

*Full Length Research Paper*

# Influence of the aryl substituent identity in 4-arylamino-3-nitrocoumarins on their antimicrobial activity

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**Two new and six previously known coumarin derivatives with promising biological properties were synthesized in moderate to good yields by reaction of 4-chloro-3-nitro-coumarin and the appropriate arylamine in ethyl acetate in the presence of triethylamine. The synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activities against pathogenic strains. A correlation between the aryl substituent identity and antimicrobial activity was discussed.**

**Key words:** 4-Arylamino-3-nitro-coumarins, synthesis, NMR spectroscopy, 4-chloro-3-nitro-coumarin, arylamines, antimicrobial activity.

## INTRODUCTION

Coumarins are known as a large group of plant secondary metabolites mainly originated from the shikimic acid pathway and having a benzopyrene core. Their function in the plant tissues are far from clear, though suggestions include waste products, plant growth regulators, fungistats and bacteriostats (Murray et al., 1982). The coumarins are extremely variable in structure, due to the various types of substitutions in their basic structure, which can influence their biological activity (Kostova, 2005).

Coumarins have attracted intense interest in recent years because of their diverse pharmacological properties. The coumarins have long been recognized to possess anti-inflammatory, antioxidant, antibacterial, antifungal, anticoagulant, antiallergic, hepatoprotective, cytotoxic and antiviral activities (Soine, 1964; O'Kennedy and Thornes, 1997; Kostova, 2005). It was suggested

that alterations in the chemical structure of coumarins could change their pharmacological and biochemical properties, and, thus the urge to acquire samples (ready for the testing of the activities in question) of synthetic and semi-synthetic simple coumarins (Kostova, 2005) originated from.

We have previously made synthetic efforts in the direction of creating coumarin derivatives with antimicrobial and/or antioxidant activities (Radulović et al., 2006, 2011; Dekić et al., 2007a, b, 2008, 2010a, b, c). Some of these compounds showed strong to moderate activity in reducing the microbial growth compared to the activity of standard antibiotics (Radulović et al., 2006). A recent QSAR study of the antimicrobial activity of some 3-nitro-coumarins has put forward some new arguments in this direction (Debeljak et al., 2007) and concluded that 4-aminoaryl substituted compounds possessed the greatest chance of being antimicrobial agents. With all of this in mind and in the continuation of our previous work, it seemed that there is further room for improvement of the observed activity of these coumarin derivatives. We

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decided to synthesize several new and structurally similar, already reported on (Dekić et al., 2010c), 4-arylamino-3-nitrocoumarins and test their *in vitro* antibacterial and antifungal activities, and compare the obtained results with the antimicrobial activity of previously synthesized 4-heteroarylamino-3-nitrocoumarins (Dekić et al., 2010a, b), in hope of reaching a somewhat broader perspective of the scope of this activity.

## EXPERIMENTAL SECTION

### General

Melting points were determined on a Kofler hot-plate apparatus and are uncorrected. HRMS(EI) spectra were recorded on a Finnigan-MAT 8230 BE mass spectrometer. The IR measurements (ATR-attenuated total reflectance) were carried out with a Thermo Nicolet model 6700 FTIR instrument. The NMR spectra were recorded on a Varian Gemini 200 ( $^1\text{H}$  NMR at 200 MHz,  $^{13}\text{C}$  NMR at 50 MHz) spectrometer, using DMSO- $d_6$  as the solvent. Chemical shifts are expressed in  $\delta$  (ppm) using TMS ( $\text{Me}_4\text{Si}$ ) as the internal standard. For thin layer chromatography (TLC), silica gel plates (Kiesel 60 F<sub>254</sub>, Merck) were used. Visualization was affected by spraying the plates with 1:1 aqueous 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 that the solvents were purified by distillation.

