Synthesis of some New Heteroarylamino-3-Nitro-2H-[1]-Benzopyran-2-ones and their Antibacterial Activity

Ramiz Hoti a\*, Idriz Vehapib, Gjyle Mulliqi-Osmanic, Hamit Ismaili and Veprim Thacia

*aFaculty of Nature Sciences – Department of Chemistry and bDepartment of Biology, University of Prishtina, “Mother Teresa” street, nn. 10000 Prishtina,*

*c Institute of Public Health of Kosovo, “Rrethi i Spitalit” nn. 10000 Prishtina, Kosovo*

 *aEmail:* *ramizhoti@yahoo.com*

*bEmail: ivehapi@yahoo.com*

**Abstract**

Novel derivatives of benzopyran-2-ones are synthesized by catalytic condensation reaction. 4-(3-Hydroxy-2-pyridinylamino)- 3-nitro-2H-[1]-benzopyran-2-one **4a**, 4-(4-methyl-3-nitro-2-pyridinylamino)- 3-nitro-2H-[1]-benzopyran-2-one, **4b**  and 4-(pyrimidinylamono)-3-nitro-2H-[1]-benzopyran-2-ones  **4(c-d )** are synthesized by condensation of 4-Chlor-3-nitro-2H-[1]-benzopyran-2-one  **2** and corresponding heteroarylamines  **3(a-d )** under reflux reaction conditions. Alkali hydrolysis of  **4(a-d )** afforded the 2-hydroxy-**ω** -nitroacetophenone  **5**. Structural characterization of the synthesized products is done on the basis of spectrometric data.Antibacterial activity of the compounds **4(a-d)** against *S. aureus*, *E. coli* and *Klebsiella* was examined by measuring the inhibition zones around the disks marked with the corresponding product solutions in N,N-DMF concentration 2 mg/mL , 4 mg/mL and 6 mg/mL.Compounds **4a** and **4c**  have shown significant antibacterial activity against *S. aureus*, compounds **4b** and **4d** exhibited significant activity against *E. coli* whereas compound **4d** was more active against *Klebsiella.*

***Keywords:*** Thiazolidin-4-one; benzopyran-2-one; condensiation; antibacterial; inhibition zones.

1. Introduction

Coumarine derivatives are very important natural extended compounds. Most of them are isolated from various plants [1,2] .They have been extensively investigated for a long time by many chemists [3,4]. Most of them have been reported [5,6] in the literature. Many of coumarinic analogues exhibited also antioxidant [7-9], anti-tubercular [10] and cytotoxic activity [11].They exhibit antifungal [12,13] , antibacterial [14] , antimicrobal [15-17] and antimalarial activity [18]. Many of coumarinic analogues exhibited also antioxidant [19,20], cytotoxic[21], anti-tubercular activity[22], sedative, analgesic and hepatoprotective [23-25] activity. For this reason, many of them have found widespread usage in pharmacies. Furthermore some of they exhibited both antibacterial and antioxidant activity[26]. Moreover the biological importance of coumarine derivatives resulted in much interest in their synthesis. Unfortunately isn’t known any general route for preparation of thesse derivates. According to that they are the object of studying and synthesis for many investigators.

In continuation of our previous studies on the synthesis of various coumarine derivatives by condensation reactions [27,28], in this study we report about preparing and structure analysing of some new substitued pyridinylamino- and pyrimidinylamino-2H-[1]-benzopyran-2-ones by condensation of 4-Chlor-3-nitro-2H-[1]-benzopyran-2 one and substituted pyridinyl- and pyrimidinylamines. In continuing alkali hydrolysis of synthetized productsis is described. Antibacterial activities of condensing products are also reported.

1. Methodsand materials

All experiments were carried out in acetonitrile as an aprotic solvent, under reflux reaction conditions. Fllowing of the reactions were monitored by TLC using Merck Kieselgel-60 (F-254) on a benzene : toluene : glac. acetic acid bath ( ratio 75 : 15 : 10 by volume, visualisation on a UW lamp ). Purification of products was done by recrystalisation from various solvents. Melting points were measured on a parafine bath in open capilary tubes and are uncorrected. 1H-NMR spectra were obtained in DMSO on UNITY plus-500 “NMR 1” Spectrometer. Chemical shifts are reported in parts per million (ppm) down field from tetramethylsilane as an internal standard (δ0,00). IR spectra were recorded in KBr discs on a Shimadzu FTIR 8400S Spectrometer with 4cm-1 resolution. Microanalyses were preformed on a Perkin-Elmer 240 B CHN analyser. Antibacterial activity of compounds were investiged applying the discs method ( d =5,5 mm, max. capacity 10 μg). The discs were wetted with N,N-DMF solutions of the synthetyzed compounds ( 2mg/ml, 4 mg/ml and 6 mg/ml ).

