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Synthesis,Characterisation and Antibacterial Activity of some [8-Amino-4,7-dihydroxy-chromen-2-one] , [N-(3-Cyano-4-ethoxy-2-oxo-2H-chromen-7-yl)-formamide] Derivatives. The Comparison with Standard Drug

Aziz Behrami[1*], Kozata Vaso[2], Sevdie Govori[3], Islam Krasniqi[3], Skender Demaku[3] Idreiz Vehapi[3]

[1*]The Geoscience and Technology Faculty Material and Metalurgy Department- Mitrovica , University of Pristine ,Kosovo
[2]Department of Chemistry, Faculty of Natural Sciences, University of Tirana , Albania
[3]Department of Chemistry, University of Pristine , Kosovo

ABSTRACT

The present study deals with the sysnthesis , struscture and antibacterial activity of [8-Amino-4,7-dihydroxy-chromen-2-one].[N-(3-Cyano-4-ethoxy-2-oxo-2H-chromen-7-yl)-formamide].Derivatives are reported Compounds 2 - Chloro - 8- hydroxyl – 7-iminomethyl - 4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione**(a_1)**.N-(4-Ethoxy-3-formyl-2-oxo-2H-chromen-7-yl)-formamide**(b_1)**.4-Ethoxy-7-formylamino-2-oxo-2H-chromene-3-carboxylic acid (2-chloro-8-hydroxy-3,6-dioxo-3,4-dihydro-2H,6H-1,5-dioxa-4-aza-phenanthren-7-ylmethyl)-amide**(c)**. 4-Ethoxy-7-[3 - (4–methoxy – phenyl)-ureido]-2-oxo-2H-chromene-3-carboxylic acid [2-chloro – 8 - (4- methoxy – phenylamino)-3,6 - dioxo-3,4-dihydro-2 H,6H-1,5-dioxa-4-aza-phenanthren-7-ylmethyl]-amide**(d).**The structures of the synthesized compounds:(a_1,b_1,c,d) were characterized by spectroscopic methods,(IR, H^1-NMR , & C^{13}-NMR) and analytical techniques (elemental analysis , melting point and TLC).The antibacterial activity of synthesized compounds was compared with antibacterial activity of the standard antibiotics cephalexine and streptomycine.The compounds shows different bacteriostatic and bactericidal activity against two cultures;Staphylococcus.aureus,Bacillus.cereus.

Keywords:[8-Amino-4,7-dihydroxy-chromen-2-one],Antibacterial activity,Staphylococcusaureus , Bacillus cereus,Cephalexine.

Corresponsing author:
Email: a.behrami_chem@live.com

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INTRODUCTION

Using [8-Amino-4,7-dihydroxy-chromen-2-one] (a), [N-(3-Cyano-4-ethoxy-2-oxo-2H-chromen-7-yl)-formamide] (b) as starting material,some new 2H-chromen-2-one (coumarin ,2H-1-benzopyran-2-one) derivatives (a_1) , (b_1) , (c) , (d) , are synthesized **(Schemes – I , II , III, IV)**.2H-Chromen-2-one derivative which are known as coumarin (specifically , a benzo-α-pyrone) derivatives are a large group of heterocyclic with oxygen as heteroatom [1-3].Coumarin and its derivatives have biological activities [4-5]. Most of them show antibacterial,bactericidal and bacteriostatic properties [6-8]. Biological activity of coumarin derivatives is linked with their structure. The different substituent in the structure of benzo-α-pyrone or benzene of coumarin has different effect in biological activity [9].

Literature shows that the biological activity of coumarin derivatives is closely linked with their influence in enzymatic processes or has an analogy of their structure with the active enzymatic centres. But , it's very important to stress that a general correlation between the structure of coumarin derivatives and their microbiological activity it's not yet found, although many efforts made by different researches [10].

These wide ranges of biological properties [11,12] have stimulated us to synthesized some new coumarin derivatives, to find optimal method, optimal conditions of the synthesis and mechanism of reaction and to investigate their antibacterial activity against two bacterial cultures Staphylococcus aureus,Bacillus.cereus.

The antibacterial activity of synthesized compounds were compared with antibacterial activity of streptomycin,andcephalexine as standard antibiotics.

