Evaluation of Antioxidant Properties of Monoaromatic Derivatives of Pulvinic Acids

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The natural mushroom pigment Norbadione A and three other pulvinic acids were shown by our group to display very efficient antioxidant properties by comparison with a collection of potent molecules including catechols, flavonoids, stilbenes, or coumarins. Despite numerous publications on robust and straightforward synthetic access to pulvinic acids by us and others, no report has been made to unravel the structure−activity relationships that govern the striking antioxidant activity. Herein is presented the synthesis of 18 diverse pulvinic acid derivatives and the study of their radical scavenging capacities by four different assays. The influence of each of the two phenyl rings, of their substituents and of the lateral chain on the antioxidant properties, was explored to reveal a simplified structure of excellent activity. These results, along with the absence of cytotoxicity, make the synthesized compounds interesting to evaluate for several biological activities and especially for anti-inflammatory effects and skin protection against UV induced oxidative stress.

Introduction

Reactive oxygen species (ROS)[a] are natural products of metabolic processes in an aerobic environment and can be generated by different pathways.[1,2] Examples of ROS species are hydrogen peroxide, hydroxyl radicals, or superoxide anion. They are produced by sources such as bioreductively activated molecules, UV exposure, or by low-molecular-weight complexes of transition state metals such as iron via the Fenton reaction. Organisms can normally defend themselves against these highly reactive species using enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, or by nonenzymatic mechanisms.[2,3] The latter involve organic molecules called antioxidants, such as vitamin C, vitamin E, or glutathione, capable of scavenging the oxidant species by hydrogen donation or breaking of an oxidation propagation chain.[4] Imbalances in the detoxification of ROS relative to their production, due to either an abnormal production of ROS or depletion of antioxidant defenses lead to the commonly called *oxidative stress* state. For many years, this state has been known to be implicated in the etiology of a large number of diseases and disorders[2,5] such as cancer,[6] coronary heart disease,[7] or rheumatoid arthritis.[8] Enhanced free radicals production and oxidative damage are also implicated in aging[1a] and related neurodegenerative diseases such as Alzheimer's,[9] Parkinson's,[10] or Huntington's diseases.[11] Thus finding therapeutic agents that can scavenge ROS is a high priority in medical research today, and many therapeutic strategies using antioxidant have already been reported in the literature or patented with promising in vivo results.[12] In this context, our group described a high-throughput screening method to search for antioxidant molecules in natural extracts. This strategy is based on competitive immunoassay techniques to rapidly evaluate the ability of different extracts or compounds to protect thymidine under different oxidative stresses.[13,14] Norbadione A (NBA) (Figure 1, left), a mushroom pigment isolated from the two mushrooms *Xerocomus badius* and *Pisolithus tinctorius*,[15] was shown to be a potent protector of thymidine under both γ irradiation and UV exposure in the presence of H_2O_2. Unfortunately, the pro-oxidant effect of NBA was observed on a plasmid-DNA under the oxidative conditions of a Fenton-like system ($FeSO_4/H_2O_2$/EDTA). Three structurally related pulvinic acids and especially di-*O*-methylatromentic acid (Figure 1, right) were then evaluated and shown to display antioxidant properties higher than that of a collection of flavonoids and similar to NBA. Furthermore, the ROS-scavenging properties of di-*O*-methylatromentic acid were confirmed in DNA protection experiments, showing no pro-oxidant effect. This pulvinic acid possesses a single hydroxyl function, which makes its structure peculiar by comparison with other potent antioxidants that were assayed. Indeed, the high efficiency of

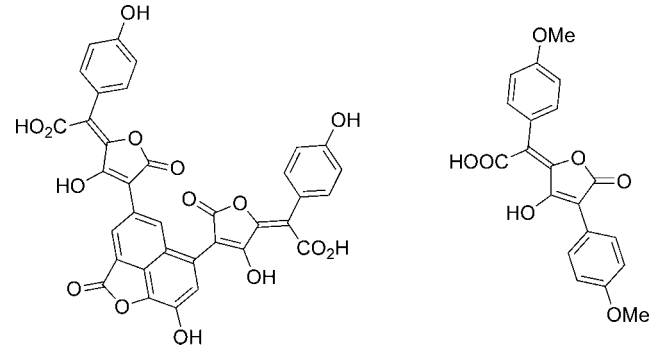

Figure 1. Structure of Norbadione A (left) and di-*O*-methylatromentic acid (right).

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† Deceased June 2nd, 2007. This communication is dedicated to the memory of Charles Mioskowski who initiated this work.

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[a] Abbreviations: ROS, reactive oxygen species; NBA, norbadione A; dThd, thymidine.

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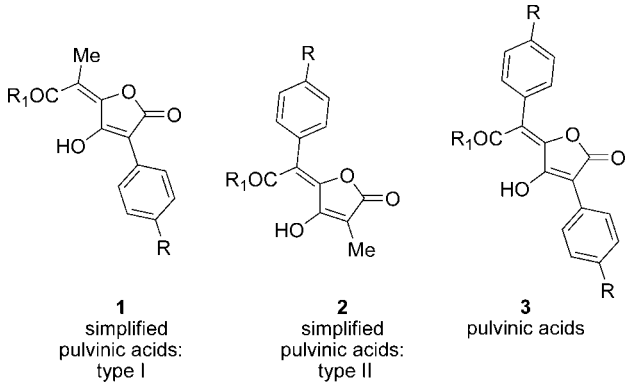


Figure 2. General structure of pulvinic acids and monoaromatic targeted molecules.

flavonoids,[16] stilbenes,[17] or coumarine derivatives[18] is known to arise from their polyhydroxylated structures.

Therefore, pulvinic acid derivatives could represent original and promising candidates as new antioxidant agents. This prompted us to further investigate this class of compounds. To efficiently explore structure−activity relationships (SARs), we based the design of the pulvinic derivatives on the known essential parameters that govern antioxidant activity of phenolic compounds. Especially it is well established that an extended aromatic structure is favorable in order to stabilize the oxygen radicals formed. For this reason, simplified analogues bearing only one aromatic ring such as type I and II compounds were designed (Figure 2). Aromatic ring substitution on phenols, particularly in the *ortho* and the *para* position, also plays a crucial role in the O−H bond dissociation enthalpy (BDE) as well as in the ionization potential (PI).[19] In the present study, the *para* substitution exploration was preferred for synthetic ease (Figure 2). Finally, variations of the lateral function R_1 in pulvinic derivatives should allow us to explore a possible effect of the intramolecular hydrogen bond between the enolic function and the carbonyl group.

We describe herein 12 examples of type I compounds, four examples of type II molecules, and two examples of symmetric pulvinic acids as well as their ROS-scavenging ability with regard to the study of the protection of thymidine and DNA and the scavenging of the superoxyde anion, along with cytotoxicity results.

Chemistry

The synthetic route followed to obtain type I compounds **1a**−**f** is summarized in Scheme 1. Addition of zinc enolate of methyl propionate on methoxymaleic anhydride **4**[20] occurred regioselectively on the carbonyl adjacent to the methoxy group, as described by Pattenden on 2-aryl-3-methoxymaleic anhydrides,[21] to give alcohol **5** (51%) as a mixture of two diastereoisomers (70/30 ratio determined by ^{1}H NMR). Dehydratation of **5** using trifluoroacetic anhydride under basic conditions yielded alkene **6** (90%) in a 90/10 mixture of *E*- and *Z*-isomers.[22] The *E*-isomer could be isolated by column chromatography, and the following transformations were performed on this isomer. Compound **6** was iodinated using iodine and cerium(IV) ammonium nitrate[21] to give **7** (94%). Then a poly ionic gel supported Pd catalyst,[23] reported in our group, was used to perform Suzuki−Miyaura cross-coupling reactions with various boronic acids. This heterogeneous catalyst allowed the efficient transformation of **7** into pulvinates **8a**−**f** (58−78%). Finally, deprotection of compounds **8** using 1 equiv of boron tribromide yielded the type I products **1a**−**f** (58−85%). Interestingly, in the case of pulvinate **8b**, deprotection of the methyl enol ether was achieved in 65% yield without significant deprotection of the aromatic methoxy group, as described on pinastric acid.[24]

Type II products **2a** and **2c** were prepared as shown in Scheme 2. Tetronic derivative **9** was prepared using described procedures[25] and protected as benzyl ether under Mitsunobu conditions (74%). Deprotonation of **10** using LDA and subsequent addition of ketoesters **11** provided alcohols **12** (66−69%), which were dehydrated using the conditions described above, albeit in lower yields (18−44%, nonoptimized). For **13a**, only the *E*-isomer was obtained, while for **13c**, both *E* and *Z* isomers were obtained as a mixture (ratio 75/25 by NMR), the *E*-isomer being isolated by column chromatography. *E*-**13** were then subjected to hydrogenolysis, affording type II products **2a** and **2c** (48 and 66% yield, respectively).

