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Antibacterial activity of some coumarine derivatives

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ABSTRACT

*4-(2-(benzyloxy) phenyl amino)-2-oxo-2H-chromene-3-carbaldehyde, **(2)**, 4-(3,5-dichloro pyridin-2-yl amino) -2-oxo -2H-chromene-3-carbaldehyde**(3)**,4-(4-methoxy benzo[d]thiazol-2-yl amino)-2-oxo-2H-chromene-3-carbaldehyde **(4)**, have been isolated in good yields by the action of corresponding aryl and heterorylamines (a-c), on 4-chloro-coumarin-3-carbaldehydes **(1)** under reflux reaction conditions. Antimicrobial properties of new coumarins **(2-4)** are investigated and results are submitted for their activities against Staphylococcus aureus, Escherichia coli, Hafnia alvei, Pseudomonas aeruginosa and Enterobacter cloacae. Applying the Agar disc diffusion technique we measured diameters of the inhibition zone around discs which are previously wetted with N, N-DMF solution of compounds, 0.1, 0.3 and 0.5 mg /ml. The inhibition zone depends from concentrations and also from sort of bacteria. The inhibition zone differ from 0 to 39 mm. Two kinds of bacteria, Hafnia alvei and Pseudomonas aeruginosa, are resistant to these new synthesized compounds. From results we may conclude that these derivates showed moderate to high activity against Staphylococcus aureus, Escherichia coli and Enterobacter cloaco. Compounds **(2-4)** are more active against Staphylococcus aureus, E.coli and Enterobacter cloaco. Compounds **(2-4)** are not active against Hafnia alvei and Pseudomonas aeruginosa in lower concentration.*

Key words: Coumarine Derivatives, Antimicrobial properties, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterobacter cloacae, Hafnia alvei.*

INTRODUCTION

Coumarins are an important group of heterocyclic compounds, that are known for their properties such as anticoagulant[1-3], antiinflamatory [4], antioxidant[5], antibacterial [6-8]. In the course of our studies on the chemistry of coumarins and related structures [9-26], we have investigated reactions for the preparation of 4-aryl and 4- heteroaryl-coumarin-3-carbaldehyde from 4-chlorocoumarin-3-carbaldehyde with aryl and heteroarylamines. Antibacterial activities of new coumarine derivatives were tested *in vitro* against bacterial strains; *Staphylococcus aureus, Escherichia coli, Hafnia alvei, Pseudomonas aeruginosa* and *Enterobacter cloacae*.

MATERIALS AND METHODS

All reactions were performed under an atmosphere of argon in oven dried glassware. Anhydrous solvents for reactions were obtained by filtration through activated alumina or by storage over molecular sieves (4 Å). Thin-layer chromatography (TLC) was performed on silicagel plates (Macherey–Nagel, 0.25 mm, UV254). Visualization was achieved either under UV light or by staining in dip solutions (vanilline (15 g), ethanol (250 mL), conc H_2SO_4 (2.5 mL) or *p*-anisaldehyde (10 mL) concentrated H_2SO_4 (10 mL), concentrated acetic acid (2 mL), ethanol (180 mL)) followed by heating with a heat gun. NMR spectra were recorded at room temperature with a Bruker AC-300 or

Bruker DRX-400 instrument. Chemical shifts (δ) are reported relative to the undeuterated residual solvent peak [$CHCl_3$: 7.27 ppm (^1H) and 77.0 ppm (^{13}C); CHD_2OD: 3.35 ppm (^1H) and 49.3 ppm (^{13}C)]. Signal assignments are based on DEPT or APT experiments, and on $^1H,^1H$- and $^1H,^{13}C$-correlation experiments (COSY/HMSC). Melting points were determined with a Buechi apparatus. The IR spectra were recorded for KBr pellets with a Perkin Elmer 1725×FT IR spectrophotometer. Elemental analyses were performed in "Ruder Boskovic" Institute, Croatia.

***4-chloro-2-oxo-2H-chromene-3-carbaldehyde* (1):** 4-hydroxycoumarine 9.72 g (60 mmol) was dissolved in DMF (46.2 ml). In this mixture and in the temp. -5 ˚C, we added slowly $POCl_3$ 27.6 g (0.18 mol). The reaction was mixed at 60˚C within 1h. After that the mixture was added in one bottle with ice. This mixture was left to stay at r.t. during the night. After filtration we get a yellow precipitate. The precipitate was washed with the solution of sodium bicarbonate 5%. It was dried in high vaacum and as product was an yelow solid 10.61 g (85%). Melting point 127˚C. IR (KBr) (υ cm^{-1}): 2920-2874 cm^{-1}(C-H alif.); 1720-1702 cm^{-1} (C=O); 1603 -1587 cm^{-1} (C=O, α-pir); 1580-1541 cm^{-1} (C=C arom). ^1H-NMR ($CDCl_3$) (ppm): 10.39 (1H, s, CHO); 8.19-7.40 (4H, m, ar-H). ^{13}C-NMR (DMSO-d_6) (ppm): 186.81, 158.44, 158.44, 53.28, 153.27, 153.27, 135.68, 125.56, 118.39, 118.22, 117.20. Elementary analysis: calculated %C (59.08), %N (3.61), experimentally %C (59.01), %N (3.59).

