i**). Among these alkenes, **4a**, which is substituted by a 4-methoxyphenyl group, was the most efficient. In general, there were not great differences in activity between an alkene **4** and the corresponding hydroxy ester **5** having the same $R^1$ group. Methyl pulvinates **6** are all substituted by the 4-methoxyphenyl group at the 3-position; however, they were usually found to be less efficient than alkene **4a**. Compounds **6g–i**, substituted by a heterocyclic ring on the exocyclic double bond ($R^2$), gave the best protection.

**Table 3**
Thymidine protection

| Compound | Thymidine protection (%) radiolysis[a] | Thymidine protection (%) UV/$H_2O_2$[b] | Thymidine protection (%) Fenton[c] |
|---|---|---|---|
| *Reference compound* | | | |
| Trolox | 5.2 ± 0.2 | 4.4 ± 1.0 | 11.7 ± 7.5 |
| *Hydroxy esters* | | | |
| **5a** | 87.4 ± 1.5 | 24.9 ± 0.2 | 25.8 ± 8.6 |
| **5b** | 62.0 ± 0.6 | 33.2 ± 2.0 | 31.0 ± 5.0 |
| **5c** | 42.7 ± 0.4 | 43.8 ± 14.7 | 7.0 ± 4.5 |
| **5d** | 60.2 ± 1.2 | 37.3 ± 1.3 | 37.6 ± 3.7 |
| **5e** | 39.0 ± 0.5 | 55.3 ± 1.0 | 55.2 ± 2.7 |
| **5f** | 37.4 ± 3.5 | 51.0 ± 1.4 | 21.1 ± 0.2 |
| **5g** | 40.3 ± 2.2 | 16.1 ± 3.6 | 0.0 ± 3.6 |
| **5h** | 60.9 ± 1.8 | 33.4 ± 0.7 | 70.7 ± 1.2 |
| **5i** | 47.9 ± 0.8 | 16.8 ± 1.9 | 2.6 ± 1.6 |
| **5j** | 5.6 ± 1.8 | 7.4 ± 2.3 | 0.0 ± 0.1 |
| **5k** | 63.7 ± 2.4 | 29.4 ± 1.9 | 93.4 ± 4.4 |
| *Alkenes* | | | |
| **4a** | 73.8 ± 0.8 | 30.7 ± 4.3 | 32.0 ± 5.0 |
| **4b** | 69.4 ± 2.9 | 37.7 ± 1.0 | 45.8 ± 6.1 |
| **4c** | 30.0 ± 0.1 | 52.7 ± 0.8 | 14.1 ± 3.2 |
| **4d** | 51.7 ± 0.5 | 41.2 ± 0.5 | 27.1 ± 0.5 |
| **4e** | 52.9 ± 1.0 | 69.3 ± 4.0 | 28.3 ± 0.7 |
| **4f** | 57.2 ± 3.4 | 32.6 ± 4.2 | 82.7 ± 2.1 |
| **4g** | 51.4 ± 3.0 | 23.6 ± 4.9 | 0.0 ± 0.6 |
| **4h** | 58.5 ± 1.7 | 31.5 ± 0.4 | 40.7 ± 0.4 |
| **4i** | 23.1 ± 2.6 | 13.9 ± 4.4 | 0.3 ± 0.4 |
| *Methyl pulvinates* | | | |
| **6a** | 46.4 ± 3.2 | 42.9 ± 1.7 | 58.0 ± 3.8 |
| **6b** | 34.2 ± 0.8 | 50.0 ± 0.1 | 91.6 ± 2.8 |
| **6c** | 22.6 ± 0.9 | 45.0 ± 1.0 | 41.7 ± 4.1 |
| **6d** | 46.3 ± 1.7 | 35.8 ± 2.1 | 33.9 ± 3.7 |
| **6e** | 17.6 ± 0.4 | 46.1 ± 3.4 | 14.8 ± 3.5 |
| **6f** | 34.3 ± 1.2 | 41.1 ± 1.2 | 41.1 ± 3.8 |
| **6g** | 76.0 ± 2.4 | 34.8 ± 0.5 | 45.2 ± 9.5 |
| **6h** | 77.0 ± 1.2 | 36.0 ± 6.2 | 13.8 ± 1.8 |
| **6i** | 62.0 ± 0.3 | 28.7 ± 1.6 | 0.0 ± 2.0 |

[a] Thymidine protection under γ-radiation exposure: [thymidine] = 15 μM, [compound] = 50 μM in 12.5 mM phosphate buffer, 340 Gy, source: $^{137}Cs$.
[b] Thymidine protection under UV/$H_2O_2$ exposure: [thymidine] = 70 μM, [compound] = 100 μM, [$H_2O_2$] = 5 mM in 12.5 mM phosphate buffer, $\lambda = 254$ nm, 1.75 J $cm^{-2}$.
[c] Thymidine protection under UV/$H_2O_2$ exposure: [thymidine] = 70 μM, [compound] = 100 μM, $Fe^{2+}$/EDTA/$H_2O_2$ (1:1:100) 640 mM in 11.4 mM phosphate buffer, 30 min. All experiments were performed in duplicate and averaged.

