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Synthesis and Electrochemical and Biological Studies of Novel Coumarin−Chalcone Hybrid Compounds

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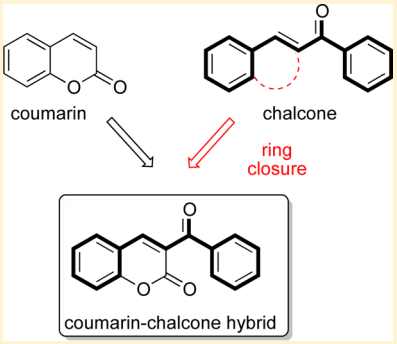
ABSTRACT: A series of novel hydroxy-coumarin−chalcone hybrid compounds **2a−i** has been synthesized by employing a simple and efficient methodology. An electrochemical characterization using cyclic voltammetry and ESR spectroscopy were carried out to characterize the oxidation mechanism for the target compounds. The antioxidant capacity and reactivity were determined by ORAC and ESR assays, respectively. Biological assays were assessed to evaluate the cytotoxicity and cytoprotection capacity against ROS/RNS on BAEC. The results revealed that all tested compounds present ORAC values that are much higher than other well-known antioxidant compounds such as quercetin and catechin. Compound **2e** showed the highest ORAC value (14.1) and also presented a low oxidation potential, good scavenging capacity against hydroxyl radicals, low cytotoxicity, and high cytoprotective activity.



INTRODUCTION

Free radicals and reactive oxygen and nitrogen species (ROS and RNS) are atoms, molecules, or ions possessing at least one unpaired electron; therefore, they are highly reactive with a wide range of other different molecules. They are constantly generated and maintained in balance in biological systems through metabolic processes, and they play important roles in a variety of normal biochemical functions such as cell signaling, apoptosis, gene expression, ion transport, and pathological processes.[1,2]

It is accepted that ROS/RNS play different roles in vivo. Some of these roles are positive and are related to their involvement in energy production, phagocytosis, regulating cell growth, intercellular signaling, and the synthesis of biologically important compounds.[3] However, the overproduction of ROS/RNS can have damaging effects on many molecules. ROS/RNS are capable of oxidizing cellular proteins, nucleic acids, and lipids because they are very small and highly reactive. For example, lipid peroxidation is a free-radical-mediated propagation of oxidative damage to polyunsaturated fatty acids involving several types of free radicals, and termination occurs through enzymatic means or by free-radical scavenging by antioxidants.[4,5] Therefore, the excess of ROS/RNS causes a disturbance in the prooxidant−antioxidant balance in favor of the former, leading to potential damage, which is known as oxidative stress.[1,6,7] Oxidative stress considerably contributes to the pathogenesis of inflammatory and cardiovascular diseases, cancer, diabetes, Alzheimer's disease, autism, and aging among others.[8−13]

The consumption of natural antioxidants, such as flavonoids, supports the endogenous antioxidant defense system. Both dietary and enzymatic antioxidants are components of interrelated systems that interact with each other to control ROS production and thereby ensuring adequate defenses against oxidative stress.[14,15]

In this sense, many studies concerning the antioxidant capacity of phenolic compounds of different types (e.g., flavonoids, coumarins, and tanins) have been established, and their physicochemical properties have been correlated with their antioxidant properties.[16] In general, free-radical scavenging, redox potential, and the antioxidant activity of these classes of compounds mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic molecules, although it is also affected by other factors.[17] Therefore, low oxidation potentials, the number of hydroxyl groups present, and the existence of electron-donating groups and/or the stabilization of a secondary radical via delocalization through conjugated systems play a key role in the antioxidant activity.[18−21]

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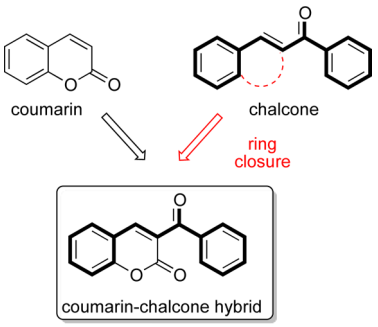
Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. Because of their ability to inhibit low-density lipoprotein (LDL) oxidation, flavonoids have demonstrated unique cardioprotective effects.[22,23] The protective effects of flavonoids in biological systems are attributed to their capacity for transferring electrons to radicals, chelating metal catalysts,[24] activating antioxidant enzymes,[25] reducing alpha-tocopherol radicals,[26] and inhibiting oxidases.[27]

Chalcones (α-phenyl-β-benzoylethylene) are prominent secondary-metabolite precursors of flavonoids and isoflavonoids in plants. Chemically, they consist of two aromatic rings linked by a three-carbon α,β-unsaturated system and are known to exhibit an impressive array of biological properties.[28−30] Chalcones have attracted much research attention because of their pharmaceutical potential resulting from their radical-scavenging,[31,32] antitumor,[33,34] anti-inflammatory,[35] and neuroprotective properties.[36] Thus, it is reasonable to explore the radical-scavenging properties of the hydroxyl derivatives of chalcones.

However, coumarins (2*H*-chromen-2-one) are a large family of compounds of both natural and synthetic origin that are important because of the pharmacological activities that these kind of compounds display,[37] such as antimicrobial,[38] monoamine oxidase (MAO) inhibition,[39−41] antitumor,[42] adenosine receptor antagonist,[43] and antioxidant[17] among others.

Therefore, coumarin and chalcones represent promising scaffolds in the medicinal chemistry realm. Taking into account the previously mentioned features[18,19] and with the aim of finding new chemical structures with antioxidant activity, we have designed a new scaffold in which the chalcone fragment is partially incorporated in the coumarin moiety. The resulting coumarin−chalcone hybrid fixes the trans-conformation of the chalcone scaffold by sharing the double bond of the pyrane ring of the coumarin nucleus (Scheme 1).

Scheme 1. Structural Rational Design of Coumarin−Chalcone Hybrid Compounds

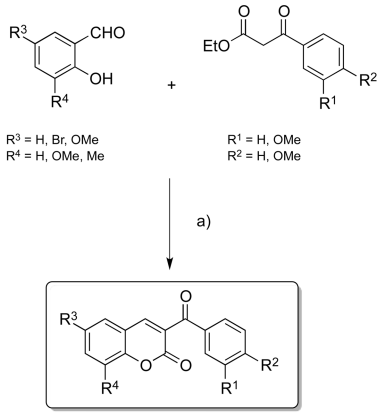


CHEMISTRY

Coumarin derivatives **2a−i** were efficiently synthesized in two steps,[40] which are briefly described as follows: (i) the synthesis of methoxy-3-benzoylcoumarins **1a−i** and (ii) the synthesis of hydroxy-3-benzoylcoumarins **2a−i**. These steps are outlined in Scheme 2.

