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Chapter

Coumarin Derivatives with Antimicrobial and Antioxidant Activities

Gabriela Tataringa and Ana Maria Zbancioc

Abstract

Coumarin derivatives are structurally interesting compounds for synthesizing antimicrobial and antioxidant agents. Starting from 4-methyl-7-hydroxycoumarin, several derivatives with these properties have been obtained through different reaction steps. Their molecular structures were established by Fourier-transform infrared spectroscopy and nuclear magnetic resonance spectroscopy. The synthesized coumarin derivatives exerted meaningful activities against Gram-positive and Gram-negative bacteria as well as strains of *Candida* spp. All compounds also exhibited high and moderate antioxidant activity in assays for DPPH inhibition, total reducing power, and nitric oxide (NO) inhibition when compared to ascorbic acid.

Keywords: 4-methyl-7-hydroxycoumarin, synthesis, coumarin derivatives, antibacterial activity, antifungal activity, antioxidant activity

1. Introduction

Natural and synthetic coumarins have drawn much attention due to its broad pharmacological activities. Literature review reveals that coumarin (2-oxo-2H-chromene) and its derivatives represent one of the most active classes of heterocyclic compounds which possess a wide spectrum of biological activities [1–9]: antitumor [1, 2], antibacterial [3, 4], antifungal [5–7], anticoagulant [8], antioxidant [9], and anti-inflammatory [10].

1.1 Coumarins as antimicrobial agents

Over the past few decades, the search for newer antimicrobials remains an area of intensive investigation in the field of medicinal chemistry due to resistance developed by microorganism to conventional antibiotics. Antimicrobials are one of most significant weapons in fighting bacterial infections. Throughout history, there has been a continual battle between humans and the multitude of microorganisms that cause infection and disease [11, 12]. Coumarin derivatives have a wide range of structural modifications [13], and they can serve as molecular templates for new drugs. Coumarin derivatives are also considered as potential antimicrobial agents [14].

Medimagh-Saidana et al. reported synthesis and antimicrobial activity of some coumarin esters (**1**) (**Figure 1**). These compounds showed good activity against

Bacillus sp. and moderate activity against *Aspergillus niger*. For the data of the antibacterial activity, these compounds were found to be active against *Pseudomonas* sp. [15].

Al-Amiery et al. have synthesized some coumarin derivatives, and their antifungal activity was determined based on the growth inhibition rates of the mycelia of strains of *Aspergillus niger* and *Candida albicans* in Potato Dextrose Broth (PDB) medium against concentrations ranging from 10 to 100 μg/ml. The compound (**2**) (**Figure 1**) showed good activity as antifungals against fluconazole as standard drug [16].

Behrami et al. synthesized 8-amino-4,7-dihydroxy-chromen-2-one coumarin derivatives. The antibacterial activities of all the compounds and standard streptomycin and cefalexine at concentrations of 2, 3, and 5 mg/ml were studied against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. One compound (**3**) (**Figure 1**) was more active than cefalexine and lesser active than streptomycin, and it was most active among synthesized compounds [17].

Some coumarin derivatives containing thiazolidin-4-one ring were synthesized by Rama Ganesh et al. and were screened for their antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria *Klebsiella pneumonia* and *Escherichia coli* at the concentration of 0.001 mol/ml compared with the standard drug ciprofloxacin. Zone of inhibition of highly active compound (**4**) (**Figure 1**) was 20 mm against *Staphylococcus aureus* and *Bacillus subtilis* [18].

1.2 Coumarins as antioxidant agents

Free radicals are molecular species capable of independent existence that contain an unpaired electron in an atomic orbital; they are usually unstable and very reactive. These species are normally produced in the human body from essential metabolic processes, but they may also occur from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals [19].

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body and to prevent the deterioration of fats and other constituents of food stuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic

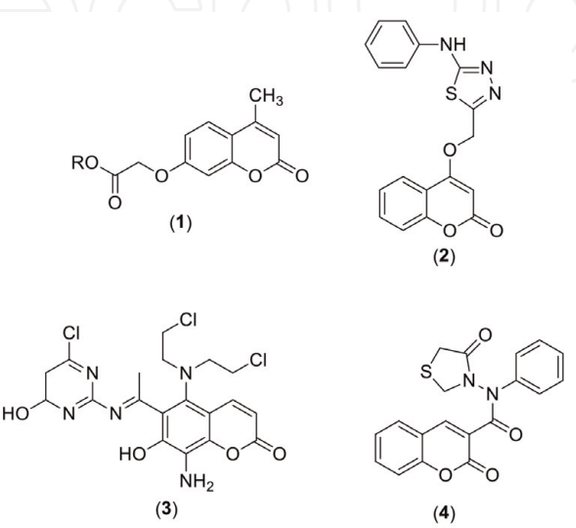


Figure 1.
Coumarin derivatives with antimicrobial activity.

