

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

Ramiz Hoti^a, Idriz Vehapi^b, Gjyle Mulliqi-Osmani^c, Hamit Ismaili^{d*}, Veprim Thaci^e

^{a,d,e}Faculty of Nature Sciences – Department of Chemistry.

^bDepartment of Biology, University of Prishtina, “Mother Teresa” street, nn. 10000 Prishtina.

^cInstitute 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; condensation; antibacterial; inhibition zones.

* Corresponding author.

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 exhibited antifungal [12,13], antibacterial [14], antimicrobial [15-17] and antimalarial activity [18]. Some of substituted coumarinic analogues with expressed antioxidant [19,20], cytotoxic [21], anti-tubercular [22], sedative, analgesic and hepatoprotective [23-25] activity are reported also. For this reason, many of them have found widespread usage in pharmacies. Furthermore some of the 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 these derivatives. 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 structural characterization of some new substituted pyridinylamino- and pyrimidinylamino-2H-[1]-benzopyran-2-ones by condensation of 4-Chloro-3-nitro-2H-[1]-benzopyran-2 one and substituted pyridinyl- and pyrimidinylamines. In continuing, alkali hydrolysis of the synthesized products is described. Antibacterial activity of condensing products are also reported.

2. Methods and materials

All experiments were carried out in acetonitrile as an aprotic solvent, under reflux reaction conditions. Following of the reactions were monitored by TLC using Merck Kieselgel-60 (F-254) on a benzene: toluene: glacial acetic acid bath (ratio 75 : 15 : 10 by volume, visualization on a UV lamp). Purification of products was done by crystallization from various solvents. Melting points were measured on a paraffin bath in open capillary tubes and values 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) downfield from tetramethylsilane as an internal standard (δ, 0.00). IR spectra were recorded in KBr discs on a Shimadzu FTIR 8400S Spectrometer with 4 cm⁻¹ resolution. Microanalyses were performed on a Perkin-Elmer 240 B CHN analyser. Antibacterial activity of compounds were investigated applying the discs method (d = 5.5 mm, max. capacity 10 µg). The discs were wetted with N,N-DMF solutions of the synthesized compounds (2 mg/mL, 4 mg/mL and 6 mg/mL).

2.1. 4-Heteroarylamino-3-nitro-2H-[1]-benzopyran-2-ones **4(a-d)** General procedure

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

2.2. 4-(3-hydroxy-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one, **4a**

To a solution of 2-amino-3-hydroxypyridine **3a** (0.5 g; 4.6 mmole) in acetonitrile (40 mL), 0.2 mL of

triethylamine was added. After that 0,27g , (1,2mmole) of 4-Chloro-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 crystalline product was filtered under vacuum, then washed with a 2 mL portion of methanol. Crystallization of residue from methanol 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%).

2.3. 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** (0,5 g, 2,26 mmole) and 4-methoxy-2-aminobenzothiazole **3b** (0,35 g, 2,26 mmole), containing triethylamine (three drops) in acetonitrile (20 mL) was refluxed under vigorously stirring for 8 h. The mixture was cooled in an ice bath for 1 h, then filtered off under vacuum and washed with a portion of 1 mL of absolute ethanol. Crystallization from methanol gave 0,4 g (52%) of brown crystalline 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%).

2.4. 4-(4-hydroxy-6-metyl-2-pyrimidinylamino)-3-nitro-2H-[1]-benzopyran-2-one, **4c**

A mixture of 4-Chloro-3-nitro-2H-1-benzopyran-2-one **2** (0,22g, 1 mmole) and 2-amino-4-hydroxy-6-methylpyrimidine **3c** (0,12 g, 1 mmole), containing triethylamine (two drops) in acetonitrile (10 mL) was refluxed on a water bath. A CaCl₂ guard tube was mounted and the reaction mixture was allowed to stir under reflux for 2 h, then cooled to room temperature and the yellow crystalline product was formed. The mixture was filtered under vacuum. The residue washed with 1mL of acetonitrile. Crystallization from methanol gave 0,21g (68%) of yellow crystalline 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%).