On the basis of the widely used Knoevenagel condensation reaction for the preparation of coumarin derivatives,[37,44] we used an efficient one-step synthesis to generate methoxy-3-benzoylcoumarin precursors **1a−i**. These compounds were prepared in good yields (74−98%) using the appropriately substituted salicylaldehyde and the corresponding β-ketoester in the presence of piperidine in ethanol under reflux for 2−5 h, obtaining the desired compound as a precipitate that was separated by filtration and further purified by recrystallization in MeOH/DCM. Final compounds **2a−i** were then synthesized by the hydrolysis of the corresponding methoxy precursors **1a−i** by employing an excess of a Lewis acid, BBr_3, in DCM at 80 °C in a Schlenk tube for 48 h followed by treatment with MeOH and purification by flash chromatography of the crude product (69−98%).

Scheme 2. Synthesis of Methoxylated (1a−i) and Hydroxylated (2a−i) 3-Benzoylcoumarin Derivatives[a]



[a]Reagents and conditions: (a) piperidine, EtOH, reflux, 2−5 h; (b) BBr_3, DCM, 80 °C, 48 h.

ELECTROCHEMICAL STUDY

Cyclic Voltammetry. The electrochemical properties of hydroxy-coumarin−chalcone derivatives **2a−i** were studied by cyclic voltammetry, employing an electrochemical cell with three electrodes and different scan rates between 0.050 and 0.75 V* s^{-1}. Under these conditions, all derivatives exhibited one (compounds **2a−g**) or two (compounds **2h** and **2i**) anodic peaks without the corresponding cathodic peak (Figure 1). The anodic peak potentials (Epa I and Epa II) obtained at a 0.75 V* s^{-1} scan rate are listed in Table 1.

Characterization of Coumarin−Chalcone Radicals by ESR Spectroscopy. To confirm the formation of semiquinone radicals from compounds **2a−i**, an electron spin resonance (ESR) spectroscopy study was performed. For compounds **2a−d** and **2g**, the spin trap PBN was used. This nitrone derivative is a diamagnetic compound that, in the presence of free radicals, produces a spin adduct (paramagnetic species), increasing the half-life of the radicals species.[45] The experiment was carried out by electrolysis of the derivatives to control the potential in situ using DMSO as the solvent and TBAP as the supporting electrolyte at room temperature. Next, the PBN spin trap was added. In the absence of the PBN spin trap, an ESR spectrum was observed for derivatives **2e**, **2f**, **2h**, and **2i**. Compound **2e**, with two hydroxyl groups, evidenced a symmetric ESR

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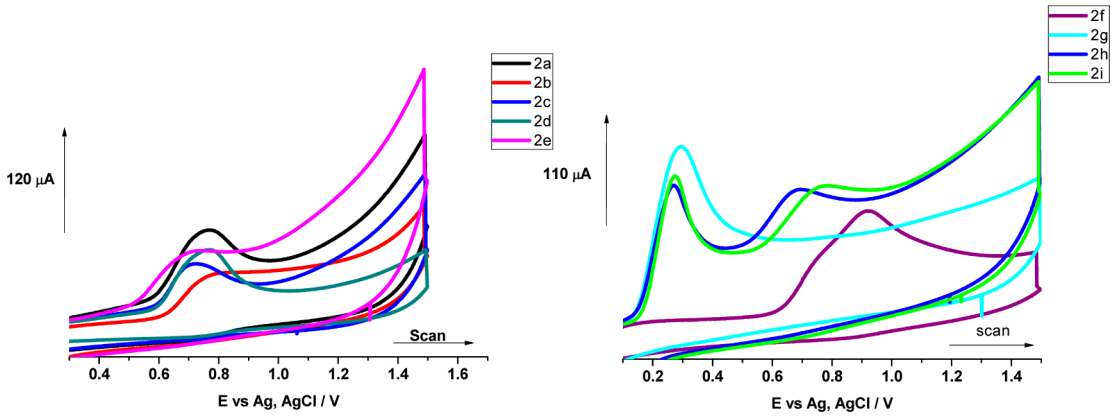

Figure 1. Cyclic voltammograms of the hydroxy-coumarin−chalcone derivatives **2a−i** in phosphate buffer, pH 7.4, at 0.75 V* s^{-1}.

Table 1. Oxidation Potential for Compounds 2a−i[a]

compound	Epa I (V)	Epa II (V)
2a		0.77
2b		0.79
2c		0.72
2d		0.77
2e		0.74
2f		0.92
2g	0.30	
2h	0.27	0.69
2i	0.27	0.77

[a]Values obtained at a scan rate of 0.75 V*s^{-1}.

spectrum with five lines (Figure 2a). Compound **2f** showed a hyperfine pattern with two lines (Figure 2b) and was simulated and characterized by two doublets resulting from two nonequivalent hydrogen atoms.

ORAC-FL Assay. The capacity for peroxyl radical scavenging by coumarin−chalcone hybrids was assessed using the oxygen-radical absorbance capacity (ORAC) assay. This methodology gives an index relative to a standard molecule (Trolox), which is a hydrosoluble analog of vitamin E. The ORAC values obtained for all derivatives are shown in Table 2. All derivatives presented an ORAC index higher than well-known antioxidants quercetin and catechin.[46] Compound **2e** presented the highest ORAC value of the studied series (14.1).

Antioxidant Reactivity by ESR Assay. The reactivity against a mixture of radical species for all of the studied coumarin derivatives was carried out by adapting the H_2O_2/NaOH/DMSO/DMPO system[47] using a noncatalytic and competitive fenton reaction in which the spin trap DMPO and the antioxidant molecule competes for the hydroxyl, superoxide anion, and methyl radicals.

Four wide hyperfine lines were observed in the control measure (labeled as "blank", indicating the absence of the tested and antioxidant molecules; Figure 3) because of the

