Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(8): 1087-1091

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

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

Aziz Behrami[1] and Florent Dobroshi[2]

Public University of Mitrovica,''Isa Boletini'' Mitrovica, Kosova

---

ABSTRACT

In present paper , we report the organic syntheses of four compounds from 4,7-Dihydroxy-chromen-2-one and describe the results of antibacterial activity of purified compounds. Compounds 4,7-Dichloro-2- oxo-2H-chromene-3-carbaldehyde (1a). 4,7-Dichloro –chromen -2-one (2a) , 4,7-Dichloro -3- iminomethyl-chromen-2-one (3a), 3-Iminomethyl -4,7-bis –(4-methoxy-phenylamino) – chromen-2-one (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 concentrations 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-NMR, ^{13}C-NMR, Streptomycine.

INTRODUCTION

Starting from Benzene – 1,3,5-triol (a); derivatives (1a,2a,3a,4a) are synthesized. Coumarin derivatives are large group of heterocyclic with oxygen as heteroatom. Coumarin is a chemical compound (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 varies biological activities. Their fame has come mainly from their antithrombic, anti-inflammatory, 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

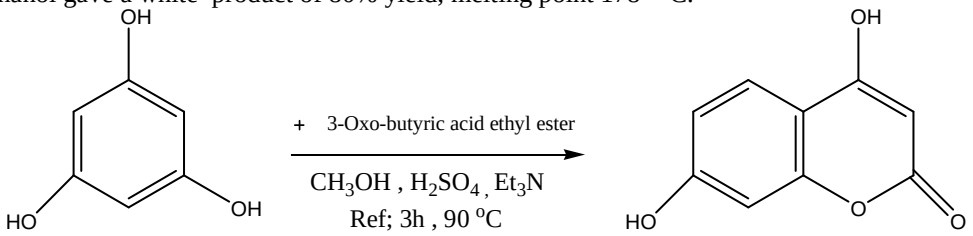
4,7-Dihydroxy -Chromen-2-one **(1a),** 4,7-Dichloro –chromen -2-one **(2a)** , 4,7-Dichloro -3- iminomethyl-chromen-2-one **(3a),** 3-Iminomethyl -4,7-bis –(4-methoxy-phenylamino) – chromen-2-one **(4a)**,

Measurement

The identification of Benzene – 1,3,5-triol 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 determined 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-d6 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.

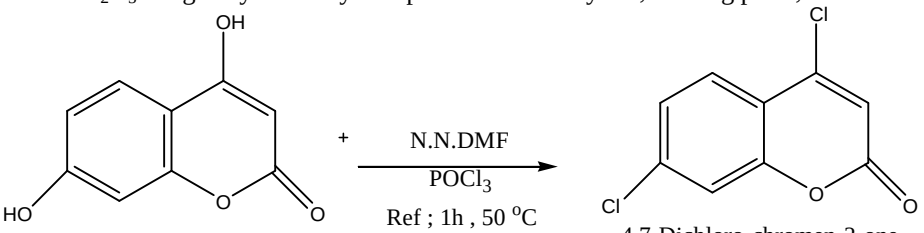
Preparation of 4,7-Dihydroxy -Chromen-2-one (1a)
For this synthesis is used as substrate Benzene – 1,3,5-triol in a 100 ml flask mixed 3 g N.NDMF , and 3g 3-oxo-butiric acid ethyl ester , 8ml CH_3OH , 2ml H_2SO_4,1ml Et_3N . The mixture was refluxed at 90 ^oC for ca. 3h. The obtained crystals white are filtered and rinsed with methanol and dried at room temperature. Recrystallization form absolute methanol gave a white product of 80% yield, melting point 178 ^oC.



Benzene-1,3,5-triol 4,7-Dihydroxy-chromen-2-one

Figure 1. Preparation of 4,7-Dihydroxy -Chromen-2-one (1a)

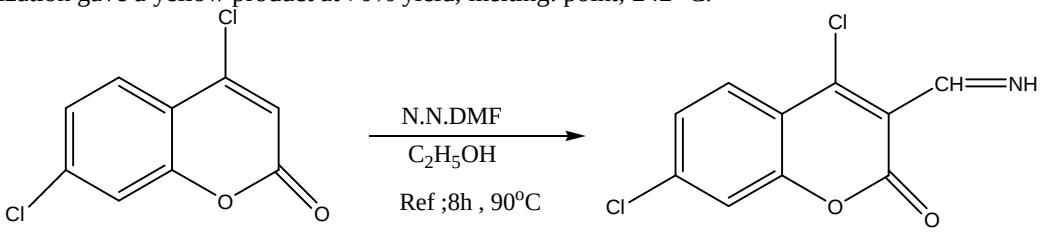
Preparation of 4,7-Dichloro –chromen -2-one **(2a)**
In a 100 ml flask were mixed 3g 4,7-Dihydroxy –Chomen- 2-one with 5ml N.NDMF , 3ml $POCl_3$ .The mixture was refluxed at 50 ^oC for ca. 1h. The obtained yellow crystals are filtered and dried at room temperature . Recrystallization form C_2H_5OH gave yellow crystals product of 70 % yield, melting point, 215 ^oC.



