Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(5):2495-2500

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antibacterial activity of coumarine derivatives synthesized from 8-amino-4,7-dihydroxy-chromen-2-one and comparison with standard drug

Aziz Behrami[1] and Islam Krasniqi[2]

[1]The Geoscience and Technology Faculty - Mitrovica, University of Pristine, Kosova
[2]Department of Chemistry, University of Pristine, Kosova

ABSTRACT

*In present paper , we report the organic syntheses of four compounds from 8-Amino-4,7-dihydroxy-chromen-2-one and describe the results of antibacterial activity of purified compounds.Compounds 3-Acetyl-8-amino-4,7-dihydroxy-chromen-2-one **(1a)**, 8-Amino-3-[1-(6- chloro-4 –hydroxy -4,5- dihydro - pyrimidin- 2- ylimino) -ethyl] -4,7-dihydroxy-chromen-2-one **(2a)**, 8-Amino-4-[bis-(2-chloro-ethyl)-amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl] -7- hydroxyl – chromen-2-one **(3a)**, 8-[Bis-(2-chloro-ethyl)-amino] -2-chloro -7- [1-(6-chloro-4- hydroxyl -4,5- dihydro – pyrimidin-2-ylimino)-ethyl]-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione **(4a)** , have been synthesized and characterized using melting points , IR spectra , ^{1}H-NMR and ^{13}C-NMR spectra. The antibacterial activity of synthesized compounds and streptomycin and cefalexine at concentractions of 2mg/ml, 3mg/ml and 5mg/ml , have been evaluated against three strains of bacterial culture; Staphylococcus aureus, E.coli and Bacillus cereus. The compounds show bacteriostatic and bactericidal activity.*

Keywords: Coumarine derivatives , antibacterial activity ,IR,^{1}H-NM ,^{13}C-NMR, Streptomycin.

INTRODUCTION

Starting from 8-Amino-4,7-dihydroxy-chromen-2-one **(a);** derivatives (1a,2a,3a,4a) are synthesized.Coumarin derivatives are large group of heterocyclic with oxygen as heteroatom.Coumarin is a chemical sompound (specifically , a benzo-α-pyrone) found in many plants 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).Coumarine and their derivatives have shown varius biological activities. Their fame has come mainly from their antithrombic, antiinflammatory, vasodilatory , and antiviral activities. Other several coumarin derivatives have antimicrobial properties (Sanghyun ; et al 1996 ; Mohareb et al 2007; Nofal et al 2000), with reflux and condensation we have synthesize some new coumarin derivatives and to investigate their antibacterial activity against Staphylococcus aureus, E.coli and Bacillus cereus.The antibacterial activity of synthesized compounds is compared with antibacterial activity of Cefalexine and Streptomycine.

EXPERIMENTAL SECTION

Experimental Chemistry

Compounds 3-Acetyl-8 – amino -4,7- dihydroxy-chromen-2-one **(1a)**, 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro -pyrimidin-2 -ylimino) - ethyl]-4,7- dihydroxy-chromen-2-one **(2a)**, 8-Amino-4-[bis-(2-chloro-ethyl)-

amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl] -7- hydroxyl – chromen-2-one **(3a)**, 8-[Bis-(2-chloro-ethyl)-amino] -2-chloro -7- [1-(6- chloro-4- hydroxyl -4,5- dihydro – pyrimidin-2-ylimino)-ethyl]-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione **(4a)** are synthesized.

Measurement

The identification of 8-Amino-4,7-dihydroxy-chromen-2-one derivatives **(1a,2a,3a,4a)** , is made by using melting point , IR , ^{1}H NMR , ^{13}C NMR spectra and elemental analysis. Melting point was determinated on a Electrothermal apparatus (Fisher Scientific 2555) in a open capillary tube and are uncorrected.Infrared spectra were recorded in cm^{-1} for KBr pellts on a FT-IR Shimadzu 8400S spectrophotometer with resolution 4 cm^{-1} . ^{1}H NMR spectra were recorded on a Bruker UNITY plus-500 'NMR 1' spectrometer using DMSO-d_6 as the solvent and TMS as the internal references standard (σ = 0,00 ppm).Chemical shifts are expressed in δ ppm.Mass spectra were taken on a LKB 9000 mass spectrometer.