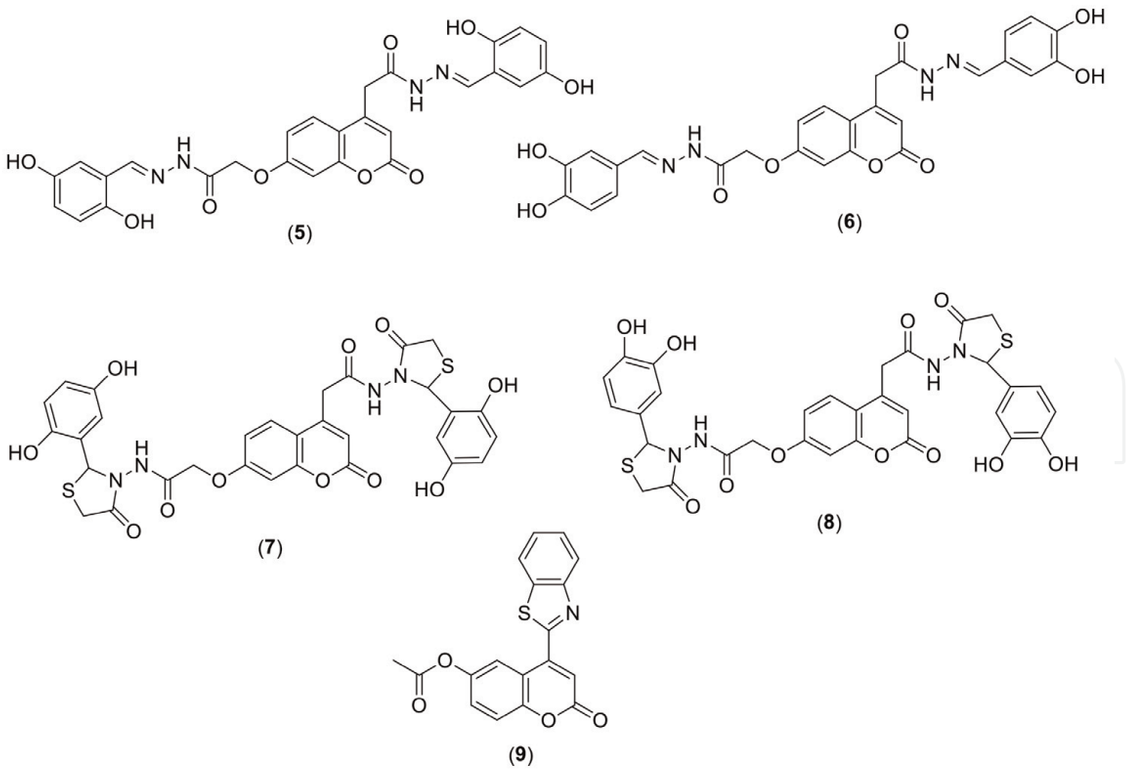


Figure 2.
Coumarin derivatives with antioxidant activity.

sources [20]. As improved antioxidant status helps to minimize the oxidative damage and thus delays or prevents pathological changes, potential antioxidant therapy should be included either as natural free-radical-scavenging antioxidant enzymes or as an agent which is capable of augmenting the activity of antioxidant enzymes [21].

The human organism possesses natural systems to annihilate these species, but when the body's ability to regulate them is overwhelmed, a condition known as oxidative stress appears, free radicals attacking important macromolecules leading to cell damage and homeostatic disruption [22].

Many coumarin derivatives have a special ability to scavenge reactive oxygen species and to influence processes involving free radical injury [23, 24].

Maja et al. synthesized a series of carbohydrazide with coumarin ring (**5**, **6**) and some coumarin derivatives with a heterocyclic ring (**7**, **8**) (**Figure 2**). All these compounds prove a good antioxidant activity [25].

Shivani et al. synthesized new coumarin-substituted derivatives of benzothiazole, and they were evaluated for antioxidant activity by DPPH radical scavenging activity. The test compound (**9**) (**Figure 2**) showed good in vitro antioxidant activity [26].

2. Synthesis of coumarin derivatives

Looking to the medicinal importance of the coumarin ring, we employed coumarin as a naturally occurring skeleton for the construction of new derivatives which might exhibit promising antimicrobial and antioxidant activities [27].

The starting materials, 4-methyl/propyl-7-hydroxycoumarin, were prepared by Pechmann synthesis which involved the condensation of resorcinol and ethylacetoacetate/ethylbutyrylacetate in the presence of H_2SO_4 concentrate [28].

2.1 Mechanism of the Pechmann condensation

The reaction is conducted with a strong Brønsted acid such as methanesulfonic acid or a Lewis acid such as $AlCl_3$. The acid catalyzes transesterification as well as keto-enol tautomerization [29]. A Michael addition leads to the formation of the coumarin skeleton. This addition is followed by rearomatization and then by elimination of water which gives the product (**Figure 3**).

The coumarin compounds have wide interest due to their diverse pharmacological properties. In particular, these biological activities make coumarin compounds more attractive and testing as novel therapeutic compounds.

As part of our aim in research of biologically active coumarin derivatives, the free hydroxyl group on the coumarin ring has allowed us to introduce some radicals that can improve the biological activity.

The fourth scheme describes the reactions of 4-methyl/propyl-7-hydroxycoumarin with ethyl bromoacetate. 2-Ethyl-((4-methy/propyl-2-oxo-2H-chromen-7-yl)oxy)acetate (**IIa–IIb**) was prepared by heating a mixture of 4-methyl/propyl-7-hydroxycoumarin and ethyl bromoacetate in the presence of K_2CO_3 anhydrous in dry acetone. After filtration, the solution was evaporated, and the solid products (**IIa** or **IIb**) were recrystallized from ethanol [30] (**Figure 4**).

The fifth scheme describes the reactions of compounds **IIa–IIb** with hydrazine hydrate. The chemistry of hydrazide and its derivatives has obtained great interest in both organic chemistry and biological science with remarkable impact. Hydrazides and hydrazones are possessing -NH-NH_2 and -NH-N=CH- groups, respectively. The availability of proton in hydrazides constitutes them as an important class of compound for new drug discovery. Therefore, researchers have shown great interest in developing these compounds as target structures for evaluating new biological activities [31].

