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A small library of 4-(alkylamino)-3-nitrocoumarin derivatives with potent antimicrobial activity against gastrointestinal pathogens

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Abstract: Due to the confirmed antimicrobial activity of both natural and synthetic coumarin derivatives, the present study was envisaged to provide further insight into the antimicrobial potential of coumarins through the screening of a designed library of nine 4-(alkylamino)-3-nitrocoumarins against a panel of 24 laboratory strains and resistant (isolates) bacterial and fungal pathogens. All compounds showed some degree of strain-selective activity that in some cases was very pronounced, reaching the value of 0.04 nmol mL^{-1} (*i.e.*, 12 ng mL^{-1}) for the minimal inhibitory concentration against *Candida albicans*. The observed activities were higher against Gram-negative strains, among which the most susceptible strain, among both ATCC strains and clinical isolates, was *Salmonella enterica* subsp. *enterica* serovar *Enteritidis*. These results indicate to a high potential of these coumarins as antimicrobials for the treatment of gastrointestinal and other infections caused by highly resistant microbial strains. Finally, a multivariate statistical analysis of the herein obtained and previous results on the antimicrobial activity of related selected coumarins was performed to allow an easier structure–activity discussion.

Keywords: 4-(alkylamino)coumarins; antimicrobial activity; *Candida albicans*; multivariate statistical analysis; *Salmonella enterica*.

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INTRODUCTION

The gastrointestinal (GI) tract represents an ideal place for the development of various microorganisms that can affect the (human) organism in both beneficial and adverse aspects, such as protection against infection, physiological functions, activation of the immune system, carcinogenesis, aging and alteration of the effect a therapeutic drug should have.[1] GI bacterial infections, which can range from mild (with few symptoms) to severe life-threatening disorders (*e.g.*, hemorrhagic colitis, intestine perforation and kidney failure) are one of the most frequent readons for patients to turn to physicians (one third of the people living in industrialized countries per year).[2] GI infections are mostly food-borne and result from inadequate cooking and inadequate storage of food, while some commensal bacteria can cause infections in immunocompromised patients (AIDS, cancer, *etc*.).[3] Hence, the search for new antimicrobial agents, renewed by the rapid and alarming development of multiresistant bacterial and fungal pathogens, has always been one of the most significant tasks of many medicinal chemists.

Coumarins are a widespread group of natural compounds present in the seeds, roots and leaves of many plant species. Their function in the plant organism is far from clear, although some authors believe that they have growth regulatory functions, or that they act as fungistatic and bacteriostatic agents.[4] This is substantiated by the extensive use of coumarin compound-containing herbal remedies in the traditional medicine of many nations due to their broad spectrum of pharmacological activities, including antibacterial and antifungal effects.[5–7]

Significant antimicrobial activity of a series of 4-aryl-substituted 3-nitrocoumarins was recently demonstrated by Debeljak and co-workers.[8] In connection with this, several arylamino- and (heteroarylamino)-3-nitrocoumarin derivatives were synthesized and their antimicrobial activity determined.[9–14] A number of these compounds showed strong activity in reducing the microbial growth comparable or even better than the activity of standard antibiotics.[9,13,14] Among them 4-(1-naphthylamino)-3-nitrocoumarin possessed the strongest antimicrobial potential as demonstrated by its action against *Salmonella enterica* in a low dose of 9 μg per disc.[15]

Additional fine-tuning of the antimicrobial activity of these coumarin derivatives could be accomplished by the introduction of an alkyl chain instead of the aromatic substituent on the nitrogen atom in position 4. In this way, the nucleophilicity and basicity would be increased that could lead to a stronger binding with potential biological target molecules. The previous statements and the fact that hitherto only the disc diffusion method was used to evaluate the activity of the synthesized coumarin derivatives, it was decided to synthesize a small library of coumarin derivatives possessing a 4-(alkylamino)-substituent on the 3-nitrocoumarin parent structure and screen the compounds for their *in vitro* antimicrobial activity against twenty four bacterial and fungal strains (both ATCC and

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clinical isolates, including three important GI pathogens *Yersinia enterocolitica*, *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* and *Shigella flexneri*) using a microdilution method. Finally, another task of this work was to compare previously published results concerning the antimicrobial activity of related coumarin derivatives and the herein obtained results using multivariate statistical analysis with the aim of finding possible structure–activity relationships.