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

According to a previously published procedure (Savel'ev et al., 1973), 4-hydroxycoumarin (1) was nitrated in glacial AcOH by 72% aqueous  $\text{HNO}_3$  to give 4-hydroxy-3-nitrocoumarin (2). Starting compound 3 was prepared from 4-hydroxy-3-nitrocoumarin (Kaljaj et al., 1987). The preparation was carried out in the following manner: 1.85 ml of *N,N*-dimethylformamide (DMF) was cooled to 10°C in an ice bath. With stirring, 4 g (26 mmol) of  $\text{POCl}_3$  was added drop wise, and the prepared mixture was stirred additionally for 15 min. Then, the ice bath was removed and the reaction proceeded at room temperature for a further 15 min. Finally, the solution of 2 (5.5 g, 26 mmol) in DMF (12.5 ml) was added drop wise. After 15 min of stirring, the reaction was stopped by adding cold water (15 ml). The precipitated solid was collected by filtration and washed with saturated sodium bicarbonate solution and water. Recrystallization from the mixture of benzene: hexane (1:1 volume ratio) yielded yellow crystals of 3 (5.1 g, 22.6 mmol) in 87% yield, mp 162-163°C.

### General synthesis of 4-arylamino-3-nitro-coumarins (5a-h)

The solution of 4-chloro-3-nitrocoumarin (3) (1 g, 4.4 mmol) and the appropriate arylamine (4a-h) (4.4 mmol) in ethyl acetate (10 ml) in the presence of triethylamine (1 ml, 7.2 mmol) was refluxed for 3 to 6 h. After cooling, the precipitated solid was filtered off, washed with ethyl acetate and water. The purity of the synthesized compounds (5a-h) was checked by TLC.

### 4-[(4-Aminophenyl)amino]-3-nitro-2H-chromen-2-one (5a)

Brown crystals, mp 228-230°C, yield 84%. HRMS(EI):  $\text{M}^+$  ( $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}_4$ ) 297.0743, requires 297.0750 ( $\Delta = -0.7$  mmu). IR and

$^1\text{H}$  and  $^{13}\text{C}$  NMR (in  $\text{CDCl}_3$ ) previously published (Dekić et al., 2010c). IR (neat,  $\text{cm}^{-1}$ ): 3371-3068 (N-H and Ar-H), 1710 (C=O), 1605 (C=C), 1549 and 1332 ( $\text{NO}_2$ ), 1240, 1056, 945, 782, 756.  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 10.07$  (brs, 1H, N-H), 8.38 (dd, 1H, H-5,  $J = 8.5, 1.5$  Hz), 7.74 (dt, 1H, H-7,  $J = 8.5, 1.5$  Hz), 7.41-7.48 (m, 2H, H-6, H-8), 6.82-6.92 (dd, 2H, H-2', H-6'  $J = 8.6, 2.0$  Hz), 6.45-6.54 (dd, 2H, H-3', H-5'  $J = 8.6, 2.0$  Hz), 5.32 (s, 2H,  $\text{NH}_2$ ).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 155.8, 151.4, 147.9, 145.7, 134.1, 125.5, 125.4$  (two C), 124.7, 124.5, 117.6, 116.3, 114.6, 113.7 (two C).

### 4-[(5-Chloro-2-hydroxyphenyl)amino]-3-nitro-2H-chromen-2-one (5b)

Yellow crystals, mp 245-246°C, yield 79%. HRMS(EI):  $\text{M}^+$  ( $\text{C}_{15}\text{H}_9\text{ClN}_2\text{O}_5$ ) 332.0213, requires 332.02 ( $\Delta = +1.3$  mmu). IR (neat,  $\text{cm}^{-1}$ ): 3382-3117 (O-H and N-H), 1684 (C=O), 1610 (C=C), 1536 and 1328 ( $\text{NO}_2$ ), 1193, 1067, 913, 760.  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 10.31$  (brs, 1H, O-H), 10.00 (brs, 1H, N-H), 8.42 (dd, 1H, H-5,  $J = 8.8, 1.6$  Hz), 7.78 (dt, 1H, H-7,  $J = 8.5, 1.6$  Hz), 7.45-7.53 (m, 2H, H-6, H-8), 7.16-7.22 (m, 2H, H-3', H-6'), 6.89 (dd, 1H, H-4',  $J = 8.3, 0.7$  Hz).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 155.3, 151.4, 151.3, 146.1, 134.3, 128.5, 126.5, 125.1, 124.9, 124.8, 122.1, 117.7, 117.6$  (two C), 114.4.

