Antioxidant Activity of Coumarins

Subjects: Pharmacology & Pharmacy
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Ubiquitously present in plant life, coumarins have multiple applications—in everyday life, in organic synthesis, in medicine and many others. They are well known for their broad spectrum of physiological effects. The specific structure of the coumarin scaffold involves a conjugated system with excellent charge and electron transport properties. The antioxidant activity of natural coumarins has been a subject of intense study for at least two decades. Significant research into the antioxidant behavior of natural/semi-synthetic coumarins and their complexes has been carried out and published in scientific literature.

antioxidant coumarins radicals' scavenging antioxidant assays

1. Introduction

Free radicals are a type of reactive species (RS), bearing an unpaired electron. They are indelibly connected with the processes of life [1]—breathing, metabolism, cell signaling [2] and many others (some prominent examples are presented in **Table 1**). For that reason, they are constantly being produced in living bodies. Such species include superoxide ion-radicals, hydroxyl radicals, hydrogen peroxide and hydroperoxyl radicals to name a few. Their impact on biological systems can be both helpful and deleterious [3][4]. Maintaining a balance between radical generation and radical elimination is a necessary requirement for good health. Disruption of that balance, caused by insufficient radical elimination, results in oxidative stress, associated with numerous pathologies—cancer [5], diabetes [6], neurodegenerative [7] and coronary artery diseases [8] and many others. That is due to the fact that free radicals are highly reactive, easily interacting with biomolecules and disrupting vital processes. Within the living body exist complex systems of free radical regulation that take into account the particular needs of the immune system and redox signaling [9]. Antioxidant defenses include both endogenous and exogenous substances. Some of these compounds are able to directly scavenge free radicals, a process that involves irreversible chemical transformation of the scavenging molecule. Others can act as chelators, preventing transition metal ions from participating in electron transfer processes, e.g., Fenton-like catalytic radical generation and subsequent peroxidation processes. A class of exogenous compounds that are associated with both scavenging and chelating antiradical action, as well as ROS-producing enzyme inhibition, are coumarins [10].

Table 1. Some examples of RS generation in living organisms.

Source of RS	Type of RS	Site of Production	Function
Nicotinamide adenine dinucleotide (NADH)	superoxide	Mitochondria	Electron transport during oxidative phosphorylation.

Source of RS	Type of RS	Site of Production	Function
dehydrogenase (Complex I)			
Coenzyme Q (Complex III)	superoxide	Mitochondria	Electron transport during oxidative phosphorylation.
Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)	superoxide	Cytosol, membranes of various organelles	Immune response, phagocytosis.
Xanthine oxidase	Superoxide, hydrogen peroxide	Liver, intestines	Catalytic conversion of hypoxanthine to xanthine and uric acid.
Nitric oxide synthase	Nitric oxide (NO), superoxide	Cytosol, cellular membranes	Synthesis of NO from L-arginine.
Myeloperoxidase	Hypochlorous acid	Neutrophils	Synthesis of hypochlorous acid during respiratory burst.
5-lipoxygenase	Indirect action	Immune cells	Leukotriene production, causing NOX stimulation and RS generation.
Peroxynitrite production	Peroxynitrite ion	Phagocytes	Produced from superoxide and NO, phagocytosis.
Fenton reaction and Haber–Weiss chain	Hydroxyl/hydroperoxyl radicals	Wherever transition metal ions such as iron and copper come in contact with hydrogen peroxide and/or superoxide.	Mostly associated with pathologies.

Coumarin (2*H*-Chromen-2-one) is the most basic member of the eponymous group of phenolic compounds. Historically, coumarins have been applied for the treatment of a variety of diseases due to their anticoagulant, anti-inflammatory, antiviral, antimicrobial, anticancer, antioxidant, etc. activities [11]. That broad spectrum of physiological effects is due to the possibility of a great variety of substitution patterns in the coumarin base structure. Generally speaking, hydrogen-donating substitutions (phenolic, amino, thiol-groups, etc.) of the benzene component of the coumarin “skeleton” tend to improve the antioxidant effect. Ortho-diacetoxy derivatives have also been reported to be effective scavengers [12]. Catecholic motifs are associated with an improvement of antioxidant action [13]. That is due, on one hand, to increased direct scavenging of radicals due to electron donation from the ortho-hydroxyl group and, on the other, bidentate metal chelation which interrupts metal ion-induced reactive species generation [14]. A number of excellent reviews have been published over the years, elucidating the structure–activity effects of coumarin substances [12][13][15][16][17].

2. Antioxidant Properties of Molecular Coumarins

2.1. Coumarins Substituted with Small Functionalities

Prahadeesh and colleagues synthesized simple coumarins: coumarin, 4-hydroxycoumarin, 7-hydroxycoumarin and 7-hydroxy-4-methylcoumarin [18]. These uncomplicated compounds were tested for peroxide scavenging (direct measurement of H_2O_2 in the presence of the compounds, 10 min of incubation) and ferric-reducing antioxidant power (FRAP). The results show that adding a hydroxyl group in position 7 improves peroxide scavenging more than threefold under the experimental conditions (IC_{50} drops from 24,902 mg/L for coumarin to 7029 mg/L for the 7-hydroxy derivative). Hydroxyl group in position 4 increases peroxide scavenging (IC_{50} = 9150 mg/L), though not as much as in position 7. Adding a methyl group at position 4 in addition to the hydroxyl group at position 7 decreases peroxide scavenging (IC_{50} = 11,014 mg/L). The standard substance used for comparison is ascorbic acid, with IC_{50} = 286 mg/L. In terms of ferric-reducing capacity, these substances' effectiveness decreases in the same order (7-hydroxy > 4-hydroxy > 7-hydroxy-4-methyl > unsubstituted coumarin). This simple experiment, involving uncomplicated structures and easy to understand model systems, serves as an excellent introduction to basic structure–activity relationships when it comes to antioxidant properties of coumarins.

