ORIGINAL ARTICLE

ANTIMICROBIAL PROSPECTION OF SOME COUMARIN DERIVATIVES

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Abstract

Coumarin and their derivatives are a large chemical family which plays an important role in the field of drugs, in recent decades a large number of studies being done on this skeleton. Despite this fact, more researches are still required in order to synthesize and identify many other biological activities for the compounds. Twenty coumarin derivatives were synthesized in good yields and their purity and chemical structures were elucidated by TLC, elemental analysis, IR and ^{1}H-NMR spectra. Their antibacterial activity against *Staphylococcus aureus, Sarcina lutea*, *Bacillus cereus*, *Escherichia coli, Pseudomonas aeruginosa* and antifungal potential against *Candida sp.* were investigated. The results showed that all of the synthesized compounds have exhibited significant antimicrobial activity.

Rezumat

Cumarina şi derivaţii săi reprezintă o clasă de compuşi chimici cu rol important în domeniul sintezei de molecule bioactive, numeroase studii fiind realizate în ultimele decenii pe compuşi care conţineau acest schelet. În ciuda acestui fapt, cercetările orientate spre sinteza şi identificarea de noi acţiuni biologice pentru derivaţii de cumarină reprezintă încă un subiect de actualitate. Douăzeci de substanţe cu nucleu cumarinic au fost sintetizate. Gradul de puritate a fost evaluat prin cromatografie pe strat subţire, iar structurile chimice au fost confirmate prin analiză elementală şi spectrală (IR şi ^{1}H-NMR). Activitatea lor antibacteriană şi antifungică a fost evaluată, rezultatele arătând un potential antimicrobian bun pentru compuşii studiaţi.

Keywords: antimicrobial, coumarin, derivatives, synthesis

Introduction

The majority of problematic infections with respect to failing antibiotic treatment are caused by multidrug resistant pathogens. In the past 20 years, the incidence of microbial infections has increased to alarming levels over the world as a result of antimicrobial resistance. This health problem demands scientists to explore and synthesize new antimicrobial compounds, effective against pathogenic microorganisms that developed resistance to the antibiotics currently used in therapy [1]. Coumarins constitute one of the most common families of green plant secondary metabolites, several of them being used in traditional medicine since ancient times. Coumarin (2*H*-α-benzopyran-2-one) and its derivatives possess a wide range of biological activities. Many products which contain a coumarin subunit have proven to be active as antimicrobial, anticancer, antifungal, anti-HIV, antioxidant, anti-inflammatory and anti-coagulant agents [2]. Coumarin ring derivatives also served as versatile precursors for many organic transformations in the synthesis of a number of drug-like molecules [3, 4]. The reactivity of the coumarin ring towards nucleophiles provides a useful route to prepare new derivatives.

Looking to the medicinal importance of the coumarin ring, in the present work, we employed coumarin as a naturally occurring skeleton for the construction of new derivatives which might exhibit promising antibacterial and antifungal activity.

Materials and Methods

Chemistry

All melting points were determined in open capillaries using a Mel-Temp apparatus and are uncorrected. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (Silica Gel 60 F254). The spots were observed by exposure to UV light. Elemental microanalyses were performed using an Elemental Exeter Analytical CE 440 Analyser. The IR spectra were recorded on a FTIR Shimadzu Prestige 8400s spectrophotometer. The NMR spectra were recorded on a Bruker Avance 500 DRX spectrometer operating at 500 MHz for ^{1}H and 100 MHz for ^{13}C. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet,

t = triplet, m = multiplet, br = broad. Chemical shifts were reported in ppm (δ-scale), coupling constants (J) in Hz.

