A Powerful Antiradiation Compound Revealed by a New High-Throughput Screening Method

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We present a new high-throughput screening method for the selection of powerful water-soluble antiradiation compounds. This method, which uses conventional immunoassay techniques, allowed the capacity of a given compound to protect thymidine from irradiation to be evaluated. By applying this assay to an antioxidant library, we showed for the first time that norbadione A, a well-known mushroom pigment, has pronounced atypical antiradiation properties.

Introduction

Oxidative damage caused by uncontrolled production of reactive oxygen species (ROS) is believed to be involved in a great number of pathologies,[1] such as cancer,[2] coronary heart disease,[3] rheumatoid arthritis,[4] and degenerative processes associated with ageing.[5] Considerable efforts have been made to find new antioxidants and to evaluate their beneficial effects with regard to preventing these diseases.[6] Antioxidants are also used as therapeutics to counter free-radical-mediated damage[7] and have found applications in the field of cancer therapy.[8] Current strategies for the discovery and evaluation of new molecules able to protect biologically relevant targets from oxidative stress involve initial screening of putative antioxidants, which are then tested for more specific biological activities.

The development of a screening method is particularly challenging because of the inherent complexity of oxidative stress, which must be reproduced as well as possible in a simple in vitro test. Numerous procedures are available for the determination of antioxidant activity[9] and all of them have advantages and drawbacks. Among the known methods, only a few achieve systematic assaying in a truly high-throughput format. The use of colored nitrogen-centered radicals such as 1,1-diphenyl-2-picrylhydrazyl or the 2,2′-azinobis-[3-ethylbenzthiazoline-2-sulfonic acid radical is one of the most popular methods[10] but oxidative stress might not be perfectly mimicked in this case. The main feature of many systems is the inhibition of the formation of an oxidized form of the target. Since oxidative stress processes involve a cascade of chemical pathways leading to a variety of byproducts, assays measuring inhibition of one given oxidized form of a selected target evaluate only part of the activity of antioxidants.

In the work reported herein, we focused on the capacity of phenol-based compounds to protect DNA-related targets from irradiation. Our approach was to irradiate a nucleobase in the presence of a putative protective agent and then to measure the amount of the nucleobase target remaining by using an analytical tool that can be applied in a high-throughput screening (HTS) format. This alternative strategy should allow rapid classification of pure compounds as well as natural extracts according to their global protective effect on a nucleobase under controlled and relevant oxidative conditions.

Results and Discussion

High-throughput screening development

The effects of ionizing radiation on the nuclear material of the cell have been the focus of numerous investigations.[11] The hydroxyl (OH) radical, generated through the radiolysis of water upon exposure to ionizing radiation, is probably the main species responsible for oxidative DNA damage. Nucleobase-centered reactive intermediates comprise the majority of the reactive species produced by ionizing radiation.[12] These intermedi-

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ates are trapped by O_2 and converted into peroxyl radicals, which generate numerous byproducts and can propagate oxidative damage in DNA. Tandem lesions induced by pyrimidine peroxyl radicals have been identified as a major form of propagation damage within DNA.[13]

The screening procedure that we have developed is based on the degradation of thymidine (dThd) by γ rays under aerobic conditions. The unmodified thymidine remaining after irradiation was quantified by using competitive enzyme immunoassay techniques (EIA), which allow rapid measurement of the concentration of a given compound in a complex mixture.

Irradiation of thymidine was conducted in a quartz 96-well microtiter plate by using a ^{137}Cs source. The crude oxidized solutions were then assayed for remaining thymidine with a specific monoclonal antibody bound to an enzyme–thymidine conjugate. As intact thymidine competes with the enzyme–thymidine conjugate for antibody binding sites, the decrease in antibody-bound-enzyme activity, which generates a colored product, is related to the protective capacity of the tested compound (Figure 1).

When an aerated aqueous solution of thymidine is exposed to γ rays, the thymidine reacts with radicals produced by water radiolysis. Addition of an OH radical to the C_5 or to the C_6 position and H-atom abstraction involving the sugar moiety or the C_5 methyl group lead to the major reactive intermediates. The carbon-centered radicals react with molecular oxygen at a diffusion-controlled rate to form peroxy radicals, which generate a multitude of stable products by inducing decomposition.[14]

Our experimental conditions for irradiation lead to continuous generation of OH radicals (0.5 μM min^{-1} for 135 min). Under these conditions, around 90% of the thymidine was destroyed and a variety of oxidized products were resolved by HPLC. When the HPLC fractions were assayed by EIA, only the fraction corresponding to intact thymidine provided a signal (Figure 2). This experiment illustrates the specificity of the an-

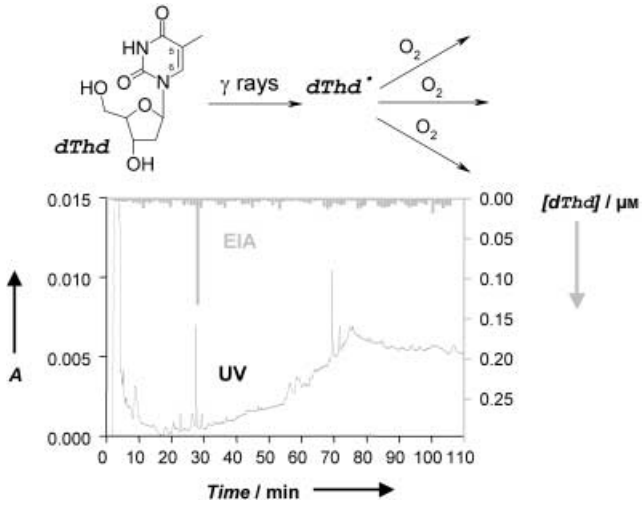


Figure 2. *Validation of the screening assay. HPLC chromatography of irradiated thymidine (135 min at 2.52 Gy min^{-1}) was conducted on a C18 analytical column (250×4 mm, 5 μm, 30°C, gradient elution from water to water/methanol (3:7), flow rate 1 mL min^{-1}). The concentration of remaining thymidine estimated by UV detection (267 nm) was 1.16±0.02 μM (92.3% degradation), and that measured by EIA detection was 1.20±0.01 μM (92.0% degradation).*