Couttolenc and colleagues performed a similar structure–activity relationship experiment on three hydroxy-substituted 4-methylcoumarin molecules (**Figure 1**) [19].

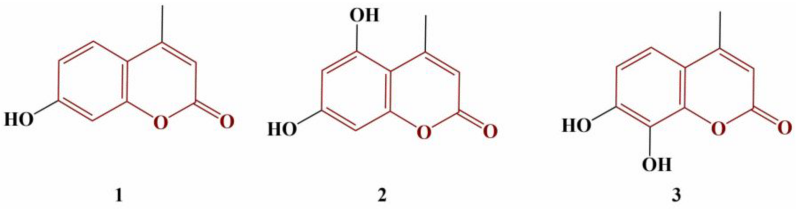


Figure 1. The 4-methylcoumarins.

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and galvinoxyl radical-scavenging were estimated. Compound 2 was the strongest scavenger of ABTS (EC_{50} = 30.83 µM), followed by Compound 3 (EC_{50} = 39.98 µM). Compound 1 had significantly lower ABTS-scavenging activity (EC_{50} = 1150 µM). EC_{50} of the standard substance Trolox was 83.50 µM. In terms of the DPPH and galvinoxyl assays, Compound 3 again performed better (EC_{50} = 150.99 µM and 13.19 µM, respectively) than Trolox (EC_{50} = 243.39.99 µM and 20.86 µM, respectively). Compounds 1 and 2 did not perform well in these stable radical model systems, with EC_{50}s above 2400 µM. The authors attribute the observed differences to the number and positioning of the hydroxyl groups. Compound 3 allows for an intramolecular hydrogen bond that stabilizes the semiquinone radical and thus improves scavenging activity [20].

A number of novel methyl-, nitro- and/or amino-substituted coumarin derivatives have been synthesized and tested for radical scavenging activity against DPPH (methanol, 1 h incubation, 37 °C) [21]. Two of the compounds, presented in **Figure 2**, behaved as strong scavengers.

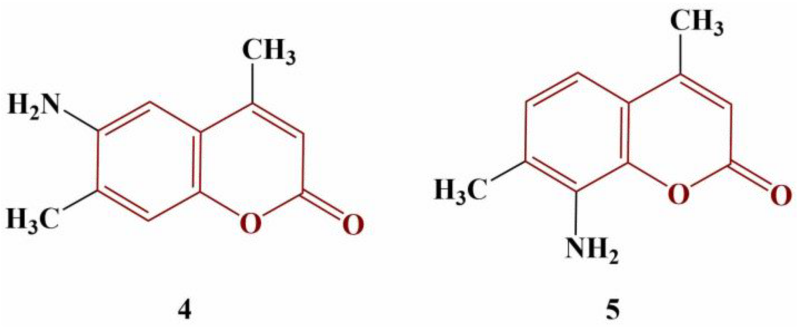


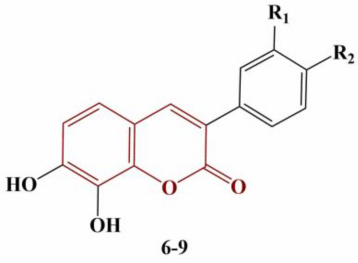
Figure 2. The two most active scavengers.

Compound 4 (IC_{50} = 10 µg/mL) was more active than ascorbic acid (IC_{50} = 33.48 µg/mL). The potency of Compound 5 was lesser than that of the standard (IC_{50} = 42.90 µg/mL). Two electron-donating amino groups at positions 6 and 8 dramatically decrease the antioxidant effect (IC_{50} = 110.20 µg/mL). Exchanging the amino groups for electron-withdrawing nitro groups further decreases DPPH scavenging activity.

2.2. Coumarins, Substituted at Positions 3 and/or 4

Ozalp and colleagues synthesized a series of hydroxycoumarins (**Figure 3**) and tested them for DPPH scavenging, FRAP, cupric-reducing antioxidant capacity (CUPRAC) and metal chelating [22].

Compound:	6	7	8	9
R_1:	H	H	CH_3	H
R_2:	H	NO_2	H	CH_3



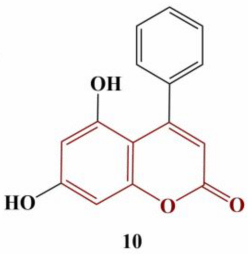


Figure 3. The hydroxycoumarins.

In terms of DPPH scavenging, all 7,8-dihydroxy members manifested significant activity (EC_{50} < 94.85 µM), equal to or higher than that of the standard compound Trolox (EC_{50} = 93.19 µM). Compound 10 had very low activity with EC_{50} = 6604.92 µM. Most active were Compounds 6 and 7 (EC_{50} = 74.70 and 64.27 µM, respectively). Compound 7 also exhibited the highest ferric-reducing and copper-reducing potentials (EC_{50} = 2.28 µM and 2.44 µM, respectively, versus EC_{50} = 1.0 µM for Trolox in both assays). Inversely, the 5,7-dihydroxo-substituted member of the series had the best ferrous-ion-chelating activity (EC_{50} = 2.702 mM), compared to Compounds 6–9 (EC_{50} = 0.728 mM up to 0.782 mM) and Trolox (EC_{50} = 1.191 mM).

Katopodi and colleagues synthesized a number of multisubstituted 3-phenylcoumarins [23]. Their common structure is presented in **Figure 4**.