The starting materials, 4-methyl/propyl-7-hydroxy-coumarin, were prepared by Pechmann synthesis which involved the condensation of resorcinol and ethylacetoacetate/ethylbutyrylacetate in the presence of H_2SO_4 concentrate [5]. 2-ethyl-((4-methy/propyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate (IIa-b) were prepared by heating a mixture of 4-methyl/propyl-7-hydroxy-coumarin (1 mole), ethyl bromoacetate (2 moles) and K_2CO_3 anhydrous in dry acetone for 16 h. After filtration, the solution was evaporated and the solid products (IIa or IIb) were recrystallized from ethanol [6]. Hydrazinolysis of compounds IIa-b gave the corresponding acetohydrazides (IIIa-b) in good yields. 1 mole IIa or IIb in ethanol was heated for 4 - 6 h with hydrazine hydrate (2 moles). After this period of time, the mixture was cooled and the precipitate was filtrate and then purified by water recrystallization [6, 7]. The reaction of the acid hydrazides (4-methyl/propyl-2-oxo-2*H*-benzopyran-7-oxyacetic acid hydrazide, 1 mole) with CS_2 (1.5 moles) in ethanol containing KOH at room temperature, gave the corresponding potassium dithiocarbazate derivatives, IVa-b [8]. To an aqueous solution of potassium 4-methyl/propyl-2-oxo-2*H*-benzopyranyl-7-oxymethyldithiocarbazate (1 mole), methyl iodide (1 mole) was added. The mixture was shook for 2 h and after this period of time a white precipitate was separated.

After recrystallization from ethanol, compounds Va-b were obtained. Potassium 4-methyl/propyl-2-oxo-2*H*-benzopyranyl-7-oxymethyldithiocarbazate (1 mole) was refluxed with acetic acid. The solid product was separated by filtration and then purified by recrystallization from acetic acid to give compounds VIa-b, 7-((5-mercapto-1,3,4-thiadiazol-2-yl)-methoxy)-4-methyl/propyl-2*H*-chromen-2-one [9, 10]. The general method for the preparation of compounds VIIa-b consisted of treating Ia-b (1 mole) with allyl bromide or methyl/ethyl iodide (1 mole), K_2CO_3 anhydrous using anhydrous acetone as solvent. A solution of compound IIa-b (1 mole) and NaOH 5% in ethanol was stirred and refluxed for 2 h. After the removal of the solvent, the residue was dissolved in water and acidified with HCl 10%, when compounds 2-(4-methyl/propyl-2-oxo-2*H*-chromen-7-yloxy))-acetic acid, VIIIa-b were separated.

Antimicrobial activity

The compounds were screened for their antibacterial and antifungal activity according to standard protocols [12]. 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, "Gr. T. Popa" University of Medicine and Pharmacy, Iaşi, Romania. Antimicrobial activity was evaluated by agar disc diffusion method [12]. 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 (10^6 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 [11]. Statistical analysis of the results included the calculation of standard deviation.

Results and Discussion

The synthesis of twenty new coumarin derivatives was carried out according to Figure 1.

In the synthesis of coumarin derivatives, elemental analysis and two basic spectroscopic techniques, infrared spectroscopy (IR) and nuclear magnetic resonance spectroscopy (NMR) were used to characterize the structures of the target compounds.

The IR spectra of all synthesized compounds showed some characteristic peaks indicating the presence of particular groups (Table I). IR, ^{1}H NMR, mass spectra, and elemental analyses of the synthesized compounds are in accordance with the assigned structures (Table II).