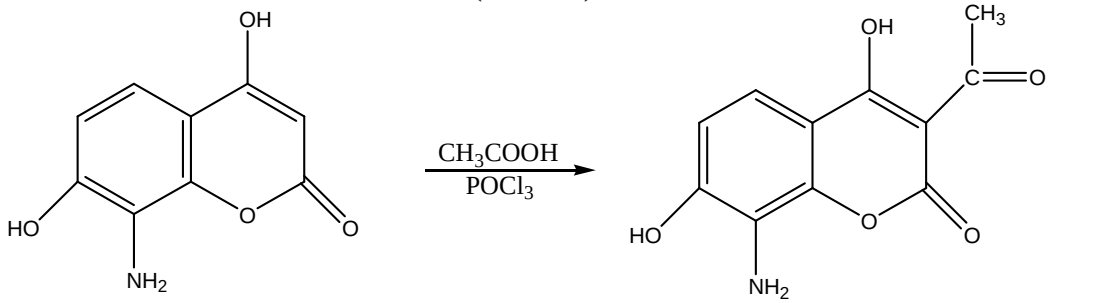
Element analysze was performed on a Perikin-Elmer 240 BCHN analyzer.The purity of the compounds (synthesized) was routinely checked by TLC using Merck Kieselgel-60 (F-254) and benzene,toluene,glacial acetic acid (80:10:10)as mobile phase.

The spots were exposed in iodine vapour for visualization.

2.2: Preparation of 3-Acetyl-8 – amino -4,7- dihydroxy-chromen-2-one **(1a)**

For this synthesis is used as substrat 8-Amino-4,7-dihydroxy-chromen-2-one in a 100 ml flask mixed 3 g of [8-Amino- 4,7- dihydroxy – chromen -2 –one] with 6ml CH_3COOH , and 1,5 ml $POCl_3$. The mixture was refluxed at 250 ^{o}C for ca. 90 min. The obtained crystals brown and white are filtred and rinsed with ethanol and dried at room temperature.Recrystallization form absolute ethanol gave a white product of 80% yield, melting point 129^{o}C.

(Scheme.1)



8-Amino-4,7-dihydroxy-chromen-2-one

3-Acetyl-8-amino-4,7-dihydroxy-chromen-2-one

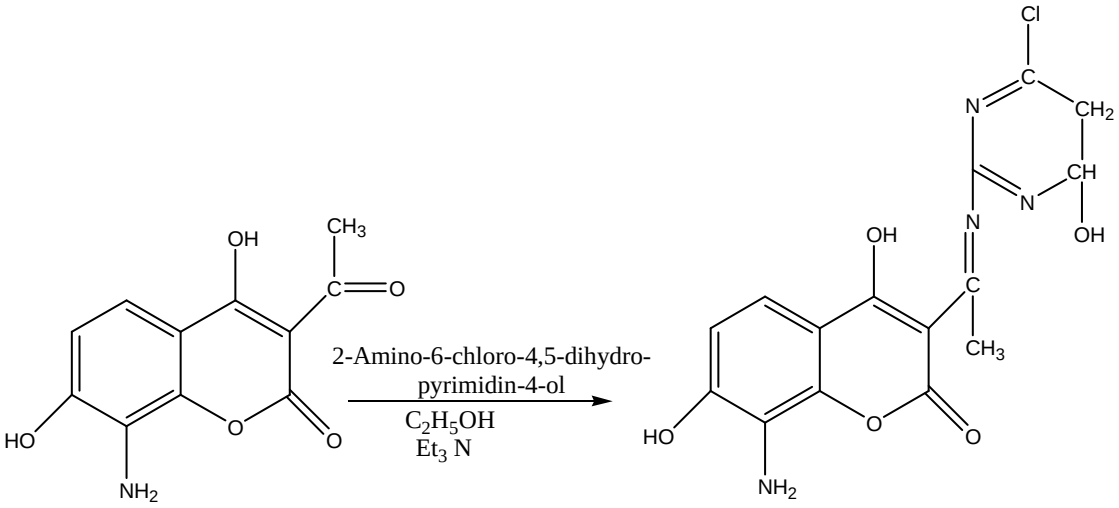
Scheme 1.Synthesis of 3-Acetyl-8 – amino -4,7- dihydroxy-chromen-2-one (1a)

