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# Synthesis of selected 3- and 4-arylcoumarin derivatives and evaluation as potent antioxidants

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**Abstract** A series of hydroxyl-, methoxy-, and acetoxy-substituted 3- and 4-arylcoumarins were synthesized. All title compounds were screened for their antioxidant capacity, ability to scavenge the 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical, and ability to chelate iron ions. Furthermore, all derivatives were assessed using molecular properties prediction and drug likeness using Molinspiration. It was found that all studied derivatives were potential candidates for further research, as they complied with Lipinski's rule of five for drug likeness. 3- or 4-arylcoumarins that possess two hydroxyl groups in *ortho* position, such as **4h**, **5b**, **h**, and **6a**, had remarkable half-maximal effective concentration ($EC_{50}$) for radical scavenging, with better performance than known antioxidants in DPPH and metal-chelating assays. In addition, the cupric-reducing antioxidant capacity and ferric-reducing antioxidant power of the synthesized compounds were investigated for antioxidant activity. Among them, **5g**, **h** and **6a**, **b** showed significantly better Trolox equivalent antioxidant capacity (TEAC) than standard compounds. The results demonstrate that the compounds with dihydroxyl groups at 6- and 7-positions of the benzopyrone ring of the arylcoumarin structure are the most active of the series as antioxidants. On the basis of these findings, these new coumarin derivatives are potential therapeutic candidates for pathogenesis of many diseases characterized by free-radical overproduction.

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## Introduction

Various natural products, especially phenolic compounds such as flavonoids and coumarins, are used in many cultures as medicinal agents and food preservatives due to their wide spectrum of pharmacological activities and low toxicity. Isolating new pharmacologically active compounds from natural sources and synthesizing their more potent analogs is a major field of research [1, 2]. Coumarin (2*H*-chromen-2-one) derivatives constitute one of the most common families of green plant secondary metabolites. Since many substitutions are possible on the core structure, coumarin derivatives have diverse pharmacological activities, including anticoagulant, anticancer, antiviral, antibacterial, antiinflammatory, and cholesterol-lowering properties [3–6]. The interesting biological properties of coumarins make these compounds very attractive targets for organic synthesis [7]. To enhance the pharmacological activities and lower the toxicity, numerous coumarin derivatives have been synthesized and published in literature [8].

All aerobic organisms produce reactive oxygen species (ROS) as byproducts of aerobic respiration [9]. Organisms have antioxidant defenses that protect against oxidative damage and numerous damage removal and repair enzymes to remove or repair damaged molecules [10]. When the balance between generation and natural elimination of ROS is broken, oxidative stress occurs [11]. ROS and oxidative stress play important roles in the pathogenesis of neurodegenerative diseases, cancer, arteriosclerosis, and diabetes [12]. For prevention and treatment of these diseases, antioxidants are often considered as drug candidates [13].

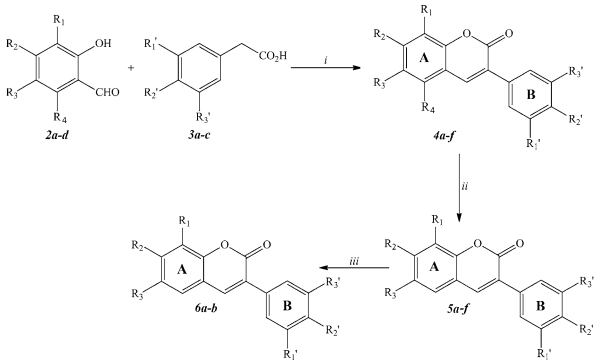
It is known that coumarin derivatives are potent antioxidants and can affect formation and scavenging of ROS, exhibiting tissue-protective antioxidant properties. These activities are likely related to their structural similarities to flavonoids and benzophenones, which interact with Fe(III) and thus inhibit hydroxyl radical and hydrogen peroxide formation by Fenton reactions [14]. However, there have been very few systematic studies reported on the relationship between antioxidant activity and coumarin structure [15–17] and even fewer studies have been published on arylcoumarins [18].

Focusing on the biological importance of hydroxyl groups for the antioxidant activity and diverse pharmacological properties of the coumarin nucleus, design and synthesis of some known and novel arylcoumarin derivatives (**4c–e**, **5e**, **f**) were carried out, and their in vitro antioxidant and radical-scavenging effects investigated. We mainly focused on the effect of the number and position of substitutions of 3- and 4-arylcoumarins possessing methoxy, hydroxy, and acetoxy groups.

## Results and discussion

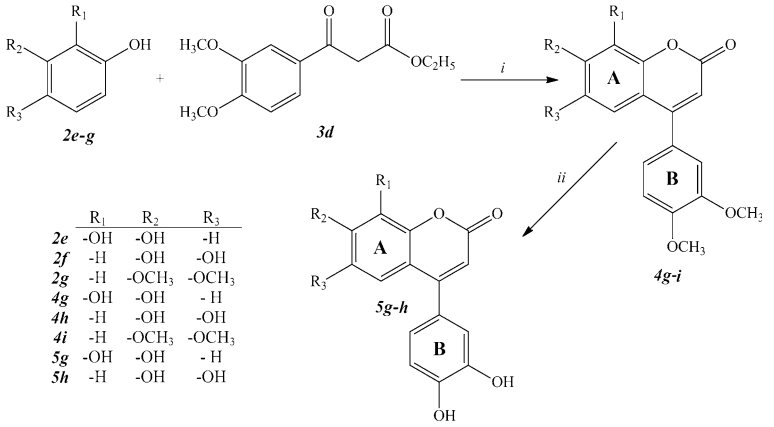
We synthesized and investigated the antioxidant effects of 29 coumarin derivatives with special focus on peripheral substituents on the A- and B-rings (Schemes 1, 2). Novel compounds (**4c**, **d**, **e**, **5e**, **f**) were efficiently synthesized according to the protocol outlined in Scheme 1. The general reaction conditions and characterization data of the new compounds are described in the "Experimental" section. The preparation of 3- and 4-arylcoumarin derivatives was performed via Perkin and Pechmann reactions, respectively, using commercially available and affordable starting materials, and newly synthesized compounds were characterized and confirmed by nuclear magnetic resonance (NMR) and mass-spectral studies.