4-(3-(benzyloxy)pyridin-2-ylamino)-2-oxo-2H-chromene-3-carbaldehyde (2): In the solution of 3-aldehydo -4-chlorocoumarine 0.5 g(2.4 mmol) in acetonitrile (15 ml) was added 2-amino-3-benzyloxypyridine 0.48 g (2.4 mmol) and was refluxed for 30 min. The formed cristaline product was washed with acetonitrile and was ricristalized from ethanol to obtain yellow solid 0.32 g (37.64%). Melting point 182.6^{0}C. IR (KBr) (υ cm^{-1}); 3350 (N-H); 3154 (C-H aromatike); 2910 (C-H alif.); 1735 (C=O, CHO); 1718 (C=O, α-pir); 1558-1420 (C=C arom); 756 (C-Carom.). ^1H-NMR ($CDCl_3$) (ppm): 9,48-9.32 dd(1H; CHO); 8.39-6.81 m(13H; aromat.); 5.51-5.18 dt(2H; CH_2O); 3.80 d(1H; NH). ^{13}C-RBM (DMSO-d_6) (ppm): 181.01, 177.76, 163.93, 162.30, 116.47, 71.21. Elementary analysis: calculated %C (71.45), %N (5.75), experimentally %C (71.30), %N (5.77).

4-(3,5-dichloropyridin-2-ylamino)-2-oxo-2H-chromene-3-carbaldehyde (3): In the solution of 3-aldehydo-4 -chlorocoumarine 0.5 g (2.4 mmol) in acetonitrile (15 ml) was added 2-amino-3,5-dichloropyridine 0.39 g (2.4 mmol) and triethylamine 2ml, and was refluxed for 30 min. After 30 min it was formed a cristaline product which we have washed with acetonitrile. Ricristalization was done with DMF. The obtained product was a yellow solid 0.39g (48.75%). Melting point 206.8^{0}C. IR (KBr) (υ cm^{-1}): 3424 (N-H); 3068 (C-H ar.); 1734-1645 (C=O, α-pir); 1609 (C=N); 1574-1418 (C=C ar.); 759 (C-C ar.). Elementary analysis: calculated %C (52.73), %N (4.98), experimentally %C (52.69), %N (4.97).

4-(4-methoxybenzo[d]thiazol-2-ylamino)-2-oxo-2H-chromene-3-carbaldehyde (4): In the solution of 3-aldehyde -4-chlorocoumarine 0.5 g(2.4 mmol) in ethanol (15 ml) was added 2-amino-4-methoxybenzothiazol 0.43 g (2.4 mmol) and triethylamine (2ml), and was refluxed for 2h. After 2h it was formed a cristaline product which we have washed with ethanol and dried with air. Ricristalization was done with acetic acid : dioksan (4:1). The obtained product was a yellow solid 0.33g (39.28%). Melting point 285.0^{0}C. IR (KBr) (υ cm^{-1}): 3372 (N-H); 1726 (C=O, α-pir.); 1611 (C=N); 1561-1405 (C=C ar); 794(C-C ar.). ^1H-NMR ($CDCl_3$) (ppm): 9.5 s(H;CHO); 8.67-6.91 m(7H ar.), 4.06 s(3H;OCH_3). ^{13}C-NMR (DMSO-d_6) (ppm): 180, 169, 165, 159, 152, 103, 56.62, 40.35, 38.68. Elementary analysis: calculated %C (62.11), %N (4.12), experimentally %C (62.15), %N (4.10).

The antimicrobial activity of the carbaldehyde coumarine derivatives **(2-4)** was determined by agar disc diffusion technique. Antimicrobial activity of newly synthesized coumarine derivatives were tested *in vitro* against bacterial strains; *Staphylococcus aureus*, *Escherichia coli*, *Hafnia alvei* and *Pseudomonas aeruginosa*. Discs are previously wetted with DMF solution of the coumarine derivatives with three different concentrations, 0.1, 0.3 and 0.5 mg/ml. The samples were incubated at 37^{0}C during 24 hr and the inhibition zones were measured.

RESULTS AND DISCUSSION

After the reaction of 4-chlorocoumarin-3-carbaldehyde **(1)** by corresponding amines **(a-c),** we have obtained the corresponding coumarin- 3-carbaldehydes **(2-4)** in good yields. (Fig. 1).

