Antimicrobial Properties of Newly Synthesized Derivatives of Coumarine

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Abstract: Problem statement: Coumarins are well known for their biological activity. On the basis of that we have synthesized some new derivatives of coumarine and investigated their antimicrobial properties. Approach: 4-Heteroaryl-coumarin-3-carbaldehydes 4(a-d) are synthesized by condensation of 4-chloro-coumarin-3-carbaldehydes 2 and corresponding heteroarylamines 3(a-d) under reflux reaction conditions. Antimicrobial properties of new coumarins 4(a-d) 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, 1, 3 and 5 mg L⁻¹. Results: The inhibition zone depends from concentrations and also from sort of bacteria. The inhibition zone differ from 0 to 30mm. Two sort of bacteria, Hafnia alvei and Pseudomonas aeruginosa, are resistant to these new synthesized compounds. Conclusion: From results we may conclude that these derivates showed moderate to high activity against Staphylococcus aureus, Escherichia coli and Enterobacter cloaco. Compounds 4(a-d) are more active against Staphylococcus aureus, E.coli and Enterobacter cloacae. Compounds 4(a-d) are not active against Hafnia alvei and Pseudomonas aeruginosa.

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

INTRODUCTION

Although coumarins have been known for many years, the research about their properties such as anticoagulant (Garazd et al., 2005, Kahritzky et al., 1996, Manolov and Danchev, 1995), antibacterial (Arora and Mathur, 1963), antinflammatory (Al-Haiza et al., 2003), antioxidant, activities has become duty and responsibility for researchers to discover new methods of synthesizing new biological active coumarins.

In the course of our studies on the chemistry of coumarins and related structures (Haber et al., 1995; Govori et al., 2002), we have investigated reactions for the preparation of 4-heteroaryl-coumarin-3-carbaldehyde from 4-chlorocoumarin-3-carbaldehyde with different 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 (Philip et al., 2009, Azadeh and Meon, 2009; Ghanarpour et al., 2010; Rusli et al., 2009) by Agar disc diffusion technique.

MATERIALS AND METHODS

Melting points were determined with a Buechi apparatus. The IR spectra were recorded for KBr pellets with a Perkin Elmer 1725×FT IR spectrophotometer, ¹H-NMR spectra were recorded on a Brucker AC 300 (300MHz) spectrometer with TMS as internal standard. Elemental analyses were performed in “Ruder Boskovic” Institute, Croatia.

General procedure for preparation of 4-heteroaryl-coumarin-3-carbaldehydes 4(a-d): The solution of 4-chlorocoumarin-3-carbaldehyde 2 (0.5g, 24 mmol) and of appropriate heteroarylamine 3(a-d) (24mmol), in acetonitrile (30 mL) in presence of catalytical amount of triethylamine was refluxed for 2-4 h. After cooling the mixture was filtered off, washed with acetonitrile and recrystallised.

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4-(4,6-dichloropyrimidin-2-ylamino)-coumarin-3-carbaldehyde (4a): Yield: 45.85%, m.p. 280 °C from mixture ethanol: DMF (3:1). IR (KBr) cm⁻¹: 3350 (N-H), 3280 (C-H arom), 1734-1664 (C = O, CHO; α-pyr.), 1610 (C = N), 1528-1420 (C = C arom), 758 (C-C arom). ¹H-RBM (CDCl₃) (ppm): 9.10 s (1H, CHO); 3.92 s (1H, N-H). ¹³C-RBM (DMSO-d₆) (ppm): 179.30 (CHO); 163.14 (C-NH); 161.57 (C-Cl); 159.65 (C = O); 154.19-104.01 (C arom). Anal: Calculated for C₁₃H₆N₄: (C, 59.99%), (H, 2.75%), (N, 12.44%). Found: (C, 60.00%), (H, 2.90%), (N, 12.38%).

4-(2-chloro-6,7-dimethoxyquinazolin-4-ylamino)-coumarin-3-carbaldehyde (4b): Yield: 43.87%, m.p. 300 °C from ethanol: DMF (3:1). IR (KBr) cm⁻¹: 3305-3122 (N-H), 3001 (C-H arom), 2920 (C-H al), 1648 (C = O), 1624 (C = N), 1564-1417 (C = C arom). ¹H-RBM (CDCl₃) (ppm): 9.60 s (1H, CHO); 8.03-6.74 m (7H, arom); 4.24 s (1H, N-H); 5.63-5.27 s (2H, 2OCH₃). Anal: Calculated for C₂₂H₂₀N₃: (C, 59.80%), (H, 4.56%), (N, 9.51%). Found: (C, 60.00%), (H, 4.29%), (N, 9.44%).

4-(5-bromotiazol-2-ylamino)-coumarin-3-carbaldehyde (4c): Yield: 51.19%, m.p. 298 °C from acetic acid. IR (KBr) cm⁻¹: 3350 (N-H), 1735-1702 (C = O, CHO), 1654 (C = N), 1560-1458 (C = C arom). ¹H-RBM (CDCl₃) (ppm): 9.32 s (1H, CHO); 8.49-6.08 m (5H, arom); 3.69-3.33 d (1H, NH). Anal: Calculated for C₁₄H₁₀N₂: (C, 45.92%), (H, 2.75%), (N, 7.65%). Found: (C, 45.27%), (H, 2.97%), (N, 7.80%).