The radical-scavenging activity of the 3- and 4-arylcoumarin derivatives was determined by investigating the scavenging of the stable free radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH); the results are given in Table 1. The DPPH radical is a stable free radical due to the extensive delocalization of the unpaired electron. Tested coumarins were able to reduce stable violet DPPH to yellow DPPH-H, with $EC_{50}$ values ranging from 0.049 mM for 6,7-dihydroxy-3-(3′,4′-dihydroxyphenyl)-2*H*-1-chromen-2-one (**6a**) to 16.411 mM for 5,7-diacetoxy-3-(3′,4′,5′-trimethoxyphenyl)-2*H*-1-chromen-2-one (**4f**). As can be seen from Table 1, compounds **4g**, **h**, **5b–e**, **g**, **h**, and **6a**, **b** are more potent agents for reduction of DPPH radical (with $EC_{50}$ values ranging from 0.049 to 0.075 mM) compared with known antioxidants Trolox, α-tocopherol, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) (with $EC_{50}$ values of 0.093, 0.132, and 0.331 mM, respectively). When 3- and 4-aryl analogs were compared, no discernible differences were found; however, 3-arylcoumarin derivatives are generally more active than their 4-arylcoumarin counterparts. These data show that, in determining the radical-scavenging activity of the arylcoumarins, the number and location of the hydroxyl groups, rather than the position of the phenyl group, is important. Similarly, Svinyarov and Bogdanov showed that the radical-scavenging activity is dependent on the number and position of the hydroxyl groups [19]. A large number of experimental and




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_1'$ | $R_2'$ | $R_3'$ |
|---|---|---|---|---|---|---|---|
| *2a* | -H | -$OCH_3$ | -$OCH_3$ | - | - | - | - |
| *2b* | -H | -OH | -OH | - | - | - | - |
| *2c* | -OH | -OH | -H | - | - | - | - |
| *2d* | -H | -OH | -H | -OH | - | - | - |
| *3a* | - | - | - | - | -OH | -OH | -H |
| *3b* | - | - | - | - | -$OCH_3$ | -$OCH_3$ | -H |
| *3c* | - | - | - | - | -$OCH_3$ | -$OCH_3$ | -$OCH_3$ |
| *4a* | -H | -$OCH_3$ | -$OCH_3$ | -H | -OAc | -OAc | -H |
| *4b* | -H | -OAc | -OAc | -H | -$OCH_3$ | -$OCH_3$ | -H |
| *4c* | -OAc | -OAc | -H | -H | -$OCH_3$ | -$OCH_3$ | -H |
| *4d* | -H | -OAc | -H | -OAc | -OAc | -OAc | -H |
| *4e* | -H | -OAc | -OAc | -H | -$OCH_3$ | -$OCH_3$ | -$OCH_3$ |
| *4f* | -H | -OAc | -H | -OAc | -$OCH_3$ | -$OCH_3$ | -$OCH_3$ |
| *5a* | -H | -$OCH_3$ | -$OCH_3$ | -H | -OH | -OH | -H |
| *5b* | -H | -OH | -OH | -H | -$OCH_3$ | -$OCH_3$ | -H |
| *5c* | -OH | -OH | -H | -H | -$OCH_3$ | -$OCH_3$ | -H |
| *5d* | -H | -OH | -H | -OH | -OH | -OH | -H |
| *5e* | -H | -OH | -OH | -H | -$OCH_3$ | -$OCH_3$ | -$OCH_3$ |
| *5f* | -H | -OH | -H | -OH | -$OCH_3$ | -$OCH_3$ | -$OCH_3$ |
| *6a* | -H | -OH | -OH | -H | -OH | -OH | -H |
| *6b* | -OH | -OH | -H | -H | -OH | -OH | -H |

**Scheme 1** Synthesis and structure of studied 3-arylcoumarin derivatives. *i* $(CH_3CO)_2O$, $CH_3COONa$, under $N_2$, 160 °C; *ii* $CH_3OH/HCl(aq)$, reflux; *iii* $BBr_3$, $CH_2Cl_2$, dry ice/$(CH_3)_2CO$




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| *2e* | -OH | -OH | -H |
| *2f* | -H | -OH | -OH |
| *2g* | -H | $-OCH_3$ | $-OCH_3$ |
| *4g* | -OH | -OH | - H |
| *4h* | -H | -OH | -OH |
| *4i* | -H | $-OCH_3$ | $-OCH_3$ |
| *5g* | -OH | -OH | - H |
| *5h* | -H | -OH | -OH |

**Scheme 2** Synthesis and structure of studied 4-arylcoumarin derivatives. *i* $CF_3COOH$, heat; *ii* $BBr_3$, $CH_2Cl_2$, dry ice/$(CH_3)_2CO$

**Table 1** Radical-scavenging and metal-chelating data obtained for tested coumarin derivatives

| Compound | DPPH[a] (mM) | Metal chelating[b] (mM) |
|---|---|---|
| 3-Arylcoumarins | | |
| **4a** | 8.651 ± 0.176 | n.a. |
| **4b** | 1.694 ± 0.020 | 3.105 ± 0.112 |
| **4c** | 1.678 ± 0.006 | 3.626 ± 0.180 |
| **4d** | 7.311 ± 0.177 | n.a. |
| **4e** | 1.313 ± 0.169 | 3.261 ± 0.102 |
| **4f** | 16.411 ± 0.467 | n.a. |
| **5a** | 0.109 ± 0.009 | 1.577 ± 0.032 |
| **5b** | 0.056 ± 0.003 | 0.847 ± 0.027 |
| **5c** | 0.072 ± 0.003 | 1.021 ± 0.027 |
| **5d** | 0.072 ± 0.002 | 1.112 ± 0.099 |
| **5e** | 0.066 ± 0.004 | 0.874 ± 0.027 |
| **5f** | 0.614 ± 0.025 | 1.214 ± 0.025 |
| **6a** | 0.049 ± 0.002 | 0.746 ± 0.031 |
| **6b** | 0.062 ± 0.002 | 0.798 ± 0.018 |
| 4-Arylcoumarins | | |
| **4g** | 0.075 ± 0.005 | 1.012 ± 0.031 |
| **4h** | 0.059 ± 0.002 | 0.857 ± 0.012 |
| **4i** | 11.514 ± 0.609 | n.a. |
| **5g** | 0.063 ± 0.002 | 0.792 ± 0.008 |
| **5h** | 0.055 ± 0.002 | 0.764 ± 0.012 |
| BHT | 1.077 ± 0.049 | 0.963 ± 0.018 |
| BHA | 0.331 ± 0.027 | 0.984 ± 0.011 |
| EDTA | n.d. | 0.185 ± 0.012 |
| Trolox | 0.093 ± 0.005 | 1.191 ± 0.017 |

Results given as mean ± standard deviation (SD, $n = 3$)

*n.a.* no activity, *n.d.* not determined

[a] Antioxidant activity measured using DPPH radical assay and data expressed as $EC_{50}$ (mM)

[b] Ferrous ion chelating effects of the compounds expressed as $EC_{50}$ (mM)

theoretical studies have revealed that coumarins containing catechol moiety offer enhanced radical-scavenging activity for natural antioxidants [20]. However, as is readily apparent by comparison of the $EC_{50}$ values in Table 1, the true difference lies in the presence or absence of two *ortho* phenolic functions. Compounds with *ortho*-dihydroxy substitutions, which are converted upon radical scavenging to *o*-quinonoid derivatives, showed the highest DPPH scavenging activity. We found that 6,7-substitution is more effective than 7,8-substitution, and similarly substitutions on the A-ring are more important than those on the B-ring. When hydroxyl groups were changed for acetoxy group, the DPPH scavenging activity was drastically reduced. When acetoxy was on the A-ring, there was low activity, but when it was present on the B-ring, the activity decreased further. Substitution with methoxy group further reduced the activity. These observations indicate that the hydroxy and acetoxy groups must be at *ortho* positions to each other for maximum activity. It is worth noting that hydroxyl or acetoxy groups at *meta* position in the A- or B- ring of the coumarin nucleus contributed very little to inhibition of the DPPH radical. Polyphenols such as pyrogallol and gallic acid, which have phenolic OH groups at *ortho* position, possess strong radical-scavenging activities. It is known that coumarin derivatives possess antioxidant activities due to their structural analogy with benzophenones and flavonoids [21]. Bailly et al. [22] investigated the DPPH radical-scavenging activity of some 3-hydroxycoumarin derivatives and demonstrated that 6,7-dihydroxycoumarins are potent scavengers. Kancheva et al. showed that the antioxidant activity of 4-methylcoumarins depends on the relative positions of the two phenolic hydroxyls. Similar to our data, they found that various substitutions on the A-ring lead to decreased antioxidant activity in the following sequence: 6,7-dihydroxy > 7,8-dihydroxy > 5,7-dihydroxy [23].