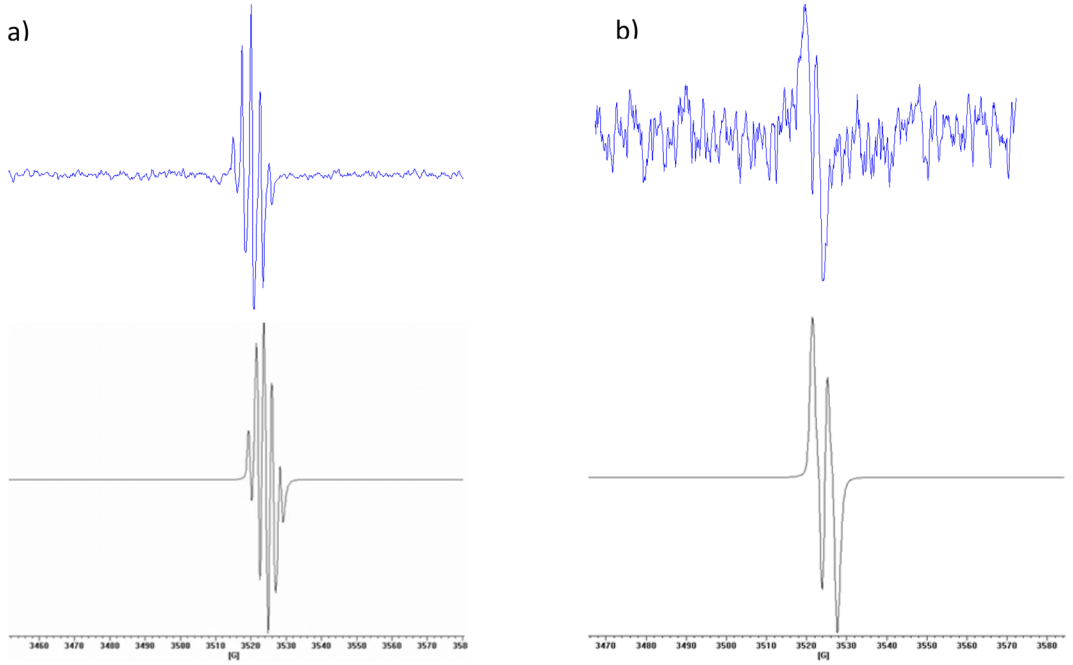

Figure 2. Experimental (blue) and simulated (black) ESR spectra for compounds **2e** (a) and **2f** (b). Simulated hyperfine splitting constants (hfscs) were a_H = 3.57 and 1.34 G for compound **2e** and a_H = 2.35, 2.35 (two equivalent hydrogen atom), and 1.75 G for compound **2f**.

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Table 2. ORAC Values and the Percentage of Radicals Scavenging for Compounds 2a–i[a]

compound	ORAC value	radical scavenging (%)
2a	9.2 ± 0.4	26
2b	11.5 ± 0.4	39
2c	9.5 ± 0.3	28
2d	10.9 ± 0.3	45
2e	14.1 ± 0.3	58
2f	8.3 ± 0.3	65
2g	13.0 ± 0.4	100
2h	8.1 ± 0.6	75
2i	8.8 ± 0.5	100
Trolox	1.0 ± 0.2	31
quercetion	7.3 ± 0.2[b]	
catechin	6.8 ± 0.2[b]	

[a]Measured by ESR spin trapping in competition with DMPO. [b]Data obtained from ref 46.

mixture of the DMPO–OH, DMPO–CH_3, and DMPO–$O_2^{\bullet-}$ adducts that formed.

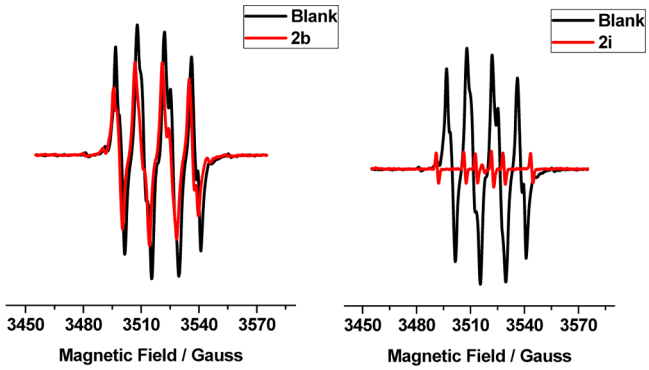

Figure 3. ESR spectra for the reactivity assays of compounds **2b** and **2i**.

BIOLOGY

Cytotoxicity Assays. The cytotoxicity of compounds **2a–i** on bovine aortic endothelial cells (BAEC) was assessed and evaluated over a concentration range of 0–50 μM by employing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.[47] The results are shown in Table 3 for concentrations of 10, 20, and 50 μM for each compound studied.

Table 3. Cytotoxicity of Compounds 2a–i tested at Three Different Concentrations on BAEC[a]

compound	10 μM	20 μM	50 μM
2a	76 ± 7	60 ± 6	44 ± 5
2b	88 ± 2	95 ± 2	86 ± 1
2c	95 ± 3	93 ± 9	104 ± 6
2d	87 ± 7	82 ± 5	101 ± 8
2e	68 ± 1	94 ± 5	93 ± 10
2f	96 ± 3	101 ± 1	93 ± 4
2g	102 ± 4	100 ± 5	92 ± 5
2h	49 ± 2	68 ± 2	63 ± 2
2i	64 ± 2	23 ± 2	71 ± 6

[a]The results are expressed as the percentage of cell viability (n = 3).

Cytoprotection Assays: ROS/RNS Scavenging Activity in Cell Systems. *Hydrogen Peroxide.* To evaluate the cytoprotection against H_2O_2, derivatives presenting high cell-viability values (compounds **2b–g**) were selected. These compounds also presented high antioxidant capacity in ORAC assays and showed notable hydroxyl-radical scavenging activity.

BAEC were incubated with two different concentrations of each derivative (10 and 20 μM) for 24 h in a 24-well plate followed by treatment with 1 mM H_2O_2 for 2 h. Cell viability was then quantified by the MTT reduction assay (Figure 4a).

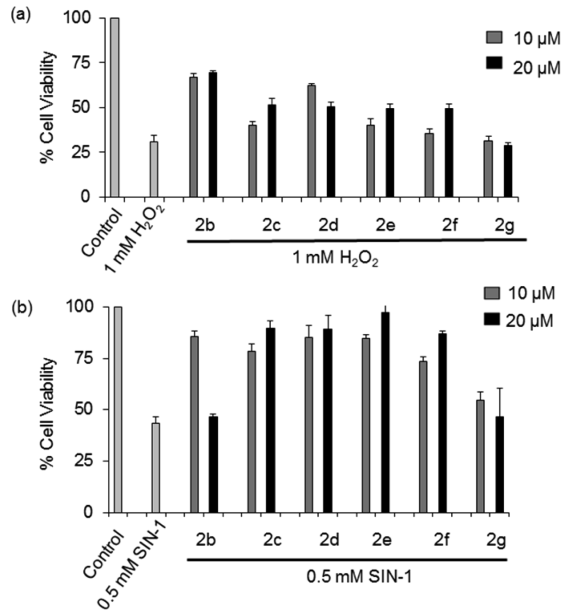

Figure 4. Cytoprotection of compounds **2b–g** at 10 and 20 μM against 1 mM H_2O_2- (a) and 0.5 mM SIN-1-induced (b) cytotoxicity of bovine aortic endothelial cells using MTT assay.

Peroxynitrite. The cytoprotective property of the compounds against peroxynitrite in BAEC was investigated by MTT assay. Peroxynitrite was generated in the cells using 3-morpholino-sydnonimine (SIN-1).[48] Figure 4b shows the results for the cytoprotection assays against peroxynitrite for compounds **2b–g**.

Theoretical Evaluation of ADME Properties. To correlate better the druglike properties of the coumarin–chalcone hybrid compounds, the lipophilicity, expressed as the octanol/water partition coefficient and herein called log *P*, as well as other theoretical calculations such as the topological polar surface area (TPSA), the number of hydrogen-bond acceptors, and the number of hydrogen-bond donors were calculated using the Molinspiration property program.[49] The theoretical prediction of the absorption, distribution, metabolism, and excretion (ADME) properties of all compounds is summarized in table 4.