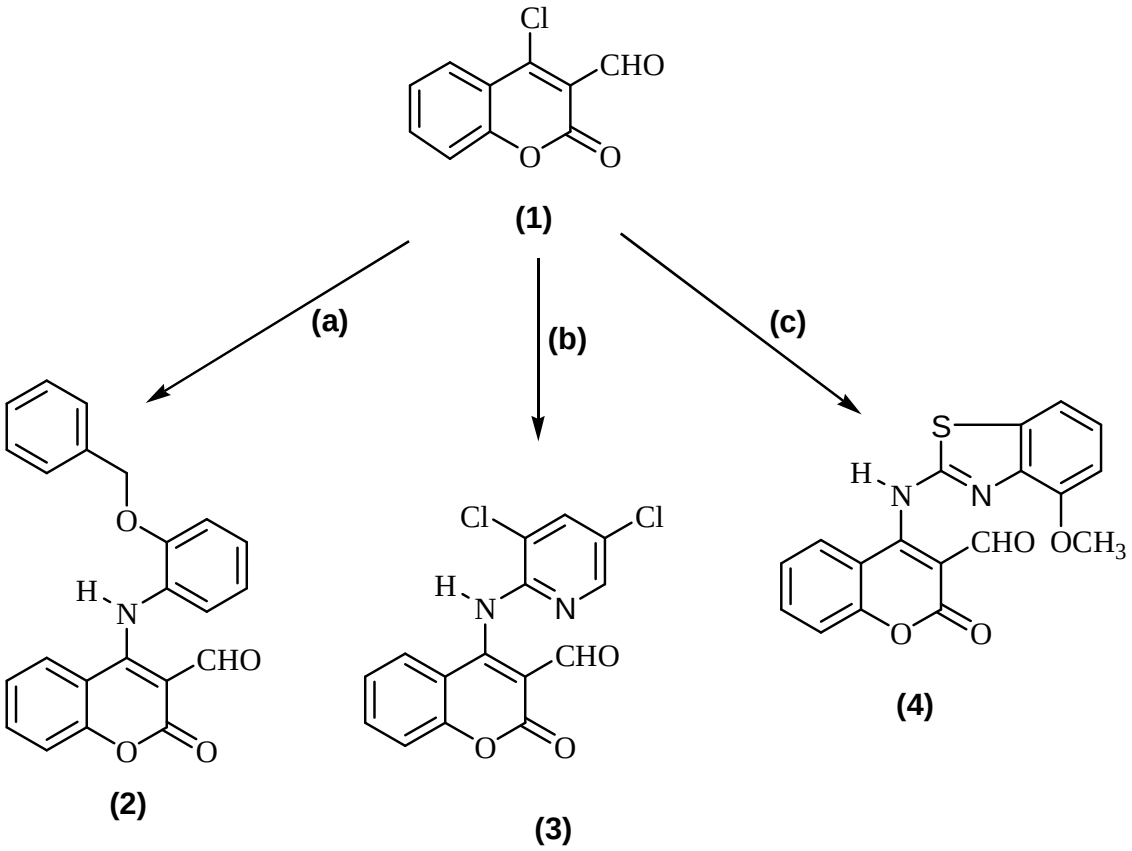

Fig. 1 .
(a)2-(benzyloxy)benzenamine; (b)3,5-dichloropyridin-2-amine;(c) 4-methoxybenzo[d]thiazol-2-amine,

The structure of synthesized coumarins **(2-4)** were determined from IR, ^{1}H-NMR, ^{13}C-NMR spectra and elementary analysis .

Continuation to our study, we examined the antimicrobial activity of the coumarine derivatives **(2-4)**. Our investigation is directed toward their activity against *Staphylococcus aureus, Escherichia coli, Hafnia alvei* and *Pseudomonas aeruginos*. Applying the agar disc diffusion technique we measured diameters of the inhibition zone around discs which are previously wetted with DMF solution of the coumarine derivatives. The antimicrobial activity of the coumarine derivatives was tested at three different concentrations : 0.1, 0.3 and 0.5 mg/ml). The antimicrobial activities of synthesized coumarins are shown in Table 3.