The DPPH scavenging reaction progress of the most potent antioxidant compounds and Trolox at the same conditions (at 0.1 mM) was also investigated and is shown in Fig. 1. It is apparent from this figure that coumarin derivatives are faster to react and the rate of DPPH consumption increases in the following sequence: **6a** > **6b** > **5h** > **5g** > Trolox. The reaction mechanism is based on an electron transfer (ET) reaction, and as occurs in other ET-based assays, the DPPH scavenging capacity is strongly influenced by steric accessibility.

The ferrozine method was used to analyze iron chelation. It was observed that, for iron chelation, two hydroxyl groups at adjacent *ortho* positions correspond to the highest iron-chelation potency. Relatively lower potency was found for *o*-acetoxy-containing coumarin derivatives. Mladenka et al. [24] found similar results for 4-methylcoumarin derivatives. To determine and compare the antioxidant activity of 3- and 4-arylcoumarin derivatives, the ferric-reducing antioxidant power (FRAP) and the cupric-reducing antioxidant power (CUPRAC) methods were employed; the results are given in Fig. 2. Coumarin compounds with an *ortho*-hydroxylation pattern on ring A showed remarkable antioxidant efficiency, far greater than that of Trolox. Compounds possessing *ortho*-hydroxyl substitutions on the A-ring were more effective than their analogs with *ortho*-hydroxyl substitutions on the B-ring. Similar observations can be made from the results of the DPPH scavenging study, but the effect of the *o*-hydroxy group's location is more apparent in the CUPRAC and FRAP assays. This difference can be explained by the increased stability of the

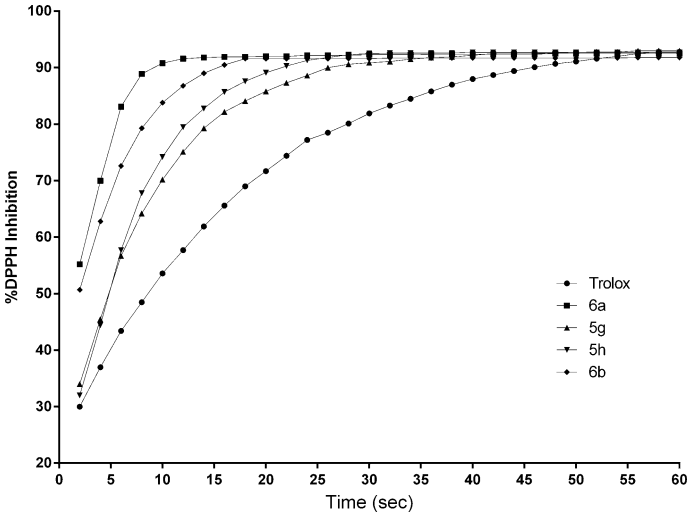



**Fig. 1** DPPH scavenging capacity of selected coumarin derivatives as a function of reaction time (s)

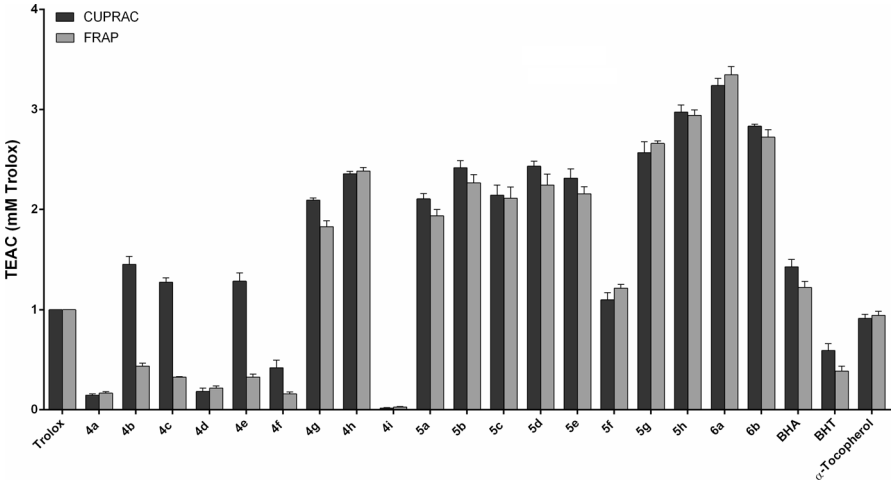



**Fig. 2** Cupric-reducing antioxidant capacity (CUPRAC) and total ferric-reducing power (FRAP) of synthesized compounds and standard antioxidants. Results expressed as Trolox equivalent antioxidant capacity (TEAC), defined as the concentration (mM) of Trolox solution having antioxidant capacity equivalent to 1.0 mM solution of each substance. Each value represents mean ± SD ($n = 3$)

aryloxyl radical of the compound due to the presence of the carbonyl group in conjugation with ring A [25]. The dissociation energy of the O–H bond is smaller due to the conjugation of the oxygen-centered unpaired *p*-orbital with a pair on the adjacent oxygen atom, which makes the transient radical more stable. As shown in Fig. 3, data obtained using the CUPRAC method are comparable to those obtained

from the FRAP assay (Spearman $r = 0.9547$), except for compounds **4b**, **c**, **e**. All three of these compounds have diacetoxy substitution on their A-ring, and their $TEAC_{FRAP}$ values are approximately three times lower than their $TEAC_{CUPRAC}$ values. The effects of the methoxy substitutions are the lowest among the tested coumarin derivatives in vitro. Roussaki et al. also demonstrated that low antioxidant efficiency of dimethoxy 3-arylcoumarins could be significantly increased by demethylation of the molecule [26]. Furthermore, orally administered phenolic compounds undergo hydroxylation and/or glucuronide and sulfate conjugation primarily by intestinal microflora and secondarily in the liver and other tissues, and it is well known that phenolic methoxyl groups of coumarins are demethylated in the liver [27]. These facts suggest that coumarin derivatives that show low activity in vitro may be converted into more active compounds during metabolism. As shown in Table 1 and Fig. 2, 6,7-dihydroxy-3-(3′,4′-dihydroxyphenylcoumarin) (**6a**) showed the strongest antioxidant activity, in both radical-scavenging and reduction power tests. Our results show that the position and type of substituent attached at the benzenoid rings of the arylcoumarin molecules had an influence on the radical-scavenging and reduction power potency. In general, substitution at 6,7-position had more influence on the properties of coumarin than substitution at 7,8-position. The effect of the hydroxyl groups in the B-ring is less than their effect in the A-ring. Bailly et al. [22] demonstrated that 6,7-hydroxycoumarin is a potent scavenger of DPPH radical. Raj et al. [28] suggested that conversion of diacetoxycoumarins to dihydroxycoumarins occurs in the presence of initiating free radicals such as DPPH, peroxy radical, or superoxide radical through formation of reactive ketene. However, Kancheva et al. [23] found that 7,8-diacetoxy-4-methylcoumarins, which do not have any free phenolic groups, do not have any chain-breaking antioxidant activity in vitro or any radical-scavenging activity towards DPPH radical. The results are consistent with a previous report showing that resonance structures of the radicals derived from coumarins with substitution at 6-position are especially stable because of the *ortho*-quinone form [27]. In addition,