* 1. **4-Heteroarylamino-3-nitro-2H-[1]-benzopyran-2-ones 4(a-d) General procedure:**

In a typical reaction, 4-Chlor-3-nitro-2H-[1]-benzopyran-2-one **2** , equimolar amount of heteroaryalmine **3(a-d)** and catalytic amount of triethylamine in acetonitrile were refluxed on a water bath for 2 – 8 h. The mixture was filtered under vacuum and the crude product purified by crystalisation.

* 1. **4-(3-hydroxy-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one, 4a**

To a solution of 2- amino-3-hydroxypyridine **3a** ( 0,5g ; 4,6 mmol ) in acetonitrile ( 40 cm3 ), 0,2 cm3 of triethylamine was added. After that 0,27g , (1,2mmol ) of 4-Chlor-3-nitro-2H-[1]-benzopyran-2-one **2** was added. The reaction mixture was refluxed for 5 h under vigorously stirring and then monitored by TLC. After that the mixture was cooled and yellow-orange crystaline product was filtered under vacuum, then washed with a 2 cm3 portion of methanole. Crystalization of residue from methanole gave 0,97g (72%) product **4a. mp** = 208 – 210 ˚C. **IR:** 3399 cm-1, 3260 cm-1, 3071 cm-1, 1696 cm-1, 1612 cm-1, 1539 cm-1, 1459 cm-1, 1373 cm-1, 1296 cm-1, 1204 cm-1, 1120 cm-1, 805 cm-1, 762 cm-1, 605 cm-1.  **1H-NMR :** δ8,80 (s, 1H), δ 7,45 – 7,60 (m, 3H), δ 7,20 – 7,30 ( m, 4H), δ 5,5 ( s, 1H). **Anal:** *Calculated for C14H9N3O5:* (C, 56,17%), (H, 3,03%), (N, 14,04%), (O, 28,76%). *Found:* (C, 56,22%), (H, 3,04%), (N, 14,03%).

* 1. **4-(4-methyl-3-nitro-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one, 4b**

A mixture of 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one **2**  ( 0,5g, 2,26mmol ) and 4-methoxy-2-aminobenzothiazole  **3b** ( 0,35g, 2,26mmol ), containing triethylamine ( three drops) in acetonitrile ( 20 cm3 ) was refluxed under vigorously stirring for 8h. The mixture was cooled in an ice bath for 1h , then filtered under vacuum and washed with a portion of 1 cm3 of apsolut ethanole. Crystalization from methanole gave 0,4g (52%) of brown crystaline product **4b**. **mp**=240 -242 ˚C. **IR:** 3298 cm-1, 3186 - 3097 cm-1, 1715 cm-1, 1665 cm-1, 1625 cm-1, 1610 cm-1, 1548 cm-1 1529 cm-1, 1459 cm-11416 cm-1, 1368 cm-1, 1294 cm-1, 1243 cm-1, 1211 cm-1 1083 cm-1, 1005 cm-1 902 cm-1, 766 cm-1 672 cm-1, 550 cm-1. **1H-NMR :** δ9,20 (s, 1H), δ7,60 ( d, 2H ), δ7,40 – 7,25 (m, 4H ), δ2,10 ( s, 3H) . **Anal:** *Calculated for C15H10N4O6 :* (C, 51,12%), (H, 2,86%), (N, 15,91%), (O, 30,21%). *Found:* (C, 50,99%), (H, 2,92%), (N, 16,01%).

* 1. **4-(4-hydroxy-6-metyl-2-pyrimidinylamino)-3-nitro-2H-[1]-benzopyran-2-one, 4c**

A mixture of 4-Chlor-3-nitro-2H-1-benzopyran-2-one **2**  ( 0,22g, 1mmol ) and 2-amino-4-hydroxy-6-metylpyrimidine  **3c** ( 0,12g, 1mmol ), containing triethylamine ( two drops) in acetonitrile ( 10 cm3 ) was refluxed on a water bath . A CaCl2 guard tube was monted and the reaction mixture was alloved stirred under reflux for 2 h. then cooled to room temperature and the yellow crystaline product was formed. The mixture was filtered under vacuum. The residue washed with 1cm3 of acetonitrile. Recrystalisation from methanole gave 0,21g (68%) of yellow crystaline product **4d**. **mp** = 217˚C. **IR:** 3153 cm-1, 2997 cm-1, 2355 cm-1, 1712 cm-1, 1647 cm-1, 1598 cm-1, 1559 cm-1 1438 cm-1, 1354 cm-1, 1184 cm-1, 762 cm-1. **1H-NMR :** δ11,76 (s, 1H), δ 7,85 ( d, 1H), δ 7,82 (t, 1H), δ 7,35-7,44 ( m, 3H), δ 5,54 (s, 1H), δ 2,08 ( s,3H). **Anal:** Calculated for C14H10N4O5: (C, 53,49%), (H, 3,21%), (N, 17,83%). Found: (C, 53,66%), (H, 3,31%), (N, 17,92%).