Hydrazinolysis of compounds **IIa–IIb** gave the corresponding acetohydrazides (**IIIa–IIIb**) in good yields [30, 32]. One mole of the compound **IIa** or **IIb** in ethanol

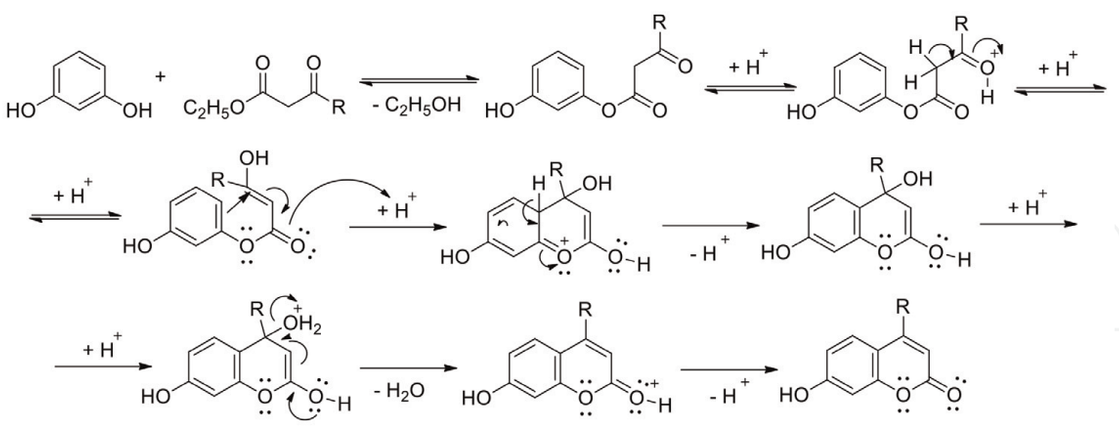


Figure 3.
Mechanism of the Pechmann condensation.

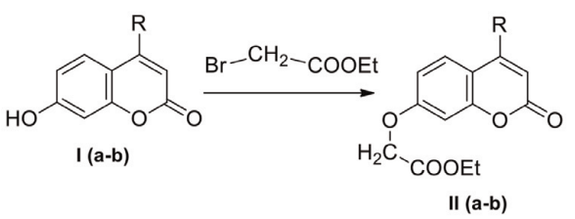


Figure 4.
*Synthesis of coumarin esters **II(a-b)**.*

was heated for 4–6 h with hydrazine hydrate (two moles). After this period of time, the mixture was cooled at room temperature, and the precipitate was filtrated and then purified by recrystallization (**Figure 5**).

The reaction of the acid hydrazides (4-methyl/propyl-2-oxo-2H-benzopyran-7-oxyacetic acid hydrazide) with CS_2 in ethanol containing KOH at room temperature has been presented in **Figure 6**. The corresponding potassium dithiocarbazate derivatives, **IVa–IVb,** are obtained [32].

The obtaining of coumarin derivatives with a thiadiazole ring has been described in **Figure 7**. Literature data show that pyrrole, pyrazole, thiadiazoles, and triazoles and their derivatives are very attractive targets due to their biological properties. In view of the above observations, we have synthesized compounds with thiadiazole ring in order to evaluate the potential antimicrobial and antioxidant activities.

These compounds were obtained following the reaction between potassium 4-methyl/propyl-2-oxo-2H-benzopyranyl-7-oxymethyldithiocarbazate and acetic acid. The reaction occurred under refluxing. The solid product was separated by filtration and then purified by recrystallization from acetic acid [33, 34].

In the synthesis of coumarin derivatives, elemental analysis and two basic spectroscopic techniques, infrared spectroscopy (IR) and nuclear magnetic resonance

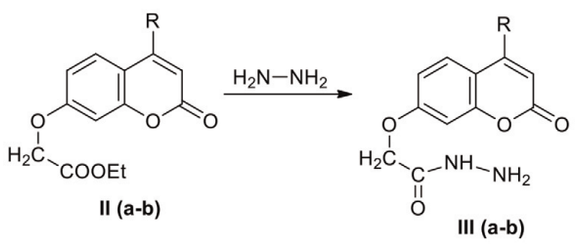


Figure 5.
*Synthesis of coumarin acetohydrazides **III(a-b)**.*

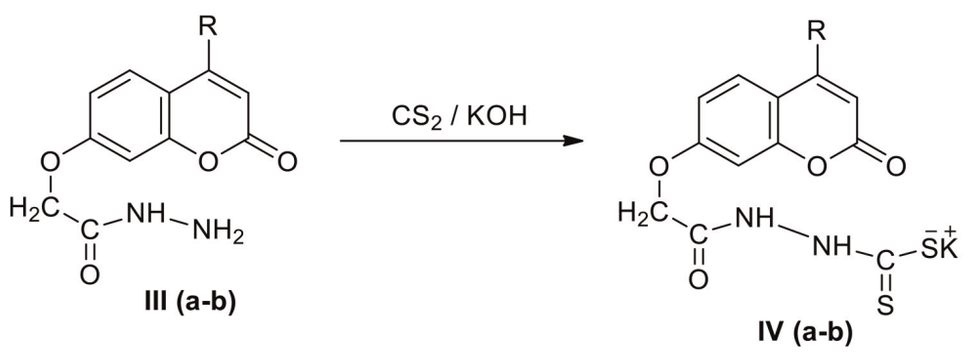


Figure 6.
*Synthesis of coumarin potassium salts **IV(a-b)**.*

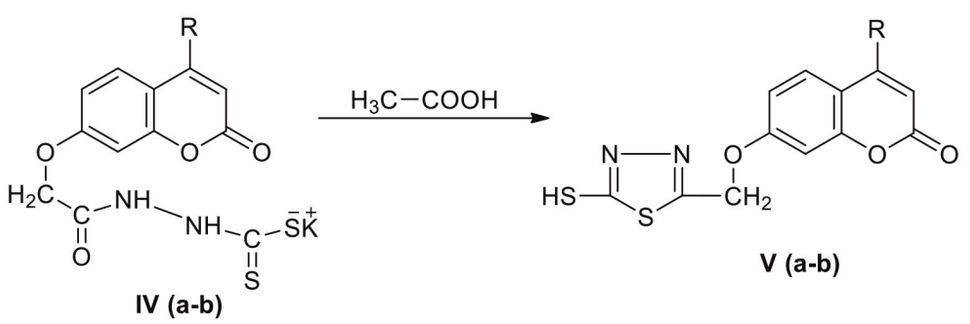
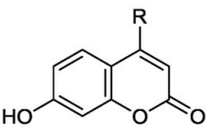


Figure 7.
*Synthesis of coumarin thiadiazoles derivatives **V(a-b)**.*

spectroscopy (NMR), were used to characterize the structures of the target compounds [35].