The results obtained for the thymidine protection when using the UV irradiation of a hydrogen peroxide solution reflects the same tendency. For example, compounds **5i** and **4i** were poorly efficient in both types of conditions. However the amplitude of the protection observed for the whole series of products was lesser than in the former tests. The best result (50.0%) was obtained for methyl pulvinate **6b**. It should be noted that the protection given by the methyl pulvinates **6** lies in a short range (about 30–50%).

In the Fenton-type oxidation, hydroxy esters **5c**, **5g**, **5i**, **5j**, bearing a $R^1$ group 4-tolyl, 2-thienyl, benzyl ester and acetyl, respectively, were found to be completely inactive, which may reveal a pro-oxidant activity for these compounds. On the contrary, 4-nitrophenyl derivative **5h** was very efficient, and phenol **5k** led to more than 90% protection. Alkenes **4g**, containing a 2-thienyl group, and **4i**, bearing a benzyl ester were inactive, as the corresponding hydroxy esters, while phenol **4f** had the best activity. Among the methyl pulvinates, compound **6b** in which $R^1$ is a 3-hydroxyphenyl group was the most efficient protector and phenyl-substituted **6a** was also quite efficient. Among these compounds, furan derivatives **6h** and **6i**, and 2-(trifluoromethyl)phenyl derivative **6e** were found to be poorly active (less than 15% protection).

In summary, the thymidine assays showed that the tested compounds which did not bear a $R^1$ aryl group displayed little activity. Usually, an electron-donating $R^1$ group was preferable, although 4-nitrophenyl-substituted **5h** protected efficiently thymidine against Fenton-type oxidation. Both 4-methoxyphenyl-substituted hydroxy ester **5a** and alkene **4a** were very active in the test involving γ-rays; it is noteworthy that compound **5a** protected thymidine as efficiently as norbadione A (84%).[10]

### 2.2.2. Scavenging activity of compounds for DPPH radical and ABTS radical cation

Both radicals DPPH· and ABTS·+ (Fig. 4) are frequently used to evaluate antioxidant activities of natural as well as synthetic compounds.

DPPH is a stable, commercially available radical, characterized by an absorption band at 515 nm. Its reduction by an antioxidant compound leads to a decolorisation which can be monitored by a spectrophotometer as described by Brand-Williams et al.[30] The assay was adapted in order to be performed in 96-well plates. For each compound, ten concentrations were employed and the percentage of remaining DPPH was determined at a steady state. The efficient concentration $EC_{50}$ or concentration necessary to decrease the initial DPPH· concentration by 50% (in mol/L of antioxidant/mol/L of DPPH·), was then obtained for each compound. The results, summarized in the Table 4, are expressed in terms of antiradical power (ARP = $1/EC_{50}$); the higher is this value, the more active is the tested compound.

Most compounds were able to scavenge the DPPH radical. Methyl pulvinates **6** were found to behave similarly, with ARP values in the range 2.97–4.08. Alkenes **4** were slightly less active, the more efficient compound being 4-methoxyphenyl derivative **4a**. 2-Thienyl derivative **4g** and 4-nitrophenyl derivative **4h** were inactive, as were hydroxy esters **5h**, **5i**, **5j**. Among the other hydroxy esters, phenyl-substituted **5b** and 4-fluorophenyl-substituted **5e** had the best activities.

The ABTS assay allows to measure the ability of an antioxidant compound to scavenge the radical cation ABTS·+, which is generated by oxidation of the ABTS salt with a strong oxidant such as potassium persulfate.[31] The reduction of the blue-green radical cation was monitored at 740 nm with a spectrophotometer. Several concentrations of compound were employed and for each concentration, the absorbance was measured until a steady state was reached. This allowed determining the $EC_{50}$ value (concentration necessary to decrease the initial absorbance at 740 nm by 50%) for each compound. The results were expressed in terms of TEAC (Trolox Equivalent Antioxidant Capacity: concentration of antioxidant that gives the same percentage change of absorbance of ABTS·+ as that of 1 mM Trolox).