EXPERIMENTAL

General

The melting points were determined on a Kofler hot-plate apparatus and are uncorrected. The HRMS(EI) spectra were recorded on a JEOL Mstation JMS 700 instrument (JEOL, Germany). The IR measurements (ATR – attenuated total reflectance) were performed using a Thermo Nicolet model 6700 FTIR instrument. The NMR spectra were recorded on a Varian Gemini 200 spectrometer (^{1}H-NMR at 200 MHz and ^{13}C-NMR at 50 MHz), using DMSO-d_6 as the solvent. The chemical shifts are expressed in δ (ppm) using TMS (Me_4Si) as the internal standard. Microanalyses of carbon, hydrogen and nitrogen were performed with a Carlo Erba 1106 microanalyser; the results agreed favorably with the calculated values. For TLC, silica gel plates (Kieselgel 60 F_{254}, Merck, Germany) were used. Visualization was affected by spraying the plates with 1:1 (*V*/*V*) aqueous solution of sulfuric acid and then heating. All the reagents and solvents were obtained from commercial sources (Aldrich, USA; Merck, Germany; Fluka, Germany) and used as received, except for the solvents that were purified by distillation.

Mass spectrometry

EI-MS and HRMS analyses were performed using a JEOL MStation JMS-700 mass spectrometer with an ionization energy of 70 eV, an ionization trap current of 300 μA and a source temperature of 230 °C. Perfluorokerosene (Sigma–Aldrich, USA) was used as the internal mass reference in the HRMS studies. The conversion of the mass reference list to a calibration was performed by the data system of the mass spectrometer. The corresponding range of mass measurements was set to include two standard peaks that encompassed the sample peak of interest. The mass resolution and scan speed used were 30,000 (10 % valley) and 60 s decade^{-1}, respectively. The accurate mass was calculated as the average of the values measured in 5–10 scans, determined from the mass centroids of $M^{+\bullet}$ and the other peaks. The error for each elemental composition data is given in units of mmu as calculated using the program installed in the data system.

*Synthesis of 4-chloro-3-nitrocoumarin (**3**)*

4-Hydroxycoumarin (**1**) was nitrated using 72 % HNO_3 in glacial AcOH to afford 4-hydroxy-3-nitrocoumarin (**2**).[16] The starting compound 4-chloro-3-nitrocoumarin (**3**) was prepared from **2** following the method of Kaljaj *et al*.[17] The preparation was carried out in the following manner: *N*,*N*-dimethylformamide (DMF, 2 mL, 26 mmol) was cooled to 10 °C in an ice bath. Under stirring, $POCl_3$ (4.0 g, 26 mmol) was added dropwise, and the obtained mixture was stirred for an additional 15 min. Then, the ice bath was removed and the reaction was left to proceed at room temperature for a further 15 min. Finally, a solution of **2** (5.4 g; 26 mmol) in DMF (12.5 mL) was added dropwise. After 15 min of stirring, the reaction was stopped by the addition of cold water (15 mL). The precipitated solid was collected by filtration and washed with saturated sodium bicarbonate solution and water. Recrystallization

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from a mixture of benzene–hexane (1:1,*V*/*V*) yielded yellow crystals of **3** (5.1 g; 22.6 mmol) in 87 % yield, m.p. 162–163 °C. The procedure was repeated twice.

*General procedure for the synthesis of 4-(alkylamino)-3-nitrocoumarins **5a–i***

The solution of 4-chloro-3-nitrocoumarin (**3**, 1.0 g, 4.4 mmol) and the appropriate amine nucleophile **4a**–**i** (4.4 mmol) in ethyl acetate (10 ml) was refluxed in the presence of triethylamine (1 ml, 7.2 mmol) for 1–3 h. After cooling, the precipitated solid was filtered off and washed with ethyl acetate and water. Purity of the synthesized compounds was checked by TLC.