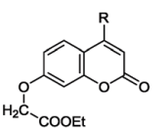
The physical constants and analytical data of compounds **Ia–Ib**, **IIa–IIb**, **IIIa–IIIb**, **IVa–IVb**, and **Va–Vb** have been given in **Tables 1**–**5**.



4-R-7-hydroxy-coumarin

Compd.	R	MP °C	Molecular formula	Analysis found (calculated) (%)	
				C	H
Ia	H_3C–	185	$C_{10}H_8O_3$	68.18 68.02	4.58 4.55
Ib	H_3C–CH_2–CH_2–	130	$C_{12}H_{12}O_3$	70.57 70.21	5.92 5.69

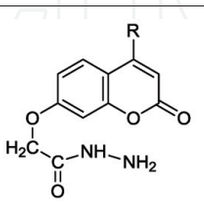
Table 1.
*Physical constants and analytical data of compounds **Ia–Ib.***



2-ethyl-((4-R-2-oxo-2H-chromen-7-yl)oxy)acetate

Compd.	R	MP °C	Molecular formula	Analysis found (calculated) (%)	
				C	H
IIa	H_3C–	100–102	$C_{14}H_{14}O_5$	64.12 64.10	5.38 5.22
IIb	H_3C–CH_2–CH_2–	98	$C_{16}H_{18}O_5$	66.19 65.89	6.25 6.05

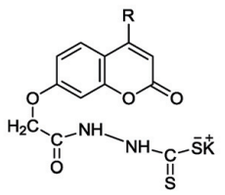
Table 2.
*Physical constants and analytical data of compounds **IIa–IIb.***



4-R-2-oxo-2H-benzopyran-7-oxyacetic acid hydrazide

Compd.	R	MP °C	Molecular formula	Analysis found (calculated) (%)	
				C	H
IIIa	H_3C–	204–205	$C_{12}H_{12}N_2O_4$	58.06 57.98	4.87 4.80
IIIb	H_3C–CH_2–CH_2–	147–148	$C_{14}H_{16}N_2O_4$	60.86 60.56	5.84 5.76

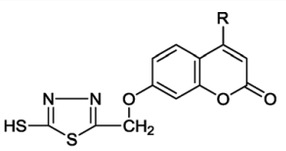
Table 3.
*Physical constants and analytical data of compounds **IIIa–IIIb.***



potassium 4-R-2-oxo-2H-benzopyranyl-7-oxymethyldithiocarbazate

Compd.	R	MP °C	Molecular formula	Analysis found (calculated) (%)	
				C	H
IVa	H_3C–	184	$C_{13}H_{11}N_2O_4KS_2$	43.08 42.87	3.06 3.01
IVb	H_3C–CH_2–CH_2–	176–178	$C_{15}H_{15}N_2O_4KS_2$	46.13 46.02	3.87 3.76

Table 4.
*Physical constants and analytical data of compounds **IVa–IVb.***



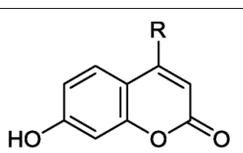
7-((5-mercapto-1,3,4-thiadiazol-2-yl)methoxy)-4-R-2H-chromen-2-one

Compd.	R	MP °C	Molecular formula	Analysis found (calculated) (%)	
				C	H
Va	H_3C–	267–268	$C_{13}H_{10}N_2O_3S_2$	50.97 50.63	3.29 3.06
Vb	H_3C–CH_2–CH_2–	172	$C_{15}H_{14}N_2O_3S_2$	53.87 53.33	4.22 4.12

Table 5.
*Physical constants and analytical data of compounds **Va–Vb.***

The IR spectra of all synthesized compounds showed some characteristic peaks indicating the presence of particular groups (**Tables 6–10**).

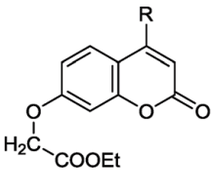
^{1}H-NMR spectra of the synthesized compounds are in accordance with the assigned structures (**Table 11**). The aliphatic protons resonated in the range of 1.20–4.77 ppm. It can be seen that all the compounds exhibited the respected proton chemical shifts in the same range.



4-R-7-hydroxy-coumarin

Compd.	R	$\upsilon_{O\text{-}H}$ cm^{-1}	$\upsilon_{C\text{-}H\ aliph}$ cm^{-1}	$\upsilon_{C=O\ lactone}$ cm^{-1}	$\upsilon_{C\text{-}O}$ cm^{-1}
Ia	H_3C–	3280	2950	1680	1150
Ib	H_3C–CH_2–CH_2–	3195	2970	1695	1140

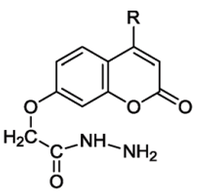
Table 6.
*IR spectral data of compounds **Ia–Ib.***



2-ethyl-((4-R-2-oxo-2H-chromen-7-yl)oxy)acetate

Compd.	R	$\upsilon_{C\text{-}H\ arom}$ cm^{-1}	$\upsilon_{C=O\ side\ chain}$ cm^{-1}	$\upsilon_{C=O\ lactone}$ cm^{-1}
IIa	H_3C–	3070	1750	1680
IIb	H_3C–CH_2–CH_2–	3080	1740	1690