RESULTS AND DISCUSSION

Taking into account the importance of the hydroxyl substituents in the antioxidant properties,[17,50] we have focused this study on hydroxy-coumarin–chalcone hybrids. All coumarin derivatives described in this Article (compounds **1a–i** and **2a–i**) were efficiently synthesized according to the simple and generalized experimental procedure outlined in

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Table 4. Theoretical Prediction[a] of the ADME Properties of Compounds 2a−i

compound	log *P*	molecular weight	TPSA (Å^2)	*n*−OH acceptors	*n*−OHNH donors	volume (Å^3)
2a	2.92	266.25	67.51	4	1	227.00
2b	2.89	266.25	67.51	4	1	227.00
2c	3.70	345.15	67.51	4	1	244.88
2d	3.32	280.28	67.51	4	1	243.56
2e	2.42	282.25	87.74	5	2	235.01
2f	3.89	345.15	67.51	4	1	244.88
2g	3.21	361.15	87.74	5	2	252.90
2h	2.16	298.25	107.97	6	3	243.03
2i	2.92	377.15	107.97	6	3	260.92

[a]TPSA, topological polar surface area; *n*−OH, number of hydrogen acceptors; *n*−OHNH, number of hydrogen bond donors. The data was determined with the Molinspiration calculation software.

Scheme 2. Electrochemical, biological, and theoretical studies were carried out for final tested compounds **2a−i**.

From the experimental results obtained by cyclic voltammetry, we observed that under these conditions all derivatives exhibited one (compounds **2a−g**) or two (compounds **2h** and **2i**) characteristic anodic peaks without the corresponding cathodic peak (Figure 1), indicating an irreversible oxidation process as a result of the oxidation of one hydroxyl group in any of the aromatic rings, forming the semiquinone radical.

The anodic peak potentials (Epa I and Epa II) obtained at a 0.75 V* s^{-1} scan rate are listed in Table 1. Monohydroxyl derivatives **2a**, **2c**, and **2d**, which contain a hydroxyl substituent at the para position of the benzoyl moiety, presented an anodic peak potential in the same positive potential range (Epa II = 0.77, 0.72, and 0.77 V, respectively). The same positive potential in derivatives **2a** and **2d** indicates that the methyl substituent at position eight of the coumarin core in **2d** is not relevant in the oxidation process. In contrast, the bromine group in derivative **2c** seems to provoke a slight decrease in the anodic peak potential (Epa II = 0.72 V). The same effect is observed for compound **2e** (Epa II = 0.74 V) that, in addition to the hydroxyl group present in the benzoyl moiety, also bears an additional hydroxyl group in the coumarin core. Compounds **2b** and **2f**, with a hydroxyl group in the coumarin moiety, presented oxidation potentials that shifted to more positive values (0.79 and 0.92 V, respectively), disfavoring the oxidation process. This effect is more pronounced for compound **2f** because of the interaction through intramolecular hydrogen bonding between the OH group in position eight and the oxygen atom of the pyrone ring as well as the electron-withdrawing properties of this pyrone system.[51]

Compounds **2g**, **2h**, and **2i** showed a characteristic peak (Epa I) attributed to the catechol moiety. This oxidation occurred at a low potential (0.30, 0.27, and 0.27 V, respectively) because of the electron-donating effect of the hydroxyl group at the 3′ position of the benzoyl moiety as well as the favorable quinone formation as the final product in these derivatives. In addition to this low-potential oxidation peak, compounds **2h** and **2i** showed an additional second peak that was assigned to the hydroxyl group at position eight of the coumarin core.

The data obtained for the characterization of the coumarin−chalcone radicals by ESR spectroscopy showed that in the absence of the spin trap *N*-*tert*-butyl-α-phenylnitrone (PBN), the oxidation of compounds **2a−d** and **2g** gave no ESR signal. However, in the presence of PBN, a hyperfine pattern with six lines was observed, corresponding to the formation of a carbon-centered semiquinone spin adduct ($a_H \approx 13.5$ and 2.3 G were found for all compounds).[52] Compound **2e**, with two hydroxyl groups, evidenced a symmetric ESR spectrum with five lines (Figure 2a), and it was interpreted as a semiquinone radical in which the hyperfine pattern corresponds to one triplet assigned to two equivalent hydrogen atoms with a_H = 2.37 G and two doublets corresponding to one non equivalent hydrogen atom with hyperfine constants a_H = 2.35 and 1.75 G.

Compound **2f** showed a hyperfine pattern with two lines (Figure 2b) and was simulated and characterized by two doublets resulting from two nonequivalent hydrogen atoms with a_H = 1.34 and 3.57 G. For the radical species of compound **2h**, the ESR spectrum (data not shown) was simulated by two doublets attributed to two nonequivalent hydrogen atoms with a_H = 4.35 and 2.37 G. Finally, for the radical species of compound **2i**, the hyperfine pattern was interpreted and simulated by four doublets resulting from four nonequivalent hydrogen atoms with hyperfine coupling constants a_H = 2.82, 2.93, 2.06, and 1.83 G as well as one triplet with a_H = 3.37 G resulting from two equivalent hydrogen atoms.

The antioxidant capacity of compounds **2a−i** was studied by ORAC-FL assays. From the data obtained, which is summarized in Table 2, we observed that the difference between the ORAC values could be related to the substituents present in these derivatives. Derivatives **2a** and **2b**, with one hydroxyl group at the para position of the benzoyl moiety and at the six position of the coumarin scaffold, respectively, presented ORAC values of 9.2 and 11.5, respectively. Compounds **2c** and **2d**, with a hydroxyl group in the same position as in compound **2a** but with an additional bromine substituent in position six of the coumarin or a methyl substituent at the position eight, respectively, presented ORAC values of 9.5 and 10.9, respectively. Derivative **2e**, which combines the substitution pattern of compounds **2a** and **2b**, was found to be the compound with the highest ORAC value (14.1).

However, by structurally comparing compounds **2c** and **2g**, we observed that the only difference is the presence of an additional hydroxyl group at the 3′ position of the benzoyl moiety in compound **2g**, forming a catechol function. This modification increases the antioxidant capacity in compound **2g** (ORAC = 13.0). ORAC values of around 8 were observed for compounds **2f**, **2h**, and **2i**. In addition to the catechol function, compounds **2h** and **2i** present an additional hydroxyl group at position eight of the coumarin ring compared to structurally related compound **2g**. This additional hydroxyl group could form a hydrogen bond with the oxygen atom of the pyrone ring, disfavoring the transfer of the hydrogen atom.

The antioxidant reactivity of compounds **2a−i** was studied by an ESR assay. With this method, it was observed that the

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intensity of the spectrum decreased in the presence of the tested coumarin−chalcone hybrid compounds that were added to the system (red line, Figure 3). This behavior was observed in all derivatives **2a−i**, and the percent of the radicals ($CH_3^{\bullet}$, $OH^{\bullet}$, $O_2^{\bullet-}$) scavenging activity is represented in Table 2. The scavenging values obtained could be related to the number and position of the hydroxyl substituents. Compounds **2g** and **2i** showed a different hyperfine pattern, which was attributed to the formation of carbon-centered spin adducts (Figure 3), demonstrating that the secondary semiquinone radical for each compound was trapped by the DMPO spin trap and the radical scavenging percentages were close to 100.