* 1. **4-(6-hydroxy-2-mercapto-4-pyrimidinylamino)-3-nitro-2H-[1]-benzopyran-2-one, 4d**

To a 4-amino-6-hydroxy-2-mercaptopyrimidine monohydrat **3d** ( 0,22g, 1,36 mmol ) in 15 cm3 of acetonitrile solution, trietylamine (4 drops) and 4-Chlor-3-nitro-2H-[1]-benzopyran-2-one **2**  ( 0,3g, 1,36mmol ) was added. The mixture was heated slightly and reflucted for 12 h at 60 – 70 ˚C then cooled i a ice bath.The crude product was filtered under vacuum, washed with 2 x 1cm3 of acetonitrile and dried. Recrystalisation from Tetrehydrofurane gave 0,29g ( 66%) of crystaline product **4d**. **mp** = 202 – 204 ˚C. **IR:** 3425 cm-1, 3294 cm-1, 3186 cm-1, 3097 cm-1, 1715 cm-1, 1665 cm-1, 1610 cm-1 1548 cm-1, 1459 cm-1, 1368 cm-1, 1294 cm-1, 1181 cm-1, 902 cm-1 766 cm-1. **1H-NMR:** δ10,55 (s, 1H), δ7,80 ( d, 1H ), δ7,50 (t, 2H ), δ7,20 ( d, 2H) , δ7,10 ( s, 1H ), δ5,60 (s, 1H ), δ2,20 ( s, 1H). **Anal:**Calculated for C13H8N4O5S: (C, 46,98%), (H, 2,43%), (N, 16,86%), (O, 24,09%), (S, 9,64%). Found: (C, 47,11%), (H, 2,36%), (N, 16,67%), (S, 9,50%).

* 1. **2-Hydroxy-ω-nitroacetophenone 5**

# Heteroarylamino-2H-1-Benzopyran-2-ones 4a , 4b , 4c and 4d (2 mmol ) was disolved to a 10 cm3 5% natrium hyodoxide water solution and heated at 95˚C for 1 h. The reaction mixture was cooled and acidified with dil.hydrochloric acid and ice to pH=1. The crude product was filtered and washed with 3 x 2 cm3 of water.Recrystalisation from ethanole gave 0,3g, ( 84 %) of product **5**. **IR:** 3400 cm-1, 3085 cm-1, 2950 cm-1, 1637 cm-1, 1613 cm-1, 1560 cm-1, 1449 cm-1 1369 cm-1, 754 cm-1. **mp**=96˚C. **1H-NMR:** δ12,92 (s, 1H), δ11,41 ( s, 1H ), δ7,87 (d, 1H ), δ7,64 ( d, 1H), δ7,18 ( q, 2H ), δ 6,28 ( s, 2H ). **Anal:** Calculated for C8H7NO4: (C, 53,04%), (H, 3,89%), (N, 7,74%), (O, 35,32%). Found: (C, 52,94%), (H, 4,18%), (N, 7.72%).

1. Results and discussion

We previously reported that catalyst condensation of 4-Chlor-3-nitro-2H-[1]-benzopyran-2 one **2** with various heterocyclic amines gives corresponding 4-Heteroarylamino-3-nitro-2H-[1]-benzopyran-2-ones[35-37]**.** According to our investigation we now report that 4-Chlor-3-nitro-2H-[1]-benzopyran-2 one **2**  react readily with various heteroarylamines to form the corresponding 4-Heteroarylamino-3-nitro-2H-[1]-benzopyran-2-ones  **4(a-d).** By reacting equimolar amounts of 4-hydroxy-3-nitro-2H-[1]-benzopyran-2-one  **1**  with phosphooxychloride and N,N-dimethylformamide [38], 4-chlor-3-nitro-2H-[1]-benzopyran-2 one  **2**  were obtained in 92% yield. Thus product  **2**  vas subjected to condensation with various substituted pyridinyl- and pyrimidinylamines  **3(a-d)** in acetonitrile under reflux to yield the respective 4-Heteroarylamino-2H-[1]-benzopyran-2-ones **4(a-d)**, (scheme 1)**.**



###### Scheme 1

By condensation of **2** with 2-amino-3-hydroxypyridine **3a**, 4-(3-hydroxy-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one  **4a** is formed in 72% yield**.** By similar treatment of  **2**  with 2-amino-3-nitro-4-methylpyridine **3b**  under reflux in acetonitrile solution gave 4-(3-nitro-4-methyl-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one **4b** in 52***%*** yield.On the other hand compound  **2**  reacts with 2-amino-4-hydroxy-6-methylpyrimidine  **3c**  and 4-amino-6-hydroxy-2-mercaptopyrimidine  **3d**  in the presence of katalitic amount of triethylamine to afford 4-(4-hydroxy-6-methyl-2-pyrimidinylamino)-3-nitro-2H-[1]-benzopyran-2-one **4c** and 4-(-6-hydroxy-2-mercapto-4- pyrimidinylamino)-3-nitro-2H-[1]-benzopyran-2-one  **4d**  respective. By alkali hydrolysis of the products **4a** , **4b** , **4c** and **4d** , everyone gave 2-Hydroxy-ω-nitroacetophenone **5** . It is believe that formaton of product **5** folloved by tautomerisation of precursors resulting to imine formation, and imine hydrolysis and decarboxylation. The structure of products were determined from their IR, 1H-NMR and 13C –NMR spectra and their elemental analysis.