### 4-[(3,5-Dibromo-4-hydroxyphenyl)amino]-3-nitro-2H-chromen-2-one (5c)

Yellow crystals, mp 232-234°C, yield 68%. HRMS(EI):  $\text{M}^+$  ( $\text{C}_{15}\text{H}_8\text{Br}_2\text{N}_2\text{O}_5$ ) 453.8782, requires 453.88 ( $\Delta = -1.8$  mmu). IR (neat,  $\text{cm}^{-1}$ ): 3440-3277 (O-H and N-H), 1694 (C=O), 1604 (C=C), 1548 and 1359 ( $\text{NO}_2$ ), 1063, 1062, 759, 748.  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 10.17$  (brs, 2H, N-H, O-H), 8.33 (d, 1H, H-5,  $J = 8.8$  Hz), 7.79 (t, 1H, H-7,  $J = 8.4$  Hz), 7.46-7.53 (m, 2H, H-6, H-8), 7.44 (s, 2H, H-2', H-6').  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 155.3, 151.6, 149.6, 145.9, 145.7, 134.5, 131.1, 127.6$  (two C), 124.9, 124.5, 117.6, 114.6, 111.7 (two C).

### 3-Nitro-4-[(3-nitrophenyl)amino]-2H-chromen-2-one (5d)

Yellow crystals, mp 253-255°C, yield 72%. HRMS(EI):  $\text{M}^+$  ( $\text{C}_{15}\text{H}_9\text{N}_3\text{O}_6$ ) 327.0474, requires 327.0491 ( $\Delta = -1.7$  mmu). IR and  $^1\text{H}$  and  $^{13}\text{C}$  NMR (in  $\text{CDCl}_3$ ) previously published (Dekić et al., 2010c). IR (neat,  $\text{cm}^{-1}$ ): 3348-3080 (N-H and Ar-H), 1689 (C=O), 1606 (C=C), 1548 and 1354 ( $\text{NO}_2$ ), 1266, 1064, 821, 761.  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 10.52$  (s, 1H, N-H), 8.37 (d, 1H, H-5,  $J = 8.0$  Hz), 8.08-8.12 (m, 2H, H-2', H-4'), 7.81 (t, 1H, H-7,  $J = 8.5$  Hz), 7.66-7.71 (m, 2H, H-5', H-6'), 7.46-7.57 (m, 2H, H-6, H-8).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 155.0, 151.8, 147.9, 145.9, 145.8, 139.2, 134.7, 130.6, 129.3, 124.9, 121.0, 118.7, 117.6, 117.5, 114.7$ .

### 2-[(3-Nitro-2-oxo-2H-chromen-4-yl)amino]benzoic acid (5e)

Yellow crystals, mp 226-228°C, yield 79%. HRMS(EI):  $\text{M}^+$  ( $\text{C}_{16}\text{H}_{10}\text{N}_2\text{O}_6$ ) 326.0560, requires 326.0539 ( $\Delta = +2.1$  mmu). IR and  $^1\text{H}$  NMR (in  $\text{CDCl}_3$ ) previously published (Savel'ev et al., 1989; Dekić et al., 2010c). IR (neat,  $\text{cm}^{-1}$ ): 3420 (O-H and N-H), 1703 (C=O), 1606 (C=C), 1553 and 1330 ( $\text{NO}_2$ ), 1289, 1032, 823, 782.  $^1\text{H}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 8.11$  (dd, 1H, H-3',  $J = 8.5, 1.5$  Hz), 7.94 (dd, 1H, H-5,  $J = 8.0, 1.5$  Hz), 7.65 (dt, 1H, H-7,  $J = 8.5, 1.5$  Hz), 7.24-7.43 (m, 3H, H-6, H-8, H-5'), 7.01 (dt, 1H, H-4',  $J = 8.5, 1.0$  Hz), 6.74 (dd, 1H, H-6',  $J = 8.0, 1.0$  Hz).  $^{13}\text{C}$  NMR (DMSO- $d_6$ , ppm):  $\delta = 168.9, 156.1, 151.9, 148.0, 147.5, 133.0, 131.6, 130.9, 124.6, 124.5, 124.4, 122.3, 121.1, 119.9, 117.2, 117.0$ .