4,7-Dihydroxy-chromen-2-one 4,7-Dichloro-chromen-2-one

Figure.2 Preparation of 4,7-Dichloro –chromen -2-one (2a)

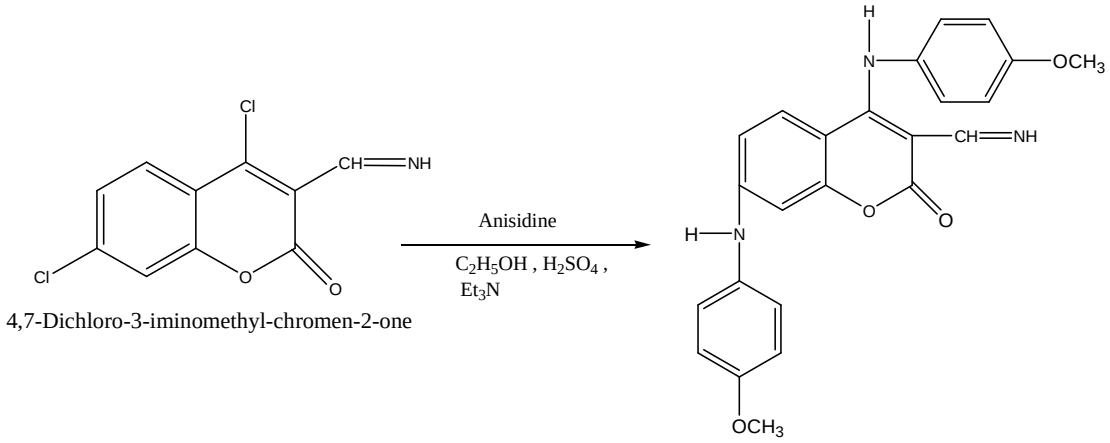
Preparation of 4,7-Dichloro -3- iminomethyl-chromen-2-one **(3a)**
In a 100 ml flask were mixed 2g of 4,7-Dichloro-chromen-2-one, 4ml NNDMF, with 8 ml C_2H_5OH and 0,2 ml Et_3N as catalyzer. The mixture was refluxed at 90 ^oC in water bath for ca.8 h .The flask was placed in an ice bath for 1h until yellow crystalline precipitate was formed. After filtration the product was recrystallized from CH_3CN .The recrystallization gave a yellow product at 70% yield, melting. point; 242 ^oC.



4,7-Dichloro-chromen-2-one 4,7-Dichloro-3-iminomethyl-chromen-2-one

Figure.3 Preparation of 4,7-Dichloro -3- iminomethyl-chromen-2-one (3a)

Preparation of 3-Iminomethyl -4,7-bis –(4-methoxy-phenylamino) – chromen-2-one **(4a)**
In a 100 ml flask were mixed 2 g 4,7-Dichloro-3-iminomethyl-chromen-2-one, 8ml C_2H_5OH, 1.5g Anisidine, 1ml H_2SO_4, 1ml Et_3N . The mixture was refluxed at 50 ^oC in sand bath for ca. 3h .The obtained red crystals are filtered and rinsed with CH_3CN and dried at room temperature. Recrystallization from CH_3CN gave a red product at 70 % yield, melting point 252 ^oC.



4,7-Dichloro-3-iminomethyl-chromen-2-one

3-Iminomethyl-4,7-bis-(4-methoxy-phenylamino)-chromen-2-one

Figure.4 Preparation of 3-Iminomethyl -4,7-bis –(4-methoxy-phenylamino) – chromen-2-one (4a)

Table 1 Analytical data

Comp'd	Yield %	m.p	M.F	Elemental analysis. Calculated :Found (calc) %				
				C	H	N	O	Cl
1a	80	178 °C	$C_9H_6O_4$	60.68 60.65	3.39 3.30		35.93 35.06	
2a	70	215 °C	$C_9H_4Cl_2O_2$	55.27 55.40	1.87 1,80		14.88 14.80	32.97 32.91
3a	70	242 °C	$C_{10}H_5Cl_2NO_2$	49.62 49.58	2.08 2.0	5.79 5.70	13.28 13.25	29.29 29.25
4a	70	415 °C	$C_{24}H_{21}N_3O_4$	69.39 69.30	5.10 5.0	10.10 10.0	15.40 15.35	

Antibacterial activity

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 or disc method (d=5.5 mm max. capacity 10 μg)

Table 2 Antibacterial activity- *Staphylococcus aureus*

Compound	Inhibition zone (mm)		
	2 mg/ml	3 mg /ml	5 mg/ml
1a	13	17	19
2a	14	20	22
3a	13	19	21
4a	14	19	20
Cephalexine	9	9	9 10 μg
Streptomycine	20	20	20 10 μg

Table 3 Antibacterial activity – *E. coli*

Compound	Inhibition zone (mm)		
	2mg/ml	3mg /ml	5mg/ml
1a	10	15	19
2a	13	19	22
3a	12	18	21
4a	11	16	21
Cephalexine	9	9	9 - 10 μg
Streptomycine	23	23	23 - 10 μg

Table 4 Antibacterial activity – *Bacillus cereus*

Compound	Inhibition zone (mm)		
	2mg/ml	3mg /ml	5mg/ml
1a	9	14	20
2a	10	15	23
3a	13	19	22
4a	11	18	21
Cephalexine	9	9	9 - 10 μg
Streptomycine	23	23	23 - 10 μg

RESULTS AND DISCUSSION

By reacting equimolar amounts of 3-oxo-butiric acid ethyl ester and corresponding reagents (according scheme 1) under reflux reaction conditions product 1a is synthesized in 80 % yield.