2.5. 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 mmole) in 15 mL of acetonitrile solution, triethylamine (4 drops) and 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one **2** (0,3g, 1,36 mmole) was added. The mixture was heated slightly and refluxed for 12 h at 60 – 70 °C then cooled in an ice bath. The crude product was filtered under vacuum, washed with 2 x 1 mL of acetonitrile and dried. Crystallization from tetrahydrofuran gave 0,29 g (66%) of crystalline 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} . **$^1\text{H-NMR}$** : δ 10,55 (s, 1H), δ 7,80 (d, 1H), δ 7,50 (t, 2H), δ 7,20 (d, 2H), δ 7,10 (s, 1H), δ 5,60 (s, 1H), δ 2,20 (s, 1H). **Anal**: Calculated for $\text{C}_{13}\text{H}_8\text{N}_4\text{O}_5\text{S}$: (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%).

2.6. 2-Hydroxy-*o*-nitroacetophenone **5**

Heteroaryl-amino-2H-1-Benzopyran-2-ones **4a**, **4b**, **4c** and **4d** (2 mmole) was dissolved in 10 mL 5% sodium hydroxide water solution and heated at 95°C for 1 h. The reaction mixture was cooled and acidified with dil. hydrochloric acid and ice to $\text{pH} = 1$. The crude product was filtered off and washed with 3 x 2 mL of water. Crystallization from ethanol gave 0,3 g, (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. **$^1\text{H-NMR}$** : δ 12,92 (s, 1H), δ 11,41 (s, 1H), δ 7,87 (d, 1H), δ 7,64 (d, 1H), δ 7,18 (q, 2H), δ 6,28 (s, 2H). **Anal**: Calculated for $\text{C}_8\text{H}_7\text{NO}_4$: (C, 53,04%), (H, 3,89%), (N, 7,74%), (O, 35,32%). Found: (C, 52,94%), (H, 4,18%), (N, 7,72%).

3. Results and discussion

We previously reported that catalyst condensation of 4-Chloro-3-nitro-2H-[1]-benzopyran-2 one **2** with various heterocyclic amines gives corresponding 4-Heteroaryl-amino-3-nitro-2H-[1]-benzopyran-2-ones^[27, 28]. According to our investigation we now report that 4-Chloro-3-nitro-2H-[1]-benzopyran-2 one **2** react readily with various heteroaryl amines to form the corresponding 4-Heteroaryl-amino-3-nitro-2H-[1]-benzopyran-2-ones **4(a-d)**. By reacting of equimolar amounts of 4-hydroxy-3-nitro-2H-[1]-benzopyran-2-one **1**, phosphoorychloride and N,N-dimethylformamide^[38], 4-chloro-3-nitro-2H-[1]-benzopyran-2 one **2** was obtained in 92% yield. Thus product **2** was subjected to condensation with various substituted pyridinyl- and pyrimidinylamines **3(a-d)** in acetonitrile under reflux to yield the respective 4-Heteroaryl-amino-2H-[1]-benzopyran-2-ones **4(a-d)**, (scheme 1).