Hydroxy esters led to results that varied largely, thus showing a strong effect of the nature of the $R^1$ group on the scavenging activity of these compounds. Derivatives containing 2-thienyl, 4-nitrophenyl and benzyl ester groups displayed only weak activities. Among the other compounds, those having a phenyl or an electron-donating $R^1$ group (compounds **5a**, **5b**, **5c**, and **5f**) led to TEAC between 1.54 and 2.16 mM. Phenol **5k** was found to be more active than Trolox. Alkenes **4** were usually less potent than the corresponding hydroxy esters **5** bearing the same substituent $R^1$.

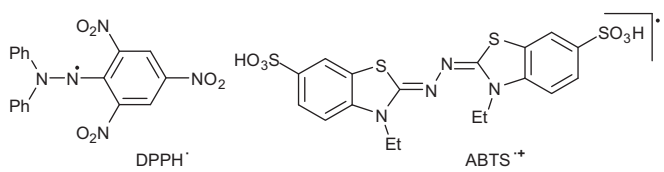



**Figure 4.** Structures of DPPH· and ABTS·+.

**Table 4**
Scavenging activity of compounds for DPPH radical and ABTS radical cation

| Compound | DPPH assay $1/EC_{50}$[a,b,c] | ABTS assay TEAC[d] (mM) |
|---|---|---|
| *Reference compounds* | | |
| Ascorbic acid | 4.56 | 0.64 ± 0.01 |
| Gallic acid | 11.72 | 1.36 ± 0.02 |
| Trolox | 4.88 | 1 |
| *Hydroxy esters* | | |
| **5a** | 3.44 | 1.55 ± 0.09 |
| **5b** | 3.57 | 1.94 ± 0.20 |
| **5c** | 3.27 | 1.54 ± 0.15 |
| **5d** | 2.81 | 4.91 ± 1.42 |
| **5e** | 4.20 | 2.94 ± 0.48 |
| **5f** | 3.12 | 2.16 ± 0.11 |
| **5g** | 2.25 | |
| **5h** | – | 24.63 ± 4.37 |
| **5i** | – | 43.34 ± 8.33 |
| **5j** | – | 82.69 ± 34.80 |
| **5k** | 3.00 | 0.60 ± 0.02 |
| *Alkenes* | | |
| **4a** | 3.60 | 1.31 ± 0.06 |
| **4b** | 3.22 | 46.19 ± 5.48 |
| **4c** | 2.56 | 6.51 ± 1.93 |
| **4d** | 1.17 | 17.71 ± 5.44 |
| **4e** | 1.97 | 15.40 ± 3.11 |
| **4f** | 2.85 | 0.48 ± 0.01 |
| **4g** | – | |
| **4h** | – | 18.70 ± 3.09 |
| **4i** | 0.93 | 18.51 ± 0.49 |
| *Methyl pulvinates* | | |
| **6a** | 3.20 | 1.67 ± 0.30 |
| **6b** | 3.68 | 1.56 ± 0.10 |
| **6c** | 3.37 | 3.97 ± 0.25 |
| **6d** | 4.08 | 2.75 ± 0.30 |
| **6e** | – | 12.11 ± 6.68 |
| **6f** | 3.27 | 6.18 ± 0.68 |
| **6g** | 2.97 | 2.69 ± 0.24 |
| **6h** | 3.35 | 1.06 ± 0.15 |
| **6i** | 3.42 | 1.16 ± 0.23 |

[a] DPPH radical scavenging capacity assay: [DPPH] = 100 μM in ethanol, [compound] = 0–1 $10^{-3}$ M in ethanol (10 concentrations), $\lambda$ = 515 nm; $EC_{50}$ = antioxidant concentration necessary to decrease the initial [DPPH·] by 50%; experiments were performed in duplicate.

[b] [DPPH]/$EC_{50}$ = antiradical power; the higher is this value, the more efficient is the antioxidant compound.

[c] No value is indicated in the cases where no steady state has been reached after 1 h.

[d] ABTS radical cation decolorisation assay: an ABTS·+ solution (absorbance = 0.70 ± 0.02 at $\lambda$ = 740 nm) and compound solutions of varying concentrations were mixed, then the absorbance was measured until a steady state is observed; the $EC_{50}$ (concentration necessary to decrease the initial absorbance at 740 nm by 50%) was determined; experiments were performed in triplicate; results are reported using TEAC.

However, 4-methoxyphenyl derivative **4a** had a TEAC of 1.31 mM and compound **4f**, which bears a phenol function, was more active than Trolox.