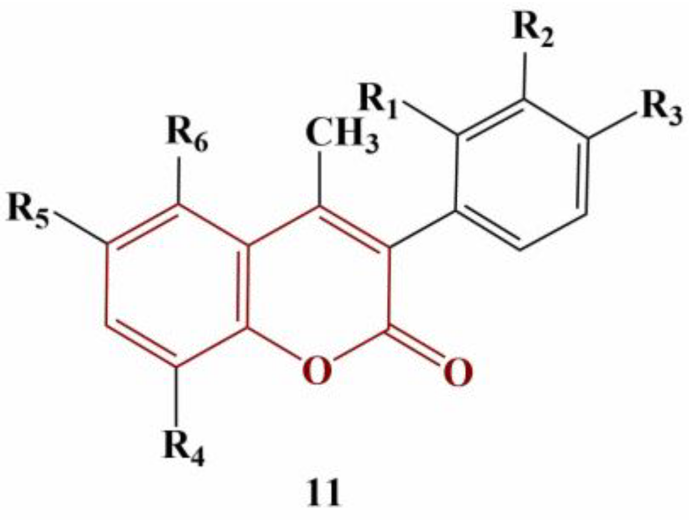
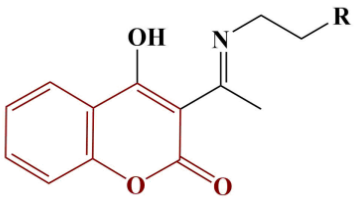


Figure 4. Common structure of the 3-phenylcoumarins.

All coumarin analogues were investigated with a panel of tests—ABTS, suppression of Fenton-generated hydroxyl radicals (DMSO competition), inhibition of 2,2′-azobis-2-methyl-propanimidamide (AAPH)-induced linoleic acid oxidation and 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) assay. All assays were performed with 100 µM concentrations of the tested compounds, with Trolox and ascorbic acid being used for comparison. Results were presented as percentage of inhibition at 100 µM. Most compounds did not reduce ABTS or did so to a very limited extent (0 to 27.8%). Adding a hydroxyl group at position 5 of the coumarin structure (R6) significantly improves activity in this model system (49.2 to 73.3%). If R3 is a hydroxyl group, ABTS scavenging is negated. An acetyloxy group at R3 or R6 improves scavenging of hydroxyl radicals and suppresses AAPH-induced oxidation. Two acetyloxy groups at R2 and R3 decrease that effect. Adding chlorine or fluorine at R1 or R2 increases scavenging of hydroxyl radicals, but the same substitution at R3 causes the opposite effect. A hydroxyl group or a fluorine atom at R3 decreases peroxide-induced intracellular ROS (as per the DCFDA assay) by 100%.

A series of novel 4-hydroxycoumarin derivatives (**Figure 5**) were synthesized and tested for antioxidant activity as part of an effort to synthesize compounds with anti-Alzheimer's activity [24]. The authors aimed to improve metal chelation by adding Schiff base functionality in addition to the phenolic hydroxyl group. A tertiary amine was added to improve anticholinergic activity and lipid–water partition coefficient.



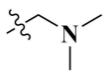
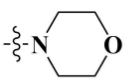
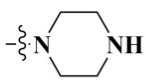
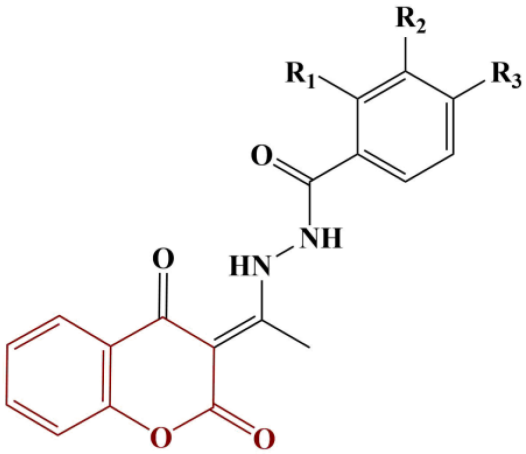
Compound:	12	13	14
R:			

Figure 5. The Schiff-base-infused coumarins.

Radical scavenging was assessed by way of Fenton-generated hydroxyl (OH) radicals and cyclic voltammetry (for assessing superoxide scavenging). All compounds manifested significant hydroxyl scavenging with IC_{50} between

2.61 and 2.94 μM. Compound 13 was the strongest scavenger and was consequently complexed with Cu(II). The novel complex was even more active with IC_{50} = 0.20 μM. The standard compound ascorbic acid had IC_{50} > 300 μM. Cyclic voltammetry showed concentration-dependent superoxide scavenging. In this case, Compound 12 seemed to completely eliminate superoxide in the model system at 5 mM.

Antonijevic and colleagues synthesized a series of coumarin–hydroxybenzohydrazide hybrids and investigated their potential antioxidant properties in vitro [25]. Additionally, theoretical investigations into the mechanisms of their activity were performed with the aid of DFT. Synthesis involved condensation of 3-acetyl-4-hydroxycoumarine with appropriate hydrazides (acetic acid as catalyst) in ethanol (heating) or n-propanol (reflux). Antioxidant activity was established with the aid of the DPPH assay. The two most active members of the series are presented in **Figure 6**.

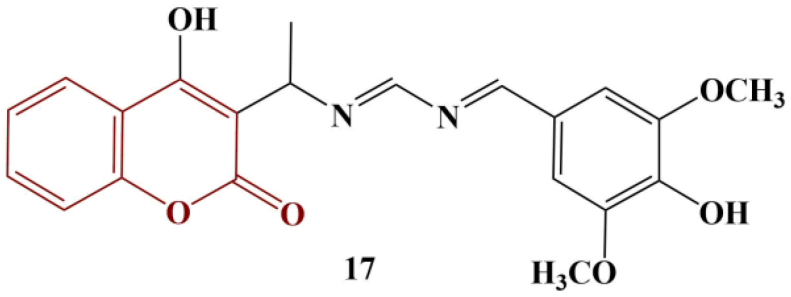


Compound:	R_1:	R_2:	R_3:
15	OH	OH	H
16	H	OH	OH

Figure 6. The coumarin–benzohydrazides.