Antimicrobial activity

The synthesized compounds were tested against a panel of microorganisms including three Gram-positive (*Bacillus cereus* (isolate from food), *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538), nineteen Gram-negative (*Escherichia coli* ATCC 8739, *E. coli* (isolate from food), *Klebsiella pneumoniae* ATCC 10031, *Salmonella enterica* subsp. *enterica* serovar *Enteritidis* ATCC 13076, *S. enterica* (5 clinical isolates), *Shigella flexneri* (5 clinical isolates) and *Yersinia enterocolitica* (5 clinical isolates), one yeast (*Candida albicans* ATCC 10231) and one mold (*Aspergillus brasiliensis* ATCC 16404) strains.

Clinical isolates of *Y. enterocolitica*, *S. enterica* subsp. *enterica* serovar *Enteritidis* and *S. flexneri* (5 isolates per strain), together with the food isolates (*B. cereus* and *E. coli*) were obtained from the Institute of Public Health, Niš, Serbia and are stored in a private microbiological collection. The isolation of the mentioned strains was performed from stool (fecal) samples of patients with a diarrheal syndrome.

Testing of antimicrobial activity

The antimicrobial activity was evaluated using the broth microdilution method.[18] Minimum inhibitory concentration (*MIC*) determinations were performed by a serial dilution method in 96-well microtitre plates. Bacterial species were cultured at 37 °C in Müller–Hinton agar (MHA) and Sabouraud dextrose agar (SDA) was the culture medium for the yeast (30 °C). After 18 h of cultivation, bacterial suspensions were made in Mueller Hinton broth and their turbidity was standardized to 0.5 McFarland scale using a densitometer (DEN-1 McFarland densitometer, Biosan). The final density of the bacterial and the yeast inoculums corresponded to 5×10^5 CFU (colony forming units). A suspension of the mould (*A. niger*) was made in Sabouraud dextrose broth (SDB) and its turbidity was confirmed by viable counting in a Thoma chamber with 1×10^4 CFU as the final size of the inoculum. The inoculums were added to all wells and the plates were cultivated at 37 °C during 24 h (bacteria) or at 30 °C for 48 h (fungi). Tetracycline, chloramphenicol and nystatin served as positive controls, while the solvent (ethanol) was used as a negative control. One inoculated well was included, to allow control of the adequacy of the broth for organism growth. One non-inoculated well, free of antimicrobial agent, was also included to ensure medium sterility.

The bacterial growth was determined by adding 20 μL of a 0.5 % aqueous triphenyltetrazolium chloride (TTC) solution.[19] The MIC was defined as the lowest concentration of the tested compound that inhibited visible growth (red colored pellet on the bottom of the wells after the addition of TTC). The experiments were performed in triplicate and mean values are presented.

Statistical analyses

Agglomerative hierarchical clustering (AHC) was performed using the Excel program plug-in XLSTAT version 2011.3.02.[20] The method was applied utilizing the *MIC* values (in

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μmol mL^{-1}) as original variables without any recalculation. AHC was determined using Pearson dissimilarity, where the aggregation criterion were simple linkage, unweighted pair-group average and complete linkage, the Euclidean distance, where the aggregation criterion were weighted pair-group average, unweighted pair-group average, and the Ward method.

RESULTS AND DISCUSSION

Chemistry

In the first reaction step, 4-hydroxycoumarin (**1**) was nitrated in glacial AcOH with HNO_3 to afford 4-hydroxy-3-nitrocoumarin (**2**). The starting 4-chloro-3-nitrocoumarin (**3**) was prepared from **2** in a reaction with phosphorus oxychloride and *N*,*N*-dimethylformamide.

The target compounds **5a**–**i** (three new – **5e**–**g**, three previously known – **5a**, **5c** and **5i**, and three commercially available but with no mention what so ever in the literature – **5b**, **5d** and **5h**), were prepared in the reaction of **3** and an alkylamine **4a**–**i**, respectively (1:1 mole ratio of starting materials) in ethyl acetate in the presence of two equivalents of triethylamine. The compounds were obtained in good yields (72–92 %). The synthesis scheme is presented in Fig. 1.`

