# Antibacterial Effects and Synthesized New Derivatives of 4-hydroxy-chromen-2-one

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***Abstract***

*In present paper , we report the organic syntheses of three compounds from 4-hydroxy-chromen-2-one and describe the results of antibacterial activity of purified compounds. Compounds 4-Hydroxy-2-oxo-2H-chromene-3-sulfonic acid (4-methoxy-phenyl)-amide (a). N-[(4-Hydroxy-2-oxo-2H-chromen-3-yl)-(2-hydroxy-phenyl)-methylene]-acetamide (b) , 4-Hydroxy-3-[(6-nitro-benzothiazol-2-ylimino)-methyl]-chromen-2-one (c), have been synthesized and characterized using melting points , IR spectra , 1H-NMR and 13C-NMR spectra. The antibacterial activity of synthesized compounds and streptomycin and cephalexin 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, FTIR-Spectroscopy, NMR.*

## I. INTRODUCTION

Starting from 4-hydroxy-chromen-2-one; derivatives (a,b,c) 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 varius biological activities. Their fame has come mainly from their antithrombic, anti-inflammatory, vasodilatory , and antiviral activities. Other several coumarin derivatives have antimicrobial properties [12-15]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 Cephalexin and Streptomycin [1-11].

## *Materials and Methods*

### *Experimental Chemistry*

4-Hydroxy-2-oxo-2H-chromene-3-sulfonic acid (4-methoxy-phenyl)-amide **(a),** N-[(4-Hydroxy-2-oxo-2H-chromen-3-yl)-(2-hydroxy-phenyl)-methylene]-acetamide **(b)** , 4-Hydroxy-3-[(6-nitro-benzothiazol-2-ylimino)-methyl]-chromen-2-one **(c),**

### *Measurement*

The identification derivatives of 4-hydroxy-chromen-2-one **(a,b,c)** , is made by using melting point , IR , 1H 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-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 analyze was performed on a Perkin-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-Hydroxy-2-oxo-2H-chromene-3-sulfonic acid (4-methoxy-phenyl)-amide **(a)**

For this synthesis is used as substrate 4g 4-hydroxy-chromen-2-one in a 100 ml flask mixed 2 g Anisidine , 8ml acetonitrile , 3ml$ClSO_3H$ and 1ml $Et_3N$ . The mixture was refluxed at 90 $^{o}$C for ca. 4h. The obtained crystals white are filtered and rinsed with methanol and dried at room temperature . Recrystallization form absolute methanol gave a white product of 70% yield, melting point 310 $^{o}$C.

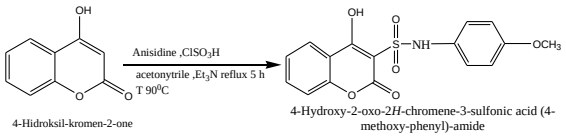


*Figure 1.* ***Preparation of*** *4-Hydroxy-2-oxo-2H-chromene-3-sulfonic acid (4-methoxy-phenyl)-amide* ***(a)***

***Preparation of*** N-[(4-Hydroxy-2-oxo-2H-chromen-3-yl)-(2-hydroxy-phenyl)-methylene]-acetamide **(b)**

In a 100 ml flask were mixed 3.5 g 4-hydroxy – Chomen- 2-one with 9ml $C_2H_5OH$, 4ml Aldehyd salicylic, 3g $CH_3COONH_4$ , 1ml HCl .The mixture

was refluxed at 100 °C for ca. 7h. The obtained yellow crystals are filtered and dried at room temperature. Recrystallization form $C_2H_5OH$ gave yellow crystals product of 78 % yield, meltingpoint, 319 °C.

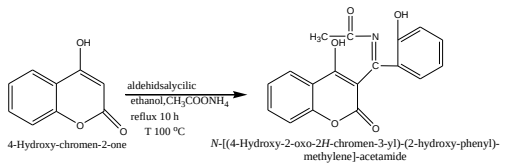


Figure.2 **Preparation of** N-{3-[Diethoxy-(2-hydroxy-phenyl)-methyl]-2-oxo-2H-chromen-4-yl}-acetamide **(b)**

***Preparation of*** 4-Hydroxy-3-[(6-nitro-benzothiazol-2-ylimino)-methyl]-chromen-2-one **(c)**

In a 100 ml flask were mixed 2.5g of 4-Hydroxy-chromen-2-one, 2g 2amino-6-nitro-benzotiazole, 8ml $C_2H_5OH$ ,with 4 ml HCOOH . The mixture was refluxed at 95 °C in water bath for ca.3 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 90% yield, melting.point;248 °C.