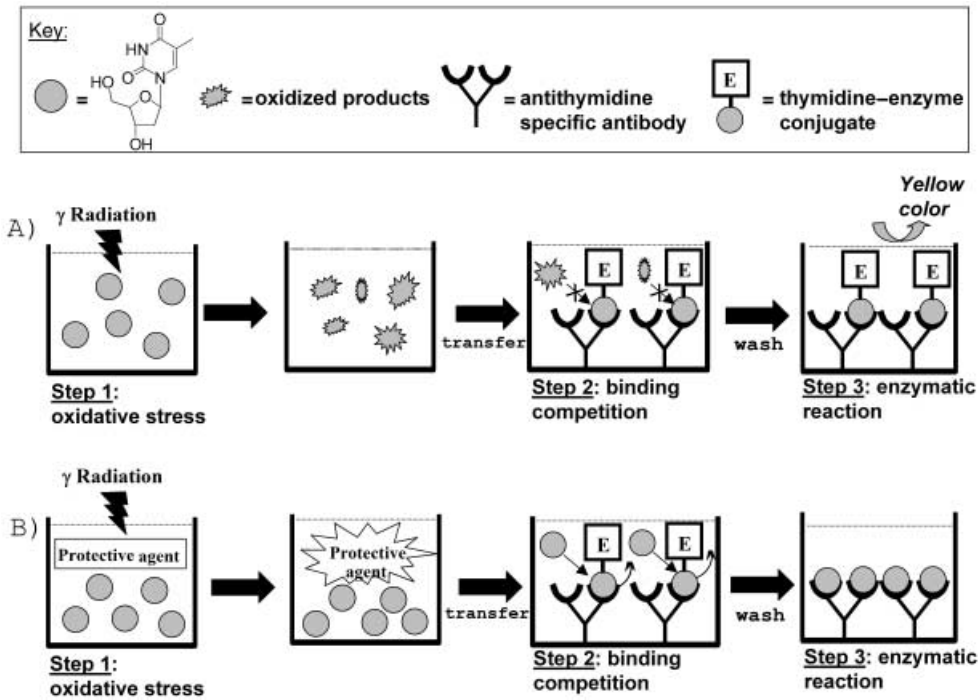


Figure 1. *Schematic representation of the thymidine protection assay. Step 1: irradiation of thymidine in the absence (A) or in the presence (B) of a protective compound; Step 2, competitive immunoassay: a sample of the irradiated solution is transferred to another plate coated with an antithymidine monoclonal antibody and containing an enzyme–thymidine conjugate; Step 3: after a washing step, the residual enzymatic activity, which is related to the thymidine concentration, is quantified by the detection of a yellow reaction product (Ellman's reagent). Results are expressed as percent thymidine protection, calculated by comparing results obtained in the presence and in the absence of the protective agent.*

tithymidine antibody as no signal interference was caused by the presence of the many products of thymidine oxidation.

Under our experimental conditions, we expect the assay described above to highlight compounds able to inhibit thymidine oxidation essentially by trapping OH radicals. Therefore, the protective effects of several compounds known as efficient OH radical scavengers were evaluated (Figure 3). The results show that the protective effect on thymidine is related to the concentration of the tested compounds and that the method allows easy comparison of protection efficiencies with high precision and good reproducibility (Figure 3A, for protection > 30%, the coefficients of variation were less than 6%).

It is well established that the OH radical trapping ability of a given compound is influenced by many parameters, such as bond dissociation enthalpy (BDE), ionization potential, chemical hardness (HOMO–LUMO gap), or spin delocalization. As a consequence, it is often not possible to correlate experimental results with a particular theoretical value. However, to allow comparison of the results obtained from our assay with reported data on OH radical scavengers, some simple phenols and flavonoids were studied (Figure 3B and C). These compounds were tested at 50 μM, the concentration that allows the best discrimination between antiradiative potencies.

The results obtained with hindered phenols **1**–**4** are in agreement with reported data on the ROS-scavenging properties of these compounds. Steric hindrance in compound **2** stabilizes the corresponding phenoxy radical, which leads to a lower BDE than that of compound **1**.[15] In contrast, the presence of two bulky *tert*-butyl groups in compound **3**, (Figure 3B and C) decreases the OH radical trapping capability of the compound, despite its lower BDE. This observation has previously been attributed to the unfavorable kinetic properties of highly hindered phenols.[16] The results for **5** and **1** reflect the known potency of catechols for the trapping of OH radicals and are a consequence of the lower BDE of **5** that results from stabilization of the phenoxyl radical through its involvment in an H-bond with the adjacent OH group.[15,17]

The protective effects measured for compounds **6**–**11** (Figure 3B and C) highlight the major determinants of the radical-scavenging capability of flavones and flavonols. In accordance with published data, we observed the beneficial effects of 1) the presence of a catechol group in ring B (see **6**–**8**); 2) the presence of a 3-hydroxy group on ring C (see **7**–**10**); 3) the presence of a hydroxy group at position 5 (see **10** and **11**).[18] Taken together, these observations lead to the conclusion that the protective effects measured by the presented HTS assay are closely related to the ROS-scavenging properties of the tested compounds.