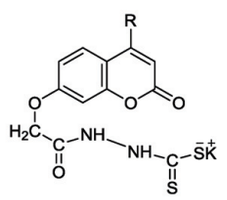
Table 7.
*IR spectral data of compounds **IIa–IIb.***



4-R-2-oxo-2H-benzopyran-7-oxyacetic acid hydrazide

Compd.	R	υ_{NH2} cm^{-1}	$\upsilon_{CO\text{-}NH}$ cm^{-1}	$\upsilon_{C\text{-}N}$ cm^{-1}
IIIa	H_3C–	3423, 3331	1612	1271
IIIb	H_3C–CH_2–CH_2–	3411, 3340	1610	1260

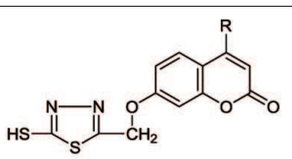
Table 8.
*IR spectral data of compounds **IIIa–IIIb.***



potassium 4-R-2-oxo-2H-benzopyranyl-7-oxymethyldithiocarbazate

Compd.	R	$\upsilon_{N\text{-}H}$ cm^{-1}	$\upsilon_{C=S}$ cm^{-1}
IVa	H_3C–	3210	1240
IVb	H_3C–CH_2–CH_2–	3150	1210

Table 9.
*IR spectral data of compounds **IVa–IVb.***



7-((5-mercapto-1,3,4-thiadiazol-2-yl)methoxy)-4-R-2H-chromen-2-one

Compd.	R	$\upsilon_{S\text{-}H}$ cm^{-1}	$\upsilon_{C=N}$ cm^{-1}	$\upsilon_{S\text{-}C}$ cm^{-1}
Va	H_3C–	2380	1610	621
Vb	H_3C–CH_2–CH_2–	2350	1620	638

Table 10.
*IR spectral data of compounds **Va–Vb.***

Compd.	R	Chemical shift (δ ppm) 500 MHz
Ia	H_3C–	2.73 ppm s, 3H: CH_3; 6.04 ppm s, H_3; 6.72 ppm s, H_8; 6.83–6.85 ppm d, H_6, $J_{H6,H5}$ = 7.5 Hz; 7.62–7.64 ppm d, H_5, $J_{H5,H6}$ = 7.5 Hz; 10.85 ppm s, 1H: OH
Ib	H_3C–CH_2–CH_2–	1.20 ppm t, 3H: CH_3 (12); 1.66 ppm m, 2H: CH_2 (11); 2.81–2.84 ppm m, 2H: CH_2 (10); 6.11 ppm s, H_3; 6.72 ppm s, H_8; 6.84–6.85 ppm d, H_6, $J_{H6,H5}$ = 7.5 Hz; 7.63–7.65 ppm d, H_5, $J_{H5,H6}$ = 7.5 Hz; 10.86 ppm s, 1H: OH
IIa	H_3C–	1.26–1.28 ppm t, 3H: CH_3 (14); 2.73 ppm s, 3H: CH_3; 4.25–4.28 ppm m, 2H: CH_2 (13); 4.61 ppm s, 2H: CH_2 (10); 6.04 ppm s, H_3; 6.84 ppm s, H_8; 7.05–7.07 ppm d H_6; 7.62–7.64 ppm d, H_5
IIb	H_3C–CH_2–CH_2–	0.95–0.98 ppm t, 3H: CH_3 (17); 1.20–1.23 ppm t, 3H: CH_3 (14); 1.60–1.65 ppm m, 2H: CH_2 (16); 2.72–2.75 ppm t, 2H: CH_2 (15); 4.15–4.20 ppm m, 2H: CH_2 (13); 4.92 ppm s, 2H: CH_2 (10); 6.17 ppm s, H_3; 6.98 ppm s, H_8; 6.96–6.97 ppm d H_6; 7.73–7.75 ppm d, H_5
IIIa	H_3C–	2.74 ppm s, 3H: CH_3; 3.86 ppm s, 2H: NH_2 (13); 4.42 ppm s, 2H: CH_2 (10); 6.03 ppm s, H_3; 6.83 ppm s, H_8; 7.05–7.07 ppm d; 7.62–7.64 ppm d, H_5; 8.02 ppm s, NH (12)
IIIb	H_3C–CH_2–CH_2–	0.95–0.98 ppm t, 3H: CH_3 (16); 1.59–1.66 ppm m, 2H: CH_2 (15); 2.72–2.75 ppm t, 2H: CH_2 (14); 4.35 ppm s, 2H: NH_2 (13); 4.61 ppm s, 2H: CH_2 (10); 6.17 ppm s, H_3; 6.98 ppm s, H_8; 6.99–7.00 ppm d H_6; 7.74–7.76 ppm d, H_5; 9.42 ppm s, NH (12)
IVa	H_3C–	2.71–2.74 ppm d, 3H: CH_3; 4.41–4.43 ppm s, 2H: CH_2 (10); 6.02–6.05 ppm q, H_3; 6.82–6.84 ppm d, H_8; 7.05–7.08 ppm q H_6; 7.62–7.64 ppm d, H_5; 9.94 ppm s, NH (12); 11.23 ppm s, NH (13)
IVb	H_3C–CH_2–CH_2–	1.20–1.22 ppm t, 3H: CH_3 (18); 1.62–1.66 ppm m, 2H: CH_2 (17); 2.79–2.82 ppm t, 2H: CH_2 (16); 4.40 ppm s, 2H: CH_2 (10); 6.10 ppm s, H_3; 6.84 ppm s, H_8; 7.04–7.08 ppm q H_6; 7.62–7.66 ppm d, H_5; 9.95 ppm s, NH (12); 11.25 ppm s, NH (13)
Va	H_3C–	2.39 ppm s, 3H: CH_3; 4.77 ppm s, 2H: CH_2 (10); 6.23 ppm s, H_3; 6.99 ppm s, H_8; 7.02–7.04 ppm d H_6; 7.70–7.72 ppm d, H_5; 10.29 ppm s, SH (16)
Vb	H_3C–CH_2–CH_2–	0.95–0.98 ppm t, 3H: CH_3 (19); 1.60–1.65 ppm m, 2H: CH_2 (18); 2.72–2.75 ppm t, 2H: CH_2 (17); 4.77 ppm s, 2H: CH_2 (10); 6.17 ppm s, H_3; 6.98 ppm s, H_8; 6.99–7.00 ppm d H_6; 7.75–7.76 ppm d, H_5; 11.27 ppm s, SH (16)