4-(2-chloropyridin-3-ylamino)-coumarin-3-carbaldehyde (4d): Yield: 54%, m.p. 215 °C from ethanol. IR (KBr) cm⁻¹: 3330 (N-H), 1735-1702 (C = O, CHO), 1516-1417 (C = C arom). ¹H-RBM (CDCl₃) (ppm): 9.32 s (1H, CHO); 8.49-6.08 m (5H, arom); 3.69-3.33 d (1H, NH). Anal: Calculated for C₁₆H₁₂N₂: (C, 60.87%), (H, 3.83%), (N, 8.87%). Found: (C, 60.04%), (H, 3.75%), (N, 8.80%).

The antimicrobial activity of compounds 4(a-d) was determined by Agar disc diffusion technique. Coumarin samples were tested in vitro against bacterial strains: Staphylococcus aureus, Escherichia coli, Hafnia alvei, Pseudomonas aeruginosa and Enterobacter cloacae, were incubated at 37°C during a period 24 hrs and the inhibition zones were measured. The compounds were tested at three different concentrations, 1, 3 and 5 mg mL⁻¹ in DMF.

**RESULTS**

Nucleophilic substitution of 4-chloro-coumarin-3-carbaldehyde (2) by corresponding aromatic amines 3(a-d), has given the corresponding 4-heteroarylamino-coumarin-3-carbaldehydes 4(a-d) in good yields. The structure of synthesized coumarins 4(a-d) were determined from IR, ¹H-NMR, ¹³C-NMR spectra and elementary analysis (Fig. 1).
Table 1: Microbiological activity of newly synthesized coumarin derivatives

| Test compound | Concentration | E. Coli ATCC 25922 | S. Aureus ATCC 25923 | Pseudomonas aeruginosa ATCC 27853 | Hafnia alvei PTCC 2005 | Enterococcus cloacae PTCC 2005 |
|---------------|---------------|---------------------|-----------------------|-----------------------------------|------------------------|-----------------------------|
| 4a            | 1             | 17                  | 23                    | 0                                  | 0                      | 17                          |
|               | 3             | 17                  | 23                    | 0                                  | 0                      | 18                          |
|               | 5             | 27                  | 27                    | 0                                  | 0                      | 18                          |
| 4b            | 1             | 20                  | 22                    | 0                                  | 0                      | 22                          |
|               | 3             | 20                  | 25                    | 0                                  | 0                      | 20                          |
|               | 5             | 20                  | 30                    | 0                                  | 0                      | 15                          |
| 4c            | 1             | 29                  | 0                     | 0                                  | 0                      | 10                          |
|               | 3             | 15                  | 27                    | 0                                  | 0                      | 15                          |
|               | 5             | 18                  | 20                    | 0                                  | 0                      | 17                          |
| 4d            | 1             | 20                  | 0                     | 0                                  | 0                      | 18                          |
|               | 3             | 20                  | 0                     | 0                                  | 0                      | 0                           |
|               | 5             | 23                  | 0                     | 0                                  | 0                      | 0                           |

Fig. 2: Graphs of microbiological activity results for compounds 4(a-d)

Continuation to our study we examined the antimicrobial activity of synthesized compounds 4(a-d). Our investigation is directed toward their activity against Staphylococcus aureus, Escherichia coli, Hafnia alvei, Pseudomonas aeruginosa and Enterobacter cloacae. Applying the disc method we have measured diameters of the inhibition zones around disc which are previously wetted with DMF solution of the samples examined (1, 3 and 5 mg mL$^{-1}$) were applied (Table 1).

**DISCUSSION**

The antimicrobial activity of synthesized coumarins is shown in Table 1 presented with graph (Fig. 2). From the results, we saw that new synthesized coumarins 4(a-g) show antibacterial activity against some bacteria. Compound 4a show activity against three bacteria, E. coli, S. Aureus and E. Cloaco. We found that if we increase the concentration of compound 4a the diameter of inhibition zone will not increase as we have expected. The Compound 4a don't show any activity against the P. Aeruginosa and H. alvei. Compound 4b show activity against E. coli, S. Aureus and E. Cloaco, but in the case of E. coli and E. Cloaco, if we increase the concentration of compound 4b, the activity will not change. But to the S. Aureus, if we increase concentration the inhibition zone will increase. Coumarine derivative 4c show antibacterial activity but in case of S. Aureus if we increase concentration of compound 4c, the activity of this compound will decrease. Compound 4d show activity against E. coli and also in the concentration of 1 mg L$^{-1}$ show activity against E. Cloaco. If we increase concentration the activity is zero against E. Cloaco.

Two bacteria, P. Aureuginosa and H. Alevei are resistant to all this compounds and also they are resistant to all this three different concentrations.
CONCLUSION

From results we may conclude that these coumarin derivatives were shown moderate to high activity against Staphylococcus aureus, Escherichia coli and Enterobacter cloacae. Compounds 4(a-d) are more active against Staphylococcus aureus, E. coli and Enterobacter cloacae.

Compounds 4(a-d) are not active against Hafnia alvei and Pseudomonas aeruginosa.

ACKNOWLEDGMENT

The researchers acknowledge the financial support received from the University of Prishtina, Kosova.

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