

## Antibacterial Activity of Compounds Synthesized From 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one

KOZETA VASO\*, AZIZ BEHRAMI† and ISLAM KRASNIQI†

Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Tirana, Albania

E-mail: kozeta\_v@yahoo.it; a.behrani\_chem@live.com

In present paper, the synthesis and the antibacterial activity of two compounds from 4-chloro-3-nitro-2H-[1]-benzopyran-2-one are reported. Compounds 4-( $\beta$ -naphthyl amino)-3-nitro-2H-[1]-benzopyran-2-one (**b<sub>1</sub>**) and 4-(4-amino-2,6-dihydroxypyrimidine)-3-nitro-2H-[1]-benzopyran-2-one (**b<sub>2</sub>**) have been characterized using melting points, IR spectra, <sup>1</sup>H and <sup>13</sup>C NMR spectra. The antibacterial activity of synthesized compounds and streptomycin at concentrations of 1, 3 and 5 mg/mL have been evaluated against three strains of bacterial culture *i.e.*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella*. The compounds show bacteriostatic and bactericidal activity.

**Key Words:** 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one, 2H-[1]-benzopyran-2-one derivatives, Antibacterial activity, *Escherichia coli*, *Klebsiella*, *Staphylococcus aureus*.

### INTRODUCTION

Starting from 4-chloro-3-nitro-2H-[1]-benzopyran-2-one (**a**); 2H-[1]-benzopyran-2-one derivatives (**b<sub>1</sub>**, **b<sub>2</sub>**) are synthesized. 2H-[1]-benzopyran-2-one derivatives known as coumarin derivatives are a large group of heterocyclic with oxygen as heteroatom<sup>1-3</sup>. Coumarin is a chemical compound (specifically, a benzo- $\alpha$ -pyrone) found in many plants<sup>1,2,4</sup> notably in high concentration in the tonka bean (*Dipteryx odorata*), vanilla grass (*Anthoxanthum odoratum*), woodruff (*Galium odoratum*), mullein (*Verbascum* spp.) and sweet grass (*Hierochloe odorata*). Coumarin and its derivatives have shown various biological activities. Their fame has come mainly from their antithrombotic, antiinflammatory, vasodilatory and antiviral activities. Other several coumarin derivatives have antimicrobial properties<sup>5,6</sup>.

These wide ranges of biological properties<sup>7-10</sup> have urged us to synthesize some new coumarine derivatives and to investigate their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella*.

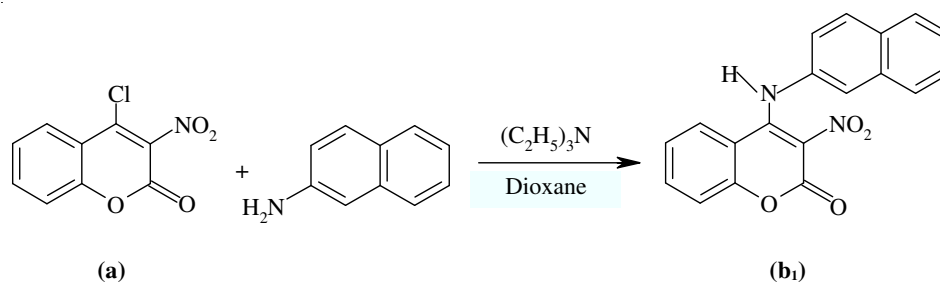
### EXPERIMENTAL

Compounds 4-( $\beta$ -naphthylamino)-3-nitro-2H-[1]-benzopyran-2-one (**b<sub>1</sub>**) and 4-(4-amino-2,6-dihydroxypyrimidine)-3-nitro-2H-[1]-benzopyran-2-one (**b<sub>2</sub>**) are

†Department of Chemistry, Faculty of Natural Science, University of Pristine, Eulex, Kosovo.

synthesized. The 2*H*-[1]-benzopyran-2-ones derivatives (**b<sub>1</sub>**, **b<sub>1</sub>**) were characterized by melting points, infrared, <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra and elemental analyses. Melting points were determined on an electrothermal apparatus in an open capillary tube and are uncorrected. Infrared spectra were recorded in cm<sup>-1</sup> for KBr pellets on a Buck Scientific Spectrophotometer. <sup>1</sup>H NMR spectra were recorded on a Varian 300 MHz spectrometer using DMSO-*d*<sub>6</sub> as the solvent and TMS as the internal reference standard. Chemical Shifts are expressed in δ ppm. Mass spectra were taken on a LKB 9000 mass spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 B CHN analyzer. The purity of the compounds (synthesized) was routinely checked by TLC using Silica G and the spots were exposed in iodine vapour for visualization.