Table 1. Antimicrobial activity of newly synthesized compound

Compound	**Conc. mg/ml**	***Escherichia Coli*** ATCC ® 25922 (mm)	***Staphyl. aureus*** ATCC ® 25923 (mm)	***Pseudom.aeruginosa*** ATCC ® 27853 (mm)	***Hafnia alvei*** PTCC ® 2005 (mm)	***Enterococcus cloace*** PTCC ® 2005 (mm)
1	0.1 0.3 0.5	18 22 22	23 23 32	0 0 20	0 0 14	20 19 20
2	0.1 0.3 0.5	0 0 23	0 0 25	0 0 0	0 0 0	0 0 0
3	0.1 0.3 0.5	11 20 25	0 0 0	0 0 0	0 0 0	20 18 17
4	0.1 0.3 0.5	15 15 15	39 35 32	0 0 0	0 0 20	0 15 0

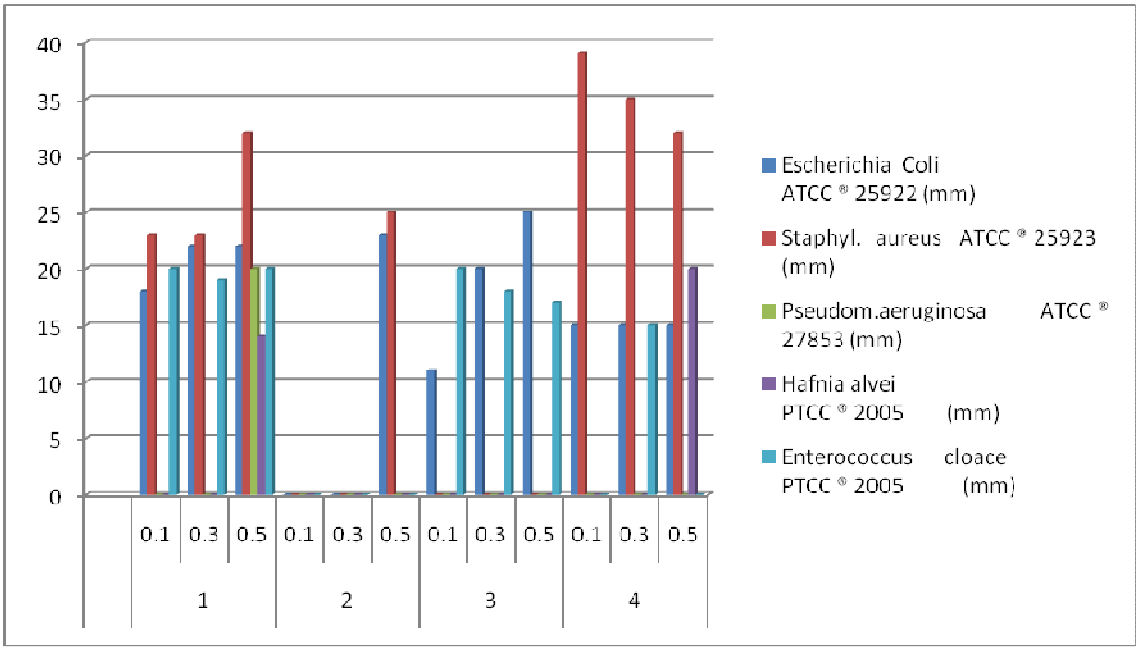

Fig.2. Graphs of antimicrobial activity resultsfor compounds (1-4).

From the results, we saw that synthesized coumarins **1-4** show antibacterial activity against some bacteria. Compound **1** show activity against *E. coli* , *S. aureus* and *E. cloaco* with three different concentration 0.1, 0.3 and 0.5 mg/ml. The compound 1 shows activity against *P. aeruginosa* and *H. alvei* only in concentration of 0.5 mg/ml. We found that if we increase the concentration of compound **1** the diameter of inhibition zone will not increase as we have expected. Compound **2** show activity against two bacteria *E. coli* and *S. aureus* only in concentration of 5 mg/ml. This compound doesn't have activity against three other bacteria. Compound **3** show activity against *E. coli* and *E. Cloaco*, but in the case of *E. coli* the activity will increase if we increase the concentration. In the case of *E. cloaco*, increasing the concentration of compound **3** the activity will not change. Coumarine derivative **4** show antibacterial activities against two kinds of bacteria *E. coli* and *S. aureus*, but in case of *S. aureus* if we increase concentration of compound **4**, the activity of this compound will decrease. Compound **4** show activity against *H. alvei* only in concentration of 0.5 mg/ml. The compound **4** doesn't have any activity against *P.auriginosa*. Two bacteria, *P. Aureiginosa* and *H. Alevei* are resistant to these compounds in lower concentration.

CONCLUSION

From results we may conclude that these coumarin derivatives were shown moderate to high activity against *Staphylococcus aureus, Escherichia coli and Enterobacter cloaco*. Compounds **1-4** are more active against *Staphylococcus aureus, E. coli and Enterobacter cloaco*. Compounds **1-4** are not active against *Hafnia alvei* and *Pseudomonas aeruginosa* in lower concentartion. As conclusion, if we change the structure of compound **1** by synthesizing its derivatives we didn't improve the microbial activity against these bacteria.

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