#### 4-[(4-Methylphenyl)amino]-3-nitro-2H-chromen-2-one (5f)

Yellow crystals, mp 192-194°C, yield 70%. HRMS(EI): M<sup>+</sup> (C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>) 296.0815, requires 296.0797 ( $\Delta$ = +1.8 mmu). IR and <sup>1</sup>H and <sup>13</sup>C NMR (in CDCl<sub>3</sub>) previously published (Tabaković et al., 1983; Dekić et al., 2010c). IR (neat, cm<sup>-1</sup>): 3276-3072 (N-H and Ar-H), 2919 (CH<sub>3</sub>), 1693 (C=O), 1610 (C=C), 1548 and 1322 (NO<sub>2</sub>), 1208, 1054, 813, 757. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, ppm):  $\delta$  = 10.24 (s, 1H, N-H), 8.42 (d, 1H, H-5, *J* = 8.1 Hz), 7.76 (t, 1H, H-7, *J* = 7.8 Hz), 7.40-7.52 (m, 2H, H-6, H-8), 7.00-7.20 (m, 4H, H-2', H-3', H-5', H-6'), 2.31 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, ppm):  $\delta$  = 155.4, 151.6, 145.7, 136.2, 135.0, 134.4, 129.6, 129.5 (two C), 124.8, 123.5 (two C), 117.6, 117.2, 114.8, 20.8.

#### 4-[(2-Methylphenyl)amino]-3-nitro-2H-chromen-2-one (5g)

Yellow crystals, mp 180-182°C, yield 81%. HRMS(EI): M<sup>+</sup> (C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>) 296.0781, requires 296.0797 ( $\Delta$ = -1.6 mmu). IR and <sup>1</sup>H and <sup>13</sup>C NMR (in CDCl<sub>3</sub>) previously published (Dekić et al., 2010c). IR (neat, cm<sup>-1</sup>): 3120-3036 (N-H and Ar-H), 2947 (CH<sub>3</sub>), 1705 (C=O), 1612 (C=C), 1556 and 1323 (NO<sub>2</sub>), 1218, 1054, 798, 749. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, ppm):  $\delta$  = 8.60 (d, 1H, H-5, *J* = 8.0 Hz), 7.77 (t, 1H, H-7, *J* = 8.0 Hz), 7.41-7.53 (m, 2H, H-6, H-8), 7.13-7.30 (m, 4H, H-3', H-4', H-5', H-6'), 2.28 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, ppm):  $\delta$  = 155.6, 151.4, 146.1, 136.2, 134.5, 134.2, 130.7, 127.9, 126.4, 125.8, 124.9, 124.8, 117.6, 116.8, 114.7, 18.0.

#### 4-[(4-Iodophenyl)amino]-3-nitro-2H-chromen-2-one (5h)

Yellow crystals, mp 250-253°C, yield 76%. HRMS(EI): M<sup>+</sup> (C<sub>15</sub>H<sub>9</sub>IN<sub>2</sub>O<sub>4</sub>) 407.9616, requires 407.9607 ( $\Delta$ = +0.9 mmu). IR and <sup>1</sup>H and <sup>13</sup>C NMR (in CDCl<sub>3</sub>) previously published (Dekić et al., 2010c). IR (neat, cm<sup>-1</sup>): 3286 (N-H), 1686 (C=O), 1604 (C=C), 1548 and 1334 (NO<sub>2</sub>), 1207, 1057, 843, 784. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, ppm):  $\delta$  = 10.27 (s, 1H, N-H), 8.39 (d, 1H, H-5, *J* = 8.0 Hz), 7.69-7.82 (m, 3H, H-7, H-3', H-5'), 7.42-7.54 (m, 2H, H-6, H-8), 6.98-7.09 (m, 2H, H-2', H-6'). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, ppm):  $\delta$  = 155.1, 151.6, 145.6, 137.9, 137.8 (two C), 137.6, 134.6, 125.4 (two C), 124.8, 117.8, 117.6, 114.6, 91.7.