2.3: Preparation of 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro -pyrimidin-2 -ylimino) - ethyl]-4,7- dihydroxy-chromen-2-one **(2a)**

In a 100 ml flask were mixed 2.5g of 3-Acetyl-8 – amino -4,7- dihydroxy-chromen-2-one,2g 2-Amino-6-chloro-4,5-dihydropyridin-4-ol with 5ml C_2H_5OH , 0.3 ml Et_3N .The mixture was refluxed at 80 ^{o}C for ca. 9h . The obtained yellow crystals are filtred and dried at room temperature . Recrystallization form C_2H_5OH gave yellow crystals product of 70% yield,meltingpoint, 245 ^{o}C. (Scheme 2) .

2.4: Preparation of 8-Amino-4-[bis-(2-chloro-ethyl) -amino] -3-[1 -(6- chloro-4 -hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl] -7- hydroxyl – chromen-2-one **(3a)**

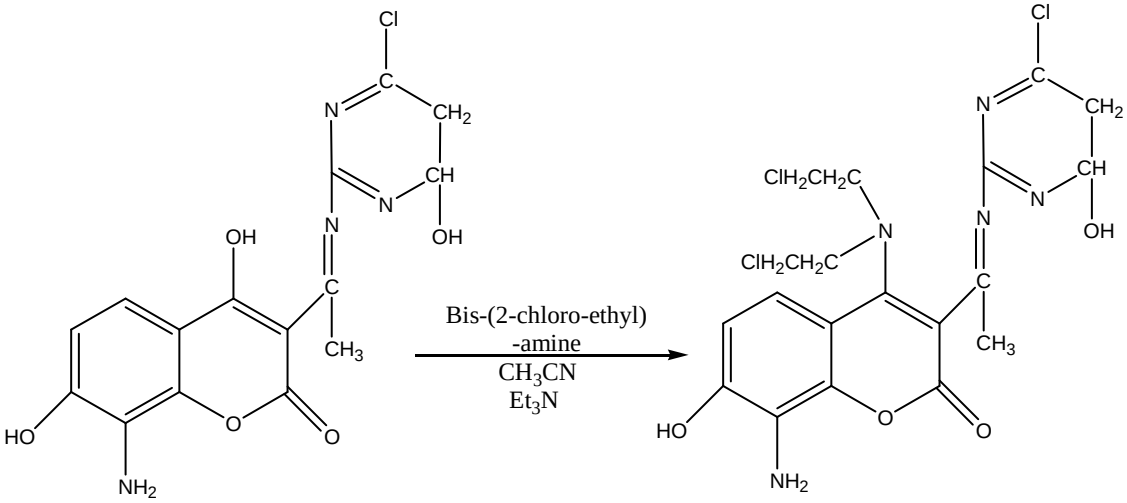
In a 100 ml flask were mixed 1.5g of 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro -pyrimidin-2 -ylimino) -ethyl]-4,7- dihydroxy-chromen-2-one ,1g Bis-(2-chloro-ethyl)-amine ,with 5 ml CH_3CN and 0,2 ml Et_3N as katalyzer. The mixture was refkuxed at 95 ^{o}C in water bath for ca. 22 h .The flask was placed in an ice bath for 1h until yellow crystalline precipitate was formed.After filtration the product was recrystallized from CH_3CN .The recrystallizacion gave a yellow product at 70% yield, melting.point;180^{o}C. (Scheme 3).



