Synthesis and characterization of some New 4-Arylamino- and 4-Pyridynilamino-3-Nitro-2H-[1]-Benzopyran-2-ones and their antibacterial activity

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Abstract
Synthesis of the new substituted 3-nitro-2H-[1]-benzopyran-2-one derivatives is presented in this study. By condensation of 4-Chloro-3-nitro-2H-1-benzopyran-2-one and corresponding arylamines 3(a-b), 4-(4'-carboxyphenylimino)-3-nitro-2H-[1]-benzopyran-2-one 4a and 4-(4'-phenylsulphonylamino)-3-nitro-2H-[1]-benzopyran-2-one 4b are synthesized in high yield. Catalytic condensation of product 2 and pyridylamines 3(c-d), afforded novel substituted 4-pyridylamino-3-nitro-2H-[1]-benzopyran-2-ones 4(c-d). Alkaline hydrolysis of 2(a-d) afforded the 2-hydroxy-ω-nitroacetophenone 5. The synthesized products are characterized 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 and results are submitted. Compounds 4c and 4a were more active against S. Aureus, 4d and 4c were more active against E. Coli whereas compound 4b showed considerable activity against Klebsiella.

Keywords: Benzopyran-2-one; condensation, antibacterial, inhibition zones

1. Introduction
2H [1]-Benzopyran-2-one derivatives are heterocyclic compounds with oxygen that are found as ingredient of the plant world, which play an important role in various life processes. Many such compounds exhibit various biological activities such as antimicrobial [1-2], antimalarial [3, 4] and antifungal [5]. Many of coumarinic analogues exhibited also antimicrobial [6-8], antioxidant [9-11] and antitumor activity [12]. It was reported that a significant number of substituted derivatives of benzopyran-2-one also show anticoagulant sedative, analgesic, anti-HIV [13-14], hepatoprotective [15-17], antiviral [18] and anti-convulsant activity [19]. For this reason, many of them have found widespread usage in pharmacies. On the other hand, many of data reported in the literature show that the ring thiazolidinone derivatives demonstrate a wide range of pharmacological activities, including those antibacterial, anti-fungal and anti-convulsant activity. The biological activity of these derivatives is conditioned by their structure. The presence of different substituents on the benzopyrone ring indicates their impact on the type and potency of biological activity. Despite continuous efforts, the relationship between structure and biological activity of these derivatives, so far has not yet been sufficiently clarified. Extraordinary biologically importance of such derivatives on the basis of thiazolidine-4-one has generated a constant interest for their synthesis and research. In continuation of our previous studies and in attempt to synthesize the new derivatives [20-22], in this study we report about optimal conditions for preparing of some new 4-arylamino- and substituted 4-pyridylamino-3-nitro-2H-1-benzopyran-2-one derivatives by condensation of 4-chloro-3-nitro-2H-[1]-benzopyran-2-one and various arylamines and pyridylamines. In continuing this report contents also alkali hydrolysis of synthetized products. Antibacterial activities 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
A typical reaction, 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one 4b (2.3 mmol) was added to a solution of 4-aminobenzoic acid 3a (0.17 g, 1 mmol) in acetonitrile (10 mL), (0.27 g, 1 mmol) of 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one 2 was obtained in 92% yield. Thus product 4b was filtered under vacuum, then washed with 1 mL portion of ethanol. Crystallization of methanol gave 0.32 g (87%) of yellow crystalline product 4d. mp = 219°C. IR: 3325 cm⁻¹, 3177 cm⁻¹, 2940 cm⁻¹, 1709 cm⁻¹, 1646 cm⁻¹, 1596 cm⁻¹, 1481 cm⁻¹, 1371 cm⁻¹, 1041 cm⁻¹, 764 cm⁻¹. ¹H-NMR (300 MHz): δ 7.67 ppm (d, 1H) and δ 1.90 ppm (s, 3H) ¹3C-NMR: δ 125.678; δ 126.638; δ 132.110; δ 134.591; δ 146.503; δ 152.366. Anal: (C, 56.80%), (H, 2.70%), (N, 7.72%).

4-(4-methoxy-2-benzothiazolylamino)-3-nitro-2H-1-benzopyran-2-one, 4d
A mixture of 4-Chloro-3-nitro-2H-1-benzopyran-2-one 2 (0.221 g, 1 mmol) and 4-methoxy-2-aminobenzothiazole 3d (0.180 g, 1 mmol), containing triethylamine (three drops) in acetonitrile (5 mL) was refluxed on a water bath. A CaCl₂ guard tube was mounted and after 15 min., the yellow crystalline product was formed. The reaction mixture was stirred under reflux for 2 h., then cooled to room temperature and filtered off under vacuum. The residue washed with 2 mL portions of acetonitrile. Crystallization from methanol gave 0.26 g (75%) of product 4c. mp > 270°C. IR: 3176 cm⁻¹, 3067 cm⁻¹, 1697 cm⁻¹, 1607 cm⁻¹, 1560 cm⁻¹, 1446 cm⁻¹, 1305 cm⁻¹, 1277 cm⁻¹, 1139 cm⁻¹, 1018 cm⁻¹, 849 cm⁻¹, 762 cm⁻¹. ¹H-NMR (300 MHz): 6.87 (2H), 6.97 (1H), 6.79 (d, 1H) δ 7.84 (d, 1H), δ 7.74 (t, 1H), δ 7.28 (m, 2H), δ 6.76 (d, 1H), δ 6.03 ppm (d, 1H), and δ 6.90 ppm (s, 1H) ¹3C-NMR: δ 118.126; δ 113.820; δ 113.431; δ 114.650; δ 115.366. Anal: Calculated for C₁₇H₁₂ClNO: (C, 55.27%), (H, 3.00%), (N, 11.37%), (O, 21.67%), (S, 8.67%). Found: (C, 55.47%), (H, 3.02%), (N, 11.26%), (S, 8.72%).