#### Testing of antimicrobial activity

The antimicrobial activity was evaluated using broth microdilution method (NCCLS, 2003). Minimum inhibitory concentrations (MIC) determination was performed by a serial dilution method in 96 well microtitre plates. Bacterial species were cultured at 37°C in Mueller Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for yeasts (30°C). After 18 h of cultivation, bacterial suspensions were made in Mueller Hinton broth and their turbidity was standardized to 0.5 McFarland. Optical density of every suspension was confirmed on a spectrophotometer (UV-VIS 1610, Shimadzu). The final density of bacterial and yeasts' inoculum was 5 x 10<sup>5</sup>. The inoculum was added to all wells and the plates were cultivated at 37°C during 24 h (bacteria) or at 30°C for 48 h (yeasts). Tetracycline 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  $\mu$ l of 0.5% triphenyl tetrazolium chloride (TTC) aqueous solution (Sartoratto et al., 2004). MIC was defined as the lowest concentration of the tested compounds that inhibited visible growth (red colored pellet on the bottom of the wells after the addition of TTC). The

experiments were done in triplicate and the mean values are presented.

#### Test microorganisms

The synthesized compounds 5a-h were tested against a panel of microorganisms including Gram positive *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* (isolate from food), *Bacillus subtilis* ATCC 6633; Gram negative *Escherichia coli* ATCC 8739, *E. coli* (isolate from food), *Klebsiella pneumoniae* ATCC 10031, *Salmonella enterica* ATCC 13076 and yeast *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. Bacterial isolates were obtained from the Institute of Public Health, Niš and are stored in the microbiological collection at the Microbiology Laboratory (Department of Biology, Faculty of Science and Mathematics, University of Niš).

#### Statistical analysis

To determine whether there is a statistically significant difference among the obtained results for antifungal and antibacterial activity assays, variance analyses were carried out using the SPSS 10.0 software package. Values of *p* < 0.05 were considered to be significantly different.

## RESULTS AND DISCUSSION

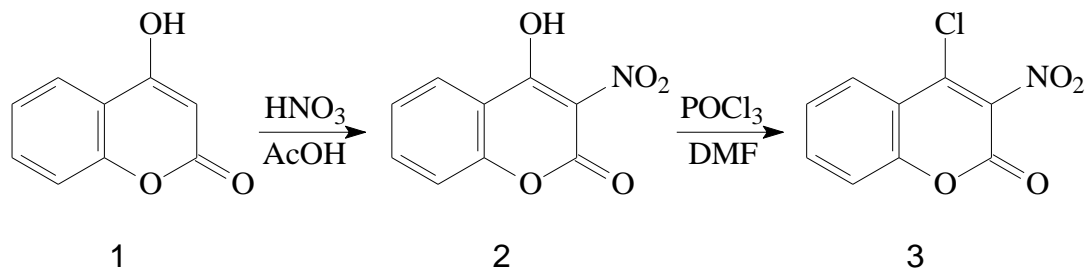
### Chemistry

Substrate in the synthesis of the coumarin derivatives, 4-chloro-3-nitrocoumarin (3), was obtained from 4-hydroxycoumarin (1), in two reaction steps and 72% yield (Scheme 1). In the first step, 4-hydroxycoumarin was nitrated in glacial AcOH with 72% HNO<sub>3</sub>(aq) to afford 4-hydroxy-3-nitrocoumarin (2). The starting compound 3, was prepared from 4-hydroxy-3-nitrocoumarin in reaction with phosphorus oxychloride and *N,N*-dimethylformamide (Scheme 1).