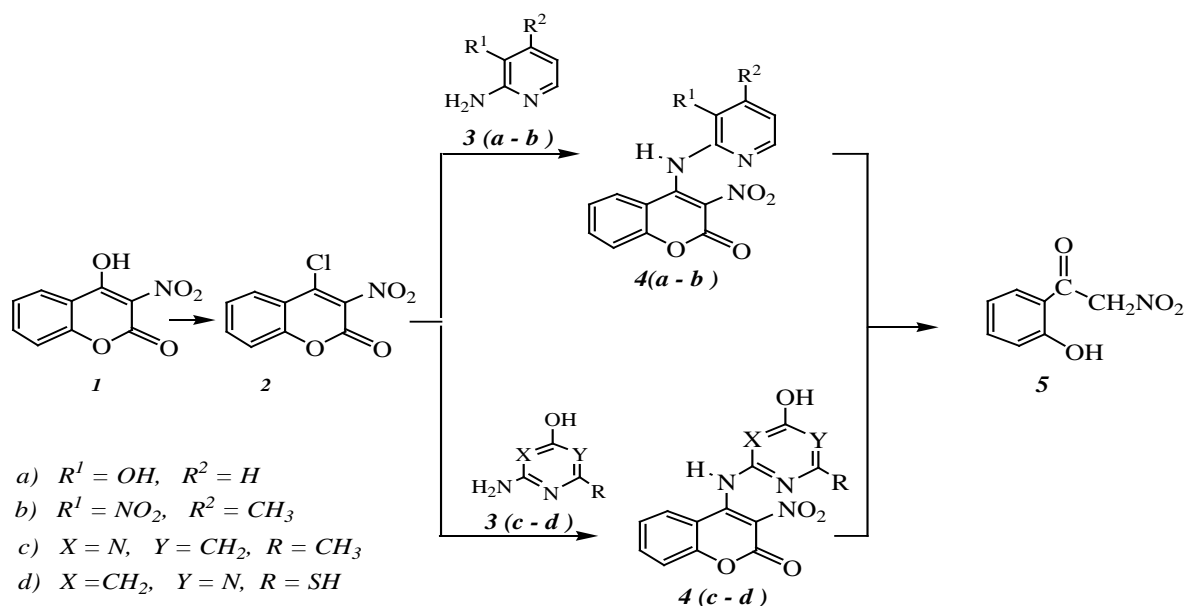


Figure 4

By condensation of **2** and 2-amino-3-hydroxypyridine **3a**, 4-(3-hydroxy-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one **4a** is obtained in 72% yield. By similar treatment of **2** and 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 catalytic 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 the formaton of product **5** followed by tautomerization of precursors resulting to imine formation, and in next step by imine hydrolysis and decarboxylation. The structure of the products were determined from their IR, $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra and their elemental analysis.

3.1. IR spectrum of **4a** showed the apsrption as a sharp peak at 3260 cm^{-1} responsible for νNH stretching, and a band at about 3390 cm^{-1} characteristic for νOH 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 νCH 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 νCO str., $\nu\text{C=N}$ and $\nu\text{C=C}$ (ar) were appeared. Two absorption at 1459 cm^{-1} and 1373 cm^{-1} attributable to νNO_2 (as) and νNO_2 (sym), and the δCH (out of plane) mode at 762 cm^{-1} also were observed. The $\nu\text{C-O}$ of six-membered lactonic system is assigned at 1296 cm^{-1} , whereas the mode at 1024 cm^{-1} resulted from $\nu\text{C-O}$ of hydroxy group. The absorption mode at 1697 cm^{-1} responsible for νCO vibration was assigned to low frequencies, may be as a result of decreasing of the respective force constant and the bond order.

3.2. The formation of **4b** is identified from $^1\text{H-NMR}$ (DMSO) spectrum where is appeared the absorption as a multiplet at $\delta 7,2-7,6\text{ ppm}$ (responsible for aromatic protons). The spectrum also displayed a singlet at $\delta 2,1\text{ ppm}$ (s, 3H, assigned for CH_3), and a singlet at $\delta 9,2\text{ ppm}$ (s, 1H responsible for NH proton apsrption). In the IR spectrum of **4b** a characteristic absorption appeared at 3294 cm^{-1} due to typical νNH stretching of secondary amines. IR spectrum of this product also showed the absorption modes at 3186 cm^{-1} and at 3097 cm^{-1} responsible for νCH stretching absorption of aromatic ring and νCH stretching of methyl group. An absorption at 1715 cm^{-1} attributable to typical νCO of unsaturated six-membered lactones was observed. The sharp peaks at 1665 cm^{-1} and 1625 cm^{-1} which are responsible for aromatic $\nu\text{C=N}$ and $\nu\text{C=C}$ vibration are also appeared. At 1548 cm^{-1} and 1294 cm^{-1} for stretching νNO_2 (as) and νNO_2 (sym), and at 766 cm^{-1} for bending δCH (ar) was also appeared.