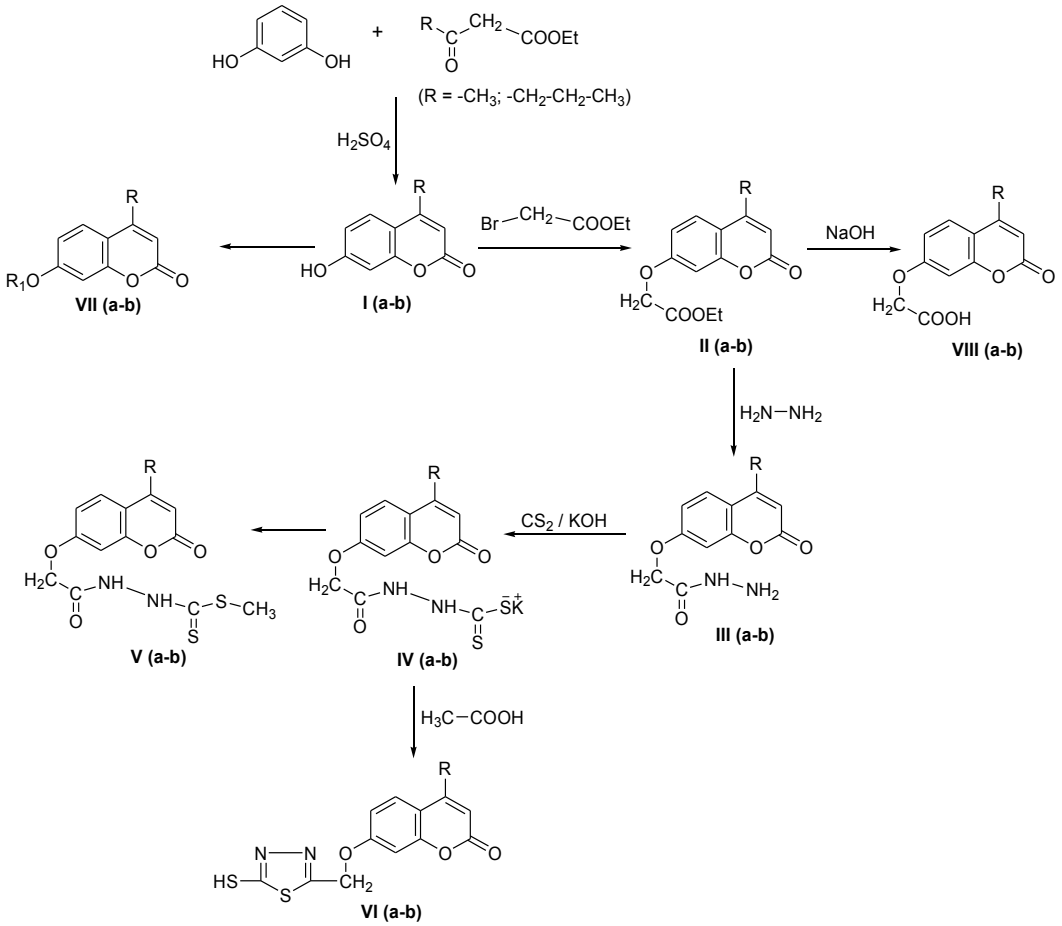

Figure 1.
Reaction scheme of the synthesized compounds

Table I
Physicochemical characteristics of the compounds I-VIII

Comp.	R	R1	Molecular formula	Molecular weight (g/mol)	Colour	Melting point (^{0}C)
Ia	H_3C-	-	$C_{10}H_8O_3$	176	pinkish	185
Ib	H_3C-CH_2-CH_2-	-	$C_{12}H_{12}O_3$	204	white	130
IIa	H_3C-	-	$C_{14}H_{14}O_5$	262	white	100-102
IIb	H_3C-CH_2-CH_2-	-	$C_{16}H_{18}O_5$	290	white	98
IIIa	H_3C-	-	$C_{12}H_{12}N_2O_4$	248	white	204-205
IIIb	H_3C-CH_2-CH_2-	-	$C_{14}H_{16}N_2O_4$	276	white	147-148
IVa	H_3C-	-	$C_{13}H_{11}N_2O_4KS_2$	362	white	184
IVb	H_3C-CH_2-CH_2-	-	$C_{15}H_{15}N_2O_4KS_2$	390	white	176-178
Va	H_3C-	-	$C_{14}H_{14}N_2O_4S_2$	338	white	117-120
Vb	H_3C-CH_2-CH_2-	-	$C_{16}H_{18}N_2O_4S_2$	366	white	174
VIa	H_3C-	-	$C_{13}H_{10}N_2O_3S_2$	306	white	267-268
VIb	H_3C-CH_2-CH_2-	-	$C_{15}H_{14}N_2O_3S_2$	334	white	172
VIIa	H_3C-	H_3C-	$C_{11}H_{10}O_3$	190	white	159-160
VIIa1	H_3C-	CH_3-CH_2-	$C_{12}H_{12}O_3$	204	white	115
VIIa2	H_3C-	CH_2=CH-CH_2-	$C_{13}H_{12}O_3$	216	white	100
VIIb	H_3C-CH_2-CH_2-	H_3C-	$C_{13}H_{14}O_3$	218	beige	185
VIIb1	H_3C-CH_2-CH_2-	H_3C-CH_2-	$C_{14}H_{16}O_3$	232	yellowish	215-218
VIIb2	H_3C-CH_2-CH_2-	CH_2=CH-CH_2-	$C_{15}H_{16}O_3$	244	oily	-
VIIIa	H_3C-	-	$C_{12}H_{10}O_4$	218	white	209
VIIIb	H_3C-CH_2-CH_2-	-	$C_{14}H_{14}O_4$	246	white	184-186

Table II

Elemental analysis and spectral data of the compounds I-VIII

Comp.	%C (t/e)	% H (t/e)	%N (t/e)	%S (t/e)	IR (cm^{-1})	^{1}H-NMR (δ, ppm)
Ia	68.18 68.02	4.58 4.55			1680 (C=O, lactone); 3280 (O-H); 1150 (C-O); 2950 (aliphatic C-H)	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	70.577 0.21	5.92 5.69			3195 (O-H); 1695 (C=O, lactone); 1140 (C-O); 2970 (aliphatic C-H)	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	64.126 4.1	5.38 5.22			1680 (C=O, lactone); 1750 (C=O, side chain); 3070 (ar. C-H)	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	66.196 5.89	6.25 6.05			1690 (C=O, lactone); 1740(C=O, side chain); 3080 (ar. C-H)	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	58.065 7.98	4.87 4.80	11.291 1.1		3423, 3331 (NH_2); 1612 (CO-NH amide); 1271 (C-N)	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	