Table 11.
^{1}H-NMR spectral data of compounds ***I–V.***

3. Pharmacological activities of coumarin derivatives

3.1 Antimicrobial activity

The compounds were screened for their antibacterial and antifungal activity according to standard protocols [36].

The antimicrobial activity was studied using Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 14579), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and pathogenic yeasts (*Candida albicans* ATCC 10231, *Candida glabrata* ATCC MYA 2950, *Candida parapsilosis* ATCC 22019). All these strains were obtained from the Culture Collection of the Department of Microbiology, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania.

Antimicrobial activity was evaluated by agar disc diffusion method (CLSI, 2014). A small amount of each microbial culture was diluted in sterile 0.9% NaCl until the turbidity was equivalent to McFarland standard no. 0.5 (106 CFU/ml). The suspensions were further diluted 1:10 in Mueller-Hinton agar for bacteria and Sabouraud agar for yeasts and then spread on sterile Petri plates (25 ml/Petri plate). Sterile stainless steel cylinders (5 mm internal diameter; 10 mm height) were applied on the agar surface in Petri plates. Then, 0.1 ml of each compound (10 mg/ml in DMSO) was added into the cylinders. The DMSO solvent was also tested in order to assess its intrinsic antimicrobial activity. Commercial available discs containing ampicillin (25 μg/disc), chloramphenicol (30 μg/disc), and nystatin (100 μg/disc) were also placed on the agar surface. The plates were incubated at 37°C for 24 h (bacteria) and at 24°C for 48 h (yeasts). After incubation the diameters of inhibition zones were read in triplicate. Statistical analysis of the results included the calculation of standard deviation (**Tables 12** and **13**).

The qualitative screening of the antimicrobial activity was performed in order to identify the antimicrobial spectrum of the tested compounds. The inhibitory effects of the synthetic compounds against Gram-positive and Gram-negative bacteria and fungi are given in **Tables 12** and **13** [35].

According to the results of the antibacterial studies, the efficacy of the tested compounds against Gram-positive bacteria was higher than that exhibited for Gram-negative bacteria. All the synthesized compounds were very active against *S. aureus* ATCC 25923, the most active compounds being **Ib**, **IIb**, **IIIb**, and **IVb**. The replacement of the methyl radical in the fourth position with the propyl group was correlated with an increased activity against *S. aureus* ATCC 25923.

The tested compounds exhibited excellent antibacterial activity against *S. lutea*, the most active derivatives being **IIb**, **IVb**, **Ib**, and **IIIb**.

Compd./reference	Diameters of the growth inhibition zone (mm)				
	***S. aureus* ATCC 25923**	***S. lutea* ATCC 9341**	***B. cereus* ATCC 14579**	***E. coli* ATCC 25922**	***Pseudomonas aeruginosa* ATCC 27853**
Ia	14 ± 0.52	25 ± 0.79	25 ± 1.52	12 ± 0.79	8 ± 0.93
Ib	27 ± 1.29	29 ± 0.83	NA	NA	NA
IIa	14 ± 0.91	22 ± 0.79	26 ± 0.79	11 ± 0.52	8 ± 1.43
IIb	27 ± 0.52	30±	NA	NA	NA
IIIa	15 ± 0.54	20 ± 0.79	22 ± 0.79	10 ± 0.79	9 ± 0.79
IIIb	25 ± 0.52	28±	NA	NA	NA
IVa	17 ± 1.08	25 ± 0.91	24 ± 1.52	12 ± 0.93	9 ± 1.43
IVb	25 ± 1.08	30 ± 0.83	NA	NA	NA
Va	14 ± 0.52	25 ± 0.52	20 ± 1.43	10 ± 1.52	8 ± 0.52
Vb	21 ± 1.43	25 ± 0.79	NA	13 ± 0.83	NA
Ampicillin (25 μg/disc)	26 ± 0.04	36 ± 0.00	NA	21 ± 0.79	NA
Chloramphenicol (30 μg/disc)	22 ± 0.00	38 ± 0.00	24 ± 0.00	21 ± 0.52	NA

Data are mean ± SD (n = 3); NA, no activity.

Table 12.
*Antibacterial activity of compounds **I–V**.*

Compd./reference	Diameters of the growth inhibition zone (mm)		
	C. albicans **ATCC 10231**	***C. glabrata*** **ATCC MYA 2950**	***C. parapsilosis*** **ATCC 22019**
Ia	24 ± 1.83	21 ± 0.52	34 ± 1.83
Ib	10 ± 0.91	10 ± 0.79	10 ± 0.54
IIa	25 ± 0.52	27 ± 0.54	35 ± 1.83
IIb	9 ± 1.83	NA	NA
IIIa	19 ± 1.79	24 ± 0.52	24 ± 1.79
IIIb	12 ± 1.83	11 ± 0.54	11 ± 0.54
IVa	23 ± 0.91	16 ± 0.52	25 ± 1.08
IVb	10 ± 0.54	12 ± 1.08	NA
Va	16 ± 1.79	21 ± 1.83	21 ± 0.54
Vb	9 ± 0.54	9 ± 0.52	NA
Nystatin (100 μg/disc)	25 ± 0.52	25 ± 0.52	24 ± 0.00

Data are mean ± SD (n = 3); NA, no activity.