MATERIAL AND METHODS

Experimental

Compounds2-Chloro-8-hydroxy-7-iminomethyl-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione **(a_1) ,** N-(4-Ethoxy-3-formyl-2-oxo-2H-chromen-7-yl)-formamide**(b_1)**, 4-Ethoxy-7-formylamino-2-oxo-2H-chromene-3-carboxylic acid (2-chloro-8-hydroxy-3,6-dioxo-3,4-dihydro-2H,6H-1,5- dioxa - 4-aza - phenanthren -7- ylmethyl)-amide**(c)**,4 - Ethoxy- 7-[3-(4-methoxy-phenyl)-ureido]-2- oxo-2H- chromene-3-carboxylic acid[2-chloro-8-(4-methoxy-phenylamino)-3,6-dioxo-3,4-dihydro- H , 6H-1,5- dioxa – 4 - aza-phenanthren-7-ylmethyl]-amide **(d),** are synthesized. The identification of 2H-chromen-2-one derivatives (a_1,b_1,c,d) , is made by using melting point , infrared , ^{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 KBrpellts 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 iodinevapour for visualization.

Synthesis of 2-Chloro-8-hydroxy-7-iminomethyl-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione **(a_1)** 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 **(a)** with 5ml $HCONH_2$, equivalent amount $ClCH_2COCl$ and 0.3 ml triethylamine as catalyzer . The mixture was refluxed at 150 ^oC for ca. 90 min. The obtained crystals yellow and orange are filtred and rinsed with ethanol and dried at room temperature.Recrystallization form absolute ethanol gave a yellow product of **(a_1)** compound at 80% yield, melting point 200^oC. **Schemel**

Synthesis of N- (4 – Ethoxy -3- formyl- 2-oxo -2H- chromen- 7-yl)- formamide**(b_1)** In a 100 ml flask were mixed 2.5g of N-(3-Cyano-4-ethoxy-2-oxo-2H-chromen-7-yl)-formamide**(b)** , with 5ml Dioxane , 1g $SnCl_2$, 0.3ml HCl .The mixture was refluxed at8 0^oC for ca. 1.5 h. The obtained yellow crystals are filtred and dried at room temperature . After obtained Aldimin added 2 ml H_2O . Then the reflux starts again for 30 min at 40^oC. The obtained white crystals are filtred and dreid room temperature.Recrystallization form dioxane gave white crystals produck of **(b_1)** compound at 70% yield,meltingpoint,157^oC.**SchemeII**

Synthesis of 4-Ethoxy-7-formylamino-2-oxo-2H-chromene-3-carboxylic acid (2-chloro-8-hydroxy-3,6-dioxo-3,4-dihydro-2H,6H-1,5-dioxa-4-aza-phenanthren-7-ylmethyl)amide **(c)** In a 100 ml flask were mixed 2 g 2-Chloro-8-hydroxy-7-iminomethyl-4H-1,5-dioxa-4-aza-phenanthrene-3,6-dione**(a_1).**N-(4-Ethoxy-3-formyl-2-oxo-2H-chromen-7-yl)-formamide**(b_1),**
with 10 ml ethanol , 0.3 ml HCl , 0,3 ml Et_3N as katalyzer. The mixture was refkuxed at 92 ^oC in water bath for ca. 7 h .The flask was placed in an ice bath for 1 h until yellow crystalline precipitate was formed.After filtration the product was recrystallized from ethanol.

The recrystallizacion from ethanol gave a yellow product of **(c)** compound at 70% yield,melting.point;180^oC.**SchemeIII**

Synthesis of4- Ethoxy – 7 - [3-(4-methoxy - phenyl) - ureido]- 2- oxo -2H-chromene-3-carboxylic acid [2-chloro-8-(4-methoxy-phenylamino)-3,6-dioxo-3,4-dihydro-2H,6H-1,5-dioxa-4-aza-phenanthren-7-ylmethyl]-amide **(d)**in a 100 ml of flask were mixed 1.5g of 4-Ethoxy-7-formylamino-2-oxo-2H-chromene-3-carboxylic acid (2-chloro-8-hydroxy-3,6-dioxo-3,4-dihydro-2H,6H-1,5-dioxa-4-aza-phenanthren-7-ylmethyl)-amide **(c),** 1.5 g C_7H_9NO (Anisidine) with 7ml CH_3CN (Acetonitrile) , 0.3 ml Et_3N.The mixture was refluxed at 95 ^oC in water bath for ca. 5 h .The obtained violet crystals are filtred and rinsed with CH_3CN and dried at room