**3.1.** IR spectrum of **4a** showed the apsorption as a sharp peak at 3260 cm-1 responsible for υ NH stretching, and a band at about 3390 cm-1 characteristic for υ OH group of pyridine system. We may suppose that appearance of this absorption mode as a inflexive form may be as a consequence of possibility of intramolecular hydrogen bonding association of this group. The CH stretching vibration from aromatic ring were appeared at 3071 cm-1. A sharp peak at 1696 cm-1 and peaks at 1612 cm-1 and 1539 cm-1 responsible for υCO str., υC=N and υC=C (ar) were appeared. Two absorption at 1459 cm-1 and 1373 cm-1 attributable to υ NO2 (as) and υ NO2 (sym), and the δCH (out of plane) mode at 762 cm-1 also were observed. The υC-O of six-membered lactonic system is assigned at 1296 cm-1, whereas the mode at 1024 cm-1 resulted from υC-O of hydroxy group. The absorption mode at 1697 cm-1 responsible for υCO vibration were assigned to low frequencies, may be as a result of decreasing of the respective force constant and the bond order [39,40] .

**3.2.** The formation of **4b** is identified from 1H-NMR (DMSO) spectrum where are appeared the absorption as a multiplet at δ7,2-7,6 ppm ( responsible for aromatic protons ). The spectrum also displayed a singlet at δ2,1 ppm ( s, 3H, assigned for CH3 ), and a singlet at δ9,2 ppm (s. 1H responsible for NH proton apsorption). In the IR spectrum of **4b** a characteristic absorption appeared at 3294 cm-1 due to typical υNH stretching of secondary amines. IR spectrum of this product also showed the absorption modes at 3186 cm-1 and at 3097cm-1 responsible for υCH stretching absorption of aromatic ring and and υCH stretching of methyl group. An absorption at 1715 cm-1 attributable to typical υCO of unsaturated six-membered lactones was observed. Sharp peaks at 1665 cm-1 and 1625 cm-1 which are responsible for aromatic υC=N and υC=C vibration are also appeared. At 1548 cm-1 and 1294 cm-1 for stretching υNO2 (as) and υNO2 (sym), and at 766 cm-1 for bending δCH (ar) was also appeared.

**3.3.** Formation of **4c** is identified from 1H-NMR ( DMSO) spectrum where are appeared the absorption as a proton doublet at δ7,85 ppm responsible for H-5 and a proton triplet at δ7,82 ppm ( H-7). The spectrum also displayed a signal at δ11,76 ppm ( s, 1H, assigned for NH ), and a multiplet signal for aromatic H-6, H-8 and H-5’ protons at δ7,35-7,44 ppm. An absorption at δ5,54 ppm is appeared as function of hydroxy proton absorption and a proton singlet at δ 2,08 ppm resulted from methyl proton vibration. 13C-NMR spectrum of **4c** showed characteristic absorptions responsible for 14 carbon atoms. In the IR spectrum of **4c** a characteristic absorption appeared at 3447 cm-1 due to typical υNH stretching of secondary amines. IR spectrum of this product also showed the absorption modes at 3153 cm-1, 2997 cm-1 and 2841 cm-1 responsible for υOH stretching absorption, υCH stretching of aromatic ring and υCH stretching of methyl group. An absorption at 1712 cm-1 attributable to typical υCO of unsaturated lactone ring is observed. Signals which are responsible for aromatic υC=N and υC=C vibrations are appeared at 1647 cm-1 and 1598 cm-1. At 1517 cm-1 and 1354 cm-1 absorptions for the stretching υNO2 (as) and υNO2 (sym), and at 762 cm-1 for the bending δCH (oop) of aromatic system were also appeared.

**3.4.** IR spectrum of **4d** showed two absorptions at 3425 cm-1 and 3294 cm-1 responsible for υNH (str) and υOH (str) vibrations. Signals at 3186 cm-1 and at 3097 cm-1 are characteristic for stretching aromatic υCH (as) and υCH (sym) vibrations. A charp peak at 1715 cm-1 and peaks at 1665 cm-1 and 1610 cm-1 responsible for υCO str., υC=N and υ C=C (ar) were appeared. Two absorption at 1548 cm-1 and 1368 cm-1 attributable to υNO2 (as) and υNO2 (sym), and the δCH bending (oop) mode at 766 cm-1 also were observed.