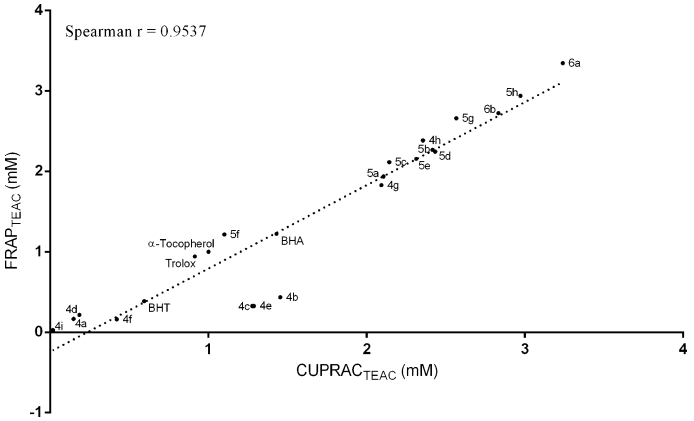



**Fig. 3** Correlation graph for $CUPRAC_{TEAC}$ and $FRAP_{TEAC}$ values of synthesized compounds. Correlation analysis performed by Spearman rho coefficient, $p < 0.0001$. *AA* ascorbic acid, *BHA* butylated hydroxyanisole, *BHT* butylated hydroxytoluene, *GA* gallic acid

the type of substituent has a strong influence on the action of coumarins as antioxidants. Our results indicate that the hydroxy group has the strongest influence, followed by the acetoxy group, and the methoxy group with least influence. A similar correlation was also reported for flavonoids [29].

Yang et al. [18] found that 6,7-dihydroxy-3-(3′,4′-dihydroxyphenyl)coumarin (**6a**) has potent antioxidative effect against 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH)-induced DNA strand breakage and exhibited specific antiproliferative activity against HL-60 cells ($EC_{50}$ = 22.1 μM) [18]. Zhang et al. [13] found that 7,8-dihydroxy-4-(3′,4′-dihydroxyphenyl)coumarin (**5g**) has potent DPPH, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and nitric oxide radical-scavenging capacity.

To integrate the patterns of data and reduce the dimension of the dataset, principal component analysis (PCA) was performed, and the resulting biplot graph, which contains score and loading plots, is shown in Fig. 4. The first two PCs accounted for 81.37 and 15.31 % of the data variance. Further components explained 2.14, and 1.19 % of the variance, respectively. The loading plot was used to assess the importance among the four antioxidant assays. In PC1, the FRAP and CUPRAC assays had positive loadings, and in PC2, all assays had positive loadings. The scores plot was used to gain an overview of the similarities or differences among the coumarin derivatives.

According to the results, it is possible to separate the tested compounds into five main groups. The most active group contains the compounds **6a**, **b** and **5g**, **h** with two *o*-hydroxyl substitutions on both the A- and B-ring of the arylcoumarin system. The second active group consists of **4g**, **h** and **5a**–**e**, which have one *o*-hydroxyl substitution on either ring A or ring B. Coumarin derivatives belonging to both of these groups showed considerably higher antioxidant activity than standard antioxidant compounds Trolox, α-tocopherol, BHA, and BHT.

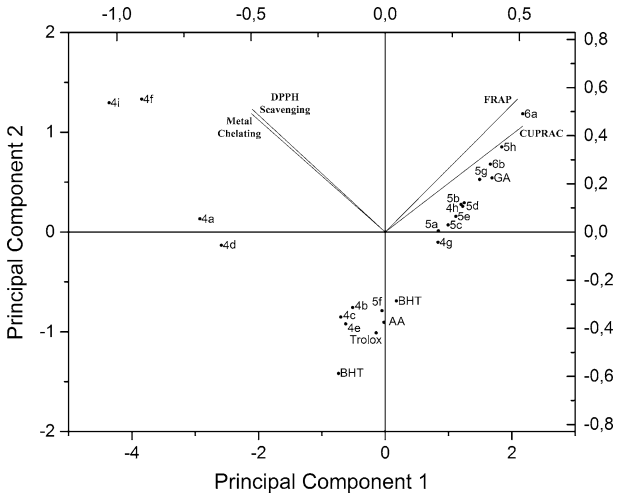



**Fig. 4** Biplot graph obtained from principal component analysis of CUPRAC, FRAP, DPPH scavenging, and metal-chelating assays. *BHA* butylated hydroxyanisole, *BHT* butylated hydroxytoluene

**Table 2** Drug likeness score for coumarin derivatives

| Compounds | Log *P* | Molecular weight | TPSA | *n* OH acceptors | *n* OHNH donors | Volume |
|---|---|---|---|---|---|---|
| **4a** | 1.997 | 398.367 | 101.289 | 8 | 0 | 340.145 |
| **4b** | 1.997 | 398.367 | 101.289 | 8 | 0 | 340.145 |
| **4c** | 2.194 | 398.367 | 101.289 | 8 | 0 | 340.145 |
| **4d** | 1.382 | 454.387 | 135.431 | 10 | 0 | 378.112 |
| **4e** | 1.981 | 428.393 | 110.523 | 9 | 0 | 365.691 |
| **4f** | 2.376 | 428.393 | 110.523 | 9 | 0 | 365.691 |
| **4g** | 2.62 | 314.293 | 89.135 | 6 | 2 | 267.122 |
| **4h** | 2.391 | 314.293 | 89.135 | 6 | 2 | 267.122 |
| **4i** | 3.006 | 342.347 | 67.147 | 6 | 0 | 302.178 |
| **5a** | 2.391 | 314.293 | 89.135 | 6 | 2 | 267.122 |
| **5b** | 2.391 | 314.293 | 89.135 | 6 | 2 | 267.122 |
| **5c** | 2.62 | 314.293 | 89.135 | 6 | 2 | 267.122 |
| **5d** | 1.974 | 286.239 | 111.123 | 6 | 4 | 232.066 |
| **5e** | 2.376 | 344.319 | 98.369 | 7 | 2 | 292.668 |
| **5f** | 2.574 | 344.319 | 98.369 | 7 | 2 | 292.668 |
| **5g** | 2.005 | 286.239 | 111.123 | 6 | 4 | 232.066 |
| **5h** | 1.776 | 286.239 | 111.123 | 6 | 4 | 232.066 |
| **6a** | 1.776 | 286.239 | 111.123 | 6 | 4 | 232.066 |
| **6b** | 2.005 | 286.239 | 111.123 | 6 | 4 | 232.066 |