In general, the MTT assay only provides qualitative trends for the cytotoxicity of the compounds and the cytoprotective properties. The cytotoxicity of compounds **2a−i** on BAEC using different concentrations (10, 20, and 50 μM) is shown in Table 3. Derivatives **2b−g** showed cell viabilities between 86 and 100% up to the maximum concentration of 50 μM. Compounds **2a**, **2h**, and **2i** showed significant toxicity at 50 μM, whereas compound **2a**, which was the most toxic, resulted in only a 44% viability. The varying degrees in the toxicity of the compounds can be accounted for by several factors, such as the differences in their compartmentalization properties, the nature and concentration of ROS produced, and their ability to scavenge radicals and/or initiate pro-apoptotic signaling pathways.

Because compounds **2a, 2i**, and **2h** demonstrated significant toxicity, they were not used for the cytoprotection assays. However, compounds **2b−g**, because of their low toxicity, were examined at lower doses of 10 and 20 μM to test their cytoprotection against hydrogen peroxide (H_2O_2) or peroxynitrite ($ONOO^-$) generated from SIN-1, and the data are shown in Figure 4 panels a and b, respectively. Compounds **2b** and **2d** showed the highest cytoprotection against H_2O_2 compared to all compounds, which presented around 40% cytoprotection at 10 μM. At 20 μM, compounds **2c−2f** were cytoprotective against $ONOO^-$ by ∼90−95%, whereas compound **2b** imparted no protection at this concentration. Compound **2g** was not cytoprotective against both H_2O_2 and $ONOO^-$ at any of the concentrations tested. All of the studied compounds showed observable cytoprotection, with remarkable results for compounds **2b−f**. In our previous work,[53] it was shown that a lypophilic spin trap such as PBN protects BAEC from H_2O_2 induced toxicity, whereas more polar DMPO protects the cells more efficiently from $ONOO^-$. The studied compounds show a better cytoprotective effect against $ONOO^-$. However, because of the complexity of the mechanisms that are directly and indirectly involved in this assay, it is difficult to determine if a correlation exists between the cytoprotection and partition coefficients because these latter values were theoretically calculated. Moreover, the oxidation potentials may not show a direct correlation with the cytotoxicity/cytoprotection data because of other cellular events that are involved such as variations in the ability of the compounds to redox cycle, subcellular compartmentalization, degradation by cellular oxido-reductants, and the ability to induce (or suppress) nonradical-mediated pro-apoptotic pathways. Therefore, although we would like to determine if a direct correlation exists between the cytotoxicity and physical data, such as the partition coefficient and redox potentials, the complexity of the mechanisms involved in cell viability makes this correlation difficult to realize.[19,54,55]

From the data obtained for the theoretical evaluation of the ADME properties, one can notice that hybrid compounds **2a−i** do not break any point of the Lipinski's rule of five, making them promising leads for drug candidates.[56] TPSA and log *P* values are compatible with those described as a predictive indicator of a drug's capacity for membrane penetration.[57]

CONCLUSIONS

In this Article, we describe the synthesis of coumarin−chalcone hybrid compounds by employing the Knoevenagel reaction as a key step for the efficient preparation of a selected series. The electrochemical properties of the studied compounds proved to be excellent, presenting low oxidation potentials, very high ORAC values (all of them were higher than the reference compounds quercetin and catechin), and a high percentage of radical scavenging. In most cases, the compounds presented low cytotoxicity for BAEC in culture even at the highest concentration tested as well as good cytoprotective effects against H_2O_2 and $ONOO^-$ induced cell death. In addition, the whole series of compounds presented good theoretical ADME properties. Therefore, on the basis of these results, the synthesized coumarin−chalcone hybrid compounds can be considered to be promising scaffolds in the studied area.

It is worth noting that compound **2e** presented the highest ORAC value (14.1), good scavenging capacity, low cytotoxicity, and high cytoprotection values, especially against $ONOO^-$ induced cell death (almost 100% cell viability at 20 μM); therefore, it can be considered to be potential candidate as a lead compound for more thorough studies of its antioxidant properties.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded with a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as the internal standard (chemical shifts are given in δ values, *J* is given in Hz). Mass spectra were obtained using a Hewlett-Packard 5988A spectrometer. Elemental analyses were performed using a PerkinElmer 240B microanalyser and were within ±0.4% of calculated values in all cases. Silica gel (Merck 60, 230−00 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm). The purity of compounds **1a−i** and **2a−i** was assessed by HPLC and was found to be higher than 95%. All of the chemical reagents employed in the synthesis process were obtained from Aldrich Chemical Co., Fluka, Across, or Merck (analytical reagent grade). All reactions were carried out under a deoxygenated and dry argon atmosphere unless otherwise indicated. Argon was dried by flowing it through $CaCl_2$ columns, NaOH stones, and P_2O_5.

General Procedure for the Synthesis of Methoxy-3-benzoylcoumarins ***1a−i.***[40] To a solution of the appropriate β-ketoester (1 mmol) and the corresponding salicylaldehyde (1 mmol) in ethanol (3 mL) was added piperidine in a catalytic amount. The mixture was refluxed for 2−5 h. After completion (indicated by TLC), the reaction was cooled, and the precipitated was filtered and washed with cold ethanol and ether to afford the desired compound. The compounds were further recrystallized from $MeOH/CH_2Cl_2$.

6-Bromo-3-(4′-methoxybenzoyl)coumarin ***(1c).*** Yellow solid. Yield: 92%. mp 222−224 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.25 (s, 1H, H-4), 8.05 (s, 1H, H-5), 7.92 (d, *J* = 8.5 Hz, 2H, H-2′, H-6′), 7.84 (d, *J* = 8.7 Hz, 1H, H-7), 7.44 (d, *J* = 8.7 Hz, 1H, H-8), 7.05 (d, *J* = 8.5 Hz, 2H, H-3′, H-5′), 3.85 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.1, 164.5, 158.1, 153.5, 143.3, 135.9, 132.7, 131.9, 129.0, 128.3, 120.6, 119.0, 116.7, 114.6, 56.2. MS (EI) *m*/*z* (%):

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359/361 (M^+, 28), 135 (100), 92 (19), 77 (20). Anal. Calcd for $C_{17}H_{11}BrO_4$: C, 56.85; H, 3.09. Found: C, 56.82; H, 3.07.

*8-Methyl-3-(4′-methoxybenzoyl)coumarin (**1d**).* White solid. Yield 93%. mp 250−252 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.34 (s, 1H, H-4), 7.91 (d, J = 8.7 Hz, 2H, H-2′, H-4′), 7.73−7.24 (m, 3H, H-5, H-6, H-7), 7.06 (d, J = 8.7 Hz, 2H, H-3′, H-5′), 3.86 (s, 3H, OCH_3), 2.41 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 164.4, 158.4, 152.9, 152.8, 144.7, 134.7, 132.3, 129.6, 127.7, 125.8, 124.7, 118.5, 114.6, 56.1, 15.0. MS (EI) m/z (%): 295 ([M + 1]$^+$, 19), 294 (M^+, 100), 135 (88), 77 (31). Anal. Calcd for $C_{18}H_{14}O_4$: C, 73.46; H, 4.79; Found: C, 73.39; H, 4.57.