Among methyl pulvinates **6**, which all contain a 4-methoxyphenyl $R^1$ group, the furan derivatives **6h** and **6i** displayed an activity similar to that of Trolox. The other compounds were less good ABTS·+ scavengers; it should be noted that the products **6c**, **6d**, **6e**, and **6f**, having an electron-withdrawing $R^2$ group, were significantly less efficient.

In these assays, compounds bearing in the 3-position of the tetronic ring a phenyl nucleus substituted by an electron-donating substituent were then the best scavengers, and phenols **5k** and **4f** were especially efficient in the ABTS assay.

### 2.2.3. β-Carotene bleaching assay

It was of interest to evaluate the efficiency of the synthesized compounds as scavengers of oxygen-centered radicals such as lipoperoxyl radicals, which play an important role in the ROS-mediated degradation of membranes, hence in atherosclerosis.

In this assay, the reaction of β-carotene with linoleic acid oxidation products in an emulsified, aqueous system, induced a color change which was monitored spectrophotometrically, at $\lambda = 470$ nm, in the presence of an antioxidant (at two concentrations: 116 μM and 24 μM).[32–34] The percentage of protection of β-carotene was determined after 4 h for each compound. It should be noted that when the oxidation of β-carotene is higher in the presence of an antioxidant than in a control experiment in the absence of compound, then the protection is negative. Selected results obtained for well-known antioxidants and for the most active compounds are summarized in Figure 5.

Among the tested compounds, methyl pulvinate **6f**, which contains a 4-acetylphenyl $R^2$ group, was the most efficient, comparable to vitamin E at 116 μM, but more active at 24 μM. Interestingly, the theoretical value of log *P*[35] for **6f** (0.54) was much lower than that of vitamin E (9.98). The partitioning of the tested compound between the lipophilic and the hydrophilic phases is an important factor to be considered in this assay, since a lipophilic antioxidant compound should be more able to scavenge the lipoperoxyl radicals. For example, hydrophilic ascorbic acid does not lead to any protection, unlike vitamin E. Hence, methyl pulvinates **6**, which are generally more lipophilic than hydroxy esters **5** and alkenes **4**, could be expected to protect β-carotene with more efficiency. Methyl pulvinate **6e**, having a 3-(trifluoromethyl)phenyl group (log *P* = 2.15), also gave a good protection at 116 μM. The other compounds that led to a protection better than 44% at this concentration are hydroxy ester **5b**, in which $R^1$ is a phenyl group, and alkene **4a**.

### 2.2.4. Discussion on antioxidant assays results

The utilization of several antioxidant tests having different characteristics was expected to afford indications on the relative properties of the three classes of compounds that were prepared, which can be viewed as analogues of pulvinic acids. The test that used thymidine as the target then informs on the capacity to protect a DNA base from oxidation, and specific informations can also be obtained from the different stresses employed in this assay. The DPPH and ABTS assays are frequently employed for the assessment of antioxidant properties of products and were thus relevant in this study. Finally, the β-carotene bleaching assay is a good way to mimic a protection effect of biological membrane. This study allowed to evaluate the respective influences of the modified substituents $R^1$ and $R^2$, and also to assess the importance of the presence of an exocyclic double bond.

The observed results are in agreement with the assumption previously made[15] that the antioxidant capacity of the pulvinic acids comes from the possibility, for the radical initially formed on the enol function oxygen atom, which is deprotonated at neutral pH, to be delocalize on the aromatic nucleus present at the 3-position of the lactone (Scheme 5). The stabilization of the radical does not appear to be sufficient when this aromatic group is replaced by a carbonyl function (acetyl or benzyl ester).

The difference of activity, in tests such as the thymidine protection against γ-radiations and the DPPH assay, between hydroxy esters **5** and the corresponding alkenes **4** bearing the same $R^1$ group was usually not very important. This may show that the presence of an exocyclic double bond is not essential to the compounds activity, at least in these tests. Hence the presence of a tetronic acid to which an aryl group is bonded in the 3-position would be the essential feature for the antioxidant property. However, more discrepancies were observed in the results obtained from the corresponding **4** and **5** products in the test involving the Fenton oxidation or the ABTS radical scavenging.

With regard to methyl pulvinates **6**, a comparison with alkene **4a**, which lacks a $R^2$ substituent, shows that the $R^2$ group does not necessary lead to an increase of activity in most assays. However, it is noteworthy that the more efficient compounds in the β-carotene bleaching assay were two methyl pulvinates **6e** and **6f**; the increased lipophilicity of these compounds as compared to hydroxy esters **5** or alkenes **4** may be accounted for this outcome.