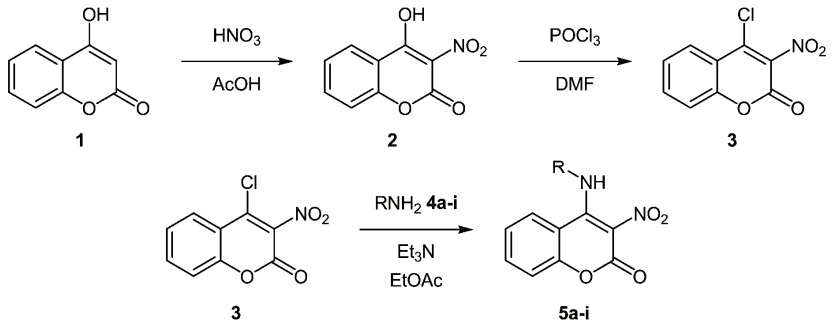

Fig. 1. Synthesis of 4-(alkylamino)-3-nitrocoumarin derivatives **5a**–**i**. (**4a**: butan-1-amine, **4b**: butan-2-amine, **4c**: 2-methylpropan-2-amine, **4d**: 3-aminopropan-1-ol, **4e**: hexan-1-amine, **4f**: octan-1-amine, **4g**: hexadecan-1-amine, **4h**: furan-2-ylmethanamine and **4i**: phenylmethanamine; R = **5a**: $CH_3(CH_2)_2CH_2$–; **5b**: $CH_3CH_2(CH_3)CH$–; **5c**: $(CH_3)_3C$–; **5d**: $HO(CH_2)_2CH_2$–; **5e**: $CH_3(CH_2)_4CH_2$–; **5f**: $CH_3(CH_2)_6CH_2$–; **5g**: $CH_3(CH_2)_{14}CH_2$–; **5h**: $C_4H_3OCH_2$– (furan-2-ylmethyl); **5i**: $C_6H_5CH_2$–.

The structures of the synthesized compounds were confirmed by analytical and spectral means (HRMS(EI), IR, ^{1}H- and ^{13}C-NMR). The physical, analytical and spectral data for compounds **5a**–**i** are given in the Supplementary material to this paper.

IR spectra of the synthesized compounds contained characteristic vibrations at 3030 to 3393 cm^{-1} corresponding to absorptions of the Ar–H and N–H bonds, and strong bands at 1677–1698 cm^{-1} corresponding to the C=O groups. The IR absorptions due to the presence of the 3-NO_2 group appeared at 1317–1364 and

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1511–1556 cm^{-1}, and the C–H bonds showed characteristic vibrations at 2925––2960 cm^{-1}.

Protons from the coumarin moiety showed very similar chemical shifts in the ^{1}H-NMR spectra of compounds **5a**–**i**. Protons in the position 5 of the coumarin core were more downfield and showed higher chemical shifts (doublet of doublets at 8.35–8.52 ppm) than the other protons attached to that ring. Significant differences in the chemical shifts of the mentioned protons existed for compounds **5b**, **5c** and **5f** where the signals for H-5 appear at 7.89–8.00 ppm. All other protons on the coumarin moiety displayed much less variation in their chemical shifts. H-7 protons appeared as a doublet of triplets or only triplets at 7.69––7.75 ppm. H-6 and H-8, the signals of which overlapped at 7.28–7.50 ppm, were more shielded. The aromatic protons of the substituent groups in compounds **5h** and **5i** showed similar values of chemical shifts and appeared as a doublet of doublets at 7.64 ppm and a multiplet at 6.34–6.45 ppm, as well as a multiplet at 7.26–7.34 ppm, respectively. The remaining protons, from the alkyl groups bonded to the coumarin moiety, were as expected more upfield.

Antimicrobial activity

The created library was first evaluated for *in vitro* antimicrobial activity against a wide range of bacteria (four Gram-negative and three Gram-positive) as well as against a yeast and a mold species. The values of their minimal inhibitory concentrations (*MIC*, nmol mL^{-1}), determined in a microdilution assay, revealed the diverse effect of these compounds on the growth of microorganisms (all strains were susceptible to the presence of these compounds in their nutritive medium with an exception of *E. coli* (isolate) and *A. brasiliensis* (Table I)). The

TABLE I. Minimal inhibitory concentrations (*MIC* / nmol mL^{-1}) of the coumarins **5a**–**i**; NT – not tested, / – not sensitive in the range of the tested concentrations; AB – reference antibiotic