Data determined using Molinspiration calculation software

*log P* octanol/water partition coefficient, *TPSA* topological polar surface area, *n OH* number of hydrogen acceptors, *n OHNH* number of hydrogen-bond donors

A traditional method to evaluate "drug likeness" is to check compliance with Lipinski's rule of five, which covers the number of hydrophilic groups, molecular weight, and hydrophobicity. From the obtained data, it is also remarkable that all the coumarin derivatives possess log *P* values (logarithm of the partition coefficient between water and 1-octanol) compatible with those required to cross membranes. Topological polar surface area (TPSA), described as a predictive indicator of membrane penetration, was also found to be positive (Table 2). In addition, it can be observed that no violations of Lipinski's rule (molecular weight, log *P*, number of hydrogen donors and acceptors) were found. This is important information regarding the promising potential of these derivatives [30].

## Conclusions

Our results show that the position and type of substituents attached at the aromatic parts of arylcoumarin derivatives influence the radical scavenging and reduction power. These dihydroxycoumarins are worth further evaluation for their pharmacological properties.

## Experimental

### Chemicals and apparatus

Starting chemicals were purchased from Aldrich (St Louis, MO, USA) or Merck (Darmstadt, Germany), unless otherwise stated. Melting points were obtained on a Gallenkamp apparatus. Infrared (IR) spectra were taken from KBr pellets with a Shimadzu FT-IR spectrometer, model 8300. $^{1}$H and $^{13}$C NMR spectra were obtained with a Varian Mercury 400 MHz spectrometer. Mass spectra were obtained with a Q-TOF LC–MS instrument (model 6200 series; Agilent, CA, USA).

*Methods*

The general methods used for synthesis of the studied compounds are summarized in Schemes 1 and 2. 3-Phenylcoumarin derivatives (**4a–f**, **5a–f**, **6a**, **b**) were prepared by reaction of substituted hydroxybenzaldehydes with corresponding arylacetic acids under traditional Perkin conditions [31], while 4-phenylcoumarin derivatives (**4g–i**, **5g**, **h**) were obtained using condensation of selected phenols with ethyl-3,4-dimethoxybenzoylacetate according to the Pechmann [32] reaction. The $^{1}$H NMR, $^{13}$C NMR, and mass spectra, and proposed structures of novel compounds are given in the Electronic Supplementary Material.

*Synthesis of 2-hydroxy-4,5-dimethoxybenzaldehyde (**2a**)* Boron tribromide (5.0 g, 20 mmol) was slowly added to a stirring solution of 2,4,5-trimethoxybenzaldehyde (**1**) (5.0 g, 25 mmol) in $CH_2Cl_2$ in a dry ice/acetone bath. After 1 h, the reaction mixture was warmed to room temperature and stirred for 3 h, then aqueous HCl (10 %; 20 mL) solution was added. The mixture was extracted with $CH_2Cl_2$ (3 × 100 mL). The organic layers were washed with NaCl solution (2 × 100 mL) and water (100 mL). The product was dried over $Na_2SO_4$ and evaporated to produce **2a** (4.3 g, 91 %). M.p.: 103 °C, lit. 104 °C [32].

*General procedure for synthesis of o-diacetoxy-3-phenylchromenones (**4a–f**)* A mixture of benzaldehyde (**2a–d**) (20 mmol), phenylacetic acids (**3a–c**) (20 mmol), and sodium acetate (50 mmol) was stirred in 40 mL acetic anhydride at 160 °C under $N_2$ atmosphere for 6 h. On reaction completion, the mixture was poured into ice-cold water (200 mL). The crude product was filtered and recrystallized from ethanol.

*6,7-Dimethoxy-3-(3′,4′-diacetoxyphenyl)coumarin (**4a**)* A mixture of 2-hydroxy-4,5-dimethoxybenzaldehyde (**2a**) (3.6 g, 20 mmol), 3,4-dihydroxyphenylacetic acid (**3a**) (3.4 g, 20 mmol), and sodium acetate (4.1 g, 50 mmol) in acetic anhydride (40 mL) was treated as described above to yield **4a** (7.7 g, 97 %). M.p.: 206 °C, lit. 207–208 °C [32]. $^{1}$H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta = 2.29$ (s, 6H, $COCH_3$), 3.89 (s, 3H, $OCH_3$), 3.90 (s, 3H, $OCH_3$), 6.85 (s, 1H), 6.93 (s, 1H), 7.22 (d,

$J = 8.2$ Hz, 1H), 7.64 (dd, $J = 8.2$, 2 Hz, 1H), 7.59 (d, $J = 2$ Hz, 1H), 7.81 (s, 1H) ppm.

*6,7-Diacetoxy-3-(3′,4′-dimethoxyphenyl)coumarin (**4b**)* A mixture of 2,4,5-trihydroxybenzaldehyde (**2b**) (3.0 g, 20 mmol), 3,4-dimethoxyphenylacetic acid (**3b**) (3.9 g, 20 mmol), and sodium acetate (4.1 g, 50 mmol) in acetic anhydride (40 mL) was treated as described above to produce **4b** (4.8 g, 60 %). M.p.: 220 °C, lit. 220 °C [32]. $^1$H NMR (400 MHz, $CD_3OD$, 25 °C): $\delta = 2.33$ (s, 3H, $COCH_3$), 2.34 (s, 3H, $COCH_3$), 3.92 (s, 3H, $OCH_3$), 3.96 (s, 3H, $OCH_3$), 6.91 (d, $J = 8.2$ Hz, 1H), 7.25 (dd, $J = 8.2$, 2.3 Hz, 1H), 7.25 (d, $J = 2.3$ Hz, 1H), 7.29 (s, 1H), 7.41 (s, 1H), 7.70 (s, 1H) ppm.

*7,8-Diacetoxy-3-(3′,4′-dimethoxyphenyl)coumarin (**4c**)* A mixture of 2,3,4-trihydroxybenzaldehyde (**2c**) (3.0 g, 20 mmol), 3,4-dimethoxyphenylacetic acid (**3b**) (3.9 g, 20 mmol), and sodium acetate (4.1 g, 50 mmol) in acetic anhydride (40 mL) was treated as described above to produce **4c** (5.2 g, 65 %). M.p.: 218 °C. $^1$H NMR (400 MHz, DMSO, TMS): $\delta$ 8.25 (s, 1H), 7.70 (d, $J = 8.7$ Hz, 1H), 7.30 (dd, $J = 7.80$ and 2.08 Hz, 1H), 7.30 (br s, 1H), 7.29 (d, $J = 7.80$ Hz, 1H), 7.03 (d, $J = 9$ Hz, 1H), 3.80 (s, 6H), 2.40 (s, 3H), 2.30 (s, 3H) ppm. MS (*m*/*z*): 398.1 $[M]^+$.