*6-Methoxy-3-(4′-methoxybenzoyl)coumarin (**1e**).* Yellow solid. Yield 80%. mp 182−184 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (s, 1H, H-4), 7.71 (d, J = 8.9 Hz, 2H, H-2′, H-6′), 7.13−6.94 (m, 3H, H-5, H-7, H-8), 6.77 (d, J = 8.9 Hz, 2H, H-3′, H-5′), 3.83 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 164.0, 158.3, 155.9, 148.6, 144.2, 132.3, 128.9, 127.3, 121.1, 118.9, 117.6, 114.2, 111.6, 55.9, 55.8. MS (EI) m/z (%): 311 ([M + 1]$^+$, 29), 310 (M^+, 92), 135 (100), 92 (22), 77 (27). Anal. Calcd for $C_{18}H_{14}O_5$: C, 69.67; H, 4.55. Found: C, 69.45; H, 4.33.

*6-Bromo-3-(3′,4′-dimethoxybenzoyl)coumarin (**1g**).* Yellow solid. Yield 98%. mp 223−224 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.23 (s, 1H, H-4), 8.05 (d, J = 2.4 Hz, 1H, H-5), 7.84 (dd, J = 8.8, 2.4 Hz, 1H, H-7), 7.56 (dd, J = 8.4, 2.0 Hz, 1H, H-6′), 7.52−7.38 (m, 2H, H-8, H-2′), 7.05 (d, J = 8.4 Hz, 1H, H-5′), 3.86 (s, 3H, 4′-OCH_3), 3.81 (s, 3H, 3′-OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.8, 157.7, 154.3, 153.2, 149.0, 142.8, 135.6, 131.6, 128.7, 127.9, 125.8, 120.4, 118.8, 116.4, 111.3, 111.1, 56.1, 55.9. MS (EI) m/z (%): 389/391 (M^+, 59), 165 (100), 71 (31). Anal. Calcd for $C_{18}H_{13}BrO_5$: C, 55.55; H, 3.37. Found: C, 55.54; H, 3.39.

*8-Methoxy-3-(3′,4′-dimethoxybenzoyl)coumarin (**1h**).* Pale-yellow solid. Yield: 95%. mp 238−240 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (s, 1H, H-4), 7.54 (dd, J = 8.2, 2.1 Hz, 1H, H-6′), 7.46 (d, J = 2.1 Hz, 1H, H-2′), 7.43−7.27 (m, 3H, H-5, H-6, H-7), 7.04 (d, J = 8.2 Hz, 1H, H-5′), 3.93 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 157.9, 154.1, 148.9, 146.6, 144.6, 143.5, 128.8, 127.1, 125.7, 124.9, 120.7, 119.0, 115.5, 111.1, 111.0, 56.3, 56.0, 55.8. MS (EI) m/z (%): 341 ([M + 1]$^+$, 25), 340 (M^+, 97), 165 (100), 137 (9), 77 (10). Anal. Calcd for $C_{19}H_{16}O_6$: C, 67.05; H, 4.74. Found: C, 67.06; H, 4.74.

*6-Bromo-8-methoxy-3-(3′,4′-dimethoxybenzoyl)coumarin (**1i**).* Pale-yellow solid. Yield 90%. mp 276−277 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.21 (s, 1H, H-4), 7.67−7.49 (m, 3H, H-5, H-7, H-6′), 7.46 (s, 1H, H-2′), 7.04 (d, J = 8.5 Hz, 1H, H-5′), 3.96 (s, 3H, 4′-OCH_3), 3.85 (s, 3H, 8-OCH_3), 3.80 (s, 3H, 3′-OCH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 195.3, 155.1, 149.9, 149.8, 148.2, 143.6, 138.1, 129.4, 128.9, 128.7, 126.0, 123.4, 119.0, 116.8, 112.6, 112.0, 59.0, 57.6, 56.7. MS (EI) m/z (%): 418/420 (M^+, 98), 165 (100), 77 (18). Anal. Calcd for $C_{19}H_{15}BrO_6$: C, 54.43; H, 3.61. Found: C, 54.43; H, 3.62.

*General Procedure for the Synthesis of Hydroxy-3-benzoylcoumarins (**2a−i**).*[40] To the corresponding methoxy-3-benzoylcoumarin (1 mmol), BBr_3 in DCM (20 mmol, 1M) was added in a Schlenk tube. The tube was sealed, and the reaction mixture was heated at 80 °C for 48 h. The resulting crude product was treated with MeOH and rotated to dryness. The obtained precipitate was recrystallized from MeOH or purified by flash chromatography using hexane/ethyl acetate mixtures as the eluent to afford the desired hydroxy derivative.

*3-(4′-Hydroxybenzoyl)coumarin (**2a**).* White solid. Yield: 78%. mp 242−243 °C (mp lit. 239 °C).[58] ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.58 (bs, 1H, OH), 8.30 (s, 1H, H-4), 7.89−7.75 (m, 3H, H-5, H-2′, H-6′), 7.78−7.64 (m, 1H, H-7), 7.51−7.37 (m, 2H, H-6, H-8), 6.86 (d, J = 8.8, 2H, H-3′,H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.1, 163.4, 158.5, 154.3, 144.2, 133.6, 132.9, 129.9, 127.8, 127.5, 125.3, 118.7, 116.7, 115.8. MS (EI) m/z (%): 267 ([M + 1]$^+$, 20), 266 (M^+, 93), 237 (25), 121 (100), 93 (22), 65 (25). Anal. Calcd for $C_{16}H_{10}O_4$: C, 72.18; H, 3.79. Found: C, 71.83; H, 3.53.

*6-Bromo-3-(4′-hydroxybenzoyl)coumarin (**2c**).* Pale-yellow solid. Yield: 73%. mp 295−298 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.61 (bs, 1H, OH), 8.22 (s, 1H, H-4), 8.05 (d, J = 2.4 Hz, 1H, H-5), 7.93−7.73 (m, 3H, H-7, H-2′, H-6′), 7.45 (d, J = 8.8 Hz, 1H, H-8), 6.86 (d, J = 8.7 Hz, 2H, H-3′, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.0, 163.6, 158.1, 153.4, 142.8, 136.2, 133.0, 131.8, 128.5, 127.6, 120.7, 119.0, 116.7, 115.9. MS (EI) m/z (%): 343/345 (M^+, 49), 121 (100). Anal. Calcd for $C_{16}H_9BrO_4$: C, 55.68; H, 2.63. Found: C, 55.30; H, 2.45.