3-Acetyl-8-amino-4,7-dihydroxy-chromen-2-one

8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-4,7-dihydroxy-chromen-2-one

Scheme 2 Synthesis of 8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro -pyrimidin-2 -ylimino) - ethyl]-4,7- dihydroxy-chromen-2-one (2a)



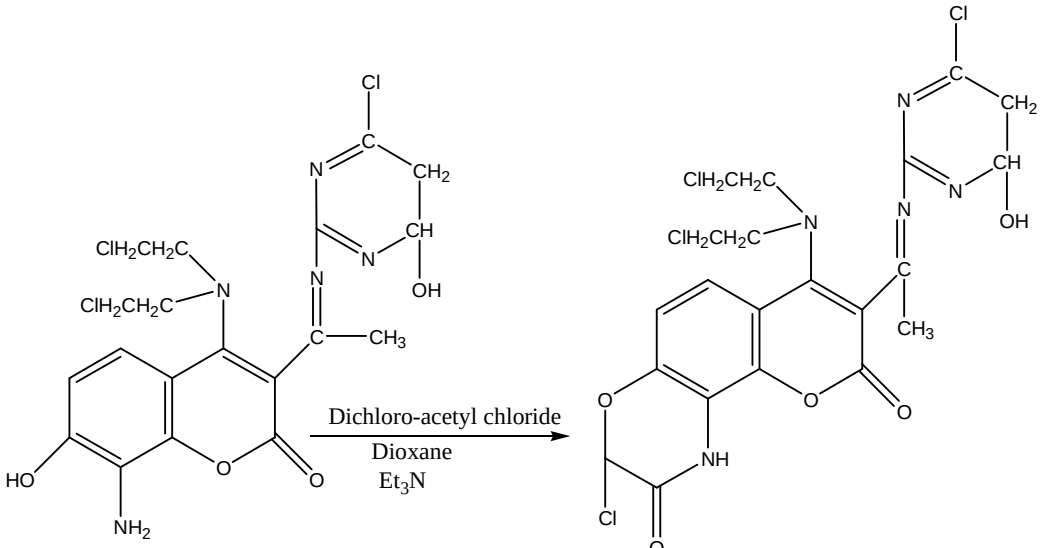
8-Amino-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-4,7-dihydroxy-chromen-2-one

8-Amino-4-[bis-(2-chloro-ethyl)-amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-7-hydroxy-chromen-2-one

Scheme 3 Synthesis of 8-Amino-4-[bis-(2-chloro-ethyl) -amino] -3-[1 -(6- chloro-4 -hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl] -7-hydroxyl – chromen-2-one (3a)

2.5: Preparation of 8-[Bis-(2-chloro-ethyl)-amino] -2-chloro -7- [1-(6- chloro-4- hydroxyl -4,5- dihydro – pyrimidin-2-ylimino)-ethyl]-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione **(4a)**

In a 100 ml flask were mixed 1g 8-Amino-4-[bis-(2-chloro-ethyl) -amino] -3-[1 -(6- chloro-4 -hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl] -7- hydroxyl – chromen-2-one **,**0.7ml Dichloro-acetyl chloride ,4ml Dioxane 0.1ml Et_3N . The mixture was refluxed at 95 ^oC in water bath for ca. 2 h .The obtained red crystals are filtred and rinsed with C_2H_5OH and dried at room temperature.Recrystallization from ethanol gave a red product at 60 % yield , melting point 204 ^oC. (Scheme 4)



8-Amino-4-[bis-(2-chloro-ethyl)-amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-7-hydroxy-chromen-2-one