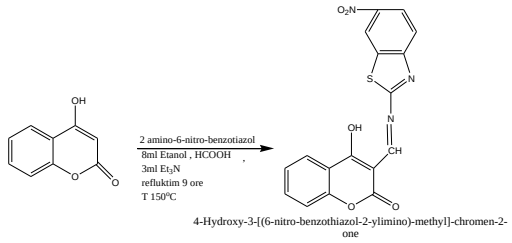


Figure. 3. **Preparation of** 4-Hydroxy-3-[(6-nitro-benzothiazol-2-ylimino)-methyl]-chromen-2-one **(c)**

**Table 1** Analytical data

| Compounds | Yeld % | m.p | M.F | Elemental analysis. Calculatet :Found (calc) % C | H | N | O | S |
|---|---|---|---|---|---|---|---|---|
| a | 70 | 310 °C | $C_{16}H_{12}ClNO_5S$ | 52.54<br>51.65 | 3.31<br>3.30 | 3.33<br>3.50 | 21.87<br>21.80 | 16.90<br>16.10 |
| b | 78 | 319 °C | $C_{22}H_{23}NO_6$ | 66.49<br>66.00 | 5.83<br>5,79 | 3.52<br>3.50 | 24.15<br>23.90 | |
| c | 90 | 248 °C | $C_{17}H_8N_3O_5S$ | 55.58<br>55.40 | 2.47<br>2.30 | 11.44<br>11.30 | 21.78<br>21.70 | |

***Antibacterial activity***

The purified synthesized compounds (a.b.c) 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 |
| a | 7 | 8,5 | 10,2 |
| b | 7,7 | 8 | 11,3 |
| c | 8,2 | 9 | 12,7 |
| | | | |
| | | | |
| Cephalexin | 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 |
| a | 10.4 | 13.7 | 18.8 |
| b | 11 | 18.8 | 21.5 |
| c | 10 | 19 | 21.0 |
| Cephalexin | 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 |
| a | 9.2 | 14.4 | 20.8 |
| b | 10.6 | 15.2 | 22.7 |
| c | 11 | 18.5 | 21.9 |
| | | | |
| | | | |
| Cephalexin | 9 | 9 | 9 - 10 µg |
| Streptomycine | 23 | 23 | 23 - 10 µg |

**Results and Discussion**

By reacting equimolar amounts of 4-hidroxyl-chromen-2-one and corresponding reagents (according scheme 1) under reflux reaction conditions product 1a is synthesized in 80 % yield.

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

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

**For (a); IR bands (KBr,cm-1)** 3428cm$^{-1}$ (N-H stretch.), 3000 cm$^{-1}$ (C-H aromatic) , 2800cm$^{-1}$ (C-H stretch.) , 1740 cm$^{-1}$ (C=0 stretch.)., 1600cm$^{-1}$(N-H); 1380cm$^{-1}$($SO_2Cl$);720cm$^{-1}$(C-H aromatic)

**$^1$H NMR (DMSO-d6) δppm** ;7.6-6.3 ( 6H aromatic) ;4.2 (H,NH) ; 3.73 (H,$CH_3$)

**$^{13}$ C NMR (DMSO) δppm** ; 162 (C,C=O) ; 163.0 ppm (C-NH) ; 150.8 (C-O); 152ppm(C-O) ;114-128.1ppm (6C aromatic);139.6ppm(C-N);56.0ppm(C,$CH_3$)

**For (b) IR bands (KBr,cm -1)** 3400 $cm^{-1}$ (OH stretch.) ; 3140 $cm^{-1}$ (C-H aromatic.) ; 2936$cm^{1}$ ( C-H alifatic .) ; 1680 $cm^{1}$ (C-O stretch.) ; 1740$cm^{-1}$(alfa pironi) ; 1380$cm^{-1}$(CONH);1220$cm^{-1}$(C-O) ; 1150 $cm^{-1}$(CO-N);748$cm^{-1}$(C-C aromatic)