60.866 0.56	5.84 5.76	10.14 9.98		3411,3340 (NH_2); 1610 (CO-NH amide); 1260 (C-N)	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	43.084 2.87	3.06 3.01	7.73 7.24	17.69 17.05	1240 (C=S); 3210 (N-H)	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	46.134 6.02	3.87 3.76	10.019 .95	16.421 6.33	1210 (C=S); 3150 (N-H)	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	49.694 9.57	4.17 4.02	8.28 8.03	18.951 8.25	656 (S-C)	2.54 ppm s, 3H: CH_3 (16); 2.73 - 2.74 ppm d, 3H: CH_3; 4.42 ppm s, 2H: CH_2 (10); 6.04 - 6.06 ppm q, H_3; 6.83 - 6.85 ppm d, H_8; 7.06 - 7.08 ppm q H_6; 7.63 - 7.64 ppm d, H_5; 9.77 ppm s, NH (12); 11.60 ppm s, NH (13)
Vb	52.445 2.40	4.95 4.77	7.64 7.59	17.5 17.31	662 (S-C)	1.20 - 1.23 ppm t, 3H: CH_3 (19); 2.09 ppm s, 3H: CH_3 (16); 2.71 - 2.74 ppm m, 2H: CH_2 (18); 3.03 - 3.06 ppm t, 2H: CH_2 (17); 4.91 ppm s, 2H: CH_2 (10); 6.14 ppm s, H_3; 6.96 - 6.98 ppm q H_6; 7.01 ppm s, H_8; 7.51 - 7.52 ppm d, H_5; 9.78 ppm s, NH (12); 11.61 ppm s, NH (13)
VIa	50.975 0.63	3.29 3.06	9.14 9.11	20.931 9.76	2380 (S-H); 1610 (C=N); 621(S-C)	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)
VIb	53.875 3.33	4.22 4.12	8.38 8.25	19.181 9.13	2350 (S-H); 1620 (C=N); 638 (S-C)	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)
VIIa	69.466 8.85	5.3 5.2			1268 (C-O)	2.73 ppm s, 3H: CH_3 (11); 3.90 ppm s, 3H: CH_3 (10); 6.02 ppm s, H_3; 6.79 ppm s, H_8; 7.02 - 7.06 ppm d, H_6; 7.61 - 7.65 ppm d, H_5
VIIa1	70.577 0.21	5.92 5.83			1284 (C-O)	2.45 ppm s, 3H: CH_3 (12); 4.57 - 4.58 ppm d, 3H: CH_3 (11); 3.94 ppm s, 2H: CH_2 (10); 6.06 ppm s, H3; 6.88 ppm s, H_8; 7.06 - 7.09 ppm d, H_6; 7.63 - 7.68 ppm d, H_5
VIIa2	72.217 2.07	5.59 5.31			1206 (C-O); 1645 (C=C)	2.38 ppm s, 3H: CH_3 (13); 4.67 - 4.68 ppm d, 2H: CH_2 (10); 5.28 - 5.30 ppm d, H_{12b}; 5.40 - 5.44 ppm d, H_{12a}; 6.01 - 6.08 ppm m, H_{11}; 6.20 ppm s, H_3; 6.96 - 6.97 ppm d, H_6; 6.98 ppm s, H_8; 7.66 - 7.68 ppm d, H_5
VIIb	71.547 1.32	6.47 6.4			1272 (C-O)	0.92 - 0.95 ppm s, 3H: CH_3 (13); 1.55 - 1.62 ppm m, 2H: CH_2 (12); 2.67 - 2.70 ppm t, 2H: CH_2 (11); 3.82 ppm s, 3H: CH_3 (10); 6.10 ppm s, H_3; 6.91 ppm s, H_8; 6.89 ppm d, H_6; 7.67 - 7.68 ppm d, H_5