8-[Bis-(2-chloro-ethyl)-amino]-2-chloro-7-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino)-ethyl]-4*H*-1,5-dioxa-4-aza-phenanthrene-3,6-dione

Scheme 4.Synthesis of 8-[Bis-(2-chloro-ethyl)-amino] -2-chloro -7- [1-(6- chloro-4- hydroxyl -4,5- dihydro – pyrimidin-2-ylimino)-ethyl]-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione

Table-1 Analytical data

Compd	Yield (%)	m.p	M.F	Elemental analysis. Calculated :Found ,**(calc)**				
				C	H	N	O	Cl
1a	80	129°C	$C_{11}H_9NO_5$	(56.17) 56.00	(3.86) 3.50	(5.96) 5.70	(34.01) 34.00	
2a	70	245°C	$C_{15}H_{13}ClN_4O_5$	(49.39) 49.70	(3.59) 3..70	(15.36) 15.10	(21.93) 21.50	(9.72) 9.60
3a	70	180°C	$C_{19}H_{20}Cl_3N_5O_4$	(46.69) 46.00	(4.12) 3.90	(14.33) 14.00	(13.09) 12.90	(21.76) 21.50
4a	60	204°C	$C_{21}H_{19}Cl_4N_5O_5$	(44.78) 45.00	(3.40) 3.00	(12.43) 12.10	(14.20) 13.85	(25.18) 24.90

BIOLOGICAL SCREENING

2.6: Antibacterial activities

The antibacterial activities of all the compounds were studied against gram-positive bacteria (Staphylococcus aureus and Bacillus.c) and gram-negative bacteria (E.coli).

The disc was watted with N,N-DMF solutions of the synthesized compounds with contreaction 2mg/ml,3mg/ml,and 5mg/ml and then are placed in petridish (d=15cm).The old subculture E.coli and B.cereus were poured and spread in petridish in Agar-Mc-Conkey while S.aureus in Agar-maltoze .The disc were incubated at 35°C for 48h,the control was also maintained with DMF ,cefalexin 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.

Table 2 Antibacterial activity- Staphylococcus aureus
Inhibition zone (mm)

Compound	2mg/ml	3mg /ml	5mg/ml
1a	13	17	19
2a	14	18	21
3a	15	18	22
4a	14	19	22
Cephalexine	9	9	9 10 μg
Streptomycine	20	20	20 10 μg

Table 3 Antibacterial activity – E.Coli

	Inhibition zone (mm)		
Compound	2mg/ml	3mg /ml	5mg/ml
1a	10	15	19
2a	11	17	21
3a	12	18	22
4a	11	16	21
Cephalexine	9	9	9 10 μg
Streptomycine	23	23	23 10 μg

Table 4 Antibacterial activity – Bacillus cereus

	Inhibition zone (mm)		
Compound	2mg/ml	3mg /ml	5mg/ml
1a	9	14	20
2a	10	15	21
3a	13	19	24
4a	11	18	21
Cephalexine	9	9	9 10 μg
Streptomycine	23	23	23 10 μg

The purified synthesized compounds (1a,2a,3a,4a) was subjected to test in vitro its antibacterial activity against three bacterial cultures ; Staphylococcus aureus,E.Coli and B.cereus. Antibacterial activity of compounds was investigated applying the Kirby-Bayer method [14] or disc method (d=5.5 mm max. capacity 10 μg).

RESULTS AND DISCUSSION

By reacting equimolar amounts of 8-Amino-4,7-dihydroxy-chromen-2-one and correspomding reagents (according scheme 1) under reflux reaction condictions product 1a is synthesized in 80 % yield.

By reacting equimolar amounts of 3-Acetyl-8-amino-4,7-dihydroxy-chromen-2-one and corresponding reagents (according scheme 2) under reflux reaction condictions product 2a is synthesized in 70 % yield.