Bacterial strain		Compound										
		5a	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	AB[a]	AB[b]
Gram-negative[c]												
E. coli	ATCC 8739	3.0	12	–	–	11	2.45	1.8	–	5.3	3.5	NT
K. pneumoniae	ATCC 10031	6.0	1.5	6.0	–	–	–	0.91	–	5.3	0.88	NT
S. enterica	ATCC 13076	6.0	95	191	189	5.4	4.9	3.6	1.4	5.3	0.44	NT
Gram-positive												
B. cereus	Isolate from food	–	–	–	–	–	–	232	44	–	0.44	NT
B. subtilis	ATCC 6633	–	–	–	–	–	4.9	29	–	/	0.22	NT
S. aureus	ATCC 6538	6.0	6.0	6.0	189	1.3	1.2	3.6	5.4	21	0.22	NT
Fungal strain[d]												
C. albicans	ATCC 10231	0.34	0.05	3.0	11.8	0.04	0.08	0.44	1.4	0.64	NT	6.8

[a]Tetracycline; [b]nystatin; [c]the food isolate of *E. coli* was resistant to all compounds in the tested range of concentrations; [d]the mold *A. brasiliensis* (ATCC 16404) was resistant to all compounds in the tested range of concentrations

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active inhibitory concentrations ranged from 0.04 to 232 nmol mL^{-1}, with **5g** being the most non-selective in its action. The best MIC value was observed for compound **5e** against *C. albicans*. The remaining compounds displayed antimicrobial activity with no special selectivity towards either Gram-positive or Gram-negative bacterial strains.

Among the Gram-positive strains, *B. cereus* was the most resistant strain, susceptible to only two out of the nine tested compounds, but at relatively high concentrations (**5h** and **5g**, Table I). *S. aureus* was the most sensitive bacterial strain inhibited by all tested compounds in a concentration range 1.2–189 nmol mL^{-1}. The activity of the compounds against Gram-negative bacteria was characterized by two extreme cases – *E. coli* (a food isolate which showed complete resistance to all the tested compounds, as well to the standard antibiotic) and *S. enterica* (an ATCC reference strain (13076) showing the highest susceptibility to the tested compounds with *MIC* values in the range (1.4 to 191 nmol mL^{-1}) similar to the ones for Gram-positive bacteria). This strong resistance of *E. coli* could be explained by its origin (food isolate, a probable cause of food-borne infections) and this was corroborated by the higher registered *MIC* values for an ATCC strain of *E. coli* (Table I). All compounds were also proven to possess good anticandidal activity (0.04–12 nmol mL^{-1}) but had no effect at the highest tested concentration on the growth of the other tested fungal organism (the mold *A. brasiliensis*) (see Table I).

Prompted by the results of this initial screening of antimicrobial activity, a selection of three compounds (based on their structural diversity, **5b** having a short *N*-alkyl substituent; **5g** having the longest *N*-alkyl substituent and among the most potent ones thus far and **5i** possessing a *N*-benzyl group) were further tested against fifteen bacterial strains representing gastrointestinal pathogenic bacteria isolated from human clinical samples (Table II). Table II also contains clinically relevant data on the patient diagnosed condition caused by the particular bacterial isolate.

Human sample isolates (five per strain) of *Y. enterocolitica*, *S. enterica* and *Sh. flexneri* showed themselves to be highly resistant to the influence of the tested coumarinic derivatives (most of which having *MIC* values higher than 1.9 nmol mL^{-1}). The differing effect on (clinical/food) isolates and ATCC strains (Tables I and II) was evident from the high resistance of the isolates to the control antibiotic used in this study as well. In the light of these facts, compound **5b** looks highly promising, since it was the only compound among the three tested, which showed inhibitory action against *S. enterica* with *MIC* values of 0.48 and 0.95 μmol mL^{-1}. Although this does not appear significant at first, this activity is more than relevant if one considers the fact that this strain was completely resistant to the highest tested concentration of the reference antibiotic (0.11 μmol mL^{-1}).