Screening of natural extracts and pure antioxidants

We applied this screening assay to several ethanol-free natural extracts, including tea and red wine extracts, which are known for their high antioxidant content.[18,19] Remarkably, the extract from the mushroom *Pisolithus tinctorius* displayed a higher protection activity than wine, tea, or fruit extracts, which contain more than 80% (wt) flavonoids, anthocyans, or teaflavins, known as powerful ROS scavengers (Figure 4A). We carried out an EIA/HPLC activity-guided fractionation of *P. tinctorius* extract

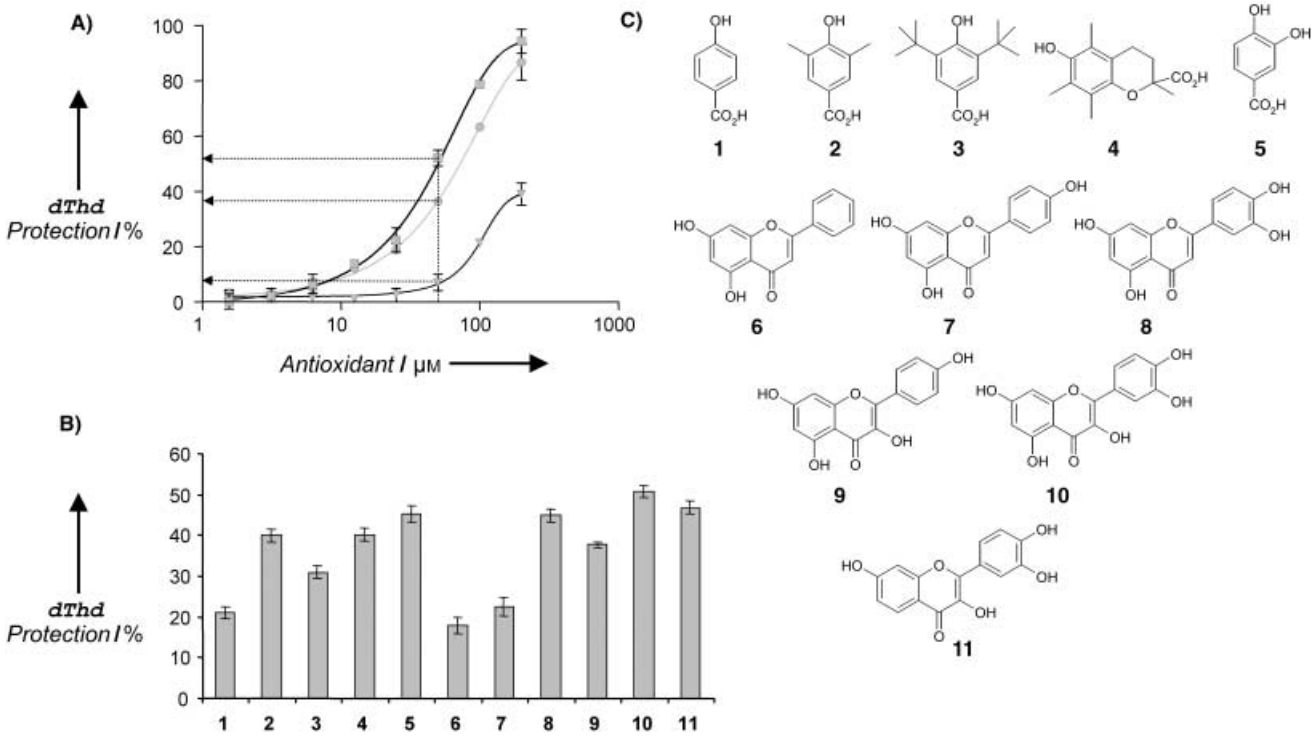


Figure 3. *Validation of the screening assay. Protection of thymidine by antioxidants. Thymidine (15 μM) in phosphate buffer (5 mM, pH 7.4) was subjected to γ irradiation (340 Gy) in the presence (A) of varying amounts of the antioxidants propyl gallate (■), trolox (●), and mannitol (▼), or in the presence (B) of each of the phenols and flavonoids (50 μM) shown in (C).*