By reacting equimolar amounts of 8-Amino-3-[1 - (6 – chloro-4 –hydroxy -4,5- dihydro - pyrimidin- 2- ylimino) - ethyl] -4,7- dihydroxy-chromen – 2 - one and corresponding reagents (according scheme 3) under reflux reaction condictions product 3a is synthesized in 70% yield.

By reacting equimolar amounts 8-Amino-4-[bis-(2-chloro-ethyl)-amino]-3-[1-(6-chloro-4-hydroxy-4,5-dihydro-pyrimidin-2-ylimino) -ethyl] -7- hydroxyl – chromen-2-one and corresponding reagents (according scheme 4) under reflux reaction condictions product 4a is synthesized in 60% yield.

The structure of 8-Amino-4,7-dihydroxy-chromen-2-one derivatives (1a,2a,3a,4a) were determined fromtheir IR, ^{1}H NMR , ^{13}C NMR spectar and their melting points as follows.

3.1: For (1a); IR bands (KBr,cm $^{-1}$) 3470(O-H vibration);3350(N-H);2850(C-H,alif);3060(C-H,ar), 1740(C=O), 1600(N-H);1570 (C=C) ;1350(N-H);690(C-H);649(C-Cl)

3.2: ^{1}H NMR (DMSO-d_6) δppm ;0.92 s(3H,CH_3),4.0d(2H,NH_2Ar),5.0s(H,OH),6.4-6.8(2H,ar) , 15.0s(H,OH)

3.3: ^{13}C NMR (DMSO) δppm ; 196(C,C=O);178(C,COH);162(C,COO);138(C,C-O);143(C,C-OH); 126.7(C,C-NH);113,2-121, 2(3C,ar);27.3(C,CH_3)

3.1.1: For (2a) IR bands (KBr,cm $^{-1}$) ;3480(OH); 3337(N-H); 3070(C-H,ar); 2950(C-H,alif) 1760(C=O), 1680(C=N); 1545(C=C), 1250(C-N);1224(C-O),700(C-H,ar);614(C-Cl)

3.2.1: ^{1}H NMR (DMSO-d_6) δppm 0.9s(3H,CH_3);1.5d(2H,CH_2);2.0s(H,OH);3.2s(H,CH); 4.0d(2H,NH_2,ar); 5.0t(H,OH), 6.4-6.8(2H,ar); 15.0s(H,OH)

3.3.1: ^{13}C NMR (DMSO) δppm 173(C,COH); 163(C=N); 164(C=N);164.5(C-Cl); 162(C,COO), 143.5(C-OH), 138(C-O), 126(C-N);118-121(3C,ar);82(C=C-C);70(C,C-OH);40,7(C,CH_2);9.8(C,CH_3)

3.1.2: For (3a) IR bands (KBr,cm $^{-1}$) 3490(OH);3418(OH);3325(NH);3050(C-H,ar);2922(C-H,alif); 1750(C=O);1655(C-N);1644(C=O);1530(C=C);1248(C-N);1222(C-O);690(C-H,ar);580(C-Cl) ;543(C-Cl)

3.2.2: ^{1}H NMR (DMSO-d_6) δppm '0.93s(3H,CH_3); 1.5d(H,CH_2); 2.0d(H,CH); 2.0d(H,OH); 2.83d(4H,2CH_2N), 3.50d(4H,2CH_2Cl); 4.0d(H,NH);5.0d(H,OH),6.4-6.8(2H,ar

3.3.2: ^{13}C NMR (DMSO) δppm ;170(C,C-N);164(C,C-Cl);164.6(C=N),163(C=N);162.5(C,COO);143(C,C-OH) ;138(C,C-O);126(C,C-N);70(C,C-OH);85(C,C=C-C),53(C,C-N);45.1(C,CH_2Cl),40.7(C,CH_2) ;9.5(C,CH_3)