Compounds 15 and 16 had IC_{50} of 2.9 ± 0.1 μM and 12.9 ± 0.4 μM, respectively. IC_{50} of the positive controls nordihydrohuaiaretic acid (NDGA) and quercetin were calculated as 1.7 ± 0.1 μM and 1.9 ± 0.1 μM, respectively. The significant antioxidant activity of these two substances was attributed by the authors to the presence of two neighboring phenolic groups. Hydrogen atom transfer (HAT) and sequential proton loss electron transfer (SPLET) mechanisms were found to be most probable in non-polar and polar solvents, respectively.

A group of asymmetric azines containing a 4-hydroxycoumarin moiety were synthesized and tested for scavenging activity toward DPPH [26]. The authors claim that most of the tested compounds did not behave as antioxidants to a significant extent, despite the presence of a hydroxyl group at position 4 in the coumarin functionality. The only exception, presented in **Figure 7**, behaved as a strong, fast scavenger of DPPH (EC_{50} = 0.53, presented as moles of antioxidant per 1 mol DPPH against EC_{50} = 0.25 for ascorbic acid).

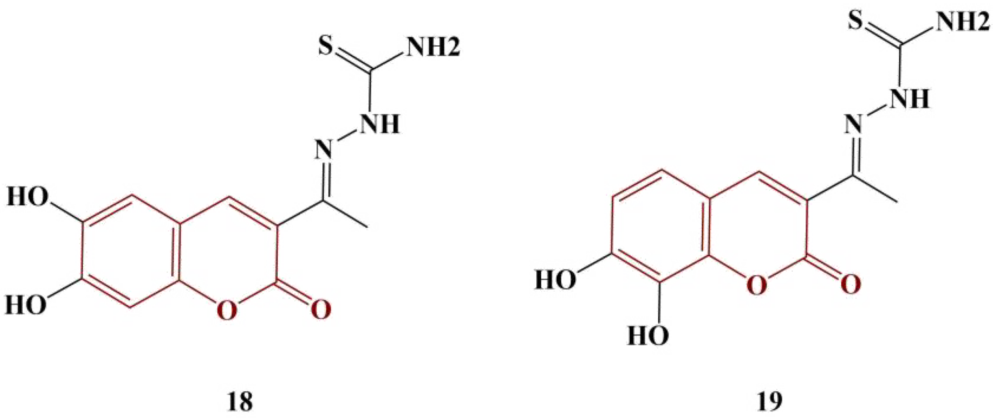


17

Figure 7. The most active asymmetric azine.

The authors proposed that the overall passivity of the hydroxyl group in position 4 was due to intramolecular hydrogen bonding. The “exceptional” compound could attribute its antioxidant activity to the hydroxyl group, present in the phenyl moiety, additionally “supported” by the neighboring electron-donating methoxy groups.

Coumarin–thiosemicarbazones were synthesized as potential antityrosinase agents and tested for antioxidant action [27]. Two of the members (**Figure 8**), bearing a catecholic motif in the coumarin fragment, manifested significant antioxidant activity.

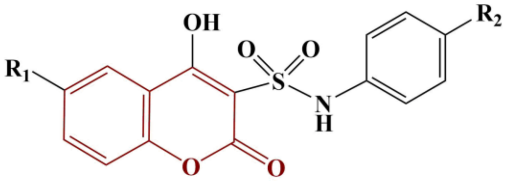


18 19

Figure 8. The most active coumarin–thiosemicarbazones.

DPPH and ABTS testing results showed that Compound 18 (IC_{50} = 7.1 μM and 9.0 μM, respectively) and Compound 19 (IC_{50} = 17.9 μM and 8.8 μM, respectively) performed better than both standard compounds—ascorbic acid (DPPH assay IC_{50} = 18.6 μM) and Trolox (ABTS assay, IC_{50} = 13.0 μM). The other members of the series bore a single hydroxyl substituent in their coumarin fragment and had more moderate scavenging effect.

A series of pyranocoumarins, coumarin-3-sulfonamides and coumarin-sulfonamide-chalcones have been synthesized and tested for antioxidant activity using the DPPH (0.05 mM) assay [28]. Pyranocoumarins exhibited very low or zero activity. The same was observed in the chalcone-substituted compounds. The most potent scavengers of DPPH are presented in **Figure 9**. Ascorbic acid was used for comparison (IC_{50} = 2.83 μg/mL)

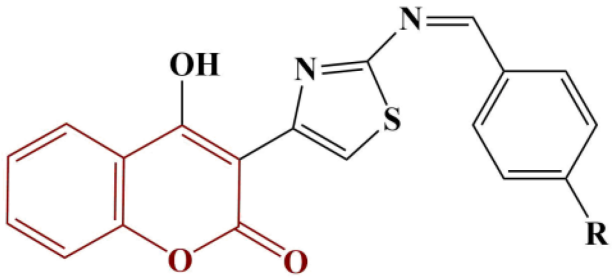


Compound:	R_1:	R_2:	IC_{50} (mcg/mL):
20	Cl	H	14.51 ± 1.827
21	CH_3	H	19.25 ± 4.171
22	Cl	OH	3.87 ± 0.409
23	CH_3	OH	4.30 ± 0.531

Figure 9. The active coumarin-3-sulfonamides.