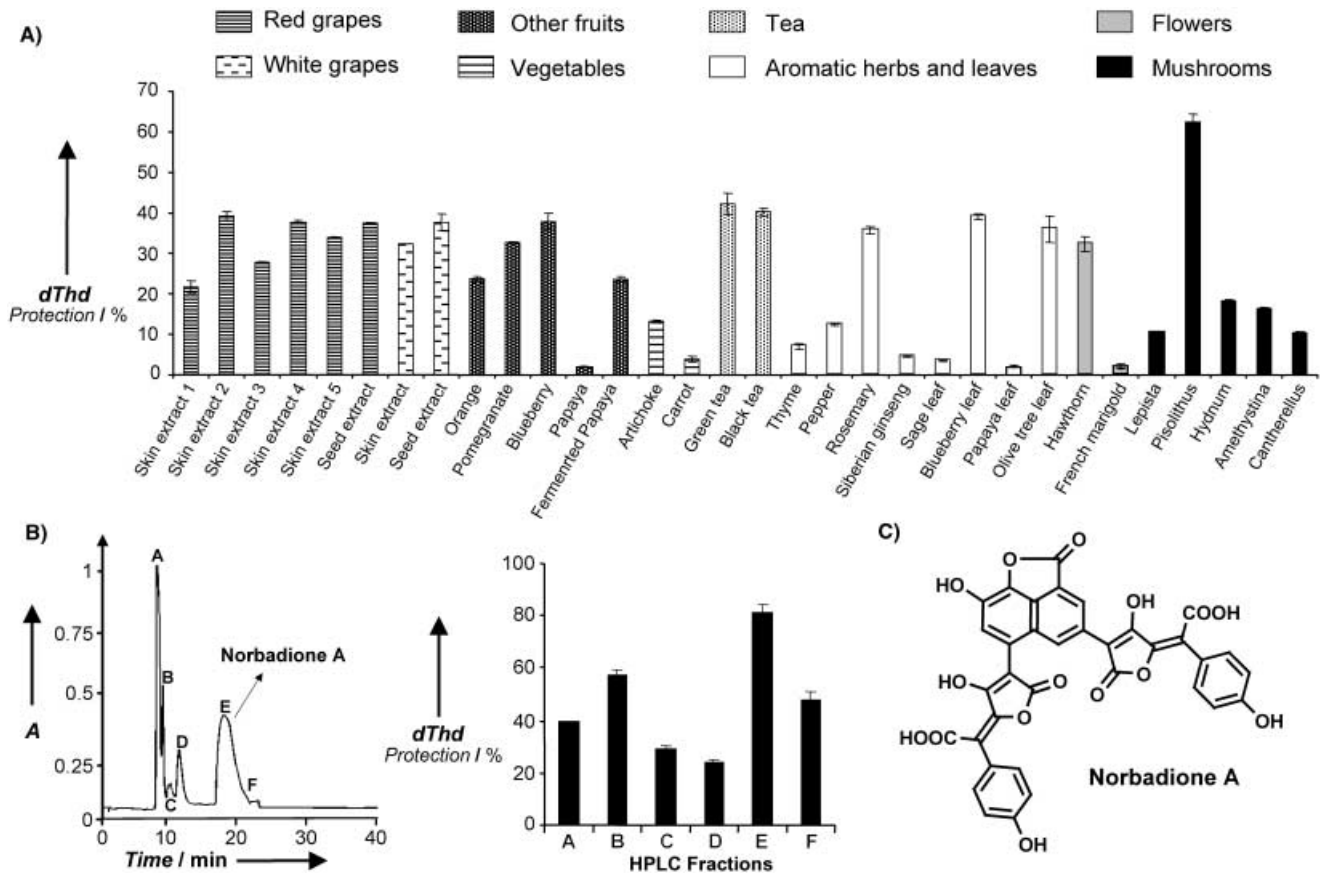
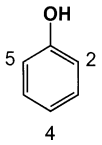


Figure 4. *Screening of natural extracts and identification of the active compound in* P. tinctorius *acetone extract. A) Thymidine (15 μM) in phosphate buffer (5 mM, pH 7.4) was subjected to γ rays (340 Gy) in the presence of a natural extract (20 μg mL^{-1}). B) HPLC separation, UV detection (265 nm), and EIA/HPLC activity-guided fractionation of* P. tinctorius *extract (see Experimental Section). Each HPLC fraction was assayed at 40 μg mL^{-1}. C) Structure of norbadione A, a pigment present in* P. tinctorius *and in several boletes.*

Table 1. *Structures of phenols* ***1A–F.***



Compound	Name	2	4	5
1A	4-hydroxybenzoic acid	H	CO_2H	H
1B	3,4-dihydroxybenzoic acid	H	CO_2H	OH
1C	gallic acid	OH	CO_2H	OH
1D	3,5-dimethyl-4-hydroxybenzoic acid	CH_3	CO_2H	CH_3
1E	3,5-di-*t*Bu-4-hydroxybenzoic acid	*t*Bu	CO_2H	*t*Bu
1F	2,4,6-tris(dimethylaminomethyl) phenol[a]	$CH_2N(CH_3)_2$	$CH_2N(CH_3)_2$	$CH_2N(CH_3)_2$

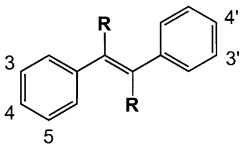
[a] tris, tris(hydroxymethyl)aminomethane.

to identify the active compound(s). Six main absorbance peaks were observed and the corresponding fractions were separated and assayed individually (Figure 4B). Peak E represents the most active fraction, which contained a unique compound that corresponded to 40% of the dry mass of the extract. This compound was identified as norbadione A by comparison with an authentic sample (Figure 4C).

Norbadione A, first isolated by W. Steglich et al. from the edible bay boletus (*Xerocomus badius* (Fr.) Kühn. ex Gilb),[20] is known to be one of the pigments responsible for the brown color of this mushroom. This compound was later isolated from *P. tinctorius* by M. Gill et al.[21] We envisioned that this new property of norbadione A could be of great interest. Therefore, we decided to compare the antiradiation properties of this molecule to those of a library of 130 strong antioxidants, phenol-based compounds or thiol derivatives known to be good radioprotectors (Tables 1–11, Scheme 1).[22] Screening was easily achieved in a few hours at a concentration of 50 μM for each compound. Representative results (on 80 antioxidants) are displayed in Figure 5.

Remarkably, norbadione A (**1H**) was found to be the best protective agent (84 ± 2% thymidine protection) among this library of compounds, which included some of the strongest known water-soluble ROS scavengers. Four other compounds

Table 2. *Structures of stilbenes* ***2A–D.***



Compound	Name	R	3	4	5	3′	4′
2A	resveratrol	H	OH	H	OH	H	OH
2B	piceatannol	H	OH	H	OH	OH	OH
2C	rhapontin	H	O-glc[a]	H	OH	OH	OCH_3
2D	diethylstilbestrol	Et	H	OH	H	H	OH

[a] glc, glucose.

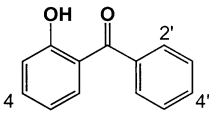
(**5A**, **5E**, **6D**, **8D**) were found to be efficient protective agents whose presence led to approximately 60% thymidine protection. All of these compounds are glycosylated polyphenols.