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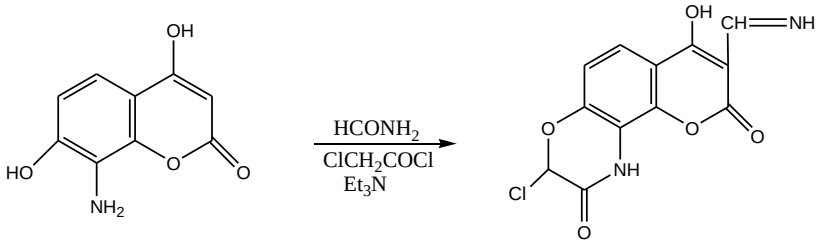
temperature.Recrystallization from ethanol gave a violet product of **(d)**product at 60% yield, melting point 186^{o}C. **Scheme IV.**

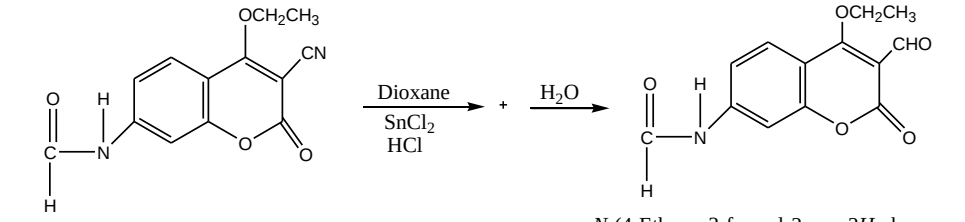
Scheme I



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

2-Chloro-8-hydroxy-7-iminomethyl -4*H*-1,5-dioxa-4-aza-phenanthrene-3,6-dione

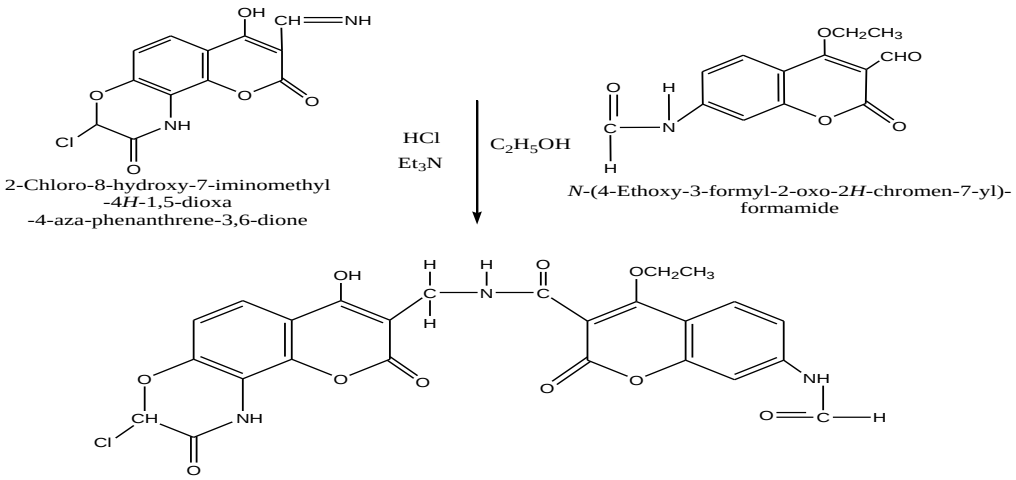
Scheme II



N-(3-Cyano-4-ethoxy-2-oxo-2*H*-chromen-7-yl)-formamide

N-(4-Ethoxy-3-formyl-2-oxo-2*H*-chromen-7-yl)-formamide

Scheme III



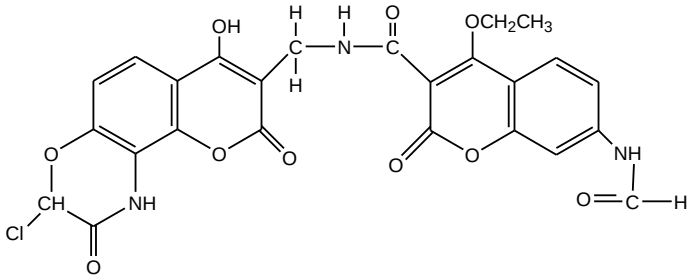
2-Chloro-8-hydroxy-7-iminomethyl -4*H*-1,5-dioxa -4-aza-phenanthrene-3,6-dione