Among hydroxy esters **5**, which are simpler analogues of pulvinic acids, the most active compound in the thymidine protection assay under γ-radiation exposure, **5a**, contained the electron-donating 4-methoxyphenyl $R^1$ substituent. The corresponding alkene **4a** was also very active in this test, as well as in the DPPH assay. Finally, hydroxy ester **5k**, which is substituted at the 3-position by a 4-phenol group, performed well in most assays, including the thymidine protection under Fenton oxidation.

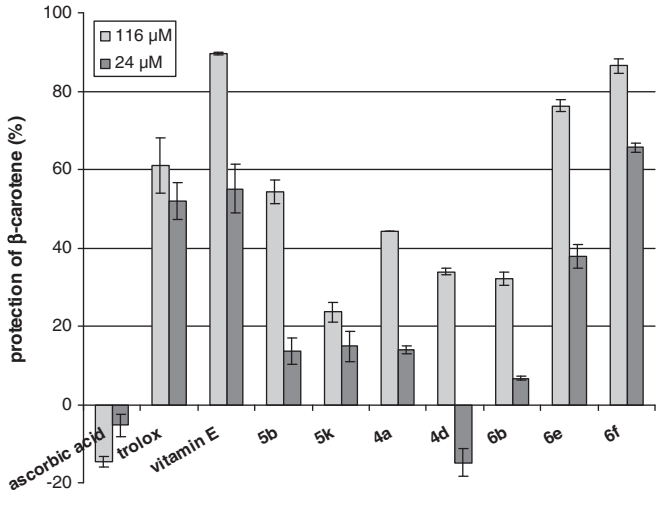
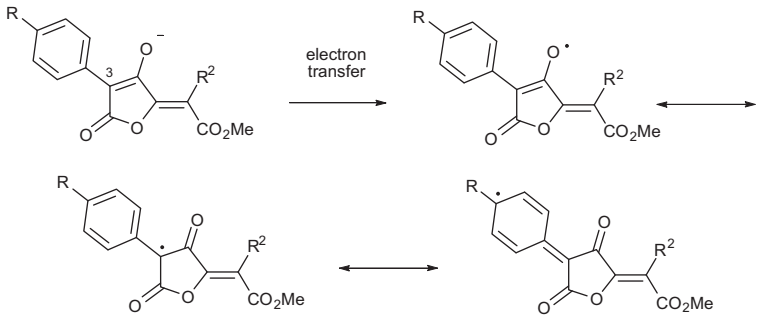



**Figure 5.** β-Carotene bleaching assay: aliquots (250 μL) of an emulsion prepared from β-carotene, linoleic acid and Tween 40 emulsifier in oxygen-saturated water were added to 50 μL of pure ethanol antioxidant compound solution at two concentrations (116 μM and 24 μM) and placed at 50 °C for 4 h. The absorbance was measured at 470 nm, and the percentage of protection of β-carotene was determined after 4 h; experiments were performed in triplicate and averaged.

## 3. Conclusions

We have reported the flexible synthesis of three types of pulvinic acids analogues, all derived from a single starting material, dimethyl L-tartrate. Various tetronic acids, containing a hydroxy ester moiety, were prepared by Lacey–Dieckmann condensation of the corresponding esters. Dehydration of these hydroxy esters then readily afforded the corresponding *Z* configurated alkenes. Differently substituted methyl pulvinates were obtained via Suzuki–Miyaura cross-couplings involving a vinyl iodide and several boronates.

Evaluation of the antioxidant properties of the synthesized compounds was then carried out using a series of tests. Most compounds have displayed a good activity. A beneficial effect of an electron-donating group at the 3-position of the tetronic acid moiety was observed. Methyl pulvinates **6** were found to be more efficient in the β-carotene bleaching assay, in accordance with their more lipophilic characteristics, as compared to the other derivatives. Compounds **4** and **5** bearing a 4-methoxyphenyl or a 4-hydroxyphenyl group were found to be very efficient in most assays. These derivatives appear very interesting for further studies owing to their good antioxidant activity. In particular, evaluation of the anti-inflammatory and radioproprotective properties of these compounds will be investigated. Lastly, it should be pointed out that structural modulations can easily be performed, as the synthesis of similar compounds can be readily achieved using the strategy presented here.




**Scheme 5.** Mechanism of pulvinic analogues oxidation.

## 4. Experimental section

### 4.1. General methods

THF was freshly distilled from sodium benzophenone ketyl. Reactions were performed under an argon atmosphere. TLC: Silica Gel $60F_{254}$ plates with detection by UV light and by an acidic ethanolic solution of phosphomolybdic acid. Column chromatography: 40–63 μm silica gel. Melting points were uncorrected. NMR: 400.133 and 100.624 MHz for $^1H$ and $^{13}C$, respectively. Chemical shifts ($\delta$) are in ppm (s = singlet, d = doublet, t = triplet, m = multiplet, b = broad), coupling constants ($J$) are in Hz.