*3-(4′-Hydroxybenzoyl)-8-methylcoumarin (**2d**).* White solid. Yield: 56%. mp 252−253 °C. ^{1}H NMR (300 MHz DMSO-d_6) δ 10.56 (bs, 1H, OH), 8.27 (s, 1H, H-4), 7.80 (d, J = 8.4 Hz, 2H, H-2′, H-6′), 7.63 (d, J = 7.5 Hz, 1H, H-5), 7.57 (d, J = 7.5 Hz, 1H, H-7), 7.30 (t, J = 7.5 Hz, 1H, H-6), 6.86 (d, J = 8.4 Hz, 2H, H-3′, H-5′), 2.40 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 163.4, 158.6, 152.6, 144.6, 134.6, 132.9, 127.8, 127.7, 127.2, 125.6, 124.8, 118.5, 115.9, 15.3. MS (EI) m/z (%): 281 ([M + 1]$^+$, 54), 280 (M+, 96), 252 (46), 251 (51), 120 (100), 93 (56), 65 (67). Anal. Calcd for $C_{17}H_{12}O_4$: C, 72.85; H, 4.32. Found: C, 72.67; H, 4.12.

*6-Hydroxy-3-(4′-hydroxybenzoyl)coumarin (**2e**).* Brown crystals. Yield: 65%. mp 301−303 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.58 (bs, 1H, 4′−OH), 9.86 (bs, 1H, 8-OH,), 8.21 (s, 1H, H-4), 7.79 (d, J = 8.8 Hz, 2H, H-2′, H-6′), 7.37−7.24 (m, 1H, H-5), 7.19−7.01 (m, 2H, H-7, H-8), 6.86 (d, J = 8.8 Hz, 2H, H-3′, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.3, 163.4, 158.8, 154.4, 147.7, 144.0, 132.9, 127.8, 127.6, 121.6, 119.2, 117.6, 115.9, 113.7. MS (EI) m/z (%): 283 ([M + 1]$^+$, 9), 282 (M^+, 51), 121 (100), 93 (15), 65 (18). Anal. Calcd for $C_{16}H_{10}O_5$: C, 68.09; H, 3.57. Found: C, 68.23; H, 3.33.

*3-Benzoyl-6-bromo-8-hydroxycoumarin (**2f**).* Pale-yellow solid. Yield: 98%. mp 293−295 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.92 (s, 1H, OH), 8.30 (s, 1H, H-4), 7.93 (dd, J = 8.1, 1.4 Hz, 2H, H-2′, H-6′), 7.79−7.63 (m, 1H, H-5), 7.63−7.44 (m, 3H, H-3′, H-4′, H-5′), 7.34−7.24 (m, 1H, H-8). ^{13}C NMR (75 MHz, DMSO-d_6) δ 191.9, 157.9, 146.3, 144.8, 142.7, 136.3, 134.5, 130.0, 129.2, 127.8, 121.9, 121.8, 120.9, 116.2. MS (EI) m/z (%): 344/346 (M^+, 46), 105 (100), 77 (59). Anal. Calcd for $C_{16}H_9BrO_5$: C, 53.21; H, 2.51. Found: C, 52.97; H, 2.41.

*6-Bromo-3-(3′,4′-dihydroxybenzoyl)coumarin (**2g**).* Yellow solid. Yield: 72%. mp 264−266 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.14 (s, 1H, OH), 9.47 (s, 1H, OH), 8.19 (s, 1H, H-4), 8.05 (d, J = 2.1 Hz, 1H, H-5), 7.84 (dd, J = 8.8, 2.2 Hz, 1H, H-7), 7.45 (d, J = 8.8 Hz, 1H, H-8), 7.39−7.20 (m, 2H, H-2′, H-6′), 6.82 (d, J = 8.1 Hz, 1H, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.6, 157.9, 153.3, 152.5, 145.9, 142.3, 135.7, 131.7, 128.4, 127.8, 124.5, 120.5, 119.0, 116.7, 116.3, 115.7. MS (EI) m/z (%): 360/362 (M^+, 87), 253 (47), 251 (45), 227 (14), 225 (18), 137 (100), 109 (44). Anal. Calcd for $C_{16}H_9BrO_5$: C, 53.21; H, 2.51. Found: C, 53.23; H, 2.52.

*8-Hydroxy-3-(3′,4′-dihydroxybenzoyl)coumarin (**2h**).* Bright-yellow solid. Yield: 81%. mp 277−279 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.35 (s, 1H, OH), 10.10 (s, 1H, OH), 9.47 (s, 1H, OH), 8.20 (s, 1H, H-4), 7.36−7.06 (m, 5H, H-5, H-6, H-7, H-2′, H-6′), 6.82 (d, J = 8.2 Hz, 1H, H-5′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 190.2, 158.4, 152.3, 145.8, 145.0, 144.2, 142.8, 128.0, 127.4, 125.3, 124.2, 119.7, 119.5, 116.4, 115.7. MS (EI) m/z (%): 299 ([M + 1]$^+$, 8), 298 (M^+, 41), 155 (16), 137 (100), 91 (31). Anal. Calcd for $C_{16}H_{10}O_6$: C, 64.43; H, 3.38. Found: 64.43; H, 3.39.

*6-Bromo-8-hydroxy-3-(3′,4′-dihydroxybenzoyl)coumarin (**2i**).* Pale-yellow solid. Yield 69%. mp 300−302 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (s, 1H, OH), 10.14 (s, 1H, OH), 9.48 (s, 1H, OH), 8.14 (s, 1H, H-4), 7.45 (d, J = 2.2 Hz, 1H, H-5), 7.38−7.12 (m, 3H, H-7, H-2′, H-5′), 6.81 (dd, J = 8.1, 3.0 Hz, 1H, H-6′). ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.3, 157.4, 152.0, 145.8, 145.5, 142.3, 141.9, 128.0, 127.4, 124.0, 121.1, 121.0, 120.5, 115.9, 115.8, 115.2. MS (EI) m/z (%): 376/378 (M^+, 100), 269 (30), 267 (24), 137 (80), 109 (74). Anal. Calcd for $C_{16}H_9BrO_6$: C, 50.95; H, 2.41. Found: C, 50.92; H, 2. 43.

Cyclic Voltammetry. Cyclic voltammetry was carried out using a potentiostat/galvanostat VersaSTAT 3 that was provided with the V3-Studio electrochemistry software package in 50 mM sodium phosphate buffer (pH 7.4) at room temperature using a three-electrode cell. A glassy-carbon electrode was used as the working electrode, a platinum wire, the auxiliary electrode, and Ag, AgCl/KCl (ca. 3.5 M), the reference electrode. All coumarin−chalcone hybrids were studied to 1 mM in 20% methanol as final concentration.

Characterization of Coumarin-Chalcone Radicals by ESR Spectroscopy. ESR measurements were carried out on an ESR spectrometer equipped with a high-sensitivity resonator at room temperature. Unless otherwise indicated, the instrument settings used for general spectral acquisition were microwave power, 10 mW, modulation amplitude, 1 G, receiver gain, 1.0×10^4 or 1.0×10^5, scan time, 21.5 s, time constant, 40.0 s, and sweep width, 120 G. The oxidation process was carried out by using 30% H_2O_2 and NaOH (25 mM) in the presence of chalcone−coumarin hybrids. Scans were integrated using the Bruker WINEPR v.2.11b software. The sample cells used were 50 μL glass capillary tubes. An automatic fitting program carried out the spectrum simulation. The hyperfine splitting constants were estimated to be accurate to within 0.05 G.