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TABLE II. Effect of the selected coumarin derivatives (**5b**, **5g** and **5i**) on growth of three gastrointestinal pathogenic bacterial strains (isolates)

Bacteria	Isolate No.	Diagnosis	*MIC* / μmol mL^{-1}			
			5b	**5g**	**5i**	Chloram-phenicol
Yersinia enterocolitica	1	Acute enterocolitis	>1.9	>1.2	>1.7	0.11
	2		>1.9	>1.2	>1.7	0.11
	3		>1.9	0.58	0.85	0.027
	4		>1.9	0.29	>1.7	0.054
	5		>1.9	>1.2	>1.7	0.11
Salmonella enterica	1	Gastroenterocolitis	0.95	>1.2	>1.7	>0.11
	2		0.95	>1.2	>1.7	>0.11
	3		0.48	>1.2	>1.7	>0.11
	4		>1.9	>1.2	>1.7	>0.11
	5		0.95	>1.2	>1.7	>0.11
Shigella flexneri	1	Shigellosis	>1.9	0.87	>1.7	0.11
	2		0.95	0.29	1.3	0.11
	3		>1.9	0.87	>1.7	0.11
	4		>1.9	>1.2	>1.7	0.11
	5		0.95	0.87	>1.7	0.11

Hitherto, a substantial amount of data on the antimicrobial potential of a number of *N*-substituted 4-amino-3-nitrocoumarins has been accumulated.[13–15,21] Using multivariate statistical analyses (MVA), in an attempt to determine their similarities and selectivity against certain pathogenic strains, the relations between the *MIC* values of the herein tested coumarin derivatives and those previously published were analyzed.[13–15,21] The compounds taken into consideration (Fig. 2) for the statistical analysis were those that were previously tested

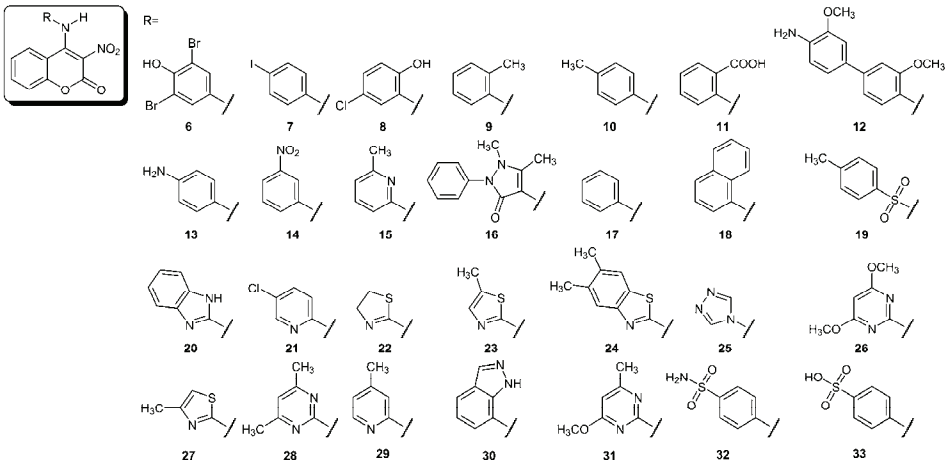

Fig. 2. Structures of coumarin derivatives (**6**–**33**) included in the multivariate statistical analyses.

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against the same panel of microorganisms as the compounds tested in this work. Agglomerative hierarchical analysis using *MIC* values against seven bacterial and two fungal strains as variables resulted in the dendrogram shown in Fig. 3 with three clearly separated groups/classes of compounds (C1–C3). Group centroids (the average representative of a group) were also plotted against microorganism strains tested (Fig. 4) to give a better picture of the distinctions existing between the groups.