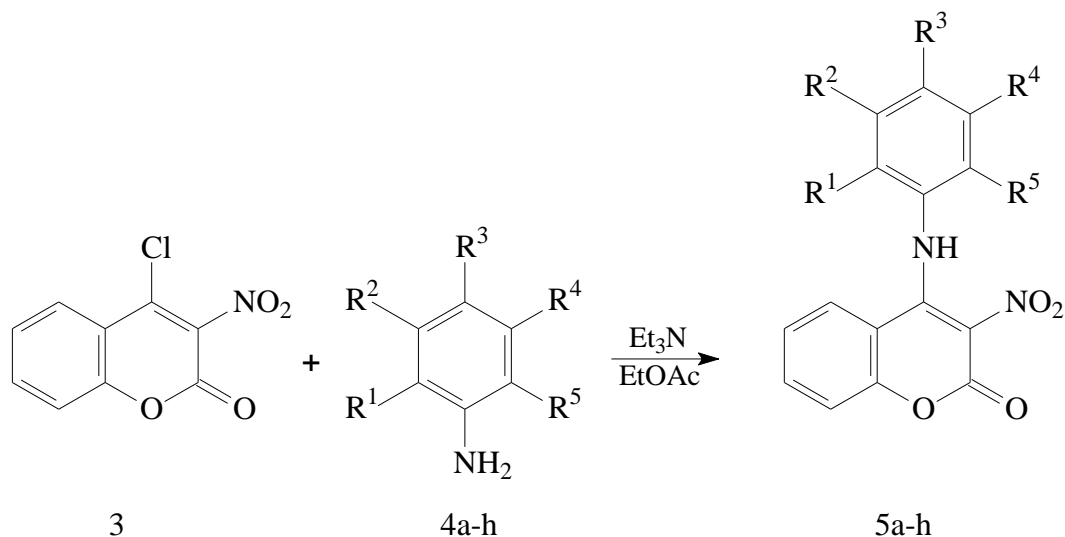
Eight coumarin derivatives (two new compounds 5b-c, and six previously known 5a, d-h, (Scheme 2) were prepared in the reaction of 4-chloro-3-nitrocoumarin (3) and an arylamine (4a-h) (4-aminoaniline, 2-amino-4-chlorophenol, 4-amino-2,6-dibromophenol, 3-nitroaniline, 2-aminobenzoic acid, *p*-toluidine, *o*-toluidine and 4-iodoaniline, respectively, 1:1 mole ratio of the starting materials) in ethyl acetate in the presence of almost two equivalents of triethylamine. The target compounds were obtained in moderate to good yields (68-84%) (Scheme 2).

The structure of compounds 5a-h was confirmed using IR and NMR spectroscopy, and HRMS. Their IR spectra contained characteristic vibrations at 1322-1359 and 1536-1556 cm<sup>-1</sup> (NO<sub>2</sub>), 1684-1710 cm<sup>-1</sup> (C=O), and 3120-3460 cm<sup>-1</sup> (N-H). Also, the IR spectra of compounds 5b, 5c and 5e showed bands at 3117-3440 cm<sup>-1</sup> corresponding to the absorption of O-H bonds.

In <sup>1</sup>H NMR spectra of 5a-d and 5f-h, when speaking of the aromatic protons of the coumarin moiety, the most



**Scheme 1.** Synthesis of 4-chloro-3-nitrocoumarin.



4a; 5a;  $R^1 = R^2 = R^4 = R^5 = \text{H}$ ,  $R^3 = \text{NH}_2$   
 4b; 5b;  $R^1 = \text{OH}$ ,  $R^4 = \text{Cl}$ ,  $R^2 = R^3 = R^5 = \text{H}$   
 4c; 5c;  $R^1 = R^5 = \text{H}$ ,  $R^2 = R^4 = \text{Br}$ ,  $R^3 = \text{OH}$   
 4d; 5d;  $R^1 = R^3 = R^4 = R^5 = \text{H}$ ,  $R^2 = \text{NO}_2$