3.1.3: For (4a) IR bands (KBr,cm $^{-1}$); 3440(OH),3390(NHCO);3035(C-H,ar);2890(C-H,alif), 1760(C=O), 1680(C=N); 1533(C=O), 1230(C-N); 1215(C-O) 700(C-H,ar);570(C-Cl,542(C-Cl)

3.2.3: ^{1}H NMR (DMSO-d_6) δppm ;0.9s(3H,CH_3);1.5d(2H,CH_2);2.0t(H,OH);2.83t(2H,CH_2N); 3.50t(2H,CH_2Cl); 6.62s(H,CH); 6.7-7.3d(2H,ar).8.0s(H,NHCO)

3.3.3: ^{13}C NMR (DMSO) δppm; 170(C,C-N),164(C,C=N);164(C,C-Cl);163(C,CONH);153.5(C,C-O); 119(C,C-N);111,118,120(3C,ar);85(C=C-C=N),70(C,C-OH);53(C,C-N);45(C,C-Cl), 40.7(C,CH_2);9.0(C,CH_3)

CONCLUSION

From the results the followin conclusion were drawn:The study provides the first evidence that compounds **(1a,2a,3a,4a)** obviously inhibit the growth of S.auerus , E.coli and B.cereus.

The compounds **(1a,2a,3a,4a)** compared with the antibacterial activity of Streptomycine in S.aureus ,E.coli and B.cereus.

This study provided the first evidence that these compounds 2a,3a,4a showed a significant antibacterial effect against S.aureus,E.coli and B.Cereus.

The chemical structures of synthesizen compounds were determined according to extensive NMR experiments and published data.

Acknowledgements
The authors thank Prof.Branko Stanovnik,University of Ljubljana and its laboratory staff for ^{1}H NMR spectrum and elemental analyses.

REFERENCES

[1] S.Govori;V.Kalaj;V.Rapic;L.Kalaj and S.Dakovic,*Heterocycel.Commun.* **2002**.,(8),129
[2] B.Stanovnik; H.Susachitzky and E.F.Scriven, Progress in Heterocyclic Chemistry,Pergamon Press ,Oxford , **1993**,(5).75-146 .
[3] S.H.Lee;D.-S.Shin; J.-S,Kim; K.-B. Oh and S.S.Kan, *Arch. Pharm. Res* **2003**., 26 .
[4]Vyas KB;Nimavat ;KS,Jani GR;Hathi MV, **2009**)Synthesisi of antimicrobial activity of coumarine derivatives metal complexes:An in vitro evalution.Orbital,(1),183-192.
[5] Abyshev AZ; Gimdein Va; Semenov EV ; Agaev EM, Abdulla-zade AA, Gueinov AB,**2006**
[6] A.Behrami;K.Vaso;I.Krasniqi, *J. Int .Environ .Appl.Sci*.**2010**, (5).247 .
[7]M.D.Aytemir ; R.C.Hider ; D.D.Erol ; M.Ozalp; and M.Ekizoglu .*Turk.J.chem.*, **2003**,445.
[8]M.M.El.Saghier;M.B.Naili;B.Kh.Rammash; N.A.Saleh and K.M.Kreddan, *Arkivoc*, **2007**,83.
[9] Z.M.Nofal ;M.El-Zahar; and S.Abd El Karim, *Molecules*, **2000**,(5).99 .
[10]Chaluvaraju KC and Ishwarbhat K.Asian , *J Chem* **2008**; (20), 4335.
[11]Rajan Ra Kali ;Jubie S,Grworamma B; and Suresh B,*Asian J Chem* **2008**:(20), 5289.
[12]Ali Mohammed Ashraf ; and Sharayar Mohammed . *Boorg Med Chem. Lett* **2009**;(17),3314.
[13]Nofal ZM; El-Zahar M; Abd El-Karim S, (**2000**) Novel coumarin derivatives with expected biological activity,*Molecules*.(5).99-113.
[14]Pandeya SN;Lakshmi VS Aandey A. *Indian J Pharma Sci* **2003**;(65):213.