### 4.2. General procedure for the synthesis of esters 9

Dicyclohexylcarbodiimide (11.58 g, 56.13 mmol) and 4-(dimethylamino)pyridine (2.74 g, 22.45 mmol) were added simultaneously to a solution of 4-methoxyphenylacetic acid (9.33 g, 56.13 mmol) and (+)-dimethyl L-tartrate (10 g, 56.13 mmol) in dry dichloromethane (400 mL) cooled at −18 °C. The solution was stirred at room temperature for 12 h. The resulting mixture was concentrated under vacuum to approximately 150 mL. Diethyl ether (300 mL) was then added and the precipitate obtained was filtered. The filtrate was washed with a saturated aqueous $NH_4Cl$ solution (3 × 100 mL), then dried ($MgSO_4$), filtered and concentrated under vacuum. Silica gel chromatography (80:20–70:30 cyclohexane/ethyl acetate) afforded ester **9a** (11.9 g, 65%) as a colorless oil.

### 4.3. Dimethyl (2*R*,3*R*)-2-hydroxy-3-(2-(4-methoxyphenyl)acetoxy)succinate (9a)

Colorless oil; $[\alpha]_D^{20}$ +29.8 (*c* 1.00, MeOH); IR (film) $\nu_{max}$: 3500, 3008, 2957, 2841, 2361, 2341, 1747, 1613, 1586, 1514, 1436, 1360, 1247, 1135, 1070, 1030, 980, 860, 822, 792, 729, 638, 588, 569 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.18 (2H, d, $J$ = 8.4 Hz, $CH_{Ar}$), 6.86 (2H, d, $J$ = 8.4 Hz, $CH_{Ar}$), 5.44 (1H, d, $J$ = 2.2 Hz, CHOC(O)), 4.74 (1H, d, $J$ = 2.2 Hz, C*H*OH), 3.80 (3H, s, $OCH_3$), 3.79 (3H, s, $OCH_3$), 3.65 (2H, s, $CH_2Ar$), 3.62 (3H, s, $OCH_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 170.9, 170.6, 167.0, 158.8, 130.4, 125.3, 114.1, 73.1, 70.4, 55.3, 53.1, 52.9, 39.8; HRMS (ESI-TOF) calcd for $C_{15}H_{18}NaO_8$ $[M+Na]^+$ 349.0899, found 349.0913.

### 4.4. General procedure for the synthesis of α-hydroxy esters 5

*Synthesis of compound* **5b**: A solution of LiHMDS (19.1 mL, 1 M in THF) was added to THF (32 mL) under nitrogen. The solution was cooled to −78 °C and then a solution of compound **9b** (1.62 g, 5.47 mmol) in THF (18 mL) was added dropwise. The reaction mixture was stirred for 1 h at −78 °C and then allowed to warm to room temperature over 2 h. After cooling at 0 °C, aqueous 2 N HCl (40 mL) was added. The two layers were separated, and the aqueous layer was extracted with AcOEt (2 × 60 mL). The combined organic layers were dried ($MgSO_4$) and filtered and concentrated under vacuum. Silica gel chromatography (89:10:1–70:30 $CH_2Cl_2$/MeOH/AcOH) afforded α-hydroxy ester **5b** (858 mg, 59%) as a white solid.

### 4.5. Methyl (*R*)-2-hydroxy-2-((*R*)-3-hydroxy-5-oxo-4-phenyl-2,5-dihydrofuran-2-yl)acetate (5b)

White solid; mp 188–190 °C; $[\alpha]_D^{20}$ +150.4 (*c* 1.0, MeOH); IR (KBr pellet) $\nu_{max}$: 3294, 3058, 3023, 2956, 2761, 1760, 1736, 1667, 1599, 1499, 1424, 1328, 1256, 1238, 1168, 1128, 1058, 1013, 883, 833, 765, 753, 699, 682 $cm^{-1}$; $^1H$ NMR (400 MHz, acetone-$d_6$) $\delta$ 7.93 (2H, d, $J$ = 7.4 Hz, $CH_{Ph}$), 7.37 (2H, t, $J$ = 7.4 Hz, $CH_{Ph}$), 7.26 (2H, t, $J$ = 7.4 Hz, $CH_{Ph}$), 5.31 (1H, d, $J$ = 1.8 Hz, CHOC(O)), 4.81 (1H, d, $J$ = 1.8 Hz, C*H*OH), 3.82 (3H, s, $OCH_3$); $^{13}C$ NMR (100 MHz, acetone-$d_6$) $\delta$ 172.2 (2C), 172.0, 131.2, 128.8, 128.2, 127.8, 102.8, 78.6, 69.6, 52.9; HRMS (ESI-TOF) calcd for $C_{13}H_{12}NaO_6$ $[M+Na]^+$ 287.0532, found 287.0530.