Studies on norbadione A

To investigate whether norbadione A can also protect DNA, a sample of supercoiled plasmid DNA, pUC18, was exposed to γ irradiation in the absence or in the presence of varying concentrations of norbadione A, then analyzed by agarose gel electrophoresis (Figure 6). After irradiation in the absence of norbadione A, the plasmid DNA migrated essentially like linear DNA (compare Lanes 3 and 4). In contrast, the DNA irradiated in the presence of norbadione A migrated like the relaxed circular form and significant amounts of the supercoiled form persisted (Lanes 5–8).

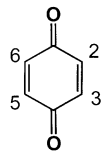
The screening results show that there is no correlation between the number of hydroxy

Table 3. *Structures of benzophenones* ***2E–H.***



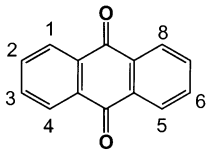
Compound	Name	4	2′	4′
2E	2,2′-dihydroxybenzophenone	H	OH	H
2F	2,4-dihydroxybenzophenone	OH	H	H
2G	2,4,4′-trihydroxybenzophenone	OH	H	OH
2H	2,2′-dihydroxy-4,4′-dimethoxybenzophenone	OCH_3	OH	OCH_3

Table 4. *Structures of quinones* ***3A*** and ***B.***



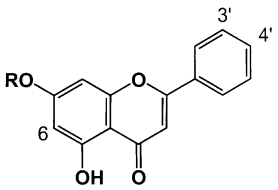
Compound	Name	2	3	5	6
3A	2,5-dihydroxy benzoquinone	OH	H	OH	H
3B	tetrahydroxy benzoquinone	OH	OH	OH	OH

Table 5. *Structures of anthraquinones* ***3C–4C.***



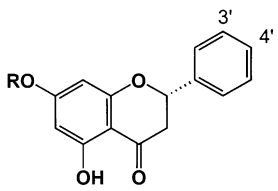
Compound	Name	1	2	3	4	5	6	8
3C	1,8-dihydroxyanthraquinone	OH	H	H	H	H	H	OH
3D	quinizarin	OH	H	H	OH	H	H	H
3E	alizarin	OH	OH	H	H	H	H	H
3F	anthrarufin	OH	H	H	H	OH	H	H
3G	anthraflavic acid	H	OH	H	H	H	OH	H
3H	purpurin	OH	OH	H	OH	H	H	H
4A	2,3-dimethylquinizarin	OH	CH_3	CH_3	OH	H	H	H
4B	emodin	OH	H	CH_3	H	H	OH	OH
4C	carminic acid	OH	glc	OH	OH	H	OH	OH

Table 6. *Structures of flavones* ***4E–5B.***



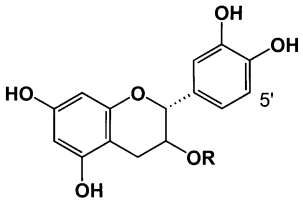
Compound	Name	6	R	3′	4′
4E	luteolin	H	H	OH	OH
4F	apigenin	H	H	H	OH
4G	chrysin	H	H	H	H
4H	baicalein	OH	H	H	H
5A	apigenin-7-glc	H	glc	H	OH
5B	apigenin-7-methoxy	H	CH_3	H	OH

Table 7. *Structures of flavonones* ***5C–E.***



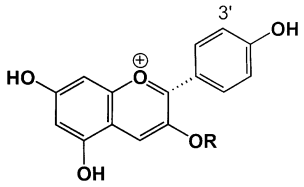
Compound	Name	R	3′	4′
5C	hesperetin	H	OH	OCH_3
5D	naringenin	H	H	OH
5E	naringin	rut	H	OH

Table 8. *Structures of flavan-3-ols* **5F–6A**.



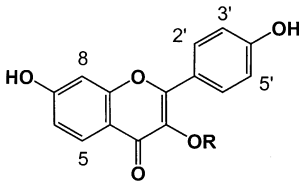
Compound	Name	R	5′
5F	(+)-catechin	H	H
5G	(−)-epicatechin	H	H
5H	(−)-gallocatechin	H	OH
6A	(−)-gallocatechin	gal	OH

Table 9. *Structures of anthocyans* **6B–E**.



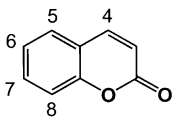
Compound	Name	R	3′
6B	Cyanidin	H	OH
6C	Pelargonidin	H	H
6D	Keracyanine	glc	OH
6E	Callistephin	glc	H

Table 10. *Structures of flavonols* **6F–7H**.



Compound	Name	R	5	8	2′	3′	5′
6F	quercetin	H	OH	H	H	OH	H
6G	kaempferol	H	OH	H	H	H	H
6H	fisetin	H	H	H	H	OH	H
7A	myricetin	H	OH	H	H	OH	OH
7B	quercitrin	rham	OH	H	H	OH	H
7C	(+)-rutin	rut	OH	H	H	OH	H
7D	morin	H	OH	H	OH	H	H
7E	gossypin	H	OH	O-glc	H	OH	H
7F	rhamnetin	H	OH	H	H	OH	H
7G	kaempferol-3-glc	glc	OH	H	H	H	H
7H	myricitrin	rham	OH	H	H	OH	OH