Radical-Generating System to Test Coumarin-Chalcone Hybrids. To the corresponding coumarin−chalcone hybrid compound (1.2 mmol) in dimethyl sulfoxide (20 μL), a hydrogen peroxide solution (50 μL, 30%) and a sodium hydroxide solution (50 μL, 25 mM) were added. The total volume of the resulting solution (300 μL) was completed with DMSO to achieve a final 4 mM solution of the tested compounds.

ORAC-FL Assay. The ORAC-FL assays were carried out on a Synergy HT multidetection microplate reader (Bio-Tek Instruments, Inc.) using white polystyrene 96-well plates. Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5 software. The oxygen radical absorbance capacity was determined as previously described[46] with slight modifications. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4) at a 200 μL final volume. FL (70 nM, final concentration) and coumarin−chalcone solutions in methanol with a range of concentration between 0.3 to 2 μM and 0.5 to 7 μM for the monohydroxy and dihydroxy derivatives, respectively, were placed in each well of the 96-well plate. This range of concentration was selected empirically to obtain a good separation between the fluorescence curves. This separation was crucial for more accurate data treatment.[59,60] The mixture was preincubated for 15 min at 37 °C before rapidly adding the AAPH solution (18 mM, final concentration). The microplate was immediately placed in the reader and automatically shaken prior to each reading. The fluorescence was recorded every 1 min for 120 min. A blank with FL and AAPH using methanol instead of the antioxidant solution as well as five calibration solutions using Trolox (0.5 to 2.5 μM) as a standard molecule were also used in each assay. The inhibition capacity was expressed as ORAC values, and it was quantified by employing eq 1. All reaction mixtures were prepared in triplicate, and at least three independent assays were performed for each sample.

$$\text{relative ORAC value} = \frac{[\text{AUC} - \text{AUC}^0]}{[\text{AUC}_{\text{trolox}} - \text{AUC}^0]} \frac{[\text{trolox}]}{[\text{derivative}]} \quad (1)$$

where AUC^0 = area under the curve blank; AUC = area under the curve derivative; $\text{AUC}_{\text{trolox}}$ = area under the curve Trolox (standard); [trolox] = Trolox concentration, molar; and [Derivative] = derivative concentration, molar

The AUC was calculated by integrating the decay of the fluorescence where F_0 is the initial fluorescence read at 0 min and F is the fluorescence read at a particular time. The AUC_{NET} corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. Data processing was performed using Origin Pro 8 SR2 (Origin Lab Corporation).

Antioxidant Reactivity by ESR Assay. The reactivity of all coumarin−chalcone hybrids against hydroxyl radicals was assessed using the noncatalytic Fenton method that was previously described.[56] In a typical experiment, 150 μL of dimethyl sulfoxide was mixed with 50 μL of NaOH (3 mM) followed by the addition of 50 μL of DMPO (30 mM) spin trap and 50 μL of hydrogen peroxide (30%). All derivatives were studied at a 4 mM final concentration and a 300 μL final volume. The mixture was positioned in the ESR cell, and the spectra were recorded after 5 min of the reaction.

Biological Studies. All chemicals and solvents used were of the highest analytical grade. For cell culture, all reagents or materials were purchased and used without further purification. Bovine aortic endothelial cells were purchased from Cell Systems (Kirkland, WA) and nonessential amino acids and Fetal Bovine Serum (FBS) were purchased from Gibco and Invitrogen, respectively.

Cell Culture. Cells were cultured in 75 cm^2 tissue-culture flasks using Dulbecco's modified Eagle's medium with 1 g/L of D-glucose and 4 mM L-glutamine and supplemented with 10% fetal bovine serum, 2.5 mg/L of endothelial cell growth supplement, and 1% nonessential amino acids in the absence of antibiotics at 37 °C in a humidified atmosphere of 5% CO_2 and 20% O_2. The medium was changed every 2 to 3 days, and the cells were subcultured once they reached 80−85% confluence.[51]

Cytotoxicity and Cytoprotection. Cytotoxicity assays in BAEC cells were performed for all coumarin−chalcone hybrids using the MTT reduction assay. BAEC were seeded in 24-well plates at a density ranging between 10^4−10^5 cells/mL per well. After the cells reached 60−70% confluence, they were incubated in the presence of 10 to 50 μM of the corresponding coumarin−chalcone derivatives for 24 h. After incubation, the medium was changed (2 mL) and 45 μL of a solution of MTT (5 mg/mL in DMEM supplemented with 0.5% FBS) was added to each well. The cells were incubated for another 2 h at 37 °C. The medium was then removed, and the wells were rinsed once with PBS. DMSO (0.6 mL) was added to each well at room temperature to solubilize the formazan crystals. The dissolved formazan was then transferred into 96-well culture plates, and the absorbance was measured at 570 nm using a UV−vis spectrophotometer.[57,58]

The percent of inhibition of cell viability was calculated using eq 2

$$\text{percent inhibition} = (1 - A_t/A_s)100 \quad (2)$$

where A_t and A_s are the absorbance of the sample solution and the solvent alone, respectively.

To assess the cytoprotective properties of the compounds against H_2O_2 and SIN-1, BAEC (10^4−10^5 cells/mL) were grown to 60−70% confluence in 24-well plates and each well was incubated with the selected derivative at 10 and 20 μM concentrations for 24 h. After incubation, the cells were washed with 1× PBS twice to remove the residual drug and exposed to 1 mM of H_2O_2 and 500 μM of SIN-1 for 2 h and 4 h, respectively. These time periods were chosen on the basis of the observed cell death (>50%) using the H_2O_2 and SIN-1 concetrations mentioned above. Finally, cell viability was evaluated by the MTT reduction assay and calculated using eq 2.

Statistical Analysis. The experimental results are expressed as the mean ± standard error of the mean (SEM) and are accompanied by the number (n) of observations. The differences between the groups were analyzed by student's t test. A p value lower than 0.05 was considered statistically significant.

Theoretical Evaluation of ADME Properties. The theoretical properties of log P, TPSA, n−OH, and n−OHNH were calculated by the methodology developed by Molinspiration using their software available on http://www.molinspiration.com/services/properties.html.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

AAPH, 2,2′-azobis(2-methylpropionamidine)dihydrochloride; BAEC, bovine aortic endothelial cells; DCM, dichloromethane; DMEM, Dulbecco's modified Eagle's medium; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; Epa, anodic peak potential; FL, fluorescein; MAO, monoamine oxidase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; ORAC, oxygen radical absorbance capacity; PBN, α-phenyl-*N*-*tert*-butylnitrone; RNS, reactive nitrogen species; SIN-1, 3-morpholinosydnonimine; TBAP, tetrabutylammonium perchlorate; TLC, thin layer chromatography; TPSA, topological polar surface area; trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

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