Comp.	%C (t/e)	% H (t/e)	%N (t/e)	%S (t/e)	IR (cm^{-1})	^{1}H-NMR (δ, ppm)
VIIb1	72.397 2.09	9.94 9.88			1254 (C-O)	1.22 - 1.25 ppm t, 3H: CH_3 (14); 1.40 - 1.46 ppm t, 3H: CH_3 (11); 1.66 - 1.70 ppm m, 2H: CH_2 (13); 2.79 - 2.83 ppm t, 2H: CH_2 (12); 3.94 - 3.99 ppm m, 2H: CH_2 (10); 6.11 ppm s, H_3; 6.86 ppm s, H_8; 7.10 - 7.12 ppm q H_6; 7.65 - 7.67 ppm d, H_5
VIIb2	73.757 3.55	6.6 6.49			1212(C-O); 1651 (C=C)	1.20 - 1.25 ppm t, 3H: CH_3 (15); 1.65 - 1.70 ppm m, 2H: CH_2 (14); 2.79 - 2.83 ppm t, 2H: CH_2 (13); 4.44 - 4.50 ppm t, 2H: CH_2 (10); 5.32 - 5.35 ppm d, 2H: CH_2 (12); 6.01 - 6.07 ppm m, CH (11); 6.15 ppm s, H_3; 6.84 ppm s, H_8; 7.05 - 7.08 ppm d H_6; 7.62 - 7.66 ppm d, H_5
VIIIa	61.54 61.42	4.3 4.25			2930 (O-H); 1710 (C=O)	2.12 ppm s, 3H: CH_3; 4.70 ppm s, 2H: CH_2 (10); 6.03 ppm s, H_3; 6.73 ppm s, H_8; 6.89 - 6.94 ppm d; 7.53 - 7.58 ppm d, H_5; 11.21 ppm s, OH (11)
VIIIb	64.12 64.06	5.38 5.33			2987 (O-H); 1724 (C=O)	0.96 - 0.99 ppm t, 3H: CH_3 (16); 1,48 - 1.51 ppm m, 2H: CH_2 (15); 2.32 -2.34 ppm t, 2H: CH_2 (14); 4.59 ppm s, 2H: CH_2 (10); 6.43 ppm s, H_3; 6.79 ppm s, H_8; 6.80 -6.82 ppm d H_6; 7.25 - 7.27 ppm d, H_5; 11.22 ppm s, OH (14)