N-(4-Ethoxy-3-formyl-2-oxo-2*H*-chromen-7-yl)-formamide

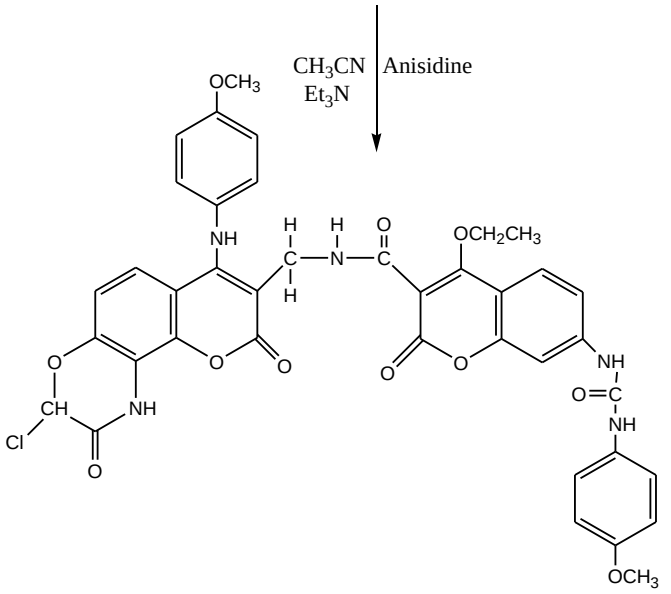
4-Ethoxy-7-formylamino-2-oxo-2*H*-chromene-3-carboxylic acid (2-chloro-8-hydroxy-3,6-dioxo-3,4-dihydro-2*H*,6*H*-1,5-dioxa-4-aza-phenanthren-7-ylmethyl)-amide

ISSN: 0975-8585

Scheme IV



4-Ethoxy-7-formylamino-2-oxo-2*H*-chromene-3-carboxylic acid (2-chloro-8-hydroxy-3,6-dioxo-3,4-dihydro-2*H*,6*H*-1,5-dioxa-4-aza-phenanthren-7-ylmethyl)-amide



4-Ethoxy-7-[3-(4-methoxy-phenyl)-ureido]-2-oxo-2*H*-chromene-3-carboxylic acid [2-chloro-8-(4-methoxy-phenylamino)-3,6-dioxo-3,4-dihydro-2*H*,6*H*-1,5-dioxa-4-aza-phenanthren-7-ylmethyl]-amide

Antibacterial activity

The purified synthesized compounds (a_1, b_1, c, d) was subjected to test in vitro its antibacterial activity against two bacterial cultures ; Staphylococcus aureus , Bacillus cerues . Antibacterial activity of compounds was investigated applying the Kirby-Bayer method [14] or disc method (d=5.5 mm max. capacity 10 µg)

Table 1 Antibacterial activity- Staphylococcus aureus Inhibition zone (mm)

Compound	2mg/ml	3mg /ml	5mg/ml
a_1	13	19	21
b_1	6	9	11
C	6	9	12
D	10	13	18
Cephalexine	7	7	7 10 µg
Streptomycine	20	20	20 10 µg

ISSN: 0975-8585

Table 2 Antibacterial activity - Bacillus cereus Inhibition zone (mm)

Compound	2mg/ml	3mg /ml	5mg/ml
a_1	14	19	20
b_1	10	13	17
C	12	15	18
D	11	18	21
Cephalexine	9	9	9 10 µg
Streptomycine	23	23	23 10 µg