Then, the monoaromatic pulvinic dilactone **14**, whose synthesis has previously been described by our group,[26] was used in order to modify the nature of the *exo*-cyclic double-bond functionalization (Table 1). By heating under reflux an alcoholic solution of **14**, the nonregioselective opening of the dilactone moiety led to a separable mixture of type I and type II ester adducts. Using methanol, type I product **1b** was obtained in 54% yield, type II adduct **2b** in 44% yield (Table 1, entry 1); similar results were obtained using isopropyl alcohol, affording products **1g** and **2d** (Table 1, entry 2). Using other nucleophiles, type I adducts could be obtained selectively. With dibutylamine and morpholine, the TBAF-mediated ring opening of **14**[26] gave access to type I amides **1h** and **1i**, respectively, in excellent yields (Table 1, entries 3 and 4). In the absence of TBAF, the opening of **14** by 1,3-diaminopropane and *N*,*O*-dimethylhydroxylamine led solely to type I adducts **1j** and **1k** in good yields (Table 1, entries 5 and 6, respectively). Such regiospecificity in the absence of TBAF by comparison with the reactivity of simple amines is not explained yet. Finally, using methyllithium (Scheme 3), only type I alcohol **1l** could be isolated with a good yield. We assume this product arose from a two-step sequence: (1) nucleophilic opening of **14** by a first equivalent of methyl anion, leading to the formation of a ketone, (2) attack of a second equivalent of methyl anion on the ketone.

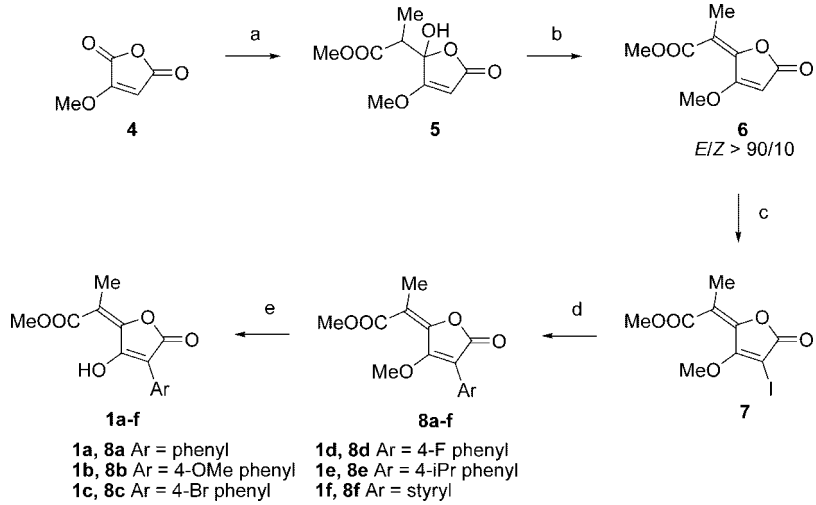
Symmetrical pulvinic acid derivatives **3a**,**b** were obtained by following procedures described by Le Gall and Mioskowski (Figure 3).[27] Interestingly, both type I adducts **1** and symmetrical pulvinic derivatives **3** are striking yellow or orange solids, while type II products **2** are pale-yellow or white solids.

Biological Results

The antioxidant activity of the synthesized pulvinic derivatives was determined using four different assays. Activity was first measured using immunoenzymatic assays, based on the study of the degradation of thymidine, under either UV-exposure or Fenton stress. The compounds were also tested for the protection of plasmidic DNA under Fenton stress. Finally, the radical scavenging abilities of superoxide anion radical were studied as well as the cytotoxicity of these new products. Structures of the tested molecules are summarized in Table 2.

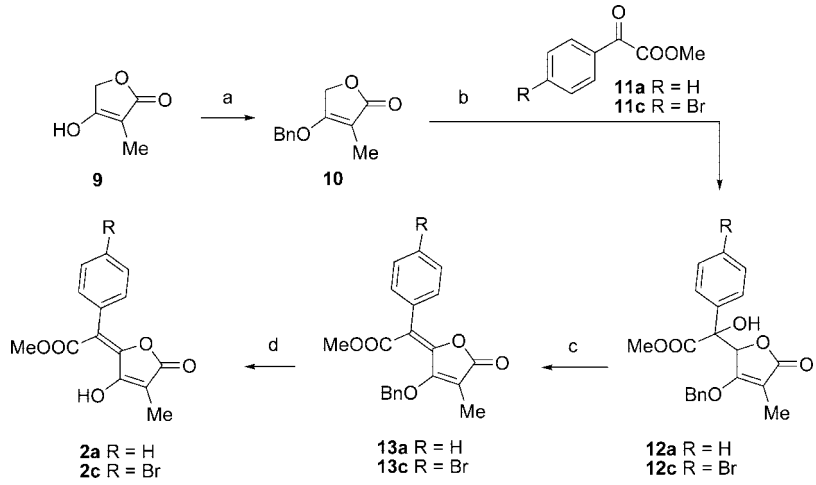
Protection of Thymidine. This test is based on the degradation of thymidine (dThd) by oxidative stress under aerobic conditions.[13,14] The unmodified thymidine remaining after this degradation step is quantified by a competitive enzyme immunoassay by using a specific antithymidine antibody. Two oxidative conditions were tested, using either a UV irradiation at 254 nm (1.75 J/cm^2) of a hydrogen peroxide solution buffered at physiological pH (Figure 4A) or a Fenton-like system, that is, in the presence of Fe^{2+}/EDTA and hydrogen peroxide (Figure

Scheme 1[a]



[a] Reagents and conditions: (a) Methyl propionate, LDA, $ZnCl_2$, THF, −78 °C, 45 min then **4**, 1 h; (b) TFAA, Et_3N, DMAP, DCM, 0 °C to rt, 12 h; (c) I_2, CAN, MeCN, 40 °C, 3 h; (d) $ArB(OH)_2$, supported Pd catalyst, iPr_2NH, MeCN, H_2O, 85 °C, 6 h 30 min; (e) BBr_3, DCM, 0 °C, 2 h.

Scheme 2[a]



[a] Reagents and conditions: (a) BnOH, DIAD, PPh_3, THF, rt, 12 h; (b) LDA, THF, −78 °C, 45 min then **11**, 1 h; (c) TFAA, Et_3N, DMAP, DCM, 0 °C, 12 h; (d) H_2, Pd/C, DCM, rt, 12 h.

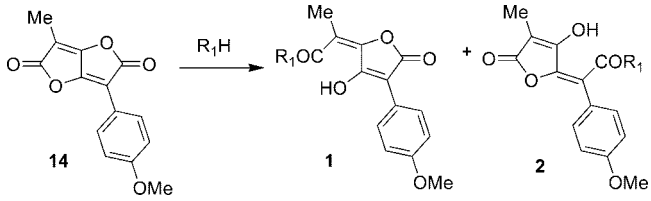
4B). Type I and type II products were evaluated, as well as pulvinic acids, NBA and Trolox, as a reference antioxidant. Each compound was assayed twice at a single concentration, either 100 μM for the UV/H_2O_2 test or 500 μM for the Fenton test. The screening results presented in Figure 4 were confirmed by the determination of EC_{50} values for six selected compounds: **1b**, **1h**, **1l**, **2a**, **2b**, and **3b**. The EC_{50} values were found to be in very good agreement with the data presented in Figure 4 (see Supporting Information for EC_{50} values and dThd protection data).

The differences in protections observed between the dThd assays under UV/H_2O_2 and Fenton-like conditions reflect the differences in the oxidative species generated in the system. Indeed, in the UV/H_2O_2 test, $HO^{\bullet}$ radicals, generated by homolytical cleavage of the oxygen−oxygen bond of H_2O_2 under UV irradiation, are probably the main species responsible for the thymidine degradation. Therefore and as previously discussed,[13] the protective effects measured by this assay are closely related to the $HO^{\bullet}$ radical-scavenging abilities of the tested compounds. On the other hand, in the case of the Fenton test, the formation of high-valence iron−oxo species is expected, along with other radical species.[28] Some molecules can strengthen the Fenton-like oxidative system and therefore display a pro-oxidant behavior, which can not be evaluated in the UV test.[14,15] These differences are clearly highlighted by our references, NBA and Trolox, which exhibit opposite activities in these two conditions (NBA being highly active in the UV/H_2O_2 test and not in the Fenton test, Trolox active in the Fenton test and not in the UV/H_2O_2 test). Indeed NBA is reported to be a potent $HO^{\bullet}$ scavenger but also to display pro-oxidant properties in the presence of iron.[13,14]

The observation of good protections in both tests for many of the assayed compounds, especially type I derivatives **1** and symmetrical pulvinic acids **3**, demonstrate that these molecules are efficient protectors in different oxidative stress conditions and indicate that they probably do not display pro-oxidant properties. These are important properties for future in vivo use. Compound **1h** displays similar protections to that of the two reference molecules, NBA in the UV/H_2O_2 test and Trolox in the Fenton test.