t = theoretically; e = experimentally

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, Gram negative bacteria and fungi are given in Tables III and IV.

Table III

Antibacterial activity of the investigated compounds

Compound/reference	Diameters of the growth inhibition zone (mm)				
	S. aureus ATCC 25923	*S. lutea* ATCC 9341	*B. cereus* ATCC 14579	*E. coli* ATCC 25922	*P. 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 ± 0.00	NA	NA	NA
IIIa	15 ± 0.54	20 ± 0.79	22 ± 0.79	10 ± 0.79	9 ± 0.79
IIIb	25 ± 0.52	28 ± 0.00	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 ± 1.79	20 ± 0.43	21 ± 0.79	11 ± 1.52	9 ± 0.79
Vb	25 ± 0.52	28 ± 0.91	NA	12 ± 0.52	NA
VIa	14 ± 0.52	25 ± 0.52	20 ± 1.43	10 ± 1.52	8 ± 0.52
VIb	21 ± 1.43	25 ± 0.79	NA	13 ± 0.83	NA
VIIa	12 ± 0.93	20 ± 1.52	22 ± 1.52	11 ± 1.79	8 ± 1.52
VIIa1	12 ± 0.93	20 ± 0.52	21 ± 1.43	12 ± 1.43	8 ± 0.79
VIIa2	13 ± 1.08	19 ± 0.54	21 ± 0.52	NA	NA
VIIb	18 ± 1.43	27 ± 0.79	NA	NA	NA
VIIb1	17 ± 0.00	29 ± 0.79	NA	NA	NA
VIIb2	17 ± 0.00	23 ± 1.43	NA	NA	NA
VIIIa	14 ± 0.91	20 ± 0.54	21 ± 0.79	11 ± 1.43	9 ± 0.52
VIIIb	20 ± 0.00	22 ± 1.43	NA	NA	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