Table 13.
Antifungal activity of compounds ***I–V****.*

We found a moderate action against *B. cereus* ATCC 14579, the most active being the umbelliferone derivatives with a methyl group attached to C4: **IIa**, **Ia**, and **IVa**.

Against *Escherichia coli* ATCC 25922, the investigated compounds had a weaker action than the controls ampicillin and chloramphenicol. The most active was the compound that contains a thiadiazole ring, **Vb**.

The presence of the methyl group attached to the coumarin ring in the fourth position had a positive influence on the anti-*Pseudomonas* ATCC 27853 potential of the compounds, all the tested 4-propyl-coumarin derivatives being inactive.

We have noticed a very important action against the investigated *Candida* strains; all tested compounds were found to be very active against fungi. The compounds **IIa** and **Ia** had a greater inhibitory potential against *C. parapsilosis* ATCC 22019 than nystatin. The introduction of the sulfur atom appeared to be correlated with a good anti-*Candida* activity.

3.2 Antioxidant activity

In order to evaluate the antioxidant activity of synthesized compounds, we use three antioxidant assays: DPPH radical inhibition, total reducing power, and nitric oxide (NO) inhibition.

The DPPH assay is based on assessing the substances' ability to reduce the stable radical (diphenylpicrylhydrazyl) to diphenylpicrylhydrazine. The DPPH free radical, bearing an odd electron, gives a strong absorption maximum at λ = 517 nm (purple color). When the odd electron of the DPPH radical pairs with a hydrogen atom from an antioxidant, the reduced form DPPH-H is created, and the color turns from purple to yellow [36, 37].

A possible mechanism that can explain the antioxidant effect of the coumarin hydrazide derivatives is related to the keto-enol forms of the substances, the enol group being capable to easily donate the hydrogen (**Figure 8**) [38].

The experimental procedure for the DPPH assay was adapted from literature [27, 28, 37], only slight modifications being made. Briefly, 2.5 ml solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical 0.1 mM in methanol was added over 0.5 ml

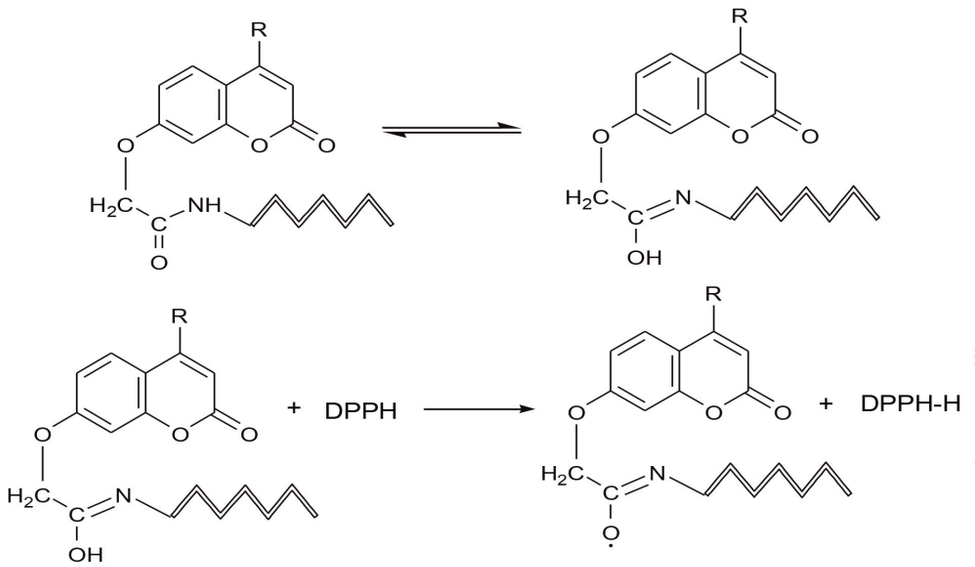


Figure 8.
Proposed mechanism for antioxidant activity of coumarin hydrazides.

of methanolic solution of the tested compound (1 mg/ml). The absorbance of the DPPH solution at 517 nm was determined spectrophotometrically before ($A_{control}$) and 15 minutes after adding the solutions of the compounds (A_{test}), and the percentage of activity was calculated. Ascorbic acid was used as a reference compound:

$$\%\ radical\ scavenging\ activity = (A_{control} - A_{test}) \times 100/A_{control}$$

where $A_{control}$ is the absorbance of the control sample (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution + test compound).

Out of the tested compounds, the most active DPPH free radical scavengers were the coumarin hydrazide derivatives (**IIIa–IIIb**, **IVa–IVb**). The activities of **IVa** were similar to that of the standard, the inhibition percentage being over 90%, the introduction of sulfur atoms in the molecule having a positive influence on the scavenging potential. Compounds **IIIa** and **IVa**, containing a methyl group, were slightly more active than their analogues with propyl radical (**Table 14**) [19].

Fe(III) reduction is often used as an indicator of electron-donating activity. In the reducing power assay, antioxidants with electron-donating abilities reduce ferricyanide to ferrocyanide by donating an electron. The amount of ferrocyanide is monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reducing ability [39]. Within this assay, EC_{50} values are the effective concentrations at which the absorbance is 0.5.

The solution of the test compound (0.5 ml) at different concentrations in methanol was mixed with phosphate buffer (1.25 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide 1% (1.25 ml), and the mixture was incubated at 50°C for 20 min. At the end of the incubation period, trichloroacetic acid 10% (1.25 ml) was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer solution was collected, and 2.5 ml were mixed with distilled water (2.5 ml) and ferric chloride 0.1% (0.5 ml). The absorbance was measured after 15 min at 700 nm against a blank. The EC50 values were calculated by linear interpolation between values above and below 50% activity. Ascorbic acid was used as reference [28, 30, 36, 37].