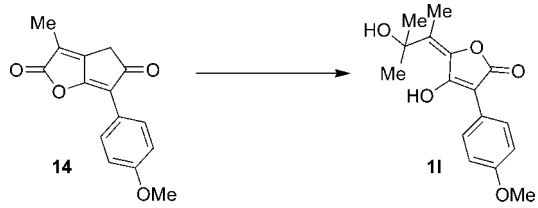
Concerning the three main series of pulvinic derivatives, the two tests demonstrate that type II compounds **2** are the less efficient protectors; good to excellent protections are obtained with type I molecules **1** and similar or slightly higher protections are observed with symmetrical derivatives **3**. Inside the type II series, almost no influence of the variation of the aromatic ring

Table 1. Ring Opening Reaction of **14**



entry	conditions	R_1	products (isolated yield)
1	Methanol, reflux, 12h	OCH_3	**1b** (54%) / **2b** (44%)
2	*Iso*-Propanol, reflux, 12h	$OCH(CH_3)_2$	**1g** (58%) / **2d** (40%)
3	Butylamine (2.5 equiv), TBAF (2 equiv), THF, -35 to -78 °C, 1h	$NH(CH_2)_3CH_3$	**1h** (83%)
4	Morpholine (2.5 equiv), TBAF (2 eq), THF, -35 to -78 °C, 1h		**1i** (96%)
5	1,3-diaminopropane (1 equiv), DCM, rt, 12h	$NH(CH_2)_3NH_2$	**1j** (64%)
6	MeNHOMe.HCl (1 equiv), Et_3N (1 equiv), DCM, 12h	$N(CH_3)OCH_3$	**1k** (90%)

Scheme 3[a]



[a] Reagents and conditions: MeLi (2 equiv), THF, −78 °C, 1 h.

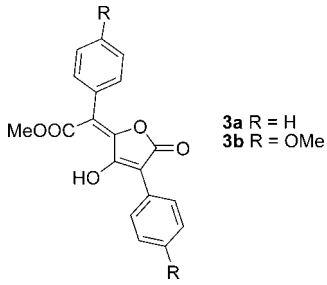


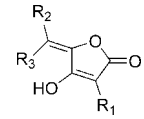
Figure 3. Structures of symmetrical pulvinic acids **3**.

substitution or the ester nature is seen because compounds **2a**−**d** show similar results in each of the two dThd protection tests.

In the type I family, both dThd-based assays show similar trends for the influence of the *para* aromatic substitution (**1a**−**1f**, Figure 4). Indeed, by comparison with the nonsubstituted **1a** compound, only the *para*-methoxy substituent of **1b** allows conservation or slight improvement of the dThd protection. The *para* aromatic substitution by a halogen (**1c** and **1d**), an alkyl (**1e**), or a vinyl chain (**1f**) decreases the protection effects on both UV/H_2O_2 and Fenton tests.

Variations of the substitution of the *exo*-cyclic double bond of type I compounds (**1g**−**1l**, Figure 4) lead also to important variations in the protections, which are similarly observed in both tests. Changing the methyl ester of **1b** into an isopropyl ester (**1g**) reduces to a large extent the protection efficiencies (from 37% to 27% in the UV/H_2O_2 test and from 88% to 37% in the Fenton test, Figure 4). Amides products **1h**−**1k** displayed

Table 2. Tested Pulvinic Derivatives



	substituent		
compd	R_1	R_2	R_3
1a	Ph	Me	CO_2Me
1b	4-OMe Ph	Me	CO_2Me
1c	4-Br Ph	Me	CO_2Me
1d	4-F Ph	Me	CO_2Me
1e	4-iPr Ph	Me	CO_2Me
1f	styryl	Me	CO_2Me
1g	4-OMe Ph	Me	$CO_2CH(CH_3)_2$
1h	4-OMe Ph	Me	$CONH(CH_2)_3CH_3$
1i	4-OMe Ph	Me	$CON(CH_2CH_2)_2O$
1j	4-OMe Ph	Me	$CONH(CH_2)_3NH_2$
1k	4-OMe Ph	Me	$CON(CH_3)OCH_3$
1l	4-OMe Ph	Me	$C(CH_3)_2OH$
2a	Me	Ph	CO_2Me
2b	Me	4-OMe Ph	CO_2Me
2c	Me	4-Br Ph	CO_2Me
2d	Me	4-OMe Ph	$CO_2CH(CH_3)_2$
3a	Ph	Ph	CO_2Me
3b	4-OMe Ph	4-OMe Ph	CO_2Me

protection activities in the same range, namely 30−48% in the UV/H_2O_2 test and 37−98% in the Fenton test. However, in both assays, the best protection results were obtained with the *n*-butyl amide **1h**. Finally, the tertiary alcohol **1l** exhibited in both assays a slightly lower potency than that of the best type I compounds tested.

Symmetrical pulvinic derivatives **3a**,**b** are very potent protectors in both tests and equivalent to the best type I compound **1h**.

In conclusion, results of UV/H_2O_2 and Fenton dThd protection tests showed that the type I and symmetrical pulvinic derivative tested are potent $HO^{\bullet}$ scavengers as well as potent protectors against an iron-based oxidative system.

Protection of Plasmid-DNA under Fenton Stress. To check that the previous results were not limited to the simple thymidine structural features, the protection of a supercoiled plasmid DNA under Fenton-type oxidation and in the presence of selected compounds was investigated. In the absence of protective agents and under the optimized oxidative conditions, supercoiled DNA (Figure 5, lane 1) is degraded, leading essentially to the formation of linear DNA (Figure 5, lane 4, only small fractions of supercoiled and circular DNA could be observed).

The protection efficiencies of the tested compounds were characterized by the detection of significant remaining amounts of supercoiled plasmid DNA after oxidation. As previously reported, NBA displayed an opposite effect because it induced a higher degree of plasmid degradation, which is an indication of its pro-oxidant effect (Figure 5, lane 5−8).[14] The symmetrical derivative **3b** and the type I product **1b** efficiently protected DNA from degradation (Figure 5, lane 9−12 and 13−16). Moreover, a positive dose−response was observed, as the proportion of supercoiled DNA increased with the concentration of products tested. On the other hand, reaction performed in the presence of type II product **2b** did not show any significant protection of DNA (Figure 7, compare lanes 4 and 17−20).

A larger set of compounds was studied for the DNA protection under the same oxidative conditions (Figure 6). Protectors were assayed at a single concentration (125 μM), and results were expressed as the remaining % of supercoiled DNA form (by light intensity integration of the three forms of

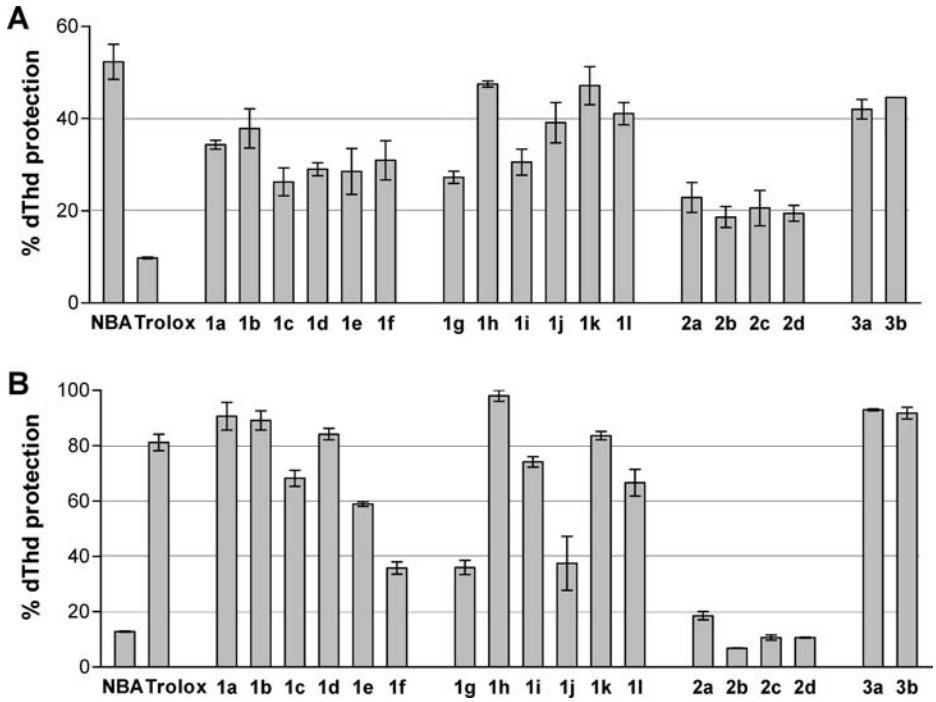


Figure 4. (A) Protective effects of pulvinic derivatives under UV exposure: dThd protection assays. [dThd] = 70 μM, [antioxidant] = 100 μM, H_2O_2 = 5 mM in phosphate buffer 25 mM (pH 7.4), 254 nm, 1.75 J/cm^2. Experiments were performed in duplicate. (B) Protective effects of pulvinic derivatives under Fenton stress: dThd protection assays. [dThd] = 70 μM, [antioxidant] = 500 μM, Fe^{2+}/EDTA/H_2O_2 (1:1:100) 700 mM in phosphate buffer 25 mM (pH 7.4), 30 min. Experiments were performed in duplicate. Results are expressed in percent thymidine protection, calculated by comparing results obtained in the presence and in the absence of protective agent.