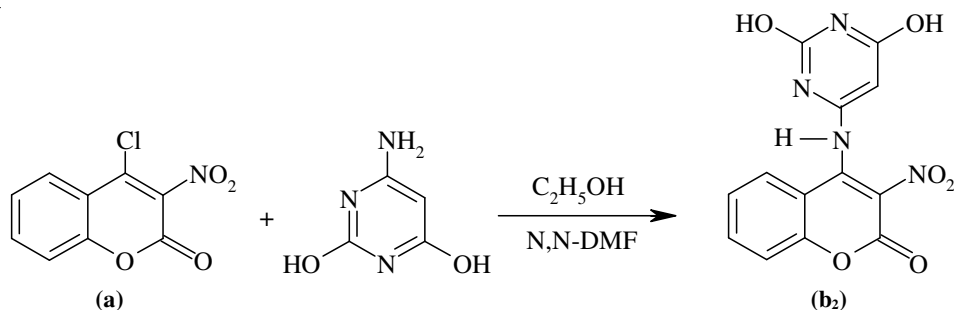
**Synthesis of 4-(β-naphthylamino)-3-nitro-2*H*-[1]-benzopyran-2-one (**b<sub>1</sub>**):** In a 100 mL flask 0.5 g of 4-chloro-3-nitro-2*H*-[1]-benzopyran-2-one diluted in 15 mL dioxane with the equivalent quantity of 0.320 g β-naphthylamine and 1 mL triethylamine as catalyzer. The mixture was refluxed for *ca.* 45 min with magnetic stirrer at room temperature. The obtained mixture was filtered, rinsed with dioxane and ether and dried at room temperature. Recrystallization from absolute ethanol gave a red brown product at 70 % yield, m.p. 175 °C (**Scheme-I**).



**Scheme-I:** Synthesis of 4-(β-naphthylamino)-3-nitro-2*H*-[1]-benzopyran-2-one (**b<sub>1</sub>**)

**Synthesis of 4-(4-amino-2,6-dihydroxypyrimidine)-3-nitro-2*H*-[1]-benzopyran-2-one (**b<sub>2</sub>**):** In a 100 mL flask 2 g of 4-chloro-3-nitro-2*H*-[1]-benzopyran-2-one mixed with the equivalent quantity of 1.50 g 4-amino-2,6-dihydroxypyrimidine. Since both reagents are solid they were dissolved first in 5 mL absolute ethanol and 2 mL N,N-DMF were added shortly. The mixture was refluxed at 90 °C, until a white yellow crystalline precipitate was formed. After filtration the product was recrystallized through a mixture of ethanol-benzene in the ratio of 1-1. The recrystallization yield was 63 %, m.p. 341 °C. (**Scheme-II**).

**Antibacterial activity:** The purified synthesized compounds **b<sub>1</sub>**, **b<sub>2</sub>** were subjected to test their antibacterial activities against bacterial cultures; *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella*. Antibacterial activity of compounds were examined by the disc method.



**Scheme-II:** Synthesis of 4-(4-amino-2,6-dihydroxypyrimidine)-3-nitro-2H-[1]-benzopyran-2-one (b<sub>2</sub>)

## RESULTS AND DISCUSSION

By reacting equimolar amounts of 4-chloro-3-nitro-2H-[1]-benzopyran-2-one (a) and corresponding reagents (Schemes I and II), under reflux reaction conditions products (b<sub>1</sub>), (b<sub>2</sub>) are synthesized in 70 and 63 % yield, respectively. The structures of 2H-[1]-benzopyran-2-one derivatives (b<sub>1</sub>), (b<sub>2</sub>) were determined from their IR, <sup>1</sup>H, <sup>13</sup>C NMR spectra and their melting points: For (b<sub>1</sub>); IR bands (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3343 (N-H vibration), 1697 (C=O,  $\alpha$ -pironi), 1607 (C=C aromatic), 776 (C-C aromatic), 2835 (C-H aromatic). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 8.9 s (1H; NH); 8.2-7.3 m (11 H aromatic). <sup>13</sup>C NMR (DMSO)  $\delta$  ppm: 133-119 m (15C aromatic); 43-38 (DMSO); 134-117 m (15 C-H aromatic). For (b<sub>2</sub>); IR bands (KBr,  $\nu_{\max}$ , cm<sup>-1</sup>): 3283 (N-H vibration), 1663 (C=O,  $\alpha$ -pironi), 1617(C=N); 1555 (C=C aromatic), 752 (C-C aromatic), 1532 (C=C aromatic). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>)  $\delta$  ppm: 10.3-9.8 s (1H; NH); 7.9-7.2m (6H aromatic); 5.0-4.2s (2H-2OH); 6.9-5.8 s (1H CH-3). <sup>13</sup>C NMR (DMSO)  $\alpha$  ppm: 164 (C-aromatic); 74 (2C-OH); 40.2-38.5 (DMSO).

**Antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella*:** The purified synthesized compounds b<sub>1</sub>, b<sub>2</sub> were subjected to test their antibacterial activities against bacterial cultures; *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella*. Antibacterial activity of compounds were examined applying the disc method (d = 5.5 mm, max. capacity 10  $\mu$ g). The disc was wetted with N,N-DMF solutions of the synthesized compounds with concentration 1, 3 and 5 mg/mL and then are placed in petridish (d = 15 cm). The old subculture *Escherichia coli* and *Klebsiella* were poured and spread in petridish in Agar-McConkey while *Staphylococcus aureus* in Agar-maltoze<sup>11</sup>. The discs were incubated at 35 °C for 48 h, the control was also maintained with DMF and streptomycin in similar manner and, the zones of inhibition of the bacterial growth were measured in mm and the results are summarized in Tables 1-3.

### Conclusion

From the results we may draw the following conclusions: (i) According to extensive NMR experiments and published data, the chemical structures of synthesized

TABLE-1  
DIAMETERS OF THE INHIBITION ZONES (mm) OF THE DISCS WET WITH VARIOUS CONCENTRATION OF THE SYNTHESIZED COUMARINE DERIVATIVES FOR *Staphylococcus aureus* AND THE COMPARISON WITH STREPTOMYCINE

Coumarine derivatives	Concentration and the inhibition zones		
	1 mg/mL	3 mg/mL	5 mg/mL
<b>b<sub>1</sub></b>	9.2 mm	5.4 mm	12.2 mm
<b>b<sub>2</sub></b>	7.6 mm	12.4 mm	18.5 mm
Streptomycine	21.0 mm	24.0 mm	25.0 mm
N,N-DMF	4.0 mm	4.0 mm	4.5 mm

TABLE-2  
DIAMETERS OF THE INHIBITION ZONES (mm) OF THE DISCS WET WITH VARIOUS CONCENTRATION OF THE SYNTHESIZED COUMARINE DERIVATIVES FOR *Escherichia coli* AND THE COMPARISON WITH STREPTOMYCINE

Coumarine derivatives	Concentration and the inhibition zones		
	1 mg/mL	3 mg/mL	5 mg/mL
<b>b<sub>1</sub></b>	14.1 mm	14.8 mm	13.5 mm
<b>b<sub>2</sub></b>	16.8 mm	16.9 mm	22.7 mm
Streptomycine	19.9 mm	24.0 mm	25.0 mm
N,N-DMF	4.0 mm	4.0 mm	4.0 mm

TABLE-3  
DIAMETERS OF THE INHIBITION ZONES (mm) OF THE DISCS WET WITH VARIOUS CONCENTRATION OF THE SYNTHESIZED COUMARINE DERIVATIVES FOR *Klebsiella* AND THE COMPARISON WITH STREPTOMYCINE

Coumarine derivatives	Concentration and the inhibition zones		
	1 mg/mL	3 mg/mL	5 mg/mL
<b>b<sub>1</sub></b>	18.1 mm	18.5 mm	19.7 mm
<b>b<sub>2</sub></b>	12.1 mm	13.4 mm	16.8 mm
Streptomycin	18.0 mm	18.5 mm	20.0 mm
N,N-DMF	4.1 mm	4.1 mm	4.1 mm

compounds were determined. (ii) This study provides the first evidence that these compounds **b<sub>1</sub>**, **b<sub>2</sub>** showed a significant antibacterial activity against *Staphylococcus aureus*, *Klebsiella* and *Escherichia coli*. (iii) Compounds **b<sub>1</sub>** and **b<sub>2</sub>** have bacteriostatic and bactericidal activity. (iv) Compound **b<sub>1</sub>** shows low antibacterial activity against *Staphylococcus aureus*, while for compound **b<sub>2</sub>** the increasing of concentration shows higher activity against these microorganisms, but always lower compared to streptomycin. (v) The increasing of concentration for compound **b<sub>1</sub>** shows bacteriostatic activity against *Escherichia coli*, while compound **b<sub>2</sub>** shows bactericidal activity. The bactericidal activity of **b<sub>2</sub>** compound is lower compared to streptomycin. (vi) Compound **b<sub>1</sub>** shows higher antibacterial activity than compound **b<sub>2</sub>** against *Klebsiella*, already equal to that of streptomycin.

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