**3.5.** The characteristic modes of product **5** appeared at 3080 – 3400cm-1 (broad band) and 2950 cm-1 which are responsible for υ OH stretching, υ OH (helat), aromatic υ CH and methylene υ CH absorptions. The characteristic peak derived from lactonic carbonyl as a result of intramolecular hydrogene bond is muved down at 1637 cm-1. IR spectrum of the hydrolysis product **5** also showed bands at 1560 cm-1 for υ C=C (ar), 1449 cm-1 for υ NO2 (as), 1369 cm-1 for υNO2 (sym) and 754 cm-1 aromatic δ CH out of plane. These values are compared with that ones from literature [41] and shown to be similar. In adition to that, the elementary analysis of obtained products **4 (a-d)** indicated in favour of described structures.

1. Antimicrobial activity

In contionuing to that we examined the antibacterial activity of synthetized compounds on the basis of Kirby-Bayer-s method [29]. Our investigation is directed toward testing their activity against Staphylococus aureus, Escherichia coli and Clebsiella. Applying the discs method we meassured diameters of the inhibition zone around discs which are previously wetted with N,N-DMF solutions of compounds, 2mg/ml, 4mg/ml and 6mg/ml. Obtained results are summarized bellow. From thesse observations resulted that these derivates were shown moderate to high activity against S. aureus, E. coli and Klebsiella. Compounds **4a** and **4c**  are more active against S. aureus. Emphatic activity against E. coli exhibited compound **4b**, whereas **4d** was more active against Klebsiella. Exept bactericide activity against S. aureus, compounds **4a** and **4c** as well vere apeared bacteriostatic activity in low concentrations. Moreower, bacteriostatic activity against Klebsiella exibited compound **4a** in low concentrations. In general increasing of concentration causes high activity against thesse microorganisma.



Fig. 1. Graphical presentation of inhibition zone diameter (mm) against *S. aureus.*



Fig. 2. Graphical presentation of inhibition zone diameter (mm) against *E. coli.*



Fig. 3. Graphical presentation of inhibition zone diameter (mm) against *klebsiella.*

1. Conclusions

Novel 4-substituted pyridinylamino- and pyrimidinylamino-3-nitro-2H-[1]-benzopyran-2-one derivates 4a-d are synthesized in the moderate and high yield via condensation reaction of 4-chloro-3-nitro-2H-[1]-benzopyran-2-one and corresponding heteroarylamines. From thesse resoults we may conclud that the tested compounds showed considerable activity against *S. aureus*, *E. coli* and *Klebsiella*. The compounds 4a and 4c express more emphatic activity against *S. Aureus*. Compounds 4b and 4d have indicate more antibacterial activity against *E. Coli*, whereas compounds 4d exhibited considerable activity against *Klebsiella*. In general, increasing the concentration of solutions, their antibacterial activity has increased.