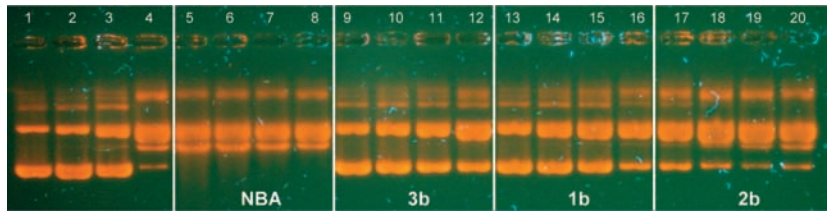


Figure 5. DNA protection by pulvinic derivatives under Fenton stress. Oxidations of supercoiled plasmid pEGFPLuc (lane 1) were carried out in the presence of 0.4 mM $Fe^{II}SO_4$/EDTA/H_2O_2 (1:1:10) for 30 min. Control experiments were performed without iron (lane 2) or without H_2O_2 (lane 3). Oxidation of DNA in the absence (lane 4) or in the presence of four concentrations (500, 250, 125, 62.5 μM respectively) of antioxidants NBA (lanes 5−8), **3b** (lanes 9−12), **1b** (lanes 13−16), and **2b** (lanes 17−20). Linear, circular, and supercoiled DNA were separated by agarose gel electrophoresis and stained with ethydium bromide.

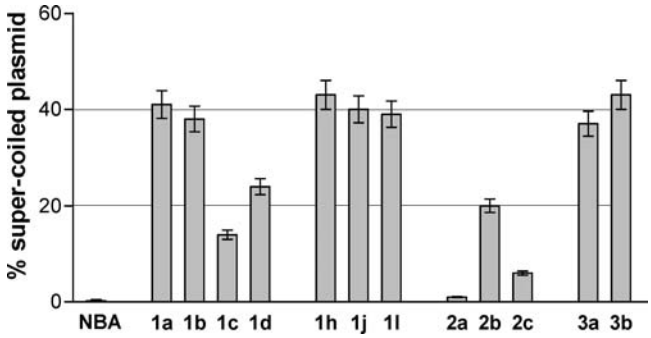


Figure 6. Protection of plasmidic-DNA by pulvinic derivatives (125 μM) under Fenton stress. The percentage of supercoiled plamid was calculated by light intensity integration of the three forms of DNA in electrophoresis gels: supercoiled, circular, and linear.

DNA in electrophoresis gels: supercoiled, circular, and linear). Results are in very good agreement with the Fenton dThd protection test because type II compounds **2a**−**2c** were found to be far less efficient protectors than type I **1a**−**1l** and symmetrical pulvinic derivatives **3a**,**b**, which were of similar potencies. For type I products, halogen-substituted derivatives were less efficient than the nonsubstituted and the methoxy-substituted products (Figure 6, compare products **1c** and **1d** to **1a** and **1b**) and the substitution of the *exo*-cyclic double bond did not seem play a crucial role in this test, all products **1b**, **1h**, **1j**, and **1l** exhibiting around 40% of protection.

Briefly, protection of thymidine and of plasmid DNA showed the same trend, indicating that type I products **1** were as efficient as the symmetrical pulvinic acids **3**. Type II products **2** protected poorly these two biological targets. No pro-oxidant effect was detected for types I and II and symmetrical compounds.

Scavenging of Superoxide Anion. The first product of univalent reduction of oxygen is the superoxide anion $O_2^{\bullet -}$. It is generated in many biological processes,[29,30] therefore compounds able to scavenge $O_2^{\bullet -}$ could be of great interest. To assess the activity of our pulvinic derivatives for the scavenging of $O_2^{\bullet -}$, photoluminescence assays were performed using the Photochem technique with standard kits ACL (Analytik Jena AG).[31,32] In these experiments, $O_2^{\bullet -}$ is generated by optical excitation (at 351 nm) of a methanolic solution of luminol used as a photosensitizer; the same molecule is used as the radical

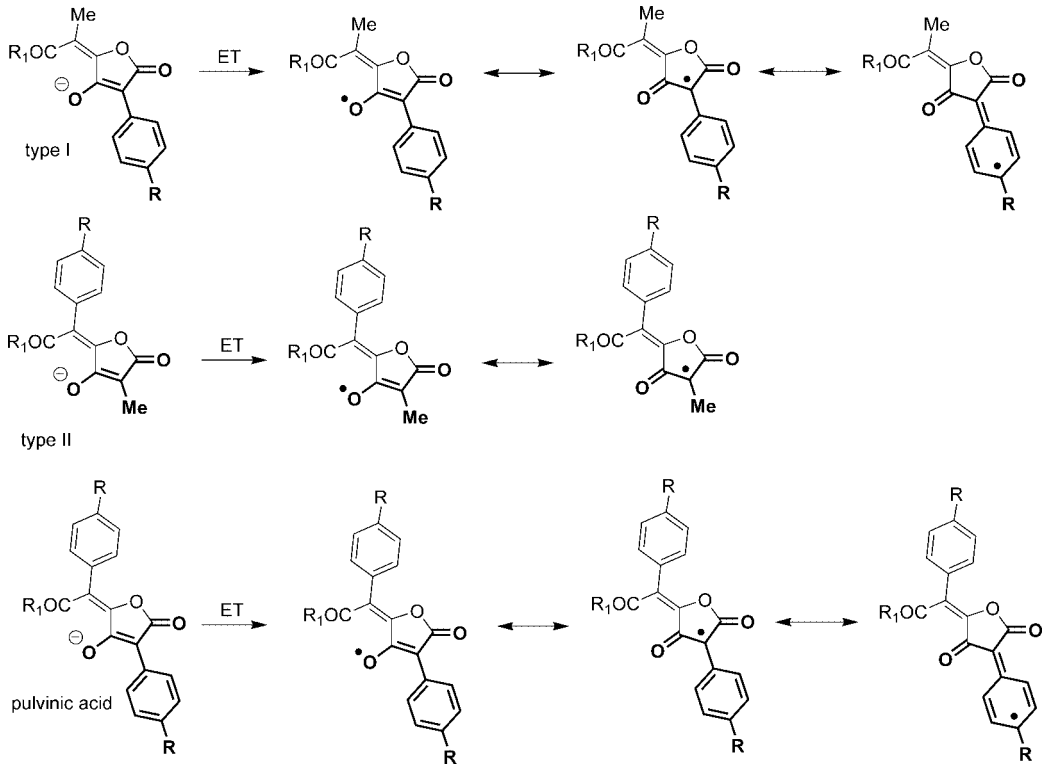


Figure 7. Suggested mechanism for the monoelectronic oxidation of pulvinic derivatives.

Table 3. Scavenging of Superoxide Anion

compd	Trolox equiv (μg)
1b	141
1c	113
1g	105
1h	86
1i	79
1j	81
2c	87
2d	77
3b	54

detecting agent by luminol-enhanced chemiluminescence.[33] Results were calculated as Trolox equivalent, which serves as a reference for this test (Table 3). Expressed values correspond to μg quantities of the tested compound needed to reach the protection equivalent to 1 μg of Trolox.

Representative compounds of each type I, type II, and symmetrical pulvinic acids were selected for this test. All tested compounds are of similar efficiency but are less potent than Trolox (in the range of 60−140 Trolox equivalents). Nevertheless, the scavenging of superoxide anion was detected in all cases. Contrary to previous tests, no clear distinction is seen here between the three series of type I, type II, and symmetrical derivatives: **1b** and **3b** on one hand and **1g** and **2d** on the other hand being of equivalent activity. The variation of the *exo*-cyclic double bond substitution has also no influence on the measured $O_2^{\bullet -}$ scavenging properties (**1b** and **1g**−**1j**, Table 3).

Cytotoxicity. The in vitro toxicity of the newly synthesized compounds was then measured on CHO cells using the MTT colorimetric assay.[34] Compounds were tested at 10, 30, and 100 μM, and none of the products, including type I products **1** (**1a, 1c**, **1i**, **1h**, **1l**), type II **2** (**2a**, **2d**), and symmetrical **3** (**3b**) showed any noticeable toxicity with respect to the control culture.

Discussion

In this section, the various protection results will be compared and a mechanistic hypothesis will be provided. Interestingly, results obtained with the two thymidine protection assays and the plasmid-DNA protection assay are very similar, demonstrating that the tested compounds retain their activity in different oxidative stress conditions and toward different biological targets. The three screening procedures clearly showed that type I and symmetrical pulvinic derivatives display similar antioxidant properties that are much higher than that of type II molecules. This observation is supported by data in Figure 4 and Figure 5 for the two sets of molecules **1a**−**3a** and **1b**−**3b**.

We propose a simple model that allows rationalizing these results (Figure 7). The first radical attack on phenolic antioxidant is known to proceed via different mechanisms.[35] The first main type of mechanism involves a hydrogen atom transfer (HAT) or a proton coupled electron transfer (PCET). The second main type of oxidative mechanism involve a sequential proton loss electron transfer (SPLET) and is known to take place under specific reaction conditions when the phenolic antioxidant can be deprotonated in the medium (Figure 7).

In the SPLET mechanism, the deprotonated phenolic function usually reacts by electron transfer with the oxidative species, faster than it does through a regular HAT mechanism. In the experimental conditions used for the thymidine and DNA protection assay (water buffered at physiological pH), and given the reported pK_a values for the enolic protons of pulvinic acids,[36] the deprotonation of the enol is very favorable, and consequently a SPLET mechanism should be preferred. Under these hypotheses, a mechanistic model is suggested in Figure 7 (starting from the deprotonated enols) in order to rationalize the results obtained in the thymidine and DNA protection assays. The prediction of simple mesomer forms give the indication that the enol radicals generated on type I and symmetric derivatives can be stabilized by delocalization on the β-cetoester fragment of the lactone and on the south aromatic ring. In the case of type II molecules, the delocalization is reduced because of the absence of the south aromatic ring, which suggests that the formation of this radical is less favorable.