The chlorine atom at position 6 of the coumarin structure improves scavenging activity compared to a methyl group in the same place. Adding a phenolic hydroxyl group to the benzene ring significantly improves activity. Notably, compounds 20–23 were also found to exhibit significant COX1/2-inhibitory activity as well, similar to Indomethacin and Celecoxib. When considering the rest of the compounds, synthesized by the authors, in terms of structure–activity relationships, sulfathiazole- and sulfanilamide-containing compounds showed higher activity than sulfadiazine-substituted substances.

New thiazolyl-coumarin derivatives were synthesized and tested for antioxidant activity (DPPH test, 0.1 mM) [29]. The most active compounds from the series are presented in **Figure 10**.



Compound:	R:
24	H
25	NO_2
26	CH_3

Figure 10. The most active thiazolyl-coumarins.

Compounds 24–26 scavenged DPPH to the same extent as gallic acid and butylated hydroxytoluene (BHT) across all tested concentrations. Adding electron-donating (hydroxyl and methoxy) groups to the side benzene ring decreases antioxidant activity in this model system. The authors also propose that the hydrazinothiazole functionality plays an important part in the antioxidant action.

Kumar and colleagues synthesized a series of hybrids, merging 1,2,3-triazoles, 1,3,4-oxadiazole and coumarins [30]. They were tested for scavenging of DPPH (ethanol, 30 min incubation, room temperature). One of the novel compounds (**Figure 11**) manifested moderate scavenging activity (28.2% inhibition) at a concentration of 40 µg/mL.

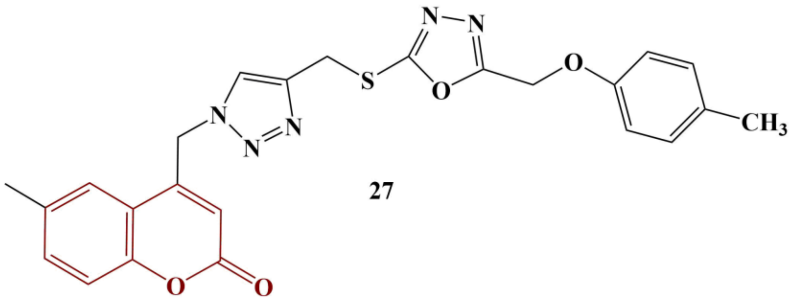


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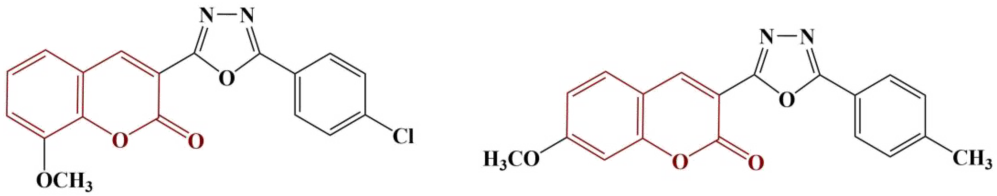
Figure 11. The most active coumarin hybrid.

Exchanging the 4-methyl group of the phenyl ring for a chlorine atom decreases scavenging to 18.2%. Adding to it a methyl group at the meta-position almost negates any activity.

Naik and colleagues synthesized a number of 3-substituted coumarin–amino acid hybrids [31]. Scavenging of DPPH (0.004% in ethanol, 30 min of incubation in dark) and nitric oxide (sodium nitroprusside with Griess reagent, 150 min incubation at room temperature) were assessed. These hybrids were generated by coupling of coumarin-3-carboxylic acid with esters of several amino acids. In terms of the DPPH assay, the best results were obtained with the coumarin–tyrosine (IC_{50} = 31.45 µg/mL) and coumarin–serine (IC_{50} = 28.23 µg/mL) hybrids compared to the standard substance (ascorbic acid, IC_{50} = 20.53 µg/mL). All compounds scavenged nitric oxide, the least pronounced effect being observed with the glycine and phenylalanine conjugates (IC_{50} = 96.94 µg/mL and IC_{50} = 66.20 µg/mL, respectively). The presence of aliphatic or aromatic hydroxyl groups increased the observed scavenging effect. For example, the IC_{50} of the tyrosine hybrid was 26.90 µg/mL. Ascorbic acid had IC_{50} = 18.40 µg/mL.

A coumarin–benzothiazole hybrid was synthesized and tested for antioxidant action with DPPH (0.2 mM, DPPH, 20 min cultivation, 28 °C) [32]. Results were compared to ascorbic acid. The novel hybrid scavenged DPPH with an IC_{50} = 591.58 µg/mL. The result for the standard compound was IC_{50} = 391.25 µg/mL.

Coumarin-tethered 1,3,4-oxadiazole analogues [33] were synthesized and tested as potential scavengers of DPPH and hydroxyl radicals. Out of ten molecules in total, two manifested significant antioxidant activity (**Figure 12**, Compounds 28 and 29).



28 29

Figure 12. The most active coumarin–oxadiazole hybrids.

The DPPH assay involved 30 min incubation at 37 °C of a number of concentrations of each substance in 0.4 mM DPPH. Compounds 28 and 29 manifested DPPH-scavenging activity with IC_{50}s of 19.47 µM and 17.19 µM, respectively. For ascorbic acid, IC_{50} = 23.80 µM. Scavenging of hydroxyl radicals was estimated using Fenton-reaction degradation of 2-deoxyribose (60 min incubation at 37 °C), followed by derivatization with thiobarbituric acid (TBA) (15 min, boiling water). Butylhydroquinone (BHA) was used as a standard. Compounds 28 and 29 manifested OH-scavenging activity with IC_{50}s of 32.62 µM and 28.51 µM, respectively. These results demonstrated similar activity to BHA (IC_{50} = 36.05 µM). Overall, improvement of the activity in both model systems was associated with adding a methoxy group in positions 7 and 8, as well as adding a chlorine atom at position 6, of the coumarin structure.