**$^{1}$H NMR (DMSO-d6) δppm** 8.0 ppm(H,N-CO) 6.72-7.63 (8H aromatic );5.0(H,OH);3.41(4H,2CH);2.2 (3H,$CH_3$)

**$^{13}$C NMR (DMSO) δppm** 167.3 ppm (C-CONH ); 162 ppm (C,C=O); 156.4ppm (C-OH);150.8 ppm (C-O) ; 144.8(C-NH) ,54,6(C,$CH_2$) ,18.5 ppm (C,$CH_3$);15.4(C,$CH_3$);121.3-129.0(8C aromatic)

**For (c) IR bands (KBr,cm -1)** 3380 $cm^{-1}$ (N-H stretch.);2990cm-$^{1}$(C-H aromatic .); 2840$cm^{-1}$(C-H aldehyde) ; 1740$cm^{-1}$ (C=O); 1620$cm^{-1}$ (N-H ),1547$cm^{-1}$(C=N );1523(Ar-$NO_2$) ; 1256ppm(C-O stretch);720ppm(C-H aromatic);650 ppm (C-S)

**$^{1}$H NMR (DMSO-d6) δppm** 9.68ppm (H,CHO ), 7.20- 9.05 (7H aromatic );4.1(H,NH)

**$^{13}$CNMR (DMSO) δppm** 190.0ppm (C ,CHO) , 162ppm (C,C=O) ; 181.1ppm ( C,C-N) ; 174.5ppm (C-C=N) 150.8ppm (C,C-O) ; 121.3-126.6(10 C aromatic)

## Conclusion

From the results the following conclusion were drawn: The study provides the first evidence that compounds (a,b,c) obviously inhibit the growth of *S. auerus*, E.coli and *B. cereus*.

The compounds (a,b,c) compared with the antibacterial activity of Streptomycin in S.aureus ,E.coli and B.cereus.

This study provided the first evidence that these compounds a,b,c 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 reported data.

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## REFERENCES

[1] Lee.S.H , Shin, D.S, Kim.J.S , K.-B. Oh and Kan.S.S, Arch. Pharm. Res, 2003; (26).

[2] Vyas.K.B, NimavatK.S, Jani.G.R, and Hathi.M.V, Orbital, 2009; 1, (183).

[3] Abyshev.A.V, GimdeinV.A, SemenovE.V, E.MAgevE.M, Abdulla A.A, Zade and Gueseinov, Pharm.Chem.J., 2006; 40.(607).

[4] Aytemir.M.D , Hider.R.C , Erol.D.D,Ozalp.M and Ekizoglu.M, Turk.J.chem., 2003; 27 : (445).

[5] El.Saghier.M.M,Naili.M.B,Rammash.M.Kh,Saleh.N.H and Kreddan.K.M, Arkivoc, 2007;(83).

[6] Nofa.Z.M ,El-Zahar.M and Abd El Karim.M ,Molecules, 2000; 5: (99).

[7] Chaluvaraju .KC and Ishwarbhat , K.Asian ,J Chem 2008; 20, (4335).

[8] Ra Kali.R , S,Grworamma.J , and Suresh.B, Asian J Chem 2008: 20: (5289).

[9] Mohammed Ashraf.A and Mohammed.SH . Boorg Med Chem. Lett 2009; 17: (3314).

[10] Nofal .ZM, El-Zahar .M, Abd El-Karim .S, Novel coumarin derivatives with expected biological activity, Molecules. 2000; 5: (99- 113).

[11] Vyas .KB, Nimavat. KS, Jani .GR, Hathi. MV, Synthesis and microbial activity of coumarin derivatives metal complex: An in vitro evaluation.Orbital, 2009; 1: (183-192).

[12] A. A. Abou-Hussein, Wolfgang Linert, Synthesis, spectroscopic studies and inhibitory activity against bactria and fungi of acyclic and macrocyclic transition metal complexes containing a triamine coumarine Schiff base ligand Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, Volume 141, 15 April 2015, Pages 223-23.

[13] Danijela Lj Stojković, Verica V. Jevtić, Nenad Vuković, Milena Vukić, Srećko R. Trifunović, Synthesis, characterization, antimicrobial and antitumor reactivity of new palladium(II) complexes with methionine and tryptophane coumarine derivatives. Journal of Molecular Structure, Volume 1157, 5 April