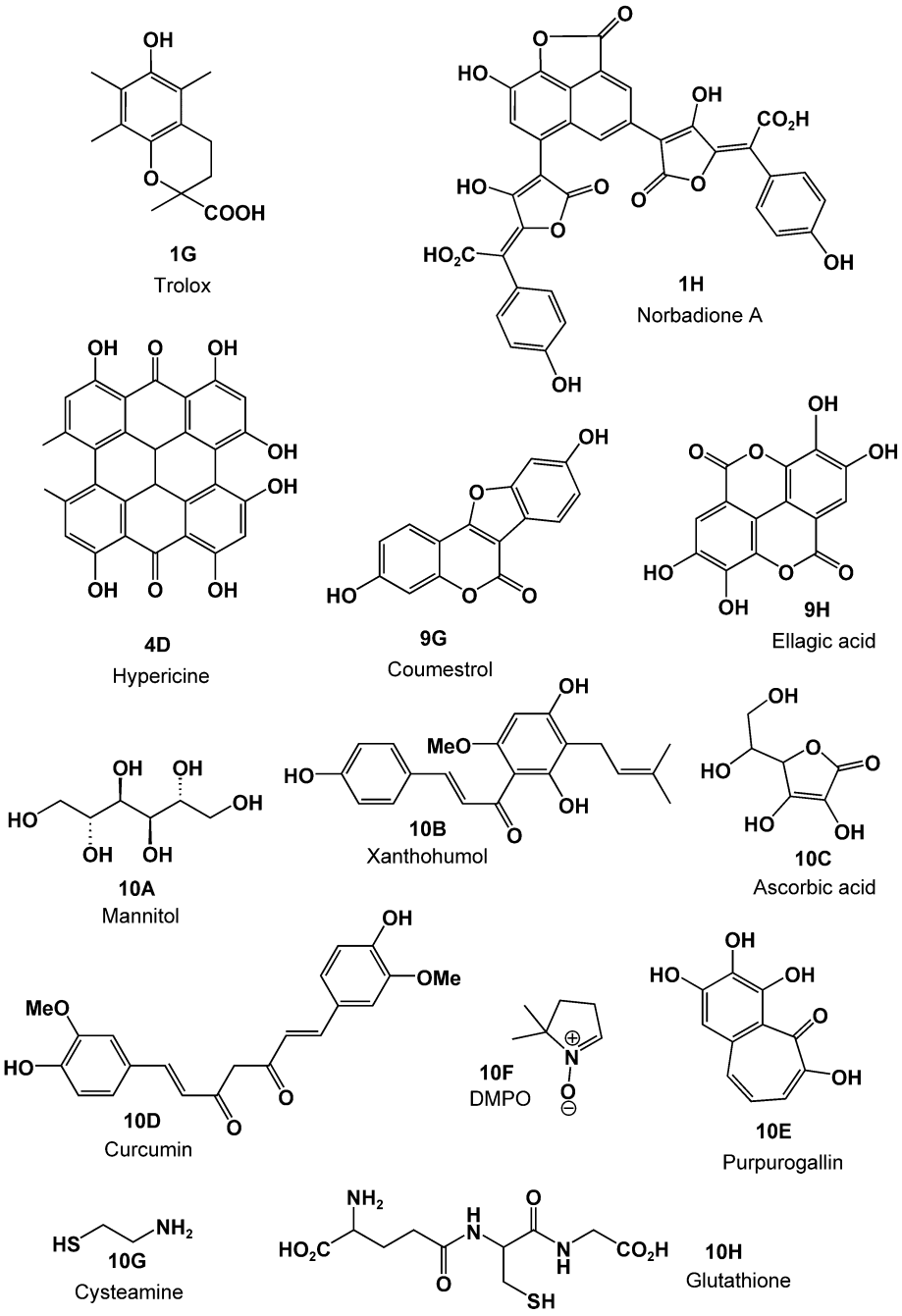
Table 11. *Structures of coumarins* **8A–9F**.



Compound	Name	4	5	6	7	8
8A	4-hydroxycoumarin	OH	H	H	H	H
8B	7-hydroxycoumarin	H	H	H	OH	H
8C	Esculetin	H	H	OH	OH	H
8D	Esculin	H	H	O-glc	OH	H
8E	Scopoletin	H	H	OCH_3	OH	H
8F	8-hydroxy-7-methoxycoumarin	H	H	H	OCH_3	OH
8G	7,8-dihydroxycoumarin	H	H	H	OH	OH
8H	4-methyldaphnetin	CH_3	H	H	OH	OH
9A	5,7-dihydroxy-4-methylcoumarin	CH_3	OH	H	OH	H
9B	7-hydroxy-4-methylcoumarin	CH_3	H	H	OH	H
9C	7-hydroxycoumarin-4-acetic acid	CH_2CO_2H	H	H	OH	H
9D	6,7-dihydroxycoumarin-4-acetic acid	CH_2CO_2H	H	OH	OH	H
9E	7-hydroxy-4-methyl-8-nitrocoumarin	CH_3	H	H	OH	NO_2
9F	7,8-dihydroxy-6-methoxycoumarin	H	H	OCH_3	OH	OH

moieties and the protective efficiency of polyphenols. Therefore, the superior activity of norbadione A must be related to other structural characteristics or to particular reaction mechanisms. The structural feature of norbadione A that makes this compound atypical is the presence of two deprotonated (at physiological pH)[23] and conjugated enol functions, which might be the origin of the remarkable antiradiation activity of this natural compound. Preliminary electrochemical experiments seem to confirm this hypothesis since cyclic voltammograms (CVs) of norbadione A in CH_3CN are characterized by two anodic signals at $E_{pa} = 0.55$ V and 0.82 V, respectively (0.1 Vs^{-1}), that indicate fully irreversible processes. This result is in agreement with the presence of two easily oxidizable moieties. The differential pulse voltammetry (DPV) electrochemical response of norbadione A has two successive well-defined anodic peaks at $E_{pa} = 0.53$ V and 0.81 V (Figure 7), values close to the CV results.