A series of novel coumarins bearing a 2,4-diaminothiazole-5-carbonyl moiety were synthesized and tested for DPPH-scavenging ability [34]. Almost all compounds manifested good scavenging ability, with IC_{50} < 50 µg/mL. The most active substance (**Figure 13**) had IC_{50} = 23.9 ug/mL—about three times higher than that of the standard substance ascorbic acid.

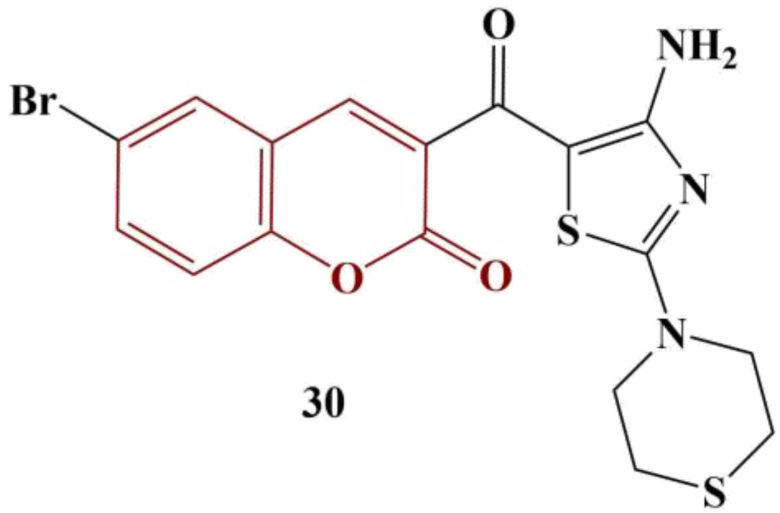


Figure 13. The most active coumarin–diaminothiazole.

Compounds with morpholine, piperidine and pyrrolidine moieties attached to the thiazole ring improved radical elimination. The introduction of Br at position 6 of the coumarin structure tended to diminish DPPH scavenging.

Li and colleagues synthesized conjugates of coumarin and hydroxytyrosol, a proven natural antioxidant. DPPH and ABTS assays were utilized to assess antioxidant activity, BHT being the control substance [35]. The most potent compound is presented in **Figure 14**.

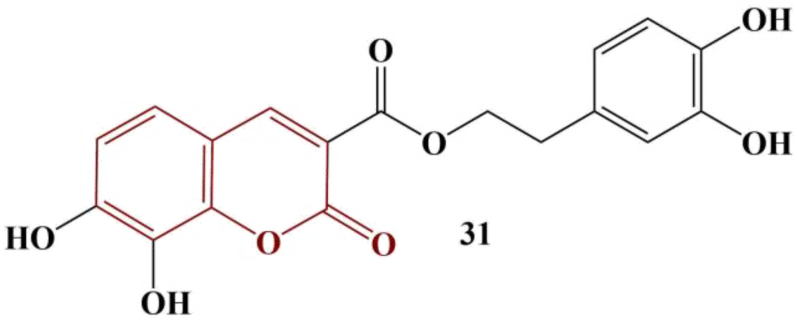


Figure 14. The most active coumarin–hydroxytyrosol hybrid.

Coumarin itself has extremely low scavenging activity (IC_{50} > 10 000 μM) in both model systems. Hydroxytyrosol manifested moderate activity (DPPH IC_{50} = 143.81 μM, ABTS IC_{50} = 170.47 μM). BHT had DPPH IC_{50} = 521.99 μM and ABTS IC_{50} = 127.07 μM. Conjugating a coumarin to hydroxytyrosol caused improved scavenging activity in both model systems. The most active substance (**Figure 14**) had DPPH IC_{50} = 26.58 μM and ABTS IC_{50} = 30.31 μM. In terms of structure–activity relationships, the authors noted that methyl, methoxy and chlorine substitutes at positions 6, 7 and/or 8 of the coumarin structure slightly improve DPPH scavenging, but diminish ABTS scavenging. Substituting hydroxyl groups at these locations improves scavenging activity in both model systems with the 7,8-dihydroxy-substituted member being the most active antioxidant.

2.3. Coumarins, Substituted at Positions 7 and/or 8

Konidala and colleagues synthesized 4-hydroxycoumarin–chalcone molecular hybrids [36] and tested them for their antimicrobial and antioxidant properties. Antioxidant activity was characterized with the aid of the DPPH assay. All compounds were tested at a single concentration (100 μg/mL). Results were presented as scavenging percentages compared to negative control (no substance present). Ascorbic acid (81.21% scavenging) and BHT (70.05% scavenging) were used for comparison. All compounds behaved as moderate scavengers of DPPH. The most active one is presented in **Figure 15** (77.92% scavenging).

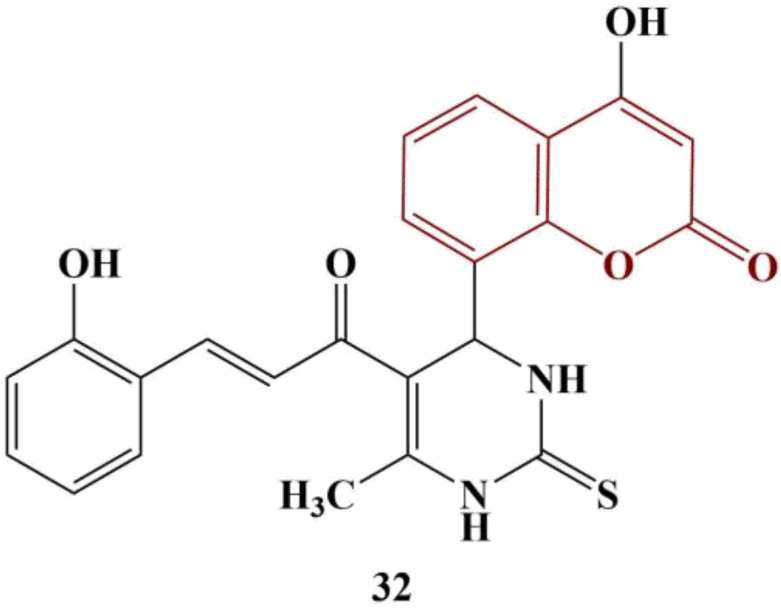


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Figure 15. The most active coumarin–chalcone hybrid.