Thus the model also clarifies why the three type II compounds **2a**−**2c**, differentiated by various *para* substituents on the north aromatic ring, have similar and low activities in the two dThd tests because this part is not expected to be involved in the first radical reaction.

On the contrary, according to the model, the *para* substitution of the south aromatic ring in type I compounds has an influence on the protection activity. This is experimentally observed with compounds **1a**−**1f** on the dThd and plasmid DNA tests. Indeed, we observed that a positive mesomer effect in the *para* position (methoxy group, **1b**) allows conservation or slight improvement of the activity compared to the nonsubstituted compound **1a**. By comparison, a positive inductive effect (*iso*-propyl group, **1e**), the presence of a halogen (**1c** and **1d**), or the styryl substitution (**1f**) is not favorable. To the best of our knowledge, no study has described so far the influence of the substitutions of the aromatic ring on the efficiency of the radical trapping via a SPLET mechanism. However, our results are relatively similar to studies on the HAT mechanism, where the influence of the substitution of aromatic rings was related to the strength of the BDE on the O−H bond of phenols. A *para*-methoxy is known to stabilize the phenoxyl radical by conjugative electron delocalization and to lower the BDE,[19a] while a *para*-halogen strengthens it.[37] *Para*-alkyl group are described to lower the BDE but less effectively than *para*-methoxy.[38] Contrarily, in our case, compound **1e** shows less satisfactory protection potency than the nonsubstituted product **1a**.

The influence of the substitution of the *exo*-cyclic double bond on the antioxidant properties (**1g**−**1l**), which is clearly exhibited in the dThd assays (Figure 4), is not explained by the proposed model. These variations could modify the solubility properties of the molecules or influence the intramolecular hydrogen bond between the enolic proton and the carbonyl and thus the pK_a values. Nevertheless, the variations of activity highlight that this is a key structural feature that needs to be optimized in order to design a potent molecule. Despite a fine understanding, the modification of the *exo*-cyclic double bond offered the most active compound tested in the dThd and DNA protection assays, namely the amide **1h**.

The complementary assay for the inhibition of $O_2^{\bullet-}$ was used to determine whether the studied compounds are general free radical scavengers or scavengers specific for the superoxide anion.[39] In several cases, a particular reactivity is associated to superoxide anion compared to other ROS because of its lower reactivity.[40] Only moderate $O_2^{\bullet-}$ scavenging activities were detected for our compounds in this assay, and the SAR main features highlighted in the previous tests were not observed (Table 3). Thus the radical trapping mechanisms on pulvinic derivatives in this assay might differ from those happening in the dThd and DNA protection assays. Indeed, we could expect SPLET to electron rich superoxide anion to be less favorable than to the electrophilic hydroxyl radicals. Under such hypothesis, an HAT mechanism should be preferred but not expected to be highly efficient for the pulvinic compounds because of the presence of strong hydrogen bonding between the reactive enolic hydrogen and the carbonyl function in pulvinic acids. Such particular intramolecular hydrogen bonds are typical of pulvinic derivatives and are present on each compound from series **1**, **2**, and **3**, which could explain the similar results obtained for all pulvinic derivatives tested in the $O_2^{\bullet-}$ scavenging test. Finally, the observed high potency of Trolox for the scavenging of $O_2^{\bullet-}$ could as well be due to a more efficient HAT phenomenon compared to pulvinic acids. Indeed the phenolic proton in Trolox is not involved in an intramolecular hydrogen bond and thus is more prone to be abstracted by the oxidative species in the solution.

Complementary experiments are necessary in order to determine the precise antioxidant mechanisms involved in the observed protection of thymidine, plasmid-DNA, and superoxide anion by pulvinic derivatives. These studies will be performed on selected compounds and will be published in due course.

Finally, the cytotoxicity results are very encouraging as a first step before in-depth studies, as all tested compounds exhibited the same pattern of harmlessness on CHO cells.

Conclusion

From two previous accounts by our group, pulvinic acids appeared to be very potent antioxidant agents, however very little was known about the SAR that dictated this interesting activity. To rapidly elucidate the main features of the SAR, our strategy was to design simplified analogues of these natural products. Following several synthetic strategies, 18 very diverse pulvinic acids derivatives were successfully synthesized. These molecules are divided into three structural categories: type I and type II, which are monoaromatic derivatives, and the symmetrical pulvinic derivatives.

Studies conducted on thymidine and plasmid DNA demonstrated that type I and symmetrical derivatives are very potent protectors of these biological targets against ROS (as efficient as NBA) and a Fenton-type oxidative system. No pro-oxidant effect was detected in the presence of iron, as it was observed for the natural pigment, norbadione A. Because the type II molecules were less potent, it can be concluded that the antioxidant properties rely on the lactone part and the south aromatic ring of pulvinic acids. SAR conclusions also highlight the influence of the *para* substituent of the south aromatic ring and of the substitution of the *exo*-cyclic double bond of pulvinic acids. Among the newly synthesized molecules, compound **1h** was found to be the most active molecule.

Our results open the route to the synthesis of simplified pulvinic acids such as type I molecules, with finely tuned physicochemical properties (molecular weight, solubility) by modulation of the phenyl and the *exo*-cyclic double bond substitutions that will retain or even improve the antioxidant properties of natural pulvinic acids. Moreover, the first demonstration of the noncytotoxicity of type I compounds encourage the study of biological applications of these molecules. Currently, amphiphilic pulvinic acid derivatives of type I are being synthesized and studied as skin protectors via anti-inflammatory effects or ROS scavenging. These dermocosmetic applications will be reported in due course.

Experimental Section

Chemistry. Flash column chromatographies were performed with silica gel (40−63 μm). Nuclear magnetic resonance spectra (^{1}H and ^{13}C) were recorded on a 200 or 300 MHz spectrometer equipped with a DUAL probe. Melting points were uncorrected. For GC-MS experiments, low-resolution mass spectra (chemical ionization) and gas chromatography retention times were recorded using a capillary column (25 mm × 0.22 mm) SGE BPX5 (5% phenyl polysilphenylenesiloxane/95% methylpolysiloxane) with helium (29 mL/min; 113 kP_a) and the following temperature conditions: interface temperature 260 °C, detector temperature 302 °C, column initial temperature 80 °C (2 min) increasing at the rate 25 °C/min. Low and high resolution mass spectrometry (MS and HRMS, respectively) data were obtained with an electrospray (ES) ion source.

Methyl 2-(2-Hydroxy-3-methoxy-5-oxo-2.5-dihydrofuran-2-yl)propanoate (5). LDA was generated by addition of *n*-BuLi (1.5 M in hexanes, 10.2 mL, 15.30 mmol, 1.1 equiv) to a solution of

iPr_2NH (2.14 mL, 15.30 mmol, 1.1 equiv) in THF (60 mL) at −78 °C. The solution was warmed to −20 °C for 15 min and then cooled back to −78 °C. A solution of methyl propionate (1.34 mL, 13.91 mmol, 1 equiv) in THF (10 mL) was added dropwise, and the mixture was stirred at −78 °C for 45 min. Zinc chloride (1 M in ether, 13.91 mL, 13.91 mmol, 1 equiv), THF (20 mL), and a solution of methoxymaleic anhydride **4**[20] (2.13 g, 16.69 mmol, 1.2 equiv) in THF (5 mL) were successively added. The mixture was slowly allowed to warm to rt and stirred 2 h. Water was added, and the aqueous phase was extracted with EtOAc. Aqueous phase was then neutralized with saturated aqueous NH_4Cl and extracted twice with EtOAc. This extract was dried over anhydrous $MgSO_4$ and concentrated to yield pure alcohol **5** (1.52 g, 51%) as a mixture a diastereoisomers (70/30 by integration in ^{1}H NMR). Major isomer: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.12 (d, J = 7.1 Hz, 3H), 3.04 (q, J = 7.1 Hz, 1H), 3.79 (s, 3H), 3.91 (s, 3H), 5.11 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 11.2, 43.3, 53.2, 60.2, 90.7, 103.3, 170.0, 173.9, 177.3. Minor isomer: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36 (d, J = 7.1 Hz, 3H), 2.93 (q, J = 7.1 Hz, 1H), 3.74 (s, 3H), 3.89 (s, 3H), 5.07 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 12.3, 45.4, 52.9, 60.2, 90.3, 102.6, 169.5, 174.5, 178.8.