3.3. Formation of **4c** is identified from $^1\text{H-NMR}$ (DMSO) spectrum where the absorption as a proton doublet at $\delta 7,85\text{ ppm}$ responsible for H-5 and a proton triplet at $\delta 7,82\text{ ppm}$ (H-7) is appeared. The spectrum also displayed a signal at $\delta 11,76\text{ ppm}$ (s, 1H, assigned for NH), and a multiplet signal for aromatic H-6, H-8 and H-5' protons at $\delta 7,35-7,44\text{ ppm}$. A signal at $\delta 5,54\text{ ppm}$ is appeared as function of hydroxy proton absorption and a proton singlet at $\delta 2,08\text{ ppm}$ resulted from methyl proton vibration. $^{13}\text{C-NMR}$ spectrum of **4c** showed characteristic absorptions responsible for respective 14 carbon atoms. In the IR spectrum of **4c** a characteristic absorption at 3447 cm^{-1} due to typical νNH stretching of secondary amines is appeared. IR spectrum of this

product also showed the absorption modes at 3153 cm^{-1} , 2997 cm^{-1} and 2841 cm^{-1} responsible for νOH stretching absorption, νCH stretching of aromatic ring and νCH stretching of methyl group. An absorption at 1712 cm^{-1} attributable to typical νCO of unsaturated lactonic ring is observed. Signals which are responsible for aromatic $\nu\text{C}=\text{N}$ and $\nu\text{C}=\text{C}$ vibrations are appeared at 1647 cm^{-1} and 1598 cm^{-1} . At 1517 cm^{-1} and 1354 cm^{-1} absorptions for the stretching νNO_2 (as) and νNO_2 (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 νNH (str) and νOH (str) vibrations. Signals at 3186 cm^{-1} and at 3097 cm^{-1} are characteristic for stretching aromatic νCH (as) and νCH (sym) vibrations. A sharp peak at 1715 cm^{-1} and peaks at 1665 cm^{-1} and 1610 cm^{-1} responsible for νCO str., $\nu\text{C}=\text{N}$ and $\nu\text{C}=\text{C}$ (ar) were appeared. Two absorption at 1548 cm^{-1} and 1368 cm^{-1} attributable to νNO_2 (as) and νNO_2 (sym), and the δCH bending (oop) mode at 766 cm^{-1} also were observed.

3.5. The characteristic modes of product **5** appeared at $3080 - 3400\text{ cm}^{-1}$ (a broad band) and 2950 cm^{-1} which are responsible for νOH stretching, νOH (chelate), aromatic νCH and methylene νCH absorptions. The characteristic peak derived from lactonic carbonyl as a result of intramolecular hydrogen bonding is moved down at 1637 cm^{-1} . IR spectrum of the hydrolysis product **5** also showed the bands at 1560 cm^{-1} for $\nu\text{C}=\text{C}$ (ar), 1449 cm^{-1} for νNO_2 (as), 1369 cm^{-1} for νNO_2 (sym) and 754 cm^{-1} aromatic δCH out of plane. In addition to that, the elementary analysis of obtained products **4 (a-d)** indicated in favour of described structures.

4. Antimicrobial activity

In continuation to that we examined the antibacterial activity of synthesized compounds on the basis of Kirby-Bauer's method [29]. Our investigation is directed toward testing their activity against *S. aureus*, *E. coli* and *Klebsiella*. Applying the discs method we measured diameters of the inhibition zone around discs which are previously marked with N,N-DMF solutions of compounds, 2mg/mL, 4mg/mL and 6mg/mL. Obtained results are summarized below. From these observations resulted that these derivatives 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*. Except bactericide activity against *S. aureus*, compounds **4a** and **4c** as well appeared bacteriostatic activity in low concentrations. Moreover, bacteriostatic activity against *Klebsiella* exhibited compound **4a** in low concentrations. In general increasing of concentration causes high activity against these microorganisms.

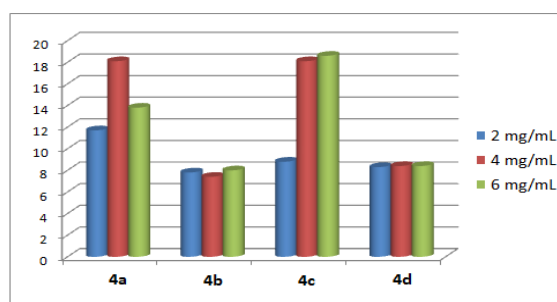


Figure 1: Graphical presentation of inhibition zone diameter (mm) against *S. aureus*.