Removing the hydroxyl group from the benzene ring slightly diminishes radical scavenging (75.22%). Moving it from the ortho- to meta-position decreases activity by more than half; a para-positioned hydroxyl group causes even further diminishing of activity. A single methoxy functionality at the para-position decreases activity (31.28% scavenging). Three methoxy groups at meta- and para-positions improve activity (71.32% scavenging).

De Souza and colleagues synthesized a series of 3-(4-(dimethylamino)phenyl)-7-aminoalcoxycoumarins [37]. They tested them for ferric-reducing potential. What they discovered was that the number of carbon atoms separating nitrogen and oxygen in the aminoalcoxy substituent impacts ferric-reducing ability. When that chain includes one carbon atom, FRAP IC_{50} was 7.49 mmol Q(quercetin)/mol of tested compound. Increasing the chain's length to two and three carbon atoms decreases FRAP values threefold.

A series of coumarin–rasagiline hybrids underwent a spectrum of tests to assess their neuroprotective, MAO-B-inhibitory and radical-scavenging properties [38]. The novel molecules were functionalized with propargyl groups in positions 3, 4 or 7 of the coumarin structure. DPPH assay (50 µM in ethanol, 30 min incubation at room temperature) yielded moderate antioxidant activities of all compounds (100 µM concentrations). Scavenging activities varied between 10–20%, with few statistically significant differences and no clear structure–activity relationship to be observed.

Joy and colleagues investigated a number of 4-methyl-7-alkynyl coumarin derivatives [39] with the DPPH assay (0.004%, 10 min cultivation, room temperature, dark conditions). BHT was used for comparison. Results were presented as percentage inhibition compared to negative control (no substance present). Adding a hydroxymethyl, 4-hydroxyphenyl or 4-aminophenyl moiety to the alkynyl substituent yields significant scavenging activity (63,3%, 74,2% and 70,8% at 100 µg/mL concentration). BHT scavenged about 90% of DPPH at 100 µg/mL. The authors

propose that the incorporation of electron-donating groups stabilizes the oxygen-centered radical, thus improving the ability of the respective compound to donate a hydrogen atom to DPPH.

Kurt and colleagues synthesized a series of carbamate-substituted coumarins [40], as shown in **Figure 16**.

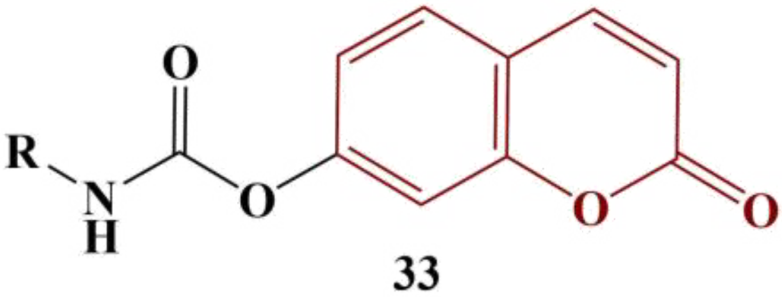

Figure 16. The coumarin–carbamates.

When the substitute R is a cyclopentyl, cyclohexyl or cyclohexylmethyl, moderate ABTS scavenging was observed (IC_{50} between 66.80 and 80.03 μM). A cycloheptyl substitute increases IC_{50} to 131.85 μM. Adding a methyl group at position 4 of the coumarin structure tends to decrease the scavenging activity about twofold. A surprising exception is the member in the molecule of which R is a cyclohehyl moiety (IC_{50} = 23.15 μM). Quercetin was used for comparison (IC_{50} = 15.149 μM).

Joy and colleagues synthesized a series of coumarins, linked to 1,2,3-triazoles, using copper-catalyzed, azide-alkyne cycloaddition [41]. The substances were screened for antimicrobial and antioxidant action. Antioxidant activity was measured with the DPPH assay. BHT was used as a standard. Results were presented as percentage inhibition compared to negative control (no substance present) at 100 μg concentration. The most active antioxidants are displayed in **Figure 17**.

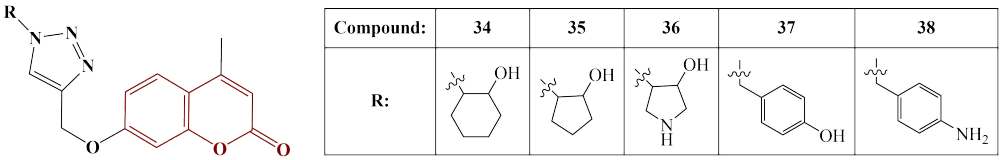

Figure 17. The most active coumarin–triazoles.

The hydrogen-donating activity of compounds 34–38 was between 61,8% and 74,2% of that for the standard BHT. The authors propose the improved antioxidant activity of these particular compounds compared to the rest of the series may be due to the electron-donating hydroxyl and amino groups incorporated in the radical R.

Popova and colleagues synthesized novel [1,3]oxazine derivatives of 7-hydroxy-6-isobornyl-4-methylcoumarin [42]. Total antioxidant activity was measured in vitro in terms of the inhibition of Fenton-reaction-induced lipid peroxidation in mouse brain homogenates (1 h incubation at 37 centigrade), followed by TBA reaction. Scavenging of DPPH (methanol solution) was also measured after 30 min of incubation at room temperature in dark conditions.