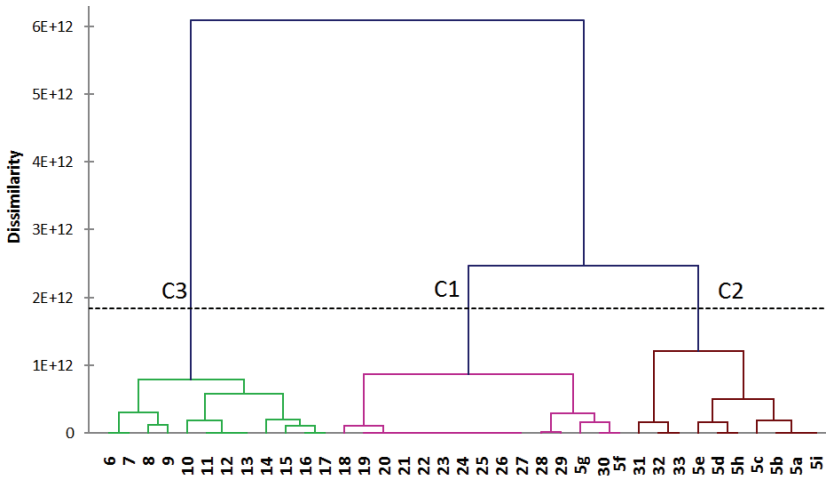

Fig. 3. Dendrogram (AHC analysis) representing the dissimilarity in the antimicrobial activity (*MIC* values of selected coumarin derivatives used as variables against five bacterial and two fungal strains) relationships of thirty-seven compounds obtained by Euclidean distance dissimilarity, using the aggregation criterion-the Ward method. Three groups of compounds were found: C1–C3.

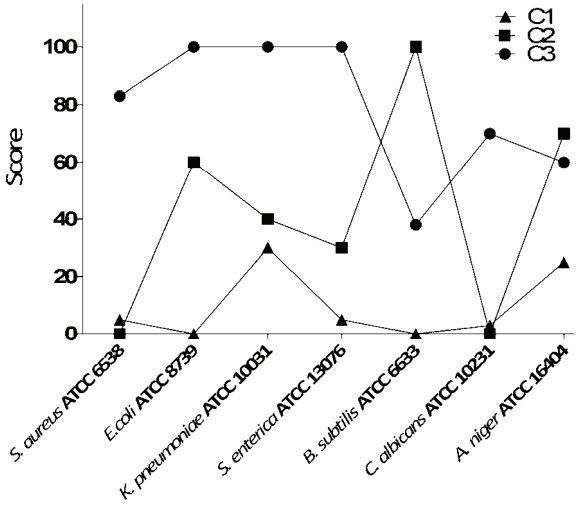

Fig. 4. Graphical representation of the scores (the lowest activity, *i.e.*, the highest value of *MIC*, was given the score 100 and the best activity value 0) of the centroids (classes C1–C3) from the performed AHC analysis (for details see Fig. 3).

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Group C1 (Figs. 3 and 4) consisted of compounds that had rather non-selective activity with the lowest *MIC* values towards *E. coli, K. pneumoniae, S. enterica, B. subtilis, C. albicans* and *A. brasiliensis*. Among these microorganisms, *K. pneumoniae* and *A. brasiliensis* were the ones more resistant to the action of compounds from group C1. Compounds **5f** and **5g** from the present paper belong to this group. The second group, designated as C2, contained all other compounds prepared in this work (**5a**–**e**, **5h** and **5i**). Group C2 compounds demonstrated a greater degree of selectivity, highly affecting the growth of *S. aureus* and *C. albicans*, while remaining relatively active against the other strains in comparison to group C1. All the other statistically compared compounds were grouped into C3 according to their overall low impact on bacterial growth (*i.e.*, high *MIC* values) – the only exception being their action against *B. subtilis* (Fig. 4).

From all of the above mentioned, it could be conclude that group 1 (C1) substances were the most potent (in terms of both their *MIC* values and broad spectrum of bacteria that the compounds affect) and group 2 substances are the most selective ones (even though they have lower *MIC* values).

Chemically speaking, compounds **5f** and **5g** differed from the rest of the compounds prepared in this work by having the longest alkyl chains attached to the nitrogen in position 4. This structural feature, a long lipophilic moiety (octyl and hexadecyl groups in **5f** and **5g**, respectively), made them less selective in their antimicrobial action, suggesting that lipophilicity, *i.e.*, an interaction with the cell membrane, might play a crucial role in their activity. It would be natural then to expect, as observed, that such compounds have an effect on more different strains (be less selective) by acting on/disrupting their membranes. Along with these *N*-alkyl derivatives (**5f** and **5g**), other members of C1 were almost invariantly *N*-heteroaryl derivatives (**20**–**30**). These could be regarded as possible hybrids of the coumarin core with other pharmacophoric groups (*i.e.*, those heterocycles that are known to possess an antimicrobial action). This hybridization appears to have resulted in the onset of the strongest observed activity.