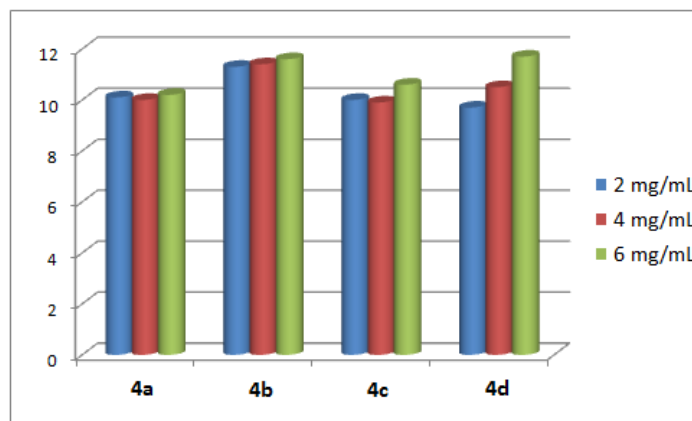


Figure 2: Graphical presentation of inhibition zone diameter (mm) against *E. coli*.

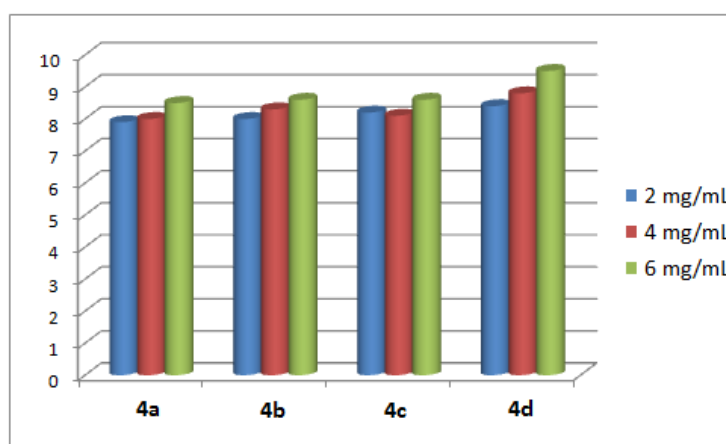


Figure 3: Graphical presentation of inhibition zone diameter (mm) against *Klebsiella*.

5. Conclusions

Novel 4-substituted pyridinylamino- and pyrimidinylamino-3-nitro-2H-[1]-benzopyran-2-one derivatives **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 these results we may conclude that the tested compounds showed considerable activity against *S. aureus*, *E. coli* and *Klebsiella*. The compounds **4a** and **4c** expressed emphatic activity against *S. Aureus*. Compounds **4b** and **4d** have showed 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.

References

- [1] F. M. Dean, Naturally Occurring Oxygen Rong Compounds, Butterworth, London, 1963.
- [2] 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.
- [3] M. M. Darla, B.S. Krishna, K. U.Rao, N. B. Reddy, M. K. Srivash, K. Adeppa, Ch. S. Sundar,