Methyl 2-(3-Methoxy-5-oxofuran-2(5*H*)-ylidene)propanoate (6). A solution of alcohol **5** (0.643 g, 2.97 mmol, 1 equiv) in DCM (30 mL) was cooled to 0 °C. Et_3N (2.49 mL, 17.85 mmol, 6 equiv) and DMAP (36 mg, 0.30 mmol, 0.1 equiv) were successively added. A solution of TFAA (1.24 mL, 8.92 mmol, 3 equiv) in DCM (5 mL) was added dropwise, and the solution was stirred 2 h at 0 °C. After addition of 1 M HCl, the mixture was extracted twice with EtOAc. The extracts were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (cyclohexane/EtOAc: 90/10 to 70/30) to afford **6** (0.525 g, 90%, *E*/*Z*: 90/10 by integration in ^{1}H NMR) as a light-yellow solid. *E*-isomer could be isolated. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (s, 3H), 3.77 (s, 3H), 3.88 (s, 3H), 5.28 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.2, 52.7, 60.0, 91.2, 114.0, 143.9, 167.4, 168.0, 169.2. MS (ES): 199.1 $[M + H]^+$.

Methyl 2-(4-Iodo-3-methoxy-5-oxofuran-2(5*H*)-ylidene)propanoate (7). To a solution of **6** (0.116 g, 0.59 mmol, 1 equiv) in MeCN (12 mL) were successively added iodine (0.45 g, 1.76 mmol, 3 equiv) and CAN (0.97 g, 1.76 mmol, 3 equiv) and the mixture was heated at 40 °C for 3 h. After addition of saturated aqueous $Na_2S_2O_3$, the mixture was extracted three times with EtOAc. The extracts were washed with saturated aqueous $Na_2S_2O_3$ and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (cyclohexane/EtOAc: 90/10 to 80/20) to afford **7** (0.197 g, 94%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (s, 3H), 3.77 (s, 3H), 4.34 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 52.2, 52.9, 60.6, 114.2, 144.0, 165.9, 167.1, 168.0. GC-MS: tr = 8.8 min, MS 325 $[M + H]^+$.

General Procedure A: Suzuki−Miyaura cross-coupling with 7; Methyl 2-(3-methoxy-5-*oxo*-4-phenyl-2(5*H*)-ylidene)propanoate (8a). Iodinated compound **7** (0.042 g, 0.15 mmol, 1 equiv), phenylboronic acid (0.18 mmol, 1.2 equiv), supported catalyst[23] (0.007 g, 0.7 mol%), and *i*Pr_2NH (0.050 mL, 0.37 mmol, 2.5 equiv) were dissolved in MeCN (3 mL) and H_2O (1 mL). The solution was degassed under an Ar flow for 1 h and then heated at 85 °C for 6 h 30 min. The mixture was then filtered on celite. After addition of H_2O, the mixture was extracted with EtOAc. The extracts were washed with H_2O, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (cyclohexane/EtOAc: 80/20) to afford **8a** (0.028 g, 78%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (s, 3H), 3.72 (s, 3H), 3.82 (s, 3H), 7.36−7.46 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.9, 52.4, 61.1, 108.3, 113.5, 128.3, 128.5, 128.8, 130.0, 142.8, 161.5, 167.6, 168.2. GC-MS: tr = 9.5 min, MS 276 $[M + H]^+$.

Methyl 2-(4-(4-Methoxyphenyl)-3-methoxy-5-oxofuran-2(5*H*)-ylidene)propanoate (8b). The target compound was prepared from **7** and 4-methoxyphenylboronic acid using general procedure A. After purification, product **8b** was obtained (0.030 g, 65%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.14 (s, 3H), 3.71 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 6.93 (d, J = 9.1 Hz, 2H), 7.41 (d, J = 9.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.0, 52.6, 55.4, 60.9, 108.8, 113.2, 114.0, 120.6, 131.2, 143.1, 160.1, 161.1, 168.0, 168.5. GC-MS: tr = 11.0 min, MS 305 $[M + H]^+$.

Methyl 2-(4-(4-Bromophenyl)-3-methoxy-5-oxofuran-2(5*H*)-ylidene)propanoate (8c). The target compound was prepared from **7** and 4-bromophenylboronic acid using general procedure A. After purification, product **8c** was obtained (0.033 g, 63%) as a pale-yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.15 (s, 3H), 3.72 (s, 3H), 3.82 (s, 3H), 7.34 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.2, 52.7, 61.3, 107.6, 114.3, 123.4, 127.6, 131.6, 131.7, 142.7, 162.0, 167.4, 168.3. GC-MS: tr = 11.1 min, MS 353, 355 $[M + H]^+$.

Methyl 2-(4-(4-Fluorophenyl)-3-methoxy-5-oxofuran-2(5*H*)-ylidene)propanoate (8d). The target compound was prepared from **7** and 4-fluorophenylboronic acid using general procedure A. After purification, product **8d** was obtained (0.029 g, 67%) as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (s, 3H), 3.72 (s, 3H), 3.82 (s, 3H), 7.09−7.15 (m, 2H), 7.45−7.50 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 52.6, 61.2, 108.1, 114.0, 115.6, 115.9, 124.6, 124.7, 131.9, 132.0, 142.9, 161.4, 161.8, 164.7, 167.6, 168.3. GC-MS: tr = 9.8 min, MS 293 $[M + H]^+$.

Methyl 2-(4-(4-Isopropylphenyl)-3-methoxy-5-oxofuran-2(5*H*)-ylidene)propanoate (8e). The target compound was prepared from **7** and 4-*iso*-propylphenylboronic acid using general procedure A. After purification, product **8e** was obtained (0.027 g, 58%) as a pale-yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25 (d, J = 6.8 Hz, 6H), 2.15 (s, 3H), 2.91 (spt, J = 6.8 Hz, 1H), 3.73 (s, 3H), 3.82 (s, 3H), 7.26 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.3 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 24.1, 34.1, 52.7, 61.1, 108.7, 113.3, 126.3, 127.5, 130.0, 143.0, 149.8, 163.8, 168.0, 168.5. GC-MS: tr = 11.0 min, MS 317 $[M + H]^+$.

Methyl 2-(4-(*E*)-Styryl-3-methoxy-5-*oxo*-furan-2(5*H*)-ylidene)propanoate (8f). The target compound was prepared from **7** and *trans*-2-phenylvinylboronic acid using general procedure A. After purification, product **8f** was obtained (0.027 g, 61%) as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.12 (s, 3H), 3.82 (s, 3H), 4.15 (s, 3H), 6.95 (d, J = 16.1 Hz, 1H), 7.27−7.43 (m, 5H), 7.63 (d, J = 16.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4, 52.8, 61.7, 107.3, 113.7, 114.3, 127.0, 129.1, 135.5, 135.9, 137.1, 143.1, 160.1, 166.9, 167.5, 168.6. GC-MS: tr = 11.3 min, MS 301 $[M + H]^+$.

General Procedure B: Deprotection of the Enol Function. A solution of compound **8** (1 equiv) was dissolved in DCM (15 mL/mmol of **8**) and cooled to 0 °C. A solution of BBr_3 (1 M in DCM, 1 equiv) was slowly added. The solution was stirred 2 h at 0 °C and then quenched by addition of 1 M HCl. After extraction with EtOAc, the extracts were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (DCM) to afford **1** as a yellow solid. Compounds **1a**−**f** were prepared following general procedure B.

Methyl 2-(3-Hydroxy-5-*oxo*-4-phenylfuran-2(5*H*)-ylidene)propanoate (1a). Yield: 85%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17 (s, 3H), 3.95 (s, 3H), 7.29−7.45 (m, 3H), 8.12 (d, J = 6.0 Hz, 2H), 13.69 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 54.3, 104.5, 111.9, 127.8, 128.2, 128.5, 129.3, 154.3, 160.2, 166.3, 172.1. HRMS (ES): calcd for $C_{14}H_{13}O_5$ 261.0757 $[M + H]^+$; found 261.0754.

Methyl 2-(3-Hydroxy-5-*oxo*-4-(4-methoxyphenyl)furan-2(5*H*)-ylidene)propanoate (1b). Yield: 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (s, 3H), 3.84 (s, 3H), 3.95 (s, 3H), 6.95 (d, J = 8.8 Hz, 2H), 8.09 (d, J = 8.8 Hz, 2H), 13.46 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 54.4, 55.6, 104.6, 111.3, 114.2, 122.3, 129.4, 154.5, 159.3, 159.6, 166.9, 172.4. MS (ES): 289.2 $[M - H]^-$. Elemental analysis for $C_{15}H_{14}O_6$: calcd, C, 62.07; H, 4.86; found C, 61.91; H, 4.89.

Methyl 2-(3-Hydroxy-5-*oxo*-4-(4-bromophenyl)furan-2(5*H*)-ylidene)propanoate (1c). Yield: 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 3H), 3.98 (s, 3H), 7.54 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 13.81 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 54.4, 103.6, 112.4, 122.3, 128.9, 129.3, 131.7, 154.2, 160.5, 166.0,

172.2. HRMS (ES): calcd for $C_{14}H_{12}BrO_5$ 338.9854, 340.9844 [M + H]$^+$; found 338.9863, 340.9842.

Methyl 2-(3-Hydroxy-5-*oxo*-4-(4-fluorophenyl)furan-2(5*H*)-ylidene)propanoate (1d). Yield: 71%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.18 (s, 3H), 3.98 (s, 3H), 7.08−7.14 (m, 2H), 8.11−8.16 (m, 2H), 13.71 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 54.4, 103.8, 112.1, 115.4, 115.7, 125.4, 129.8, 154.3, 159.6, 160.7, 164.0, 166.3, 172.2. HRMS (ES): calcd for $C_{14}H_{12}FO_5$ 279.0663 [M + H]$^+$; found 279.0657.