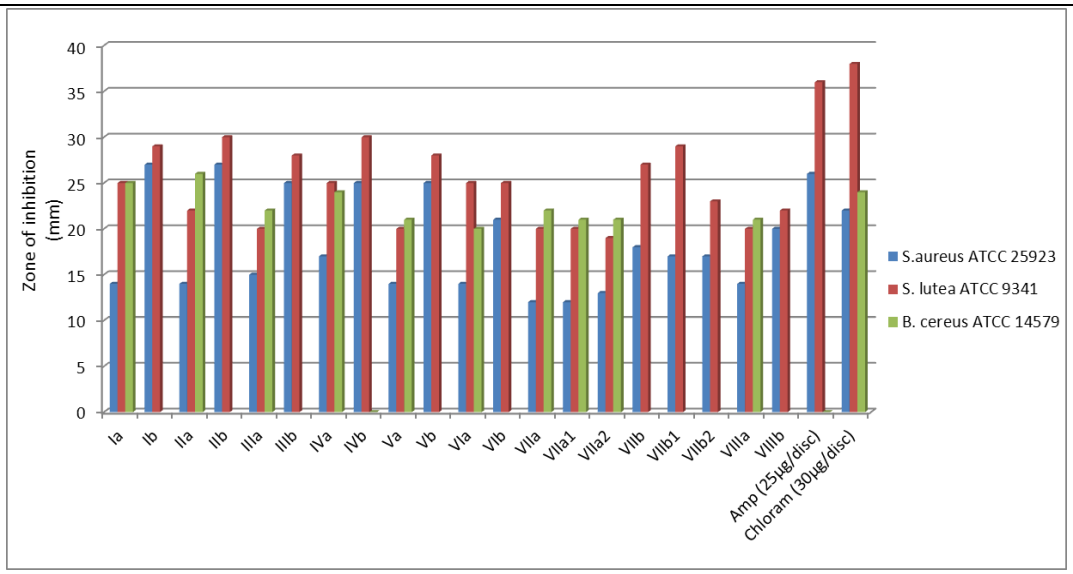

Figure 2.
Relationship between inhibition zones for the tested compounds and Gram positive bacteria strains

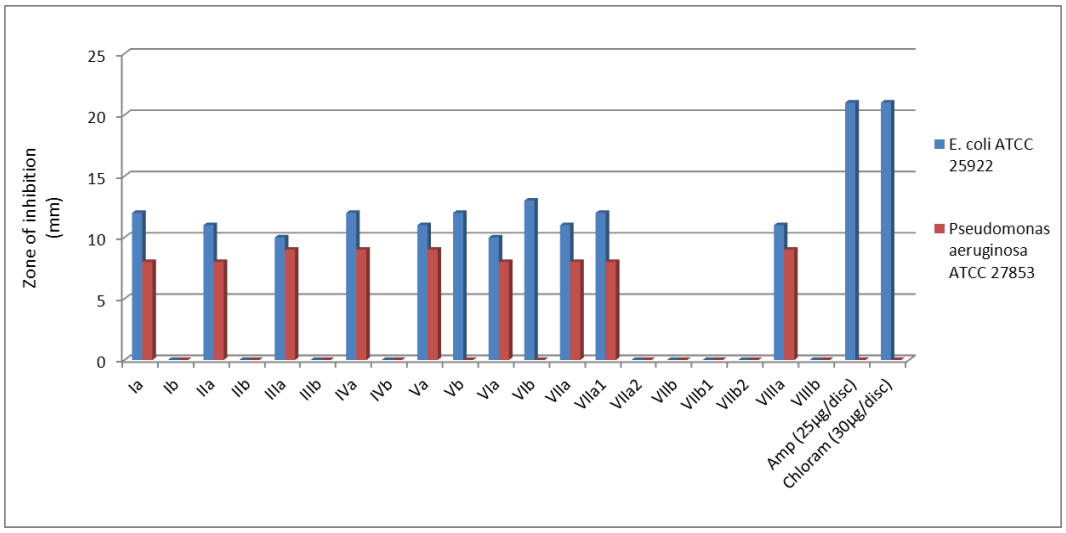

Figure 3.
Relationship between inhibition zones for the tested compounds and Gram negative bacteria strains