1. References
2. F. M. Dean, *Naturally Occurring Oxygen Rong Compounds,* Butterworth, London, 1963.
3. D. M. X. Donnelly, and Boland, *in The Flavonoids*: Advances in Research since 1986, ed. J. B. Harborne, Chapman and Hall, London, 1994, 239 – 258.
4. [M. M. Darla](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-1), [B.S. Krishna](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-2), [K. U.Rao](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-3), [N. B. Reddy](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-4), [M. K. Srivash](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-5), [K. Adeppa](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-6), [Ch. S. Sundar](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-7), [C. S. Reddy](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-8), [K. Misra](http://link.springer.com/article/10.1007/s11164-013-1258-1#author-details-9), Synthesis and bio-evaluation of novel 7-hydroxy coumarin derivatives via Knoevenagel reaction, [*Research on Chemical Intermediates*](http://link.springer.com/journal/11164)*,* Vol. 41, [Issue 2](http://link.springer.com/journal/11164/41/2/page/1), pp 1115–1133, 2015.
5. [X. He](http://www.sciencedirect.com/science/article/pii/S0968089614003423)[a](http://www.sciencedirect.com/science/article/pii/S0968089614003423#af005), [Y. Y. Chen](http://www.sciencedirect.com/science/article/pii/S0968089614003423)[a](http://www.sciencedirect.com/science/article/pii/S0968089614003423#af005), [J. B. Shi](http://www.sciencedirect.com/science/article/pii/S0968089614003423), [W. J Tang](http://www.sciencedirect.com/science/article/pii/S0968089614003423), [Z. X. Pan](http://www.sciencedirect.com/science/article/pii/S0968089614003423), [Z. Q. Dong](http://www.sciencedirect.com/science/article/pii/S0968089614003423), [B. A. Song](http://www.sciencedirect.com/science/article/pii/S0968089614003423), [J. Li](http://www.sciencedirect.com/science/article/pii/S0968089614003423), , [X. H. Liu](http://www.sciencedirect.com/science/article/pii/S0968089614003423),New coumarin derivatives: Design, synthesis and use as inhibitors of hMAO, [*Bioorganic & Medicinal Chemistry*](http://www.sciencedirect.com/science/journal/09680896)*,* [Vol. 22, Issue 14](http://www.sciencedirect.com/science/journal/09680896/22/14), pp 3732–3738, 2014.
6. R.D.H. Murray, J.Hendez, S.A.Brown, *The Natural Coumarins,* Willey, New York, 1982.
7. [D. M. Manidhar](https://www.researchgate.net/profile/Darla_Manidhar), [R. Kesharwani](https://www.researchgate.net/profile/Rajesh_Kesharwani), [N. Bakthavatchala](https://www.researchgate.net/researcher/2027141602_N_Bakthavatchala_Reddy), [K. Misra](https://www.researchgate.net/profile/Krishna_Misra), Designing, synthesis, and characterization of some novel coumarin derivatives as probable anticancer drugs, *Med Chem Res.* 22: 4146–4157, 2013.
8. Y.K. Tyagi, A. Kumar, H.G. Raj, P. Vohra, G. Gupta, R.K. Gupta, Synthesis of novel amino and acetyl amino-4-methylcoumarins and evaluation of their antioxidant activity. *Eur. J. Med. Chem.* 40, 413–420, 2005.
9. [H. Osman](http://link.springer.com/search?facet-author=%22Hasnah+Osman%22), [A. Arshad](http://link.springer.com/search?facet-author=%22Afsheen+Arshad%22), [C. K. Lam](http://link.springer.com/search?facet-author=%22Chan+Kit+Lam%22), [M. C. Bagley](http://link.springer.com/search?facet-author=%22Mark+C+Bagley%22), Microwave-assisted synthesis and antioxidant properties of hydrazinyl thiazolyl coumarin derivatives, [*Chemistry Central Journal*](http://link.springer.com/journal/13065)**,** 6:32, 1186/1752-153X, 2012.
10. [S.V. Rodriguez](http://pubs.rsc.org/en/results?searchtext=Author%3ASaleta%20Vazquez-Rodriguez),  [R. F.Guinez](http://pubs.rsc.org/en/results?searchtext=Author%3ARoberto%20Figueroa-Gu%C3%AD%C3%B1ez),  [M. J. Matos](http://pubs.rsc.org/en/results?searchtext=Author%3AMaria%20Jo%C3%A3o%20Matos),   [L. Santana](http://pubs.rsc.org/en/results?searchtext=Author%3ALourdes%20Santana),   [E. Uriarte](http://pubs.rsc.org/en/results?searchtext=Author%3AEugenio%20Uriarte),  [M. Lapier](http://pubs.rsc.org/en/results?searchtext=Author%3AMichel%20Lapier),   [J. D. Maya](http://pubs.rsc.org/en/results?searchtext=Author%3AJuan%20Diego%20Maya),  [C. O. Azar](http://pubs.rsc.org/en/results?searchtext=Author%3AClaudio%20Olea-Azar),  Synthesis of coumarin–chalcone hybrids and evaluation of their antioxidant and trypanocidal properties, ***Med. Chem. Commun*., 4**, 993-1000. 2013.