### 4.6. General procedure for the synthesis of alkenes 4

Triethylamine (2.2 mL, 15.9 mmol) was added to a suspension of α-hydroxyester **5b** (700 mg, 2.65 mmol) and DMAP (16.2 mg, 0.13 mmol) in dry dichloromethane (160 mL) cooled at −10 °C. Trifluoroacetic anhydride (0.75 mL, 7.95 mmol) was added dropwise over 15 min. The reaction mixture was then allowed to warm to room temperature. After stirring for 16 h, 3 N HCl (3 mL) was added. After stirring for 1 h at room temperature, the two layers were separated, and the aqueous layer was extracted with AcOEt (2 × 10 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. Silica gel chromatography (90:10, then 50:50, then 30:70 cyclohexane/acetone) afforded alkene (*Z*)-**4b** as an orange solid (410 mg, 62%).

### 4.7. Methyl (*Z*)-2-(3-hydroxy-5-oxo-4-phenylfuran-2(5*H*)-ylidene)acetate (4b)

Orange solid; mp 210–212 °C; IR (KBr pellet) $\nu_{max}$: 3230, 3100, 2961, 1771, 1702, 1661, 1641, 1439, 1404, 1373, 1257, 1215, 1184, 1136, 1026, 960, 900, 843, 786, 772, 695, 597 $cm^{-1}$; $^1H$ NMR (400 MHz, acetone-$d_6$) $\delta$ 7.91–7.88 (2H, m, $CH_{Ph}$), 7.47–7.42 (2H, m, $CH_{Ph}$), 7.39–7.35 (1H, m, $CH_{Ph}$), 5.95 (1H, s, C*H*$CO_2Me$), 3.76 (3H, s, $OCH_3$); $^{13}C$ NMR (100 MHz, acetone-$d_6$) $\delta$ 167.8, 164, 162.1, 153.1, 129.6, 129.2 (2C), 129.1, 105.7, 96.2, 52.0; HRMS (ESI-TOF) calcd for $C_{13}H_{10}NaO_5$ $[M+Na]^+$ 269.0426, found 269.0427.

### 4.8. General procedure for the Suzuki–Miyaura cross-coupling

All the solvents were degassed. To a solution of iodide **11** (150 mg, 0.373 mmol) in THF (19 mL) were added $Pd(PPh_3)_2Cl_2$ (13 mg, 0.019 mmol, 5 mol %), a solution of 3-hydroxyphenylbo-

ronic acid pinacol ester **12b** (123 mg, 0.559 mmol, 1.5 equiv) in THF (7 mL), and 2 M aqueous $Na_2CO_3$ (8.2 mL). The reaction mixture was refluxed for 2 h under argon. After cooling to room temperature, water (5 mL) and aqueous 3 N HCl (3 mL) were added. The aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated under vacuum. Silica gel chromatography (70:30 cyclohexane/AcOEt) afforded **6b** as a yellow solid (100 mg, 73%).

### 4.9. Methyl (*E*)-2-(3-hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-2-(3-hydroxyphenyl)acetate (6b)

Yellow solid; mp 180–181 °C; IR (KBr pellet) $\nu_{max}$: 3366, 2958, 2839, 2408, 1746, 1671, 1600, 1475, 1439, 1374, 1310, 1280, 1254, 1182, 1070, 838 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 13.56 (1H, s, OH), 8.12 (2H, d, $J$ = 9.1 Hz, $CH_{Ar}$), 7.27 (1H, t, $J$ = 7.9 Hz, $CH_{Ar}$), 6.97 (2H, d, $J$ = 9.1 Hz, $CH_{Ar}$), 6.86 (1H, ddd, $J$ = 7.9, 2.4, 0.9 Hz, $CH_{Ar}$), 6.81 (1H, ddd, $J$ = 7.9, 1.6, 0.9 Hz, $CH_{Ar}$), 6.74 (1H, dd, $J$ = 2.4, 1.6 Hz, $CH_{Ar}$), 3.87 (3H, s, $OCH_3$), 3.85 (3H, s, $OCH_3$); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 171.7, 166.4, 159.7, 158.7, 155.4, 155.1, 133.5, 129.5 (3C), 122.6, 121.7, 117.2, 115.8, 115.0, 114.1 (2C), 105.5, 55.4, 54.5; HRMS (ESI-TOF) calcd for $C_{20}H_{16}NaO_7$ $[M+Na]^+$ 391.0794, found 391.0793.