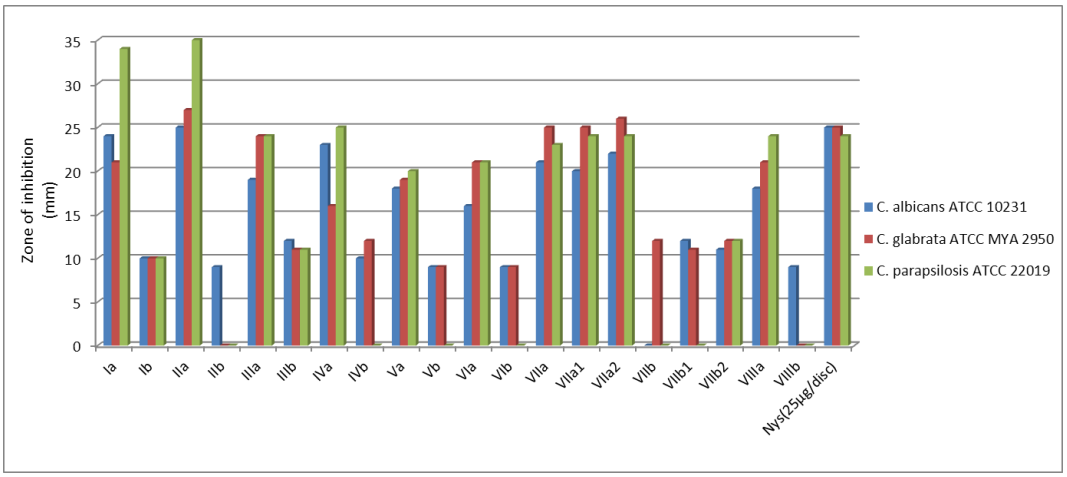

Figure 4.
Relationship between inhibition zones for the tested compounds and *Candida sp.* strains

Table IV

Antifungal activity of the tested compounds

Compound/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	18 ± 0.54	19 ± 0.52	20 ± 0.52
Vb	9 ± 1.83	9 ± 0.54	NA
VIa	16 ± 1.79	21 ± 1.83	21 ± 0.54
VIb	9 ± 0.54	9 ± 0.52	NA
VIIa	21 ± 2.59	25 ± 0.54	23 ± 0.79
VIIa1	20 ± 0.52	25 ± 0.52	24 ± 0.79
VIIa2	22 ± 0.79	26 ± 0.54	24 ± 0.52
VIIb	NA	12 ± 1.08	NA
VIIb1	12 ± 0.79	11 ± 1.08	NA
VIIb2	11 ± 0.54	12 ± 0.52	12 ± 1.08
VIIIa	18 ± 0.52	21 ± 0.79	24 ± 0.52
VIIIb	9 ± 1.08	NA	NA
Nystatin (100 µg/disc)	25 ± 0.52	25 ± 0.52	24 ± 0.00

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

The screened derivatives showed very interesting biological activity against the mentioned strains. 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*, the most active compounds being: Ib, IIb, IIIb, IVb and Vb. The replacing of the methyl radical in the 4th position with propyl group was correlated with an increased activity against *S. aureus*. The tested compounds exhibited excellent antibacterial activity against *Sarcina lutea*, the most active derivatives being IIb, IVb, Ib, IIIb, Vb, VIIb1. We found a moderate action against *B. cereus*, the most active being the umbelliferone derivatives with a methyl group attached to C4: IIa, Ia IVa and VIII (Figure 2).

Against *Escherichia coli*, the investigated compounds had a weaker action compared to the controls: ampicillin and chloramphenicol. The most active was the compound with a thiadiazole ring, VIb. Out of the twenty synthesized substances, nine exhibited slight inhibitory effects against *Pseudomonas aeruginosa*. The presence of the methyl group attached to the coumarin ring in the 4th position had a positive influence on the anti- *Pseudomonas* potential of the compounds, all the tested 4-propyl-coumarin derivatives being inactive (Figure 3).

We 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* compared to nystatin. The introduction of the sulphur atom appeared to be correlated with a good anti-*Candida* activity (Figure 4).

Conclusions

Our present study was focused on the synthesis, spectral analysis and antimicrobial activities of some coumarin derivatives. Twenty coumarin derivatives were obtained and their structures were confirmed by elemental and spectral analyses. The antibacterial and antifungal potentials of the synthesized compounds were assessed against Gram positive and Gram negative bacteria and against three strains of *Candida sp.* Some of the compounds were very effective as anti-microbial and antifungal agents.

The most susceptible bacterial strains were *S. aureus* and *Sarcina lutea*. Five of the tested substances (Ib, IIb, IIIb, IVb, Vb) were very active against these two Gram positive bacteria, their diameters of the growth inhibition zone for *S. aureus* being larger than those recorded for chloramphenicol for the same micro-organism.

The results of the research were promising and some of the synthesized derivatives represent good candidates for MIC and MBC determination during future studies.

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