11. D. Yu, M. Suzuki, L. Xie, S.L. Natschke, K.H. Lee, Recent progress in the development of coumarin derivatives as potent anti-HIV agents. *Med. Res. Reiv.,* 23, 322–345, 2003.
12. J. N. Modranka, E. Nawrot, J. Graczik, In vivo antitumor, in vitro anti- bacterial activity and alkylating properties of phosphorohydrazine derivatives of coumarin and chromone. *Eur. J. Med. Chem.*, 41 1301–1309, 2006.
13. R. S. [Araújo](http://www.ncbi.nlm.nih.gov/pubmed?term=de%20Ara%C3%BAjo%20RS%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), F. Q. [Guerra](http://www.ncbi.nlm.nih.gov/pubmed?term=Guerra%20FQ%5BAuthor%5D&cauthor=true&cauthor_uid=23306152),  [E. O. Lima](http://www.ncbi.nlm.nih.gov/pubmed?term=de%20O%20Lima%20E%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), C. A. [Simone](http://www.ncbi.nlm.nih.gov/pubmed?term=de%20Simone%20CA%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), J. F. [Tavares](http://www.ncbi.nlm.nih.gov/pubmed?term=Tavares%20JF%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), L. [Scotti](http://www.ncbi.nlm.nih.gov/pubmed?term=Scotti%20L%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), M. T. [Scotti](http://www.ncbi.nlm.nih.gov/pubmed?term=Scotti%20MT%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), T. M. [Aquino](http://www.ncbi.nlm.nih.gov/pubmed?term=de%20Aquino%20TM%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), R. O. [Moura](http://www.ncbi.nlm.nih.gov/pubmed?term=de%20Moura%20RO%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), F. J. [Mendonça](http://www.ncbi.nlm.nih.gov/pubmed?term=Mendon%C3%A7a%20FJ%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), J. M. [Barbosa-Filho](http://www.ncbi.nlm.nih.gov/pubmed?term=Barbosa-Filho%20JM%5BAuthor%5D&cauthor=true&cauthor_uid=23306152), Synthesis, Structure-Activity Relationships (SAR) and in Silico Studies of Coumarin Derivatives with Antifungal Activity*,* [*Int J Mol Sci.*](http://www.ncbi.nlm.nih.gov/pubmed/23306152)  10;14(1):1293-309, 2013.
14. M. Mazzei, E. Nieddu, M. Miele, A. Balbi, M. Ferrone, M. Fermeglia, et al., Synthesis of Mannich bases of 7-hydroxycoumarin and screened against Fla- viviridae, *Bioorg. Med. Chem.* 16 2591–2605, 2008.
15. S. A. Mayekar, V. V. Mulwad, Synthesis and antibacterial activity of 6-(5phenyl- {1,3,4}thiadiazol-2-ylimino)-benzopyran-2-ones. *Indian J. Chem.* 47 (B) 1438–1442, 2008.
16. N. C. Desai, H. M. Satodiya, K. M. Rajpara, V. V. Joshi, H. V. Vaghani, Microwave assisted synthesis of new coumarin based 3-cyanopyridine scaffolds bearing sulfonamide group having antimicrobial activity, *Indian Journal of Chemistry,* 52B, 904-914, 2013.
17. [S. Rehman](http://link.springer.com/search?facet-author=%22Sadia+Rehman%22), [M. Ikram](http://link.springer.com/search?facet-author=%22Muhammad+Ikram%22), [R. J. Baker](http://link.springer.com/search?facet-author=%22Robert+J+Baker%22), [M. Zubair](http://link.springer.com/search?facet-author=%22Muhammad+Zubair%22), [E. Azad](http://link.springer.com/search?facet-author=%22Effat+Azad%22), [S. Min](http://link.springer.com/search?facet-author=%22Soyoung+Min%22), [K. Riaz](http://link.springer.com/search?facet-author=%22Kashif+Riaz%22), [K. H. Mok](http://link.springer.com/search?facet-author=%22KH+Mok%22), [S. Rehman](http://link.springer.com/search?facet-author=%22Saeed-Ur+Rehman%22), Synthesis, characterization, in vitro antimicrobial, and U2OS tumoricidal activities of different coumarin derivatives, [*Chemistry Central Journal*](http://link.springer.com/journal/13065)*,* 7:68, [1186/1752-153X](http://ccj.springeropen.com/articles/10.1186/1752-153X-7-68), 2013.
18. S. A. Mayekar, V. V. Mulwad, Synthesis and antibacterial activity of 6-(5phenyl- {1,3,4}thiadiazol-2-ylimino)-benzopyran-2-ones, *Indian J. Chem.* 47 (B) 1438–1442, 2008.
19. K. V. [Sashidhara](http://www.ncbi.nlm.nih.gov/pubmed?term=Sashidhara%20KV%5BAuthor%5D&cauthor=true&cauthor_uid=22607674), A. [Kumar](http://www.ncbi.nlm.nih.gov/pubmed?term=Kumar%20A%5BAuthor%5D&cauthor=true&cauthor_uid=22607674), R. P. [Dodda](http://www.ncbi.nlm.nih.gov/pubmed?term=Dodda%20RP%5BAuthor%5D&cauthor=true&cauthor_uid=22607674), N. N. [Krishna](http://www.ncbi.nlm.nih.gov/pubmed?term=Krishna%20NN%5BAuthor%5D&cauthor=true&cauthor_uid=22607674), P. [Agarwal](http://www.ncbi.nlm.nih.gov/pubmed?term=Agarwal%20P%5BAuthor%5D&cauthor=true&cauthor_uid=22607674), K. [Srivastava](http://www.ncbi.nlm.nih.gov/pubmed?term=Srivastava%20K%5BAuthor%5D&cauthor=true&cauthor_uid=22607674), S. K. [Puri](http://www.ncbi.nlm.nih.gov/pubmed?term=Puri%20SK%5BAuthor%5D&cauthor=true&cauthor_uid=22607674), Coumarin-trioxane hybrids: synthesis and evaluation as a new class of antimalarial scaffolds, [*Bioorg Med Chem Lett*.](http://www.ncbi.nlm.nih.gov/pubmed/22607674)  15;22(12):3926-3930, 2012.