Methyl 2-(3-Hydroxy-5-*oxo*-4-(4-*iso*-propylphenyl)furan-2(5*H*)-ylidene)propanoate (1e). Yield: 69%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (d, J = 7.0 Hz, 6H), 2.18 (s, 3H), 2.93 (spt, J = 7.0 Hz, 1H), 3.96 (s, 3H), 7.29 (d, J = 8.5 Hz, 2H), 8.03 (d, J = 8.5 Hz, 2H), 13.57 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 24.0, 34.2, 54.3, 104.9, 111.6, 126.6, 126.7, 127.9, 149.2, 154.5, 159.6, 166.4, 172.2. HRMS (ES): calcd for $C_{17}H_{19}O_5$ 303.1227 [M + H]$^+$; found 303.1220.

Methyl 2-(3-Hydroxy-5-*oxo*-4-(*E*)-styrylfuran-2-(5*H*)-ylidene)propanoate (1f). Yield: 61%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.15 (s, 3H), 3.96 (s, 3H), 6.88 (d, J = 16.5 Hz, 1H), 7.26−7.35 (m, 3H), 7.50−7.53 (m, 3H), 7.58 (d, J = 16.1 Hz, 1H), 13.34 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.9, 54.2, 105.3, 111.7, 114.7, 126.8, 128.3, 128.8, 133.7, 137.4, 154.2, 159.0, 166.2, 172.0. HRMS (ES): calcd for $C_{16}H_{15}O_5$ 287.0914 [M + H]$^+$; found 287.0907.

4-Benzyloxy-3-methylfuran-2(5*H*)-one (10). Tetronic derivative **9**[25] (0.932 g, 8.17 mmol, 1 equiv) was suspended in THF (40 mL). Benzyl alcohol (1.01 mL, 9.80 mmol, 1.2 equiv) and PPh_3 (2.57 g, 9.80 mmol, 1.2 equiv) were successively added. The solution was cooled to 0 °C and DIAD (1.94 mL, 9.80 mmol, 1.2 equiv) was slowly added and the mixture was stirred overnight at rt. After concentration, the residue was purified by silica gel column chromatography (DCM) to yield compound **10** (1.23 g, 74%), as a pale-yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.86 (s, 3H), 4.63 (s, 2H), 5.22 (s, 2H), 7.33−7.46 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 7.2, 65.9, 72.1, 99.3, 127.3, 128.8, 135.0, 171.5, 175.2. MS (ES): 205.1 [M + H]$^+$.

Methyl 2-(3-(Benzyloxy)-4-methyl-5-*oxo*-2.5-dihydrofuran-2-yl)-2-hydroxy-2-phenyl Acetate (12a). LDA was generated by addition of *n*-BuLi (1.5 M in hexanes, 3.74 mL, 5.61 mmol, 1.5 equiv) to a solution of *i*Pr_2NH 0.79 mL, 5.61 mmol, 1.5 equiv) in THF (20 mL) at −78 °C. The solution was warmed to −20 °C for 15 min and then cooled back to −78 °C. A solution of **9** (0.764 g, 3.74 mmol, 1 equiv) in THF (10 mL) was slowly added. After 30 min, ketoester **11a** (1.6 mL, 11.23 mmol, 3 equiv) was added. The mixture was then allowed to warm to rt. After addition of saturated aqueous NH_4Cl, the mixture was extracted with EtOAc. The extract was dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (cyclohexane/AcOEt: 90/10 to 100% AcOEt) to afford alcohol **12a** (0.950 g, 69%) as a yellow oil, mixture of diastereoisomeric forms (65/35 by integration in ^{1}H NMR). Major isomer could be isolated. ^{1}H NMR (200 MHz, DMSO-d_6): δ: 1.94 (s, 3H), 3.29 (s, 3H), 5.47 (dd, J = 11.0 Hz, J = 4.2 Hz, 2H), 5.75 (s, 1H), 6.36 (s, 1H), 7.33−7.44 (m, 8H), 7.62 (d, J = 7.8 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.5, 52.1, 72.9, 77.3, 80.1, 98.4, 126.1, 127.8, 128.0, 128.1, 128.3, 128.4, 135.7, 139.3, 169.5, 171.3, 174.1. MS (ES): 391.0 [M + Na]$^+$.

Methyl 2-(3-(benzyloxy)-4-methyl-5-*oxo*-2.5-dihydrofuran-2-yl)-2-(4-bromophenyl)-2-hydroxyacetate (12c). The target compound was prepared from **9** (0.226 g, 1.11 mmol, 1 equiv) and ketoester **11c** (0.807 mL, 11.23 mmol, 3 equiv), following the procedure described for compound **12a**. Purification of the residue (cyclohexane/AcOEt: 90/10 to 50/50) afforded alcohol **12c** (0.329 g, 69%) as a yellow oil, equimolar mixture of diastereoisomeric forms (^{1}H NMR). ^{1}H NMR (200 MHz, $CDCl_3$, mixture) δ 2.02 (s, 3H), 2.06 (s, 3H), 3.37 (s, 3H), 3.86 (s, 3H), 5.12 (s, 2H), 5.34 (s, 2H), 5.41 (s, 1H), 6.70 (s, 1H), 7.27−7.55 (m, 18H). MS (ES): 447.1, 449.0 [M + H]$^+$.

Methyl 2-(3-(Benzyloxy)-4-methyl-5-oxofuran-2(5*H*)-ylidene)-2-phenylacetate (13a). Following the procedure described for compound **6**, dehydratation of alcohol **12a** (0.356 g, 0.89 mmol) afforded product **13a** (0.055 g, 18%), *E*-isomer, as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.14 (s, 3H), 3.29 (s, 3H), 5.40 (s, 2H), 7.32−7.60 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 9.3, 52.3, 74.4, 101.8, 115.4, 128.5, 128.8, 129.0, 129.2, 129.3, 131.3, 134.6, 141.4, 161.3, 166.8, 169.9. MS (ES): 351.1 [M + H]$^+$.

Methyl 2-(3-(Benzyloxy)-4-methyl-5-oxofuran-2(5*H*)-ylidene)-2-(4-bromo)phenylacetate (13c). Following the procedure described for compound **6**, dehydratation of alcohol **12c** (0.089 g, 0.20 mmol) afforded product **13c** (0.038 g, 44%) as a white solid, mixture of *E* and *Z* forms. *E*-isomer could be isolated. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.14 (s, 3H), 3.27 (s, 3H), 5.40 (s, 2H), 7.31−7.38 (m, 2H), 7.42−7.52 (m, 8H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 9.3, 52.4, 74.5, 102.2, 114.3, 123.6, 127.3, 128.7, 129.0, 129.2, 130.3, 131.1, 132.0, 141.9, 161.2, 166.5, 169.6. MS (ES, pos): 429.2, 431.2 [M + H]$^+$.

Methyl 2-(3-Hydroxy-4-methyl-5-oxofuran-2(5*H*)-ylidene)-2-phenylacetate (2a). To a solution of compound **12a** (0.121 g, 0.35 mmol) in DCM (10 mL) was added Pd/C (10%, 0.17 g, 0.1 equiv). H_2 was then introduced and the mixture was stirred overnight at rt. After filtration on celite and concentration, the residue was purified by silica gel column chromatography (DCM) to afford product **2a** (0.046 g, 48%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.90 (s, 3H), 3.85 (s, 3H), 7.21−7.27 (m, 2H), 7.38−7.43 (m, 3H), 12.86 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.6, 54.3, 104.4, 114.8, 128.1, 128.5, 130.0, 132.0, 155.2, 161.5, 168.0, 171.4. HRMS (ES): calcd for $C_{14}H_{13}O_5$ 261.0757 [M + H]$^+$; found 261.0753.

Methyl 2-(4-Bromophenyl)-2-(3-hydroxy-4-methyl-5-oxofuran-2(5*H*)-ylidene)acetate (2c). Following the procedure described for compound **2a**, catalytic hydrogenation of **12c** (0.035 g, 0.08 mmol) afforded compound **2c** (0.018 g, 66%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.89 (s, 3H), 3.85 (s, 3H), 7.09 (d, J = 8.6 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 12.79 (s, 1H). MS (ES): 339.0, 341.0 [M + H]$^+$. HRMS (ES): calcd for $C_{14}H_{11}BrO_5$ 338.9863, 340.9844 [M + H]$^+$; found 338.9872, 340.9858.

Methyl 2-(3-Hydroxy-4-methyl-5-oxofuran-2(5*H*)-ylidene)2-(4-methoxyphenyl)acetate (2b). A solution of dilactone **14**[26] (35.4 mg, 0.14 mmol) in MeOH (5 mL) was heated under reflux overnight. After concentration, the residue was purified by silica gel column chromatography (cyclohexane/AcOEt: 80/20 to 70/30) to afford product **1c** (21.9 mg, 54%) as a yellow solid and product **2c** (17.9 mg, 44%) as a white solid. **2b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.88 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 6.91 (d, J = 8.7 Hz, 2H), 7.16 (d, J = 8.8 Hz, 2H), 12.84 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.6, 54.3, 55.4, 104.4, 113.7, 114.7, 124.2, 131.5, 155.0, 159.8, 161.6, 168.2, 171.7. HRMS (ES): calcd for $C_{15}H_{15}O_6$ 291.0863 [M + H]$^+$; found 291.0865.