4e; 5e;  $R^1 = \text{COOH}$ ,  $R^2 = R^3 = R^4 = R^5 = \text{H}$   
 4f; 5f;  $R^1 = R^2 = R^4 = R^5 = \text{H}$ ,  $R^3 = \text{Me}$   
 4g; 5g;  $R^1 = \text{Me}$ ,  $R^2 = R^3 = R^4 = R^5 = \text{H}$   
 4h; 5h;  $R^1 = R^2 = R^4 = R^5 = \text{H}$ ,  $R^3 = \text{I}$

**Scheme 2.** Reaction scheme of the synthesis of the new coumarin derivatives.

shielded protons were H-6 and H-8, which overlapped at 7.40 to 7.57 ppm. The protons in positions 5 and 7 appeared as doublets or doublet of doublets at  $\delta$  8.33 to 8.60 ppm and triplets or doublet of triplets at 7.69 to 7.82 ppm, respectively. The  $^1\text{H}$  NMR spectrum (coumarin moiety) of 5e was analogous to the ones mentioned in all but one chemical shift - a doublet of doublets at  $\delta$  7.94 ppm, corresponding to the H-5 proton that was shifted up-field. The observed chemical shift difference for the compound 5e could be rationalized as the consequence of the presence and orientation (a conformational change could have also influenced this) of the carboxylic group in the arylamino moiety of this molecule.

The values of chemical shifts in both the  $^1\text{H}$ - and  $^{13}\text{C}$

NMR spectra recorded in  $\text{CDCl}_3$  (Dekić et al., 2010c) differed somewhat from the ones obtained in the present study owing to the change of the solvent employed for the NMR measurements (and a discussion of such is outside the scope of this publication). In this work we decided to use  $\text{DMSO-}d_6$  instead of chloroform due to the higher solubility of the synthesized compounds in the later. Thus, all of the reported NMR data in this study (in  $\text{DMSO-}d_6$ ) are reported here for the first time.

### Pharmacology

The synthesized compounds 5a–h were screened for

their *in vitro* antimicrobial activity against a panel of microorganisms including Gram-positive and Gram-negative bacteria, as well as two fungal strains. The minimal inhibitory concentration (MIC,  $\mu\text{g/ml}$ ) was determined, taking Tetracycline and Nystatine as the reference drugs. The bioactivity data are summarized in Table 1.

A consideration of the obtained results of *in vitro* antibacterial activity of the synthesized compounds points to their greater effect (showing, thus, selectivity) towards Gram-positive bacteria. Gram-negative bacteria were almost completely unsusceptible to the synthesized compounds in the studied concentration range. Only a weak activity against a G(-) strain for compounds 5e and 5g, against *E. coli* (a food isolate), was noted. Gram-positive bacteria were more sensitive to the synthesized compounds, although to much higher doses compared to the standards used as the positive control (Tetracycline). Worth noting is the effect of compound 5f against *S. aureus*. For the fungi, a greater susceptibility of *C. albicans* compared to *A. niger* was observed. Compound 5h showed the greatest anticandidal activity. A certain degree of activity of compounds 5c and 5h against *A. niger* was also noted. The synthesized 4-arylamino-3-nitrocoumarins showed less pronounced activity compared to the previously synthesized 4-heteroaryl-amino-3-nitrocoumarins (Dekić et al., 2010a, b). Based on this we could conclude that a significant role in the onset of antimicrobial activity of these compounds play the heteroatoms in the heteroaryl ring substituent bounded to the coumarin framework. In support of this goes the fact that the activity of these compounds was significantly reduced or even completely lost in the case of sterically-hindered heteroatoms (Dekić et al., 2010b).

## Conclusion

In summary, this paper describes the synthesis and characterization of eight coumarin derivatives possessing a 4-arylamino substituent. The compounds (two new and six previously known) were obtained by condensation of 4-chloro-3-nitrocoumarin with the appropriate arylamine. 4-Chloro-3-nitrocoumarin proved to be a good substrate in these reactions. The synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activities against pathogenic strains. The current results together with previous ones (Dekić et al., 2010a,b; Radulović et al., 2006, 2011) showed that a heteroatom needs to be present in the arylamino substituent for such a derivative to show any significant antimicrobial activity.

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