The amplitude of the current corresponding to the first DPV or CV peak strongly suggests a two-electron transfer. The irreversibility of the electrochemical process is associated with the existence of chemical steps coupled to the electron transfer, as previously described for polyphenols.[24] Electrochemical ex-



Scheme 1. *Antioxidants. DMPO, 5,5-Dimethyl-1-parroline-*N*-oxide.*

periments performed in an aqueous medium confirm the easily oxidizable character of norbadione A. At pH 7, the DPV curve displayed three successive electrochemical signals at 0.44 V, 0.75 V, and 1.00 V. All the processes represented by these signals are irreversible, as judged from CV experiments. The E_{pa} value of norbadione A in aqueous medium at pH 7 compares well with those of other polyphenols. For instance, the E_{pa} values for resorcinol, vanillic acid, chrysin, ellagic acid, and curcumin are 0.63, 0.52, 0.79, 0.36, and 0.50 V versus Ag/AgCl, respectively,[24] whilst hypericine and emodine have values of 0.55 V and 0.60 V, respectively.[25]

Preliminary experiments involving the irradiation of a solution of norbadione A suggest the formation of pisoquinone, among several other unidentified products. The presence of this molecule in mushroom extracts has been described by M. Gill et al.[26] Pisoquinone was identified by comparison of data for experimental samples with published NMR data,[26] as well as with the experimentally obtained NMR spectrum of an extract containing this compound. Although the mechanism of this oxidation is not clear at this stage, the electrochemical properties of norbadione A suggest oxidation of two functions is possible. This observation allowed us to propose a mechanism involving a deprotonated enol on norbadione A (Scheme 2).

Conclusion

The presented screening method proved to be very suitable for rapid evaluation of the antiradiation capabilities of water-soluble ROS-scavenging agents and has the potential to allow analysis of more than 1000 samples per day. The underlying principle of the procedure is generally applicable and could be successfully extended to other types of targets than thymidine, or to other types of oxidative stress.

Norbadione A was found to be the most potent protective compound among a collection of highly powerful anti-ROS agents. The remarkable antiradiation properties of this compound seem to be related to its particular structural features, although complementary experiments are needed to definitively establish the mechanism of oxidation. Recent investigations in our group indicate that this compound displays not only a strong in vitro activity but also interesting biological properties. These properties and the possibility of producing very significant amounts of this compound from *P. tinctorius*, a mushroom commonly used in silviculture, make norbadione A, in our opinion, a very promising compound for future development.

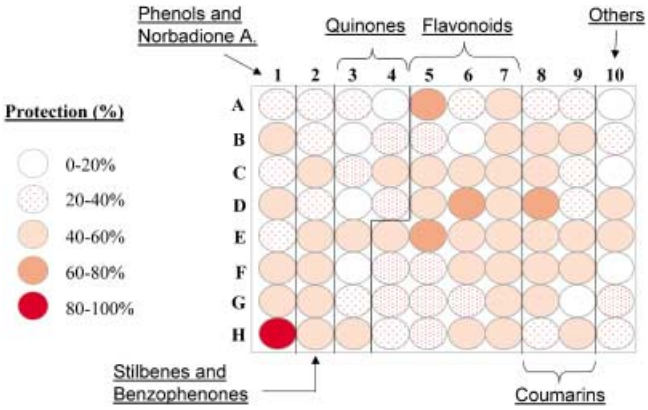


Figure 5. *Screening results. Thymidine (15 μM) in phosphate buffer (5 mM, pH 7.4) was subjected to γ rays (340 Gy) in the presence of pure antioxidants (50 μM). The extent of thymidine protection is represented by color-coding for clarity. Norbadione A was in well* ***1 H****.*

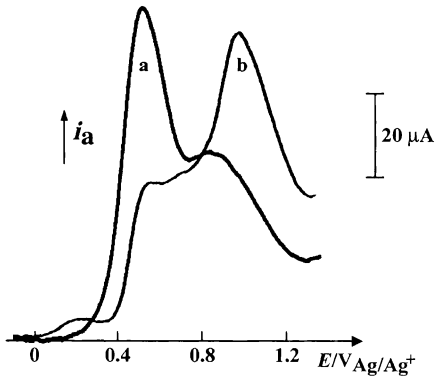


Figure 7. *DPV curve recorded at a Pt electrode in a CH_3CN + tetra-nbutylammonium perchlorate (TBAP) solution (0.1 M) of norbadione A (0.8 mg mL^{-1}). a) Norbadione A dipotassium salt; b) Norbadione A, acidic form. v = 10 mV s^{-1}, pulse height = 25 mV, step time = 0.2 s.*