*5,7-Diacetoxy-3-(3′,4′-diacetoxyphenyl)coumarin (**4d**)* A mixture of 2,4,6-trihydroxybenzaldehyde (**2c**) (3.0 g, 20 mmol), 3,4-dihydroxyphenylacetic acid (**3a**) (3.4 g, 20 mmol), and sodium acetate (4.1 g, 50 mmol) in acetic anhydride (40 mL) was treated as described above to produce **4d** (5.2 g, 65 %). M.p.: 185–187 °C. $^1$H NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 8.13$ (s, 1H), 7.61 (d, $J = 2.1$ Hz, 1H), 7.60 (d, $J = 8.2$ Hz, 1H), 7.34 (dd, $J = 8.8$ and 2.1 Hz, 1H), 7.26 (d, $J = 2.1$ Hz, 1H), 7.11 (d, $J = 2.1$ Hz, 1H), 2.38 (s, 3H, -$COCH_3$), 2.29 (s, 6H, –$COCH_3$), 2.28 (s, 3H, –$COCH_3$). $^{13}$C NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 169.4$, 169.0, 168.7, 168.6, 159.4, 154.1, 152.9, 148.0, 142.8, 142.0, 134.6, 133.2, 127.7, 125.9, 124.4, 123.9, 113.6, 111.7, 108.1, 21.4, 21.3, 20.8, 20.7. MS (*m*/*z*): 454.1 $[M]^+$.

*6,7-Diacetoxy-3-(3′,4′,5′-trimethoxyphenyl)coumarin (**4e**)* A mixture of 2,4,5-trihydroxybenzaldehyde (**2b**) (3.0 g, 20 mmol), 3,4,5-trimethoxyphenylacetic acid (**3c**) (3.4 g, 20 mmol), and sodium acetate (4.1 g, 50 mmol) in acetic anhydride (40 mL) was treated as described above to produce **4e** (5.2 g, 65 %). M.p.: 185–188 °C. $^1$H NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 8.22$ (s, 1H), 7.66 (s, 1H), 7.47 (s, 1H), 7.04 (br s, 2H), 3.80 (s, 6H, $OCH_3$), 3.69 (s, 3H, $OCH_3$), 2.31 (s, 3H, –$COCH_3$), 2.30 (s, 3H, –$COCH_3$). $^{13}$C NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 168.8$, 168.3, 159.6, 153.0, 150.9, 144.7, 139.6, 139.0, 138.6, 130.2, 127.1, 122.7, 118.1, 111.9, 106.7, 60.5, 56.5, 20.8, 20.7. MS (*m*/*z*): 428.1 $[M]^+$.

*5,7-Diacetoxy-3-(3′,4′,5′-trimethoxyphenyl)coumarin (**4f**)* A mixture of 2,4,6-trihydroxybenzaldehyde (**2d**) (3.0 g, 20 mmol), 3,4,5-trimethoxyphenylacetic acid (**3c**) (3.4 g, 20 mmol), and sodium acetate (4.1 g, 50 mmol) in acetic anhydride (40 mL) was treated as described above to produce **4f** (4.9 g, 61 %). M.p.:

185–188 °C, lit. 186–187 °C [33]. $^{1}$H NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 8.04$ (s, 1H), 7.24 (d, $J = 2.1$ Hz, 1H), 7.10 (d, $J = 2.1$ Hz, 1H), 6.96 (br s, 2H), 3.80 (s, 6H, $OCH_3$), 3.68 (s, 3H, $OCH_3$), 2.39 (s, 3H, $-COCH_3$), 2.29 (s, 3H, $-COCH_3$). $^{13}$C NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 169.4$, 169.0, 159.5, 153.9, 152.9, 152.7, 147.8, 138.7, 134.0, 130.2, 127.3, 113.5, 111.7, 108.0, 107.1, 60.5, 56.5, 21.3, 21.2.

*General procedure for synthesis of o-dihydroxy-3-phenylchromenones (**5a–f**)* The *o*-diacetoxy-3-phenylchromenone derivatives (**4a–f**) were refluxed with MeOH/HCl (aq) for 3 h, and methanol was removed using distillation. The crude product was obtained after ethanol recrystallization and filtration.

*6,7-Dimethoxy-3-(3′,4′-dihydroxyphenyl)chromenone (**5a**)* Compound **4a** (6.4 g, 16 mmol) was treated as described above to yield **5a** (3.5 g, 70 %). M.p.: 219 °C, lit. 220–221 °C [32]. $^{1}$H NMR (400 MHz, $CD_3OD$): $\delta = 3.77$ (s, 3H, $OCH_3$), 3.83(s, 3H, $OCH_3$), 6.71 (d, $J = 8.4$ Hz, 1H), 6.79 (s, 1H), 6.88 (dd, $J = 8.2$, 1.8 Hz, 1H), 7.10 (d, $J = 1.8$ Hz, 1H), 7.13 (s, 1H), 7.61 (s, 1H) ppm.

*6,7-Dihydroxy-3-(3′,4′-dimethoxyphenyl)chromenone (**5b**)* Compound **4b** (4.7 g, 12 mmol) was treated as described above to yield **5b** (2.9 g, 77 %). M.p.: 255 °C, lit. 255–256 °C [32]. $^{1}$H NMR (400 MHz, $CD_3OD$, 25 °C): $\delta = 3.79$ (s, 3H, $OCH_3$), 3.85 (s, 3H, $OCH_3$), 6.79 (s, 1H), 6.94 (d, $J = 8.9$ Hz, 1H), 7.11 (s, 1H), 7.31 (dd, $J = 8.4$, 2 Hz, 1H), 7.35 (d, $J = 2$ Hz, 1H), 7.85 (s, 1H) ppm.

*7,8-Dihydroxy-3-(3′,4′-dimethoxyphenyl)chromenone (**5c**)* Compound **4c** (4.7 g, 12 mmol) was treated as described above to yield **5c** (2,3 g, 72 %). M.p.: 220–221 °C, lit. 220 °C [34]. $^{1}$H NMR (400 MHz, $CD_3OD$, 25 °C): $\delta = 3.75$ (s, 3H, $OCH_3$), 3.81 (s, 3H, $OCH_3$), 6.71 (d, $J = 8.4$ Hz, 1H), 6,87 (d, J = 8.4 Hz, 1H), 6.92 (d, $J = 8.4$ Hz, 1H), 7.15 (dd, $J = 8.5$ and 2 Hz, 1H), 7.22 (d, $J = 2$ Hz, 1H), 7.80 (s, 1H) ppm.