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

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

By reacting equimolar amounts 4,7-Dichloro -3- iminomethyl-chromen-2-one and corresponding reagents (according scheme 4) under reflux reaction conditions product 4a is synthesized in 70% yield.

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

For (1a); IR bands (KBr, cm^{-1}) 3300-3600 cm^{-1} (OH vibration.), 1720 cm^{-1} (C=O), 1600 (C=C stretch.), 750 cm^{-1} (C-H bend.). **^{1}H NMR (DMSO-d6) δ ppm**; 5, 7 ppm (H, OH), 6, 4, 6.7, 7.5 (H, aromatic), 15.0 ppm (H, OH). **^{13}C NMR (DMSO) δ ppm**; 156.9 (C, C-OH); 162.0 ppm (C, C=O); 173.3 (C, COOH).

For (2a) IR bands (KBr, cm^{-1}) 1720 cm^{-1} (C=O stretch.); 1600 cm^{-1} (C=C stretch.); 750 cm^{-1} (C-H bend.); 600 cm^{-1} (C-Cl stretch.). **^{1}H NMR (DMSO-d6) δ ppm:** 7.21 ppm -7.53, t (H aromatic). **^{13}C NMR (DMSO) δ ppm:** 166.9 ppm (C-Cl); 162 ppm (C, COO); 15 2ppm (C, C-O); 133.4 ppm (C, C-Cl); 121, 7, 125, 6, 128 ppm (3C aromatic).

For (3a) IR bands (KBr, cm^{-1}) 3428 cm^{-1} (N-H stretch.); 3000cm-^{1}(C-H aromatic.); 1730cm^{-1}(C=O); 1650cm^{-1} (H, CH=NH); 1590cm^{-1} (C=C aromatic), 740cm^{-1}(C-H aromatic). **^{1}H NMR (DMSO-d6) δ ppm:** 8.5ppm (H, CH=NH), 7.2, 7.2, 7.6 t(3H aromatic). **^{13}CNMR (DMSO) δ ppm:** 121.7ppm, 125.6.ppm, 128ppm (3C aromatic), 133ppm (C-Cl); 152ppm (C, C-O); 162ppm (C, C=O), 163.7ppm (C, C-NH).

For (4a) IR bands (KBr, cm^{-1}); 3428cm^{-1}(N-H stretch.), 3000cm^{-1}(C-H aromatic.); 2870cm^{-1}(C-H stretch.); 1730cm^{-1}(C=O stretch.); 1650cm^{-1}(C=N stretch.); 1600cm^{-1} (N-H bend), 740cm^{-1} (C-H aromatic). **^{1}HNMR (DMSO-d6) δ ppm:** 4.0ppm (3H, CH_3); 4.0ppm (2H, NH), 6.35-6.52 m(1, 1H aromatic), 6.52ppm ^{2}H, CH=NH. **^{13}CNMR (DMSO) δ ppm;** 56ppm (C, CH_3); 115-127 ppm(11C aromatic), 151.7ppm (C, C-O); 162ppm (C, C=O), 142.8ppm (C, C-NH); 162ppm (C, C-NH); 114ppm (C, C=NH).

CONCLUSION

From the results the following conclusions 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 1a, 2a, 3a, 4a, 5a showed a significant antibacterial effect against *S.aureus, E.coli* and *B.Cereus.*
The chemical structures of synthesized 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]Stanovnik B.; Susachitzky *H.* Scriven EF, (**1993**) Progress in Heterocyclic Chemistry, Pergamon Press, Oxford,(5).75-146 .
[2]Vyas KB;Nimavat ;KS,Jani GR;Hathi MV, (**2009**) *Orbital*,(1),183-192.
[3]Behrami A.; Vaso K.; Krasniqi I., (2010) *J. Int .Environ .Appl.Sci.*, (5).247 .
[4]Mishra D.K., Mishra A.P., **(2011)** *Int. J. Pharm. Res. Dev*., **3(5),** 24-31

[5]Ali Mohammed Ashraf ; and Sharayar Mohammed . *Boorg Med Chem. Lett* (**2009)**;(17),3314.
Nofal ZM; El-Zahar M; Abd El-Karim S, (**2000***) Molecules*.(5).99-113.
[6]Chandra S. Kumar P., **(2005)** *Spectrochimica Acta Part A*, **61**, 219-224