***Iso*-propyl 2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)propanoate (1g) and *Iso*-propyl 2-(3-Hydroxy-4-methyl-5-oxofuran-2(5*H*)-ylidene)2-(4-methoxy phenyl)acetate (2d).** A solution of dilactone **14**[26] (18.8 mg, 0.07 mmol) in *i*-PrOH (3 mL) was heated under reflux overnight. After concentration, the residue was purified by silica gel column chromatography (cyclohexane/AcOEt: 80/20 to 70/30) to afford product **1g** (13.4 mg, 58%) as a yellow solid and product **2d** (8.9 mg, 40%) as a white solid. **1g**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (d, J = 6,4 Hz, 6H), 2.14 (s, 3H), 3.83 (s, 3H), 5.21 (spt, J = 6.4 Hz, 1H), 6.95 (d, J = 9.1 Hz, 2H), 8.10 (d, J = 9.1 Hz, 2H), 13.74 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 21.7, 55.3, 72.0, 104.3, 112.1, 113.9, 122.1, 129.2, 154.2, 158.9, 159.3, 166.7, 171.2; HRMS (ES): calcd for $C_{17}H_{19}O_6$ [M + H]$^+$ 319.1149; found 319.1167. **2d**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (d, J = 6.4 Hz, 6H), 1.87 (s, 3H), 3.83 (s, 3H), 5.18 (spt, J = 6.4 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 8.10 (d, J = 8.7 Hz, 2H), 13.03 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.6, 21.5, 55.4, 72.1, 103.9, 113.4, 115.6, 124.4, 131.6, 154.4, 159.6, 161.8, 168.4, 170.7; MS (ES): 319.1 [M + H]$^+$; HRMS (ES): calcd for $C_{17}H_{19}O_6$ 319.1176 [M + H]$^+$; found 319.1188.

***N*-Butyl-2-(3-hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)propanamide (1h).** To a solution of dilactone **14**[26] (23.6 mg, 0.09 mmol, 1equiv) in 1.5 mL of THF was slowly added a solution of TBAF (1 M solution in THF, 0,18 mL, 0,18 mmol, 2

equiv) at −35 °C. The mixture was stirred for 15 min and then cooled to −78 °C. A solution of butylamine (23 μL, 0.22 mmol, 2.5 equiv) in 1 mL of THF was added. After 15 min of stirring at −78 °C, the mixture was allowed to warm to room temperature. After concentration in vacuo, the crude residue was purified by chromatography on silica gel (DCM) to furnish amide **1h** (27.4 mg, 83%) as an orange solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.97 (t, J = 7.4 Hz, 3H), 1.65−1.25 (m, 4H), 2.18 (s, 3H), 3.50−3.40 (m, 2H), 3.83 (s, 3H), 6.32 (brs, 1H), 6.95 (d, J = 9.0 Hz, 2H), 8.11 (d, J = 9.0 Hz, 2H), 15.82 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.7, 13.8, 20.2, 31.2, 40.8, 55.4, 102.8, 111.3, 113.9, 122.6, 128.9, 152.9, 159.1, 160.8, 167.5, 169.0. HRMS (ES) calcd for $C_{18}H_{22}NO_5$ $[M + H]^+$ 332.1466; found 332.1480.

(*E*)-4-Hydroxy-3-(4-methoxyphenyl)-5-(1-morpholino-1-oxopropan-2-ylidene)furan-2(5*H*)-one (1i). To a solution of dilactone **14**[26] (25.5 mg, 0.10 mmol, 1equiv) in 1.5 mL of THF was slowly added a solution of TBAF (1 M solution in THF, 0,20 mL, 0,20 mmol, 2 equiv) at −35 °C. The mixture was stirred 15 min and then cooled to −78 °C. A solution of morpholine (22 μL, 0.25 mmol, 2.5 equiv) in 1 mL of THF was added. After 15 min of stirring at −78 °C, the mixture was allowed to warm to room temperature. After concentration in vacuo, the crude residue was purified by chromatography on silica gel (DCM) to furnish amide **1i** (32.9 mg, 96%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.15 (s, 3H), 3.63−3.70 (m, 4H), 3.70−3.75 (m, 4H), 3.83 (s, 3H), 6.94 (d, J = 9.1 Hz, 2H), 8.04 (d, J = 9.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.3, 46.2, 55.4, 66.9, 104.6, 112.6, 114.0, 121.9, 129.2, 150.3, 158.8, 159.3, 166.9, 171.2. HRMS (ES) calcd for $C_{18}H_{20}NO_6$ $[M + H]^+$ 346.1285; found 346.1291.

***N*-(3-Aminopropyl)-2-(3-hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)ylidene) Propanamide (1j).** To a solution of dilactone **14**[26] (89.1 mg, 0.34 mmol, 1 equiv) in DCM (3 mL) was added 1.3-diaminopropane (0,14 mL, 0.17 mmol, 0.5 equiv) and the solution was stirred overnight at rt. After concentration, the residue was purified by silica gel column chromatography (DCM/MeOH: 100/0 to 80/20) to afford product **21** (36 mg, 64%), as a yellow solid. ^{1}H NMR (200 MHz, MeOD) δ 1.87−1.97 (m, 2H), 2.07 (s, 3H), 3.03 (t, J = 7.3 Hz, 2H), 3.42 (t, J = 6.1 Hz, 2H), 3.78 (s, 3H), 6.86 (d, J = 9.0 Hz, 2H), 7.95 (d, J = 9.0 Hz, 2H). MS (ES): 333.0 $[M + H]^+$. HRMS (ES): calcd for $C_{17}H_{21}N_2O_5$ 333.1445 $[M + H]^+$; found 333.1449.

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene-*N*-methoxy-*N*-methyl Propanamide (1k). To a solution of dilactone **14**[26] (0.183 g, 0.71 mmol, 1 equiv) in DCM (4 mL) were added *N*,*O*-dimethylhydroxylamine hydrochloride (0.069 g, 0.71 mmol, 1 equiv) and triethylamine (0.1 mL, 0.71 mmol, 1 equiv). After 2 h stirring at rt, the mixture was hydrolyzed by addition of 1 M HCl solution and extracted twice with EtOAc. The extracts were dried over anhydrous magnesium sulfate and concentrated. The residue was purified by chromatography on silica gel (DCM/MeOH: 100/0 to 90/10) to furnish Weinreb amide **1k** (0.204 g, 90%) as a yellow solid. ^{1}H NMR (200 MHz, MeOD) δ 2.24 (s, 3H), 3.39 (s, 3H), 3.76 (s, 3H), 3.83 (s, 3H), 6.94 (d, J = 8.8 Hz, 2H), 8.07 (d, J = 8.8 Hz, 2H), 12.32 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.4, 34.0, 55.2, 62.0, 103.9, 113.5, 113.7, 122.0, 129.0, 151.0, 159.0, 166.9, 170.1. MS (ES): 318,1 $[M - H]^-$. HRMS (ES): calcd for $C_{16}H_{16}NO_6$ 318.0983 $[M - H]^-$; found 318.0974.

4-Hydroxy-5-(3-hydroxy-3-methlybutan-2-ylidene)-3-(4-methoxyphenyl)furan-2(5*H*)-one (1l). To a solution of dilactone **14**[26] (75.8 mg, 0.29 mmol, 1 equiv) in THF (2 mL) was slowly added a solution of MeLi (1.6 M in ether, 0.27 mL, 0.44 mmol, 1.5 equiv) at −78 °C. The solution was allowed to warm to rt and the reaction was quenched by addition of 1 M HCl. The mixture was then extracted twice with EtOAc and the extracts were dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (cyclohexane/AcOEt: 50/50) to afford alcohol **1l** (42.0 mg, 64%), as a pale-orange solid. ^{1}H NMR (300 MHz, MeOD) δ 1.53 (s, 6H), 2.03 (s, 3H), 3.81 (s, 3H), 6.93 (d, J = 9.1 Hz, 2H), 7.99 (d, J = 9.1 Hz, 2H). ^{13}C NMR (50 MHz, MeOD) δ 16.2, 29.1, 55.6, 75.3, 102.9, 114.5, 124.2, 126.6, 132.7, 140.9, 160.0, 162.0, 170.8. MS (ES): 291.1 $[M + H]^+$. HRMS (ES): calcd for $C_{16}H_{19}O_5$ 291.1227 $[M + H]^+$; found 291.1224.

Biology. Thymidine Protection Assay under UV Exposure and Fenton Stress. These procedures have been previously described.[14]

Scavenging of Superoxide Anion. Antioxidant capacity was mesured by Photochem apparatus from Analytik Jena. ACL kits of reagents (ref 400−803 from Analytik Jena) were used as well as PCL soft to enable results analysis: kinetic and nanomol equivalent of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) calculated by integration of the curves on a typical interval of 180 s. Results are expressed in μg of compound equivalent to the activity of 1 μg of standard (Trolox).

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Supporting Information Available: Detailed descriptions of HPLC methods and purity values of final compounds; EC_{50} measurements for selected compounds in the protection of thymidine assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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