*5,7-Dihydroxy-3-(3′,4′-dihydroxyphenyl)chromenone (**5d**)* Compound **4d** (4.7 g, 12 mmol) was treated as described above to yield **5d** (3,1 g, 79 %). M.p.: >300 °C, lit. 339 °C [35]. $^{1}$H NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 10.60$ (s, 1H, –OH), 10.30 (s, 1H, –OH), 8.98 (br s, 2H, –OH), 7.89 (s, 1H), 7.10 (d, $J = 2.1$ Hz, 1H), 6.92 (dd, $J = 8.2$ and 2.1 Hz, 1H), 6.72 (d, $J = 8.2$ Hz, 1H), 6.24 (d, $J = 2.3$ Hz, 1H) 6.18 (d, $J = 2.3$ Hz, 1H). $^{13}$C NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 161.9$, 160.8, 156.2, 155.8, 145.9, 145.2, 134.3, 126.9, 120.2, 119.7, 116.0, 115.8, 102.8, 98.7, 94.0.

*6,7-Dihydroxy-3-(3′,4′,5′-trimethoxyphenyl)chromenone (**5e**)* Compound **4e** (4.7 g, 12 mmol) was treated as described above to yield **5e** (2.9 g, 82 %). M.p.: 220–221 °C. $^{1}$H NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 9.80$ (br s, 2H, -OH), 8.08 (s, 1H), 7.02 (s, 1H), 6.99 (br s, 2H), 6.75 (s, 1H), 3.79 (s, 6H, $OCH_3$), 3.67 (s, 3H, $OCH_3$). $^{13}$C NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 160.7$, 152.9, 150.9, 148.4,

143.5, 141.3, 137.9, 131.2, 122.4, 112.7, 111.9, 106.4, 102.6, 60.5, 56.4. MS (*m*/*z*): 344.1 $[M]^+$.

*5,7-Dihydroxy-3-(3′,4′,5′-trimethoxyphenyl)chromenone (**5f**)* Compound **4f** (4.7 g, 12 mmol) was treated as described above to yield **5f** (2,7 g, 76 %). M.p.: 220–221 °C. $^1$H NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 10.68$ (s, 1H, –OH), 10.39 (s, 1H, –OH), 8.04 (s, 1H), 6.92 (br s, 2H), 6.27 (d, $J = 2.1$ Hz, 1H), 6.21 (d, $J = 2.0$ Hz, 1H), 3.79 (s, 6H, $OCH_3$), 3.67 (s, 3H, $OCH_3$). $^{13}$C NMR (400 MHz, $d_6$-DMSO, ppm): $\delta = 162.4$, 160.4, 156.6, 156.1, 152.9, 137.8, 136.1, 131.4, 120.0, 106.3, 102.7, 98.8, 94.1, 60.5, 56.4. MS (*m*/*z*): 344.1 $[M]^+$.

*Synthesis of 6,7-dihydroxy-3-(3′,4′-dihydroxyphenyl)chromenone (**6a**)* As described previously, boron tribromide (3.0 g, 12 mmol) was slowly added to a stirring solution of **5a** (2.9 g, 10 mmol) in $CH_2Cl_2$ in a dry ice/acetone bath. After 1 h, the reaction mixture was warmed to room temperature and stirred for 3 h, then aqueous HCl (10 %; 20 mL) solution was added. The mixture was extracted with $CH_2Cl_2$ (3 × 100 mL). The organic layers were washed with NaCl solution (2 × 100 mL) and water (100 mL). The product was dried over $Na_2SO_4$ and evaporated to produce **6a** (2.1 g, 72.4 %). M.p.: 298 °C, lit. >300 °C [36]. $^1$H NMR (400 MHz, DMSO, TMS): $\delta$ 7.89 (s, 1H), 7.14 (d, $J = 2.2$ Hz, 1H), 7.01 (s, 1H), 6.95 (dd, $J = 8.2$ and 2.2 Hz, 1H), 6.75 (d, $J = 8.2$ Hz, 1H), 6.73 (s, 1H).

*Synthesis of 7,8-dihydroxy-3-(3′,4′-dihydroxyphenyl)chromenone (**6b**)* Compound **5c** (2.9 g, 10 mmol) was treated as described above to yield **6b** (2,3 g, 79,2 %). M.p.: 294 °C, lit. 295 °C [37]. $^1$H NMR (400 MHz, DMSO): $\delta = 6{,}69$ (d, $J = 8.4$ Hz, 1H), 6.85 (d, $J = 8.4$ Hz, 1H), 6.95 (dd, $J = 8.4$ Hz and 2.0 Hz, 1H), 7.17 (d, $J = 8.4$ Hz, 1H), 7.25 (d, $J = 2$ Hz, 1H) 7.91 (s, 1H), 9.07 (s, 1H), 9.13 (1H), 9.36 (s, 1H), 9.91 (s, 1H) ppm.

*General procedure for synthesis of o-methoxy-4-phenylchromenones (**4g–i**)* Using a typical procedure for cyclization reaction, a mixture of phenols (**2e–g**) (47 mmol), ethyl-3,4-dimethoxybenzoylacetate (**3d**) (47 mmol), and $CF_3COOH$ (15 mL) was refluxed for 6 h, cooled, collected by filtration, and washed with water, and solvent was evaporated under vacuum.

*7,8-Dihydroxy-4-(3′,4′-dimethoxyphenyl)chromenone (**4g**)* A mixture of 1,2,3-benzenetriol (**2e**; 5.99 g, 47 mmol), ethyl-3,4-dimethoxybenzoylacetate (**3d**; 11.97 g, 47 mmol), and $CF_3COOH$ (15 mL) was refluxed for 6 h, cooled, collected by filtration, washed with water, and dried under vacuum to yield **4g** (7.89 g, 52 %). M.p.: 271–272 °C, lit. 274.1–274.4 °C [38]. $^1$H NMR (400 MHz, $CD_3OD$, 25 °C): $\delta = 3.78$ (s, 3H, $OCH_3$), 3.80 (s, 3H, $OCH_3$), 6.10 (s, 1H), 6.72 (d, $J = 2.0$ Hz, 1H), 6.85 (d, $J = 8.4$ Hz, 1H), 6.89 (d, $J = 8.6$ Hz, 1H), 6.92 (dd, $J = 8.5$, 1.6 Hz, 1H).

*6,7-Dihydroxy-4-(3′,4′-dimethoxyphenyl)chromenone (**4h**)* A mixture of 1,2,4-benzenetriol (**2f**; 5.99 g, 47 mmol), ethyl-3,4-dimethoxybenzoylacetate (**3d**;

11.97 g, 47 mmol), and $CF_3COOH$ (15 mL) was treated as described above to produce **4h** (7.28 g, 48 %). M.p.: >300 °C, lit. >300 °C [31]. $^1H$ NMR (400 MHz, $CD_3OD$, 25 °C): $\delta = 3.79$ (s, 3H, $OCH_3$), 3.82 (s, 3H, $OCH_3$), 6.01 (s, 1H), 6.70 (d, $J = 2.0$ Hz, 1H), 6.84 (d, $J = 8.2$ Hz, 1H), 6.86 (s, 1H), 7.0 (dd, $J = 8.5, 1.6$ Hz, 1H).