### 4.10. Antioxidant assays

#### 4.10.1. Thymidine protection assay under γ-rays, UV/$H_2O_2$ exposure, and Fenton oxidation

These procedures have been previously described.[10,13]

#### 4.10.2. DPPH radical scavenging capacity assay

The DPPH· method of Brand-Williams et al.[30] was modified for this assay. The assay was performed in a 96-well plate. A 200 μM solution of DPPH· was prepared in pure ethanol. In each well were added 100 μL of a sample at varying concentrations in ethanol (0–1 $10^{-3}$ M) and 100 μL of the DPPH· solution. Samples were prepared in duplicate, and ten different concentrations were employed. The plate was read every 5 min at 515 nm for a period of 1 h, using a spectrophotometer SpectraMax Plus$^{384}$ (Molecular Devices). For each concentration, a kinetic curve was plotted. The percentages of remaining DPPH· at the steady state were determined. Hence, the measurement of antioxidant activity was possible only for compounds for which the kinetics of reaction made it possible to reach a steady state before 1 h. Then for each compound, a curve showing the percentage of remaining DPPH· versus the molar ratio of antioxidant to DPPH· was plotted, allowing the determination of the $EC_{50}$ values ($EC_{50}$ antioxidant concentration necessary to decrease the initial DPPH· concentration by 50%). As recommended by Brand-Williams et al., the results are presented in the terms of antiradical power (ARP = $1/EC_{50}$).

#### 4.10.3. ABTS radical cation decolorisation assay

A 7 mM solution of ABTS salt in water was added to a 140 mM solution of potassium persulfate to obtain a final concentration of 3.5 mM. The mixture was stirred at room temperature in the dark for one night to form ABTS radical cation. Before use, the solution was diluted in pure ethanol so as to obtain an absorbance of 0.70 (0.02) at 740 nm, at 30 °C (Multiskan FC, Thermofischer microplate lector). The resulting solution was stable when maintained in the dark. An $ABTS^{\cdot+}$ solution (100 μL) was added to solutions of the compound (200 μL in pure ethanol) at variable concentrations. For each concentration, the absorbance was measured until a steady state was observed. The percentage of reduction of $ABTS^{\cdot+}$ was calculated according to the following equation: $Q = (A_0 - A_C)/A_0 \times 100$, where $A_0$ = initial absorbance, without the tested compound, and $A_C$ = measured absorbance. All determinations were made in triplicate and then averaged. A curve representing the percentage of reduction of $ABTS^{\cdot+}$ as a function of the concentration of tested compound was then drawn. This allowed to determine the $EC_{50}$ (concentration for which the initial absorbance at 740 nm decreased by 50%). For greater clarity, the results will be presented on charts using the term TEAC (Trolox Equivalent Antioxidant Capacity; the lower is this value, the more active is the compound).

#### 4.10.4. β-Carotene bleaching assay

The method described by Marco[32] and modified by Miller[33] and Taga et al.[34] was adapted for a microplate lector. In a flask were introduced 2 mL of a 2 g $L^{-1}$ dichloromethane solution of β-carotene), 90 mg of linoleic acid and 240 mg of Tween 40 emulsifier. The solution was then mixed and evaporated under argon flow. Oxygen-saturated water (50 mL) was added to the residue and then the solution was vortexed for 30 s. Aliquots (250 μL) of this emulsion were transferred to microplates well containing 50 μL of pure ethanol antioxidant compound solution. A zero reading was taken at 470 nm immediately after adding the emulsion to the antioxidant solution. The reaction was thermostated at 50 °C and the kinetics was plotted over 4 h. The last point of the measure was taken after 4 h. (Multiskan FC, Thermofischer microplate lector).

The antioxidant activity was expressed by the percentage of inhibition of β-carotene bleaching with regard to the control by the following equation: $P = [1 - (A_0 - A_t)/(A_{c0} - A_{ct})] \times 100$, where $A_0$ is the initial absorbance, $A_t$ is the absorbance after 4 h, $A_{c0}$ is the initial absorbance without antioxidant compound, $A_{ct}$ is the absorbance after 4 h without antioxidant compound. All determinations were made in triplicate and then averaged. The evaluation was performed using two concentrations (24 μM, 116 μM) of tested compound.

## Acknowledgments

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## Supplementary data

Supplementary data (physical and spectroscopic characteristics of synthesized compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.09.037.

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35. log *P* = logarithm of the partition coefficient (octanol/water); log *P* indicated are theoretical values obtained using ChemDraw software.