- C. S. Reddy, K. Misra, Synthesis and bio-evaluation of novel 7-hydroxy coumarin derivatives via Knoevenagel reaction, *Research on Chemical Intermediates*, Vol. 41, Issue 2, pp 1115–1133, 2015.
- [4] X. He^a, Y. Y. Chen^a, J. B. Shi, W. J Tang, Z. X. Pan, Z. Q. Dong, B. A. Song, J. Li^a, X. H. Liu, New coumarin derivatives: Design, synthesis and use as inhibitors of hMAO, *Bioorganic & Medicinal Chemistry*, Vol. 22, Issue 14, pp 3732–3738, 2014.
- [5] R.D.H. Murray, J.Hendez, S.A.Brown, *The Natural Coumarins*, Willey, New York, 1982.
- [6] D. M. Manidhar, R. Kesharwani, N. Bakthavatchala, K. Misra, Designing, synthesis, and characterization of some novel coumarin derivatives as probable anticancer drugs, *Med Chem Res.* 22: 4146–4157, 2013.
- [7] 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.
- [8] H. Osman, A. Arshad, C. K. Lam, M. C. Bagley, Microwave-assisted synthesis and antioxidant properties of hydrazinyl thiazolyl coumarin derivatives, *Chemistry Central Journal*, 6:32, 1186/1752-153X, 2012.
- [9] S.V. Rodriguez, R. F.Guinez, M. J. Matos, L. Santana, E. Uriarte, M. Lapier, J. D. Maya, C. O. Azar, Synthesis of coumarin–chalcone hybrids and evaluation of their antioxidant and trypanocidal properties, *Med. Chem. Commun.*, 4, 993-1000. 2013.
- [10] 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.
- [11] 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.
- [12] R. S. Araújo, F. Q. Guerra, E. O. Lima, C. A. Simone, J. F. Tavares, L. Scotti, M. T. Scotti, T. M. Aquino, R. O. Moura, F. J. Mendonça, J. M. Barbosa-Filho, Synthesis, Structure-Activity Relationships (SAR) and in Silico Studies of Coumarin Derivatives with Antifungal Activity, *Int J Mol Sci.* 10;14(1):1293-309, 2013.
- [13] 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.
- [14] 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.
- [15] 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.
- [16] S. Rehman, M. Ikram, R. J. Baker, M. Zubair, E. Azad, S. Min, K. Riaz, K. H. Mok, S. Rehman, Synthesis, characterization, in vitro antimicrobial, and U2OS tumoricidal activities of different coumarin derivatives, *Chemistry Central Journal*, 7:68, 1186/1752-153X, 2013.
- [17] 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.

- [18] K. V. Sashidhara, A. Kumar, R. P. Dodda, N. N. Krishna, P. Agarwal, K. Srivastava, S. K. Puri, Coumarin-trioxane hybrids: synthesis and evaluation as a new class of antimalarial scaffolds, *Bioorg Med Chem Lett.* 15;22(12):3926-3930, 2012.
- [19] H. Osman, A. Arshad, C. K. Lam, M. C. Bagley, Microwave-assisted synthesis and antioxidant properties of hydrazinyl thiazolyl coumarin derivatives, *Chemistry Central Journal*, 6:32, 1186/1752-153X, 2012.
- [20] S. V. Rodriguez, R. F. Guinez, M. J. Matos, L. Santana, E. Uriarte, M. Lapier, J. D. Maya, C. O. Azar, Synthesis of coumarin-chalcone hybrids and evaluation of their antioxidant and trypanocidal properties, *Med. Chem. Commun.*, 4, 993-1000, 2013.
- [21] 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.
- [22] 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.
- [23] M. Atmaca, H. M. Bilgin, B. D. Obay, H. Diken, M. Kelle, E. Kale, The hepatoprotective effect of coumarin and coumarin derivatives on carbon tetrachloride-induced hepatic injury by antioxidative activities in rats. *J Physiol Biochem.* 67(4), 569-76, 2011.
- [24] B. Ahmed, S. A. Khan, T. Alam, Synthesis and antihepatotoxic activity of some heterocyclic compounds containing the 1,4-dioxane ring system. *Pharmazie*, 58(3), 173-176, 2003.
- [25] T. Okamoto, T. Kobayashi, S. Yoshida, Synthetic Derivatives of Osthole for the Prevention of Hepatitis, *Medicinal Chemistry*, 3, 35-44, 2007.
- [26] 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.
- [27] R. Hoti, V. Kalaj, I. Vehapi, H. Ismaili, V. Thaçi, M. Bicaj, Novel Pyrimidin-2-yl-benzylidene imines and 2-[(pyrimidin-ylimino)-methyl]-phenols and their antibacterial activity, *The FASEB Journal, Experimental Biology*, 2010, 1b487, Anaheim Ca. Apr. 2010,
- [28] R. Hoti, A. Nura-Lama, G. 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.
- [29] Bauer, A. W. et al. , Antibiotic susceptibility testing by standardized single disc method, *American journal of clinical pathology*, 44, , 493: 496, 1966.