20. [H. Osman](http://link.springer.com/search?facet-author=%22Hasnah+Osman%22), [A. Arshad](http://link.springer.com/search?facet-author=%22Afsheen+Arshad%22), [C. K. Lam](http://link.springer.com/search?facet-author=%22Chan+Kit+Lam%22), [M. C. Bagley](http://link.springer.com/search?facet-author=%22Mark+C+Bagley%22), Microwave-assisted synthesis and antioxidant properties of hydrazinyl thiazolyl coumarin derivatives, [*Chemistry Central Journal*](http://link.springer.com/journal/13065)***,*** 6:32, 1186/1752-153X, 2012.
21. [S. V. Rodriguez](http://pubs.rsc.org/en/results?searchtext=Author%3ASaleta%20Vazquez-Rodriguez),  [R. F. Guinez](http://pubs.rsc.org/en/results?searchtext=Author%3ARoberto%20Figueroa-Gu%C3%AD%C3%B1ez),  [M. J. Matos](http://pubs.rsc.org/en/results?searchtext=Author%3AMaria%20Jo%C3%A3o%20Matos),   [L. Santana](http://pubs.rsc.org/en/results?searchtext=Author%3ALourdes%20Santana),   [E. Uriarte](http://pubs.rsc.org/en/results?searchtext=Author%3AEugenio%20Uriarte),  [M. Lapier](http://pubs.rsc.org/en/results?searchtext=Author%3AMichel%20Lapier),   [J. D. Maya](http://pubs.rsc.org/en/results?searchtext=Author%3AJuan%20Diego%20Maya),  [C. O. Azar](http://pubs.rsc.org/en/results?searchtext=Author%3AClaudio%20Olea-Azar),  Synthesis of coumarin–chalcone hybrids and evaluation of their antioxidant and trypanocidal properties, ***Med. Chem. Commun.,* 4**, 993-1000, 2013.
22. J. Nawrot-Modranka, E. Nawrot, J. Graczik, In vivo antitumor, in vitro anti- bacterial activity and alkylating properties of phosphorohydrazine derivatives of coumarin and chromone. *Eur. J. Med. Chem. 41* 1301–1309, 2006.
23. D. Yu, M. Suzuki, L. Xie, S. L. Natschke, K. H. Lee, Recent progress in the development of coumarin derivatives as potent anti-HIV agents. *Med. Res. Reiv.* 23 322–345, 2003.
24. M. [Atmaca](http://www.ncbi.nlm.nih.gov/pubmed?term=Atmaca%20M%5BAuthor%5D&cauthor=true&cauthor_uid=21656273), H. M. [Bilgin](http://www.ncbi.nlm.nih.gov/pubmed?term=Bilgin%20HM%5BAuthor%5D&cauthor=true&cauthor_uid=21656273), B. D. [Obay](http://www.ncbi.nlm.nih.gov/pubmed?term=Obay%20BD%5BAuthor%5D&cauthor=true&cauthor_uid=21656273), H. [Diken](http://www.ncbi.nlm.nih.gov/pubmed?term=Diken%20H%5BAuthor%5D&cauthor=true&cauthor_uid=21656273), M. [Kelle](http://www.ncbi.nlm.nih.gov/pubmed?term=Kelle%20M%5BAuthor%5D&cauthor=true&cauthor_uid=21656273), E. [Kale](http://www.ncbi.nlm.nih.gov/pubmed?term=Kale%20E%5BAuthor%5D&cauthor=true&cauthor_uid=21656273), The hepatoprotective effect of coumarin and coumarin derivates on carbon tetrachloride-induced hepatic injury by antioxidative activities in rats. [*J Physiol Biochem.*](http://www.ncbi.nlm.nih.gov/pubmed/21656273) 67(4), 569-76, 2011.
25. B. [Ahmed](http://www.ncbi.nlm.nih.gov/pubmed?term=Ahmed%20B%5BAuthor%5D&cauthor=true&cauthor_uid=12685811), S. A. [Khan](http://www.ncbi.nlm.nih.gov/pubmed?term=Khan%20SA%5BAuthor%5D&cauthor=true&cauthor_uid=12685811), T. [Alam](http://www.ncbi.nlm.nih.gov/pubmed?term=Alam%20T%5BAuthor%5D&cauthor=true&cauthor_uid=12685811), Synthesis and antihepatotoxic activity of some heterocyclic compounds containing the 1,4-dioxane ring system. [*Pharmazie*](http://www.ncbi.nlm.nih.gov/pubmed/12685811)*,* 58(3), 173-176, 2003.
26. T. Okamoto, T. Kobayashi, S. Yoshida, Synthetic Derivatives of Osthole for the Prevention of Hepatitis, *Medicinal Chemistry,* 3, 35-44, 2007.
27. S. Rajasekaran, G. K. Rao, S. Pai, A. Rajan, Design, synthesis, antibacterial and in vitro antioxidant activity of substituted 2H-benzopyran-2-one derivatives*, International Journal of ChemTech Research*, 3, 2, 555-559, 2011.
28. R. Hoti, V. Kalaj, I. Vehapi,H. Ismail**i,** V. Thaçi, M. Bicaj, Novel Pyrimidin-2-yl-benzylidene imines and 2-[(pyrimidin-ylimino)-methyl]-phenoles and their antibacterial activity, *The FASEB Journal,Experimental Biology,* 2010, lb487, Anaheim Ca. Apr. 2010,
29. R. Hoti, A. Nura-Lama, Gj. Mulliqi-Osmani, N. Tronia, F. Gashi, H. Ismaili V. Thaçi, Synthesis of 4-Triazolylamino- and 4-Benzothiazolylamino-3- nitro-2H-[1]-Benzopyran-2-ones and their Antimicrobial Activity, *Orbital: The Electronic Journal of Chemistry,* Vol. 6, No.3, July-September, 2014.
30. Bauer, A. W. et al. , Antibiotic suscepetibility testing by standardized single disc method, *American journal of clinical pathology,* 44, , 493: 496, 1966.