*6,7-Dimethoxy-4-(3′,4′-dimethoxyphenyl)chromenone (**4i**)* A mixture of 3,4-dimethoxyphenol (**2g**; 7.2 g, 47 mmol), ethyl-3,4-dimethoxybenzoylacetate (**3d**; 11.97 g, 47 mmol), and $CF_3COOH$ (15 mL) was treated as described above to produce **4i** (8.24 g, 42.9 %). M.p.: 221 °C, lit. 222–223 °C [39]. $^1H$ NMR ($CDCl_3$): $\delta = 7.07$ (dd, $J = 8.3$, 1.9 Hz, 1H), 7.02 (d, $J = 8.3$ Hz, 1H), 6.98 (d, 1H, $J = 1.9$ Hz), 6.97 (s, 1H), 6.92 (s, 1H), 6.25 (s, 1H), 3.98 (s, 6H), 3.92 (s, 3H), 3.79 (s, 3H).

*Synthesis of 7,8-dihydroxy-4-(3′,4′-dihydroxyphenyl)chromenone (**5g**)* As described in 5.1.1, boron tribromide (4.5 g, 18 mmol) was slowly added to a stirring solution of **4g** (4.35 g, 15 mmol) in $CH_2Cl_2$ in a dry ice/acetone bath. After 1 h, the reaction mixture was warmed to room temperature and stirred for 3 h, then aqueous HCl (10 %; 30 mL) solution was added. The mixture was extracted with $CH_2Cl_2$ (3 × 100 mL). The organic layers were washed with NaCl solution (2 × 100 mL) and water (100 mL). The product was dried over $Na_2SO_4$ and evaporated to produce **5g** (3.6 g, 86 %). M.p.: 301 °C, lit. >300 °C [38]. $^1H$ NMR (500 MHz, DMSO, TMS): $\delta = 6.91$ (br d, $J = 8.75$ Hz, 1H), 6.86–6.84 (m, 2H),6.77 (s, 1H), 6.76 (dd, $J = 8.90$ and 1.50 Hz, 1H), 6.50 (br s, 1H), 6.00 (d, $J = 1.5$ Hz, 1H).

*Synthesis of 6,7-dihydroxy-4-(3′,4′-dihydroxyphenyl)chromenone (**5h**)* Compound **4h** (4.35 g, 15 mmol) was treated as described above to yield **5h** (3.4 g, 81 %). M.p.: 304 °C, lit. >300 °C [40]. $^1H$ NMR (500 MHz, DMSO, TMS): $\delta = 6.94$ (s, 1H), 6.87 (d, $J = 8.1$ Hz, 1H), 6.85 (d, $J = 2.0$ Hz, 1H), 6.77 (s, 1H), 6.76 (dd, $J = 8.1$ and 2.0 Hz, 1H), 5.96 (s, 1H).

## Antioxidant assays

### *DPPH radical-scavenging activity*

The scavenging activity was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method described by Blois [41] with slight modifications. In summary, 1 mL DPPH (in methanol, 0.1 mM) and 250 µL coumarin derivative (in methanol containing 5 µL DMSO, conc. 10–100 µg/mL) were shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517 nm. The capacity for scavenging free radicals was calculated from the following formula:

$$\% \ \text{Inhibition} = (A_c - A_s / A_c) \times 100,$$

where $A_c$ is the absorbance of the control and $A_s$ is the absorbance of the test compound. The radical-scavenging activity of the samples is expressed in terms of

$EC_{50}$ (concentration required for a 50 % decrease in absorbance of DPPH radical). $EC_{50}$ values were calculated from the graph of % scavenging activity against the various concentrations of the samples plotted using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA. USA). A lower $EC_{50}$ (mM) value indicates greater antioxidant activity. To investigate the reaction progress of the DPPH scavenging, 250 μL of compounds **6a–g**, **5g**, **h**, and Trolox (0.1 mM) and 1 mL DPPH (in methanol, 0.1 mM) were shaken briefly and the absorbance was measured at 517 nm at 2-s intervals for 60 s.

*Ferric-reducing antioxidant power (FRAP) assay*

Ferric ion ($Fe^{3+}$) reducing power was evaluated using the method developed by Oyaizu with slight modifications [42]. Sample (100 μL, dissolved in phosphate buffer 50 μM, pH 6.6, containing 5 μL DMSO) was mixed with phosphate buffer (200 μL, 50 μM, pH 6.6) and $K_3Fe(CN)_6$ (200 μL, 1 %). The mixture was incubated at 50 °C for 20 min. A portion (250 μL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 10,000*g* for 10 min. The upper layer of the solution (700 μL) was mixed with $FeCl_3$ (150 μL, 0.1 %), and the absorbance was measured at 700 nm. BHT, BHA, and Trolox were used as positive controls. Results are expressed as Trolox equivalent antioxidant capacity (TEAC), defined as the concentration (mM) of Trolox solution having antioxidant capacity equivalent to 1.0 mM solution of the substance under investigation. A higher TEAC value indicates greater reducing power ability.

*Cupric-reducing antioxidant capacity (CUPRAC) assay*

Cupric-reducing antioxidant capacity (CUPRAC) was determined as described by Apak et al., with slight changes [43]. Briefly, 800 μL of coumarin derivatives was dissolved in $NH_4Ac$ buffer pH 7, containing 5 μL DMSO, and mixed with 160 μL $NH_4Ac$ buffer pH 7, 160 μL $CuCl_2$ (10 mM), 160 μL neocuproine (7.5 mM). After 30 min, absorbance was measured at 450 nm. Results are expressed as TEAC values. A higher TEAC value indicates greater reducing power ability.

*Metal chelating*

Ferrous ion ($Fe^{2+}$) chelating activity was determined by inhibiting ferrous–ferrozine complex formation after treatment of test material with ferrous ion ($Fe^{2+}$). Compounds at concentrations of 20–100 μg/mL in 500 μL methanol were added to a solution of 2 mM $FeCl_2$ (500 μL). The reaction was initiated by addition of 5 mM ferrozine (200 μL) in methanol. Then, the mixture was shaken vigorously at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm [44]. The percentage inhibition of ferrozine–$Fe^{2+}$ complex formation was calculated as

$$[(A_0 - A_s)/A_s] \times 100,$$

where $A_0$ is the absorbance of the control and $A_s$ is the absorbance of the extract/standard. $Na_2EDTA$ was used as positive control [45]. The ferrous ion chelating effects of the compounds are expressed in terms of $EC_{50}$ (concentration required for chelating 50 % of ferrous ions). A lower $EC_{50}$ (mM) value indicates greater antioxidant activity.

*Theoretical evaluation of ADME properties*

The most widely used absorption, distribution, metabolism, and excretion (ADME) model is Lipinski's rule of five [46], which is based on four simple rules related to molecular properties. To better understand the overall properties of the described compounds, theoretical prediction of ADME properties [molecular weight, lipophilicity (expressed as log *P*), number of hydrogen donors and acceptors] of all the compounds was carried out and is presented in Table 2 [47].

*Statistical analysis*

All experiments were performed in triplicate, and data obtained from experiments were analyzed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Results are expressed as mean ± SD. The level of significance was defined as $p < 0.05$. The results from the different antioxidant assays were subjected to principal component analysis using OriginPro 9.1 (OriginLab Corporation, Northampton, MA).

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**Compliance with ethical standards**

**Conflict of interest** Authors declare that there is no conflict of interest.

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