Table 3

Compound	IR (cm^{-1})	^{1}H NMR ppm	^{13}C NMR ppm
a_1	3450(OH),3330 (NHCO), 1740(C=O),1680(CH=NH), 1660(NHCO),1600(C=C)Ar 1040(C-O-C),657(C-Cl)	δ.6.22 (s,H,CHCl) 6.7,7.3 (d,2H,Ar) 7.50(s,H,CH=NH) 8.0(s,H,NH) 11.0(s,H,OH)	δ.111,119,120,123,(C,Ar) 82(CH_2),105(CHCl) 153(C,CO),162(C,COO) 163(C,CO-NH) 163.7(C,CH=NH) 173(C,CH-OH)
b_1	3220(NHCO),2850(C-H) 2720(CHO),1728(C=O) 1700(CHO),1620(NHCO) 1600(C=C)Ar,1100(C-O)	δ.1.0,1.17,1.22(t,3H,CH_3) 3.98,4.0,(d,2,$HOCH_2$) 4.1,(s,H,NH) 7.60,7.61,7,58(m,3H,Ar) 8.21,8.23,(d,H,HCONH) 9.68,(s,H,CHO)	δ.15.1,(C,CH_3)60,(C,CH_2O) 97.1,(C,=C=CH) 113,117,126,123(C,Ar) 137,(C,CNH),57(C,C=O) 162(C,COO),190(C,CHO)
C	3400(OH),3372(NH) 3200(NHCO),2830(C-H) 2720(CHO),1732(C=O) 1718(CHO),1628(NHCO) 1620(NHCO),1600(NHCO) 1073(NH-C),1050(C-O) 732(C=C)618(C-Cl)	δ.1.07,1.20(d,3H,CH_3) 2.50,3,63(m,H,N-CH_2) 3.76(d,H,O-CH_2),4(sH,NH) 6.62(s,H,CHCl),6,7- 7.60(m,5H,Ar).8.29- 8.51(m,3H,NHC=O) 9.09-9.91(d,H,CHO) 10.84(s.H.OH)	δ.15.1(C,CH_3),59.7(C,OCH_2) 35.0(c,CH_2NH),92(c,=C- CH_2),111,113,117,120,123,126 (6C,Ar),105(C-Cl) 119(C-N) 153(C,CO),162(C,COO) 160.5(C,CONH), 163,2(C,CONH) 166.8(C,CONH) 180.2(C,C-O)
D	3350(NHCO),3200(NHC) 1720(C=O),1620, 1615(NHCO),1600(C=C) 1250,1050(C-O) 780(C=C)Ar,620(C-Cl)	δ.0.9,1.17,1,25(t,3H,CH_3) 2.27-2.74(m,2H-CH_2NH) 3.08-3,63(m,H,nHCH_2) 3,73,3,75(d,6H,2OCH_3) 3.78,3.80,3,81(t,H,NH) 4.0(s,2H,OCH_2) 5.65,5.95(d,2H,2NHCO) 6.35-7.65(m,9H,Ar) 8.23-8.41(d,2H.NHCO)	δ.15(C,CH_3),36.4(C,CH_2NH) 56(C,OCH_3),59.7(C,OCH_2) 105.6(C,CHCl),111,113,114.9, 114,116,117,120,(11C,Ar) 119.2(C,C-NH),139(C,C-NH) 143.7(C-O),152.2(C,CONH) 162(C,COO),162.3(C,CONH) 166.8(C,CONH)

ISSN: 0975-8585

Table-4 Analytical data

Compd	Yield (%)	m.p	M.F	Elemental alalysis.Calculated (found) (%)				
				C	H	N	O	Cl
a_1	80	200	$C_{12}H_7ClN_2O_5$	48.92 (48.37)	2.39 (2.29)	9.51 (9,30)	27.15 (26.80)	12.03 (11.94)
b_1	70	157	$C_{13}H_{11}NO_5$	59.77 (59.0)	4.24 (3.90)	5.36 (4.90)	30.62 (29.0)	
C	80	180	$C_{25}H_{18}ClN_3O_{10}$	54.02 (54.0)	3.26 (2.95)	7.56 (7.50)	28.78 (28.70)	6.38 (6.30)
D	60	186	$C_{39}H_{32}ClN_5O_{11}$	59.89 (59.0)	4.12 (4.00)	8.95 (8.80)	22.50 (21.80)	4.53 (3.90)

CONCLUSION

From the results the followin conclusion were drawn:The study provides the first evidence that compounds (a_1,b_1,c,d) obviously inhibit the growth of Staphyllococcusauerus and Bacillus cereus.

Antibacterial activity of **a_1**, **b_1,c,d**compounds is stronger than of Cephalexine in S.aureus and B.cerues .The compounds **a_1**,**b_1**,**c,d** compared with the antibacterial activity of Streptomycine in S.aureus and B.cerues.

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

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