Group C2 compounds demonstrated that the presence of shorter alkyl substituents on N-4 lowers the activity but also that the nucleophilicity/basic nature of this nitrogen could be relevant for the overall noted activity. The weakly nucleophilic/basic anilino-type nitrogen, present in all compounds from group C3, might be the reason why these compounds showed much lower potency in inhibiting the growth of microorganisms when compared to the activity of the much more basic/nucleophilic (at N-4) compounds from C2. Not even the presence of a phenol group in compounds **6** and **8** (known to be the carrier of antimicrobial activity of many natural compounds, thymol, for example) had a significant impact on the activity of the *N*-aryl coumarin derivatives grouped in C3. Moreover, the size of the *N*-substituent seems not to be of importance in the appearance of antimicrobial activity, as exemplified by the low activity of compounds **12** and **18**.

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Bearing all of this in mind, it could be concluded that the nature of the *N*-substituent at position 4 profoundly affects the antimicrobial potency of 3-nitrocoumarins. The respective activity decreases in the following order *N*-long alkyl chains ≈ *N*-heteroaryl > *N*-short alkyl chains >> *N*-aryl derivatives.

CONCLUSION

In the present paper, a wide range of activities, ranging from completely inactive to active against antibiotic-resistant strains, was registered for the studied 4-(alkylamino)-substituted 3-nitrocoumarins. The most important information, relevant for any possible future applications of these compounds, is the fact that the tested coumarins exhibited activity against strains isolated from fecal samples of the patients with acute diarrheal syndrome. Bacterial infections of the digestive tract are considered the major cause of morbidity and mortality, especially in children, the elderly and immunosuppressed patients, both in industrialized and developing countries.[22] These results demonstrate the substantial antimicrobial potential of these coumarins in the therapy of GI and other infections caused by highly resistant strains. Furthermore, the activity observed against *S. aureus* was quite significant, knowing that this bacterial strain can be multidrug resistant.[23] A multivariate treatment of the results of the antimicrobial assays obtained in this work and those previously attained enabled the tested compounds to be classified according to their overall activity, selectivity and structural requirements for the observed activities to be discussed. A general conclusion could be reached that both a greater lipophilicity of the *N*-substituent, and nucleophilicity and basicity of this nitrogen have beneficial effects on the antimicrobial activity of these compounds. Such results and conclusions allow and direct further studies focused on an even larger enhancement of the here obtained activity by additional chemical modification of these coumarin antimicrobials.

SUPPLEMENTARY MATERIAL

Physical, analytic and spectral data for the prepared derivatives are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

МАЛА СИНТЕТСКА БИБЛИОТЕКА 4-(АЛКИЛАМИНО)-3-НИТРОКУМАРИНА СА ЈАКОМ АНТИМИКРОБНОМ АКТИВНОШЋУ ПРОТИВ ГАСТРОИНТЕСТИНАЛНИХ ПАТОГЕНА

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Инфекције изазване микроорганизмима са развијеном резистентношћу према антибиотицима тренутно представљају главни узрок озбиљних здравствених компликација код пацијената. Стога су истраживања која за циљ имају откриће нових и/или активнијих антимикробних агенаса веома важна. Имајући у виду потврђену антимикробну активност природних и синтетичких деривата кумарина, овај рад треба да пружи додатни увид у антимикробни потенцијал кумаринских једињења испитивањем активности библиотеке од девет нових 4-(алкиламино)-3-нитрокумарина против 24 соја микроорганизама, који представљају лабораторијске сојеве и резистентне (изолати) бактеријске и фунгалне патогене. Сва једињења су показала одређени степен селективне активности, која је у неким случајевима била нарочито изражена достижући минималну инхибициону концентрацију од 0,04 nmol mL^{-1} (12 ng mL^{-1}) против гљивице *Candida albicans*. Утврђена активност је већа према Грам-негативним сојевима, а најосетљивија, и међу ATCC сојевима и клиничким изолатима, је била *Salmonella enterica* subsp. *enterica* serovar *Enteritidis*. Ови резултати указују на потенцијал ових кумарина као антимикробних агенаса (већина је активна у концентрацијама реда nmol/ml) у третману гастроинтестиналних и других инфекција изазваних високо резистентним сојевима микроорганизама. Коначно, ради лакшег повезивања структуре и активности урађена је мултиваријантна статистичка анализа података добијених у овом раду и оних из претходних студија антимикробне активности структурно сличних кумарина.

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