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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 heterorylamines 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 cloaco*. 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 researche about their properties such as anticoagulant (Garazd *et al*., 2005; Katritzky *et al*., 1996; Manolov and Danchev, 1995), antibacterial (Arora and Mathur, 1963), antiinflamatory (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; Ghanbarpour *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, ^{1}H-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-chlorocumarin-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 filtred 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), 3120 (C-H arom), 1734-1664 (C = O, CHO;α-pir.);1610 (C = N);1528-1420 (C=C arom.), 758 (C-C arom).^{1}H-RBM($CDCl_3$) (ppm):9-9.10 s (1H,CHO);8.42-6.46 m (5H;arom), 3.92 s (1H;N-H), ^{13}C-RBM (DMSO-d_6) (ppm):CHO(179.30);C-NH(163.14);C-Cl(161.57);C=O (159.65);C arom.(154.19- 104.01);DMSO (40.34-34.43). Anal: Calculated for $C_{13}H_6N_4$: (C,59.99%), (H, 2.75%), (N,12.44%) Found: (C,60.00%), (H,2.90%), (N, 12.38%).

4-(2-chloro-6,7-dymethoxyquinazolin-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),3483-3122 (N-H), 3001 (C-H arom), 2920 (C-H al), 1648 (C = O), 1624 (C = N), 1516-1417(C = C arom). ^{1}H-RBM($CDCl_3$) (ppm):9.60 s (1H;CHO);8.03-6.74 m (7H;ar.); 4.24 s (1H;N-H);5.63-5.27 ss (6H;2OCH3). Anal: Calculated for $C_{22}H_{20}N_3$: (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). ^{1}H-RBM($CDCl_3$) (ppm):9.32 sd (1H;CHO); 8.49-6.08 m (5H;arom); 3.69-3.33 d (1H;NH). Anal: Calculated for $C_{14}H_{10}N_2$: (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), 3055 (C-H arom), 1735 (C=O, CHO; α-piron), 1610 (C = N) 1508-1458 (C=C aromatic). ^{1}H-RBM($CDCl_3$) (ppm):9.32 sd (^{1}H;CHO);8.49-6.08 m (5H;arom); 3.69-3.33 d (1H;NH). Anal: Calculated for $C_{16}H_{12}N_2$: (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 cloaco*, 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

Nucleophylic 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, ^{1}H-NMR, ^{13}C-NMR spectra and elementary analysis (Fig. 1).

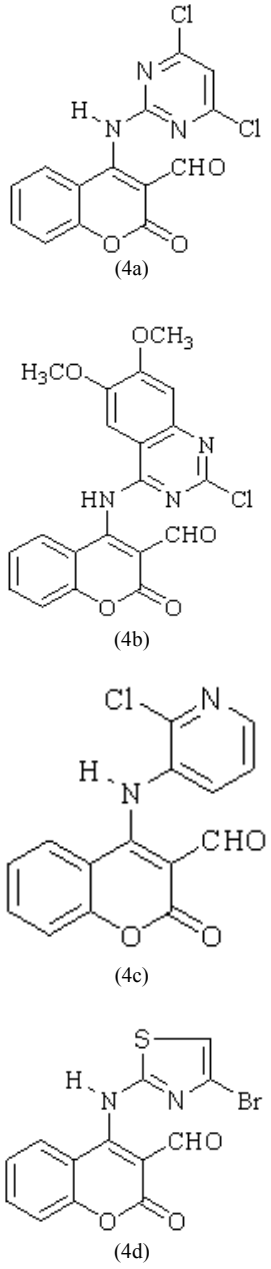

Fig. 1: Structure of Coumarine derivatives 4 (a-d)

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	0	29	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

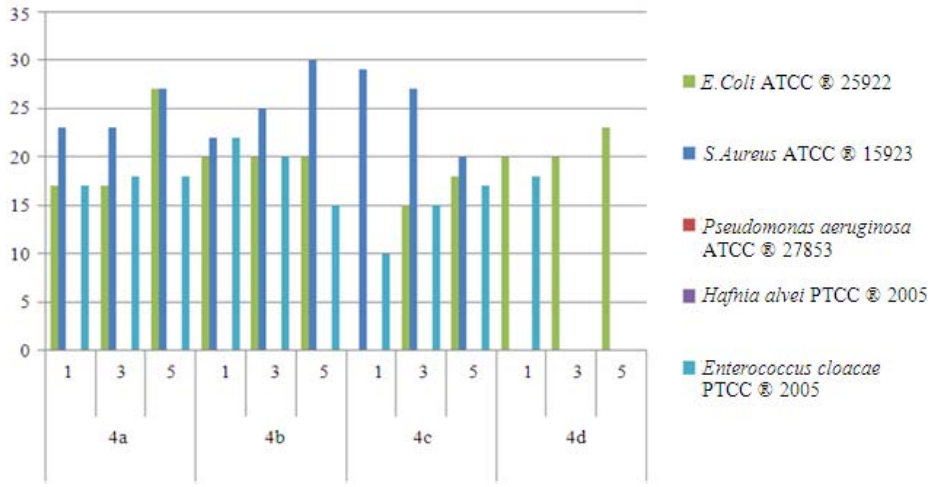

Fig. 2: Graphs of microbiological activity results for compounds 4(a-d)

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 cloaco*. 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-d) 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. Aeuriginosa 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 1mg L^{-1} show activity against *E. Cloaco*. If we increase concentration the activity is zero against *E. Cloaco*.

Two bacteria, *P. Aureiginosa* 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 cloaco*. Compounds 4(a-d) are more active against *Staphylococcus aureus, E. coli and Enterobacter cloaco*.

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

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