In both cases, Trolox was used as a reference. In terms of DPPH, all novel compounds manifested very low scavenging activity (between 5 and 20 times lower than Trolox) at 10 μM and 100 μM. On the other hand, all of them inhibit lipid peroxidation in mouse brain homogenates to a higher extent than Trolox. The authors propose that the observed effect may be due to the lipophilicity of the substances and the specific properties of the model system—an emulsion that causes molecules to aggregate at the phase boundary. This hypothesis is confirmed by the fact that increasing the hydrophilicity of the compounds causes a decrease in the peroxidation-inhibitory activity.

A series of coumarin hybrids with 1,2,3-triazoles were synthesized and tested for antioxidant activity (DPPH assay) [43]. Two classes of compounds were generated: 4-substituted and 7-substituted coumarins (**Figure 18**: 39 and 40).

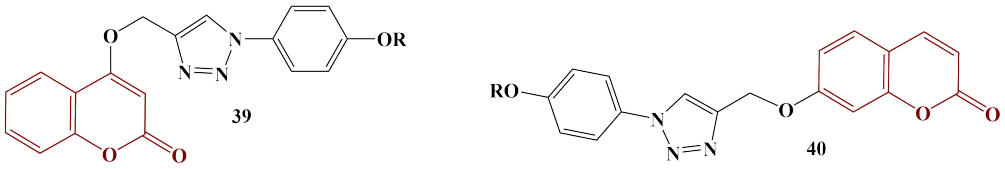

Figure 18. The coumarin–triazole hybrids.

Both series of compounds were substituted with the same functionalities (a hydrogen atom or a variety of substituted benzyl moieties), allowing for a direct comparison in terms of antioxidant properties of similar substances, differing only in the particular site of coumarin substitution. Generally, the 7-substituted coumarins performed better than their 4-substituted counterparts. Monomethyl-substituted benzyl moieties tended to improve overall antioxidant activity, particularly the meta-substituted ones. Fluorine substitution produced better activity than chloro- or bromo-substitution. Nitro-substitution either did not change or impaired antioxidant activity compared to compounds where the benzyl moiety was unsubstituted. All compounds were less active (IC_{50} between 3.33 and 9.74 μg/mL) compared to the standard ascorbic acid (IC_{50} = 1.23 μg/mL).

A series of coumarin-coupled thiazines (**Figure 19**) were synthesized and underwent antioxidant testing [44]. DPPH (180 μg/mL, methanol, 25 min cultivation) and ABTS were applied. The best results with DPPH were obtained when the moiety R was hydrogen, 2-Cl and 4-Cl (IC_{50} between 35.35 and 40.02 μg/mL; ascorbic acid had IC_{50} = 36.22 μg/mL). Radical-scavenging activity is reduced by 4-OH and 4-F substitution.

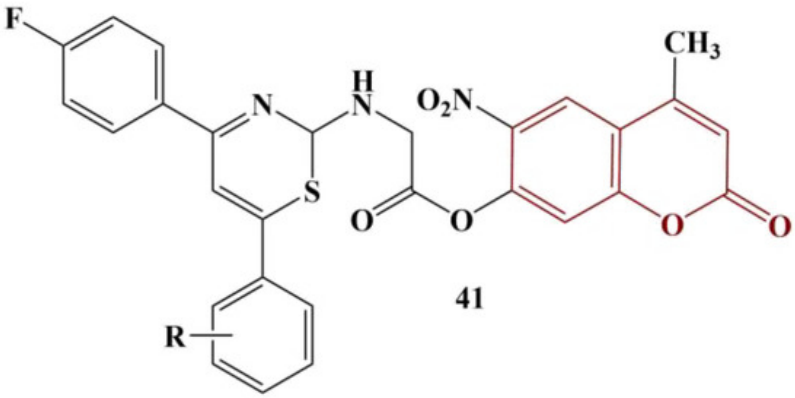

Figure 19. Structure of the thiazine–coumarin hybrids.

On the other hand, 4-F and 4-CH_3 substitution provided the best result with the ABTS test (IC_{50}= 53.92 and 52.00 µg/mL respectively, ascorbic acid had IC_{50}= 22.64 µg/mL). Contrary to DPPH, 2-Cl, 4-Cl and hydrogen substitution in the benzene ring decreases ABTS scavenging.

Xue and coworkers performed theoretical investigations of a number of 7-hydroxycoumarin – chalcone hybrids[45]. The chalcone moiety was attached at position 8 of the coumarin structure. The results obtained demonstrated that the 7-OH group in ring B of the coumarin structure is less favorable as a donor of hydrogen atom compared to differently positioned OH groups in the same ring. Theoretical calculations demonstrated that the position 7 hydroxyl group forms an intramolecular hydrogen bond with the chalcone carbonyl oxygen atom. The presence of such a bond increases the bond dissociation energy of the 7-OH and makes it less favourable for participation in HAT, compared to OH groups at the chalcone benzene ring. In gas phase/benzene medium the HAT mechanism was calculated to be thermodynamically more favourable.

Gunduz and coworkers synthesized a new coumarin derivative - 7-((8-(4-benzylpiperidin-1-yl)octyl)oxy)-4-methyl-2H-chromen-2-one[46]. The novel compound was tested for antiproliferative and antioxidant activity. DPPH assay showed moderate activity (concentrations between 0.03125 and 1.0 mg/mL), weaker than that of the standard BHT at the same concentrations. Polar media (ethanol or water) seemed to favor the SPLET mechanism, as solvation was calculated to decrease proton affinities.

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