The reducing power of the tested compounds was modest, and the results are presented in **Table 15**. The only substances that were moderately active were the hydrazide derivatives **IIIa** and **IVa**, but their activity was inferior to that exhibited by the reference substance (ascorbic acid) [19].

Compd.	Inhibition percentage (%)
Ia	21.4
Ib	21.2
IIa	19
IIb	19.83
IIIa	73
IIIb	53.56
IVa	95
IVb	87.67
Va	31.51
Vb	25.13
Ascorbic acid 1 mg/ml	96.8

Table 14.
***DPPH** inhibition percentages of compounds **I–V** (1 mg/ml).*

Compd.		Extinction
Ia		0.0599
Ib		0.0105
IIa		0.0234
IIb		0.0010
IIIa	(1 mg/ml)	1.3432
	(0.8 mg/ml)	1.1237
	(0.6 mg/ml)	1.0821
	(0.4 mg/ml)	0.9674
	(0.2 mg/ml)	0.5499
IVa	(1 mg/ml)	0.6592
	(0.8 mg/ml)	0.5900
	(0.6 mg/ml)	0.4854
	(0.4 mg/ml)	0.4258
	(0.2 mg/ml)	0.2785
IVb		0.4887
Va		0.0472
Vb		0.3063
Ascorbic acid 1 mg/ml		2.8261

Table 15.
*The reducing power of compounds **I–V**.*

The calculated values for EC_{50} are shown in **Table 16**. This method could not be applied to compound **IIIb** due to the formation of an abundant precipitate in the process.

Nitric oxide is involved in a variety of biological functions (neurotransmission, vascular homeostasis, antimicrobial and antitumor activities). NO was primarily

Compd.	EC_{50} (mg/ml)
Ia	>>1
Ib	>>1
IIa	>>1
IIb	>>1
IIIa	0.176
IVa	0.627
IVb	1.02
Va	>>1
Vb	>1
Ascorbic acid 1 mg/ml	0.049

Table 16.
The calculated values of EC_{50}.

described as a regulator of vascular tones in the cardiovascular system. Beyond this function it can prevent platelet activation, limit leukocyte adhesion to the endothelium, and regulate myocardial contractility, and it is involved in immune system reactions.

Despite the possible beneficial effects of NO, it also contributes to oxidative damage. In general, the overwhelming production of NO contributes to the pathogenesis of both acute and chronic inflammatory processes, and NO has been recognized as one of the main signaling molecules involved in these processes [23, 40]. Therefore, compounds that act like nitric oxide inhibitors have beneficial effects.

The NO inhibition assay is based on the diazotization of sulfanilic acid at acid pH by nitric oxide. The reaction product is subsequently coupled stoichiometrically with N-(1-naphthyl)ethylenediamine, forming a colored azo compound which is measured spectrophotometrically at a peak absorbance of 548 nm [36].

0.5 ml of the tested coumarin derivative solution, as well as ascorbic acid (standard compound), was taken in separate tubes, and 2.0 ml of sodium nitroprusside

Compd.	NO inhibition (%)
Ia	15.2
Ib	19.8
IIa	12.11
IIb	14.7
IIIa	22.8
IIIb	55.5
IVa	28.45
IVb	29.44
Va	22.6
Vb	26
Ascorbic acid 50 mg/ml	77.19

Table 17.
*NO inhibition activity of compounds **I–V**.*

(10 mM) and 0.5 ml phosphate buffer saline (pH = 7.4) were added to each tube. The solutions were incubated at 25°C for 150 minutes. After the incubation, over 0.5 ml of the incubated solution 1 ml of sulfanilic acid 0.33% was added, and the mixture was left for 5 min at room temperature; after this period of time, 1 ml naphthylethylene diamine (NED) HCl reagent 0.1% was added, and the solutions were incubated for another 30 min. The absorbance was measured at 546 nm [38].

Most of the investigated compounds were moderate NO inhibitors (**Table 17**) [19].

4. Concluding remarks

We have synthesized some coumarin derivatives starting from 4-methyl-7-hydroxycoumarin with antimicrobial and antioxidant activities to different reaction steps. The IR and NMR spectra of the synthesized compounds were in accordance with the assigned structures. All the synthesized compounds were very active against *S. aureus* ATCC 25923, and they exhibited excellent antibacterial activity against *S. lutea*. The presence of the methyl group attached to the coumarin ring in the fourth position had a positive influence on the anti-*Pseudomonas* ATCC 27853 potential of the compounds, all the tested 4-propyl-coumarin derivatives being inactive. Against the investigated *Candida* strains, all tested compounds were found to be very active. The introduction of the sulfur atom appeared to be correlated with a good anti-*Candida* activity. The most active DPPH free radical scavengers were the coumarin hydrazide derivatives, the activities of these being similar to that of the standard. The reducing power of the tested compounds was modest, and only the hydrazide derivatives were moderately active. Most of the investigated compounds were moderate NO inhibitors.

The interest in the synthesis of coumarin derivatives has been gaining importance over the last decades, reflecting the importance of such compounds in both medical and chemical research. Future goals for this field of research include the discovery, synthesis, and development of compounds which display increased potency, as well as fueling structure–activity relationship studies aimed at understanding the modes of action of the most biologically active members of these classes of products.

Although coumarin is a simple molecule and many of its derivatives have been known for more than a century, it continues to maintain the interest of researchers being a plentiful source of potential drug candidate because of their significant therapeutic potential.

Conflict of interest

The authors declare no conflict of interest.

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Author details

Gabriela Tataringa* and Ana Maria Zbancioc
University of Medicine and Pharmacy "Grigore T. Popa", Iasi, Romania

*Address all correspondence to: gtataringa22@yahoo.com

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