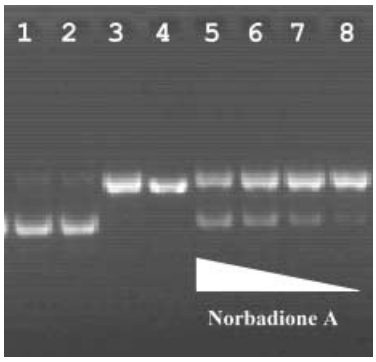


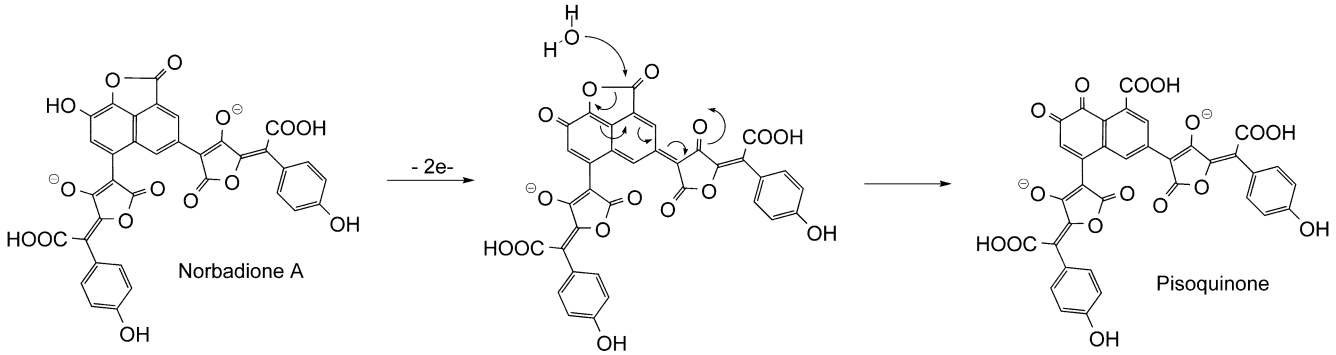
Figure 6. *DNA protection by norbadione A. Supercoiled plasmid pUC18 DNA (Lanes 1 and 2) was irradiated at 2.52 Gy min^{-1} for 30 min in the absence (Lane 3) or the presence of 62.5 μM (Lane 8), 125 μM (Lane 7), 250 μM (Lane 6), or 500 μM (Lane 5) norbadione A. Lane 4, control linear plasmid DNA (digested by Hind III). Linear and supercoiled DNA were separated by agarose gel electrophoresis and stained with ethydium bromide.*

Experimental Section

Mushroom extraction protocol:[21] Fresh fruit bodies (50 g) of fungi were finely chopped and soaked in acetone (200 mL) at room temperature for 3 h. The acetone extract was decanted from the fungal material, which was further soaked in acetone (2× 200 mL) for 5 and 18 h. The combined extracts were evaporated to dryness under reduced pressure to give a dark gum or powder, which was dissolved in water (4 mg mL^{-1}) and assayed directly for its antioxidant properties.

HPLC fractionation of *P. tinctorius* extract: The crude extract of *P. tinctorius* (Pers.) Coker et Couch (288 mg) was applied to a Hypersil BDS column (250×50 mm) and eluted with methanol/water/trifluoroacetic acid (50:50:0.1) at a flow rate of 85 mL min^{-1}. Absorbance was monitored at 265 nm. The eluted fractions were concentrated by evaporation to dryness, dissolved in water at 40 μg mL^{-1}, and then assayed by using the thymidine protection assay.

Thymidine protection assay: Thymidine (15 μM, Aldrich) and antioxidant (50 μM) or natural extract (40 μg mL^{-1}, obtained from Naturex, Ferco, or Osato International Inc.) in phosphate buffer (150 μL, 5 mM, pH 7.4) were added to each well of a quartz microtiter plate. The plates were irradiated for 135 min at 2.52 Gy min^{-1} in a γ irradiator (IBL 637, equipped with a ^{137}Cs source). Samples of the irradiated solutions (25 μL) were quenched by the addition of EIA buffer (25 μL) containing bovine serum albumin (1 mg mL^{-1}, Sigma; in 100 mM phosphate buffer, pH 7.4) then transferred to a second plate (Maxisorb–Nunc) coated with polyclonal goat anti-mouse antibody (Jackson Immuno. Research Laboratories Inc.) and supplemented with acetylcholinesterase–thymidine conjugate (50 μL, prepared and conserved as previously described)[27] and specific monoclonal antithymidine antibody (50 μL) in EIA buffer.



Scheme 2. *Proposed mechanism for the formation of pisoquinone from norbadione A under γ radiation.*

After incubation for 2 h at room temperature, the plates were washed and Ellman's reagent was added. The absorbance related to the activity of the solid-phase-bound AChE was measured at 414 nm. Results are expressed as percent thymidine protection. Thymidine quantification was achieved by using a calibration curve (fitted by linear log-logit transformation) obtained with pure thymidine. All measurements were made in duplicate.

Electrochemical experiments: For the experiments performed in organic medium, acetonitrile (Rathburn, HPLC grade) was used as received. TBAP from Fluka was dried under vacuum at 80 °C for 3 days before it was used as the supporting electrolyte. The reference electrode was $Ag/AgNO_3$ (10 mM in CH_3CN containing 0.1 M TBAP). The potential of the standard ferrocene/ferrocenium (Fc/Fc^+) redox couple in acetonitrile is 0.07 V under our experimental conditions. For the experiments performed in aqueous medium, $LiClO_4$ (0.1 M) was used as the supporting electrolyte, and the reference electrode was a saturated KCl standard calomel electrode. The pH value was adjusted by controlled addition of small amounts of concentrated NaOH or $HClO_4$ aqueous stock solutions to the electrochemical cell. Electrochemical experiments were conducted in a conventional three-electrode cell under an argon atmosphere at 20 °C. Rotating disc electrode (RDE) voltammetry was carried out at a rotation rate of 600 rpm. Cyclic voltammetry curves were recorded at a scan rate of 0.1 Vs^{-1} and differential pulse voltammetry (DPV) curves were recorded at a 10-mV s^{-1} scan rate with a pulse height of 25 mV and a step time of 0.2 s. The working electrode was a Pt disc (5 mm in diameter for DPV and CV or 2 mm for RDE voltammetry) polished with diamond paste (1 μm) before each measurement.

Irradiation of norbadione A: A sample of norbadione A (13 mg in 130 mL H_2O) was irradiated (66.5 Gy and 133 Gy). The resulting mixture was lyophilized and analyzed by ^1H NMR spectroscopy. The formation of pisoquinone was followed by observation of three characteristic NMR signals corresponding to the proton of the naphtoquinone part of the molecule, as previously described: $\delta = 8.6$ (d, $J = 1.8$ Hz), 8.24 (d, $J = 1.8$ Hz), 6.51 ppm.

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Keywords: antioxidants · high-throughput screening · immunoassays · natural products · radiolysis

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