2.6 2-Hydroxy-6-nitroacetophenone 5
Heteroarylamino-2H-1-Benzopyran-2-ones 4a, 4b, 4c and 4d (2 mmol) was dissolved to a 10 mL 5% natrium hydoxide water solution and heated at 95°C for 1 h. The reaction mixture was cooled and acidified with diluted hydrochloric acid and ice to pH = 1. The crude product was filtered and washed with 3 mL of water. Crystallization from ethanol gave 0.3 g, (84%) of product 5. mp = 96°C. IR: 3400 cm⁻¹, 3085 cm⁻¹, 2950 cm⁻¹, 1637 cm⁻¹, 1613 cm⁻¹, 1550 cm⁻¹, 1499 cm⁻¹ 1369 cm⁻¹, 754 cm⁻¹. ¹H-NMR: δ 11.29 (2H), δ 11.41 (s, 1H), δ 8.77 (d, 1H), 8.76 (d, 1H), 8.75 (d, 2H), 6.72 (s, 2H). Anal: Calculated for C₁₅H₁₂NO: (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
According to previous investigation we now report that 4-Chloro-3-nitro-2H-1-benzopyran-2-one 2 react readily with various arylamines and substituted pyridylamines to form the corresponding 4-arylamino-, respectively 4-pyridylamino-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 and phosphor oxychloride and N,N-dimethylformamide [18], 4-chloro-3-nitro-2H-1-benzopyran-2-one 2 was obtained in 92% yield. Thus product 2 was subjected to condensation with arylamines 3(a-b) and substituted pyridylamines 3(c-d) in acetonitrile under reflux to yield the respective 4-arylamino-3-nitro-2H-1-benzopyran-2-ones 4(a-b) and 4-pyridylamino-3-nitro-2H-1-benzopyran-2-ones 4(c-d) (scheme 1).
By condensation of 2 and 4-aminobenzoic acid 3a in acetonitrile solution under reflux, 4-(4’-carboxyphenylamino)-3-nitro-2H-[1]-benzopyran-2-one 4a is obtained. By similar treatment of 2 with 4-sulphanilic acid 3b gave 4-(4’-phenylsulphonylamino)-3-nitro-2H-[1]-benzopyran-2-one 4b in 68% yield. On the other hand compound 2 reacts with 2-amino-3-hydroxypyridine 3c and 2-amino-4-methyl-3-nitropyridine 3d in the presence of catalytic amount of triethylamine, to afford 4-(3-hydroxy-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one 4c and 4-(4-methyl-3-nitro-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one 4d respectively. Alkaline hydrolysis of the products 4a, 4b, 4c and 4d, afforded 2-Hydroxy-ω-benzopyran-2-one 4d respectively. Alkaline hydrolysis of the products 4a, 4b, 4c and 4d, afforded 2-Hydroxy-ω-nitroacetophenone 5. Formation of product 5 followed by tautomerization of precursors resulting to imine formation, and imine hydrolysis and in the next step by decarboxylation. The structure of products were determined from their IR, 1H-NMR and 13C–NMR spectra and their elemental analysis.

IR spectrum of 4a exhibited a broad band at 3535-3250 cm\(^{-1}\) as a result of \(\nu_{\text{NH}}\) stretching absorption. The absorption bands at 3438 cm\(^{-1}\) and 3401 cm\(^{-1}\) responsible for \(\nu_{\text{NH}}\) and COOH proton vibrations. Absorption signals at \(\delta 8.7\) ppm and 11.3 ppm correspond to \(\delta 7.3–7.8\) ppm responsible for aromatic protons, and \(\delta 7.2–7.6\) ppm for C–O–C of lactonic system appears at 1214 cm\(^{-1}\). At 1176 cm\(^{-1}\) and 701 cm\(^{-1}\) were appeared. The \(\nu_{\text{C–O}}\) of six-membered lactonic system is observed. Peaks at 1726 cm\(^{-1}\) and 1709 cm\(^{-1}\) are assigned for \(\nu_{\text{CO str.}}\) of this group. The \(\nu_{\text{C–C (ar)}}\) were appeared, two absorption at 1459 cm\(^{-1}\) and 1373 cm\(^{-1}\) attributable to \(\nu_{\text{NO2}}\) (as) and \(\nu_{\text{NO2}}\) (sym), and the \(\delta_{\text{CH (out of plane)}}\) at 762 cm\(^{-1}\) were also observed. The \(\nu_{\text{C–O}}\) of six-membered lactonic system was 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 \(\nu_{\text{CO}}\) vibration were assigned to low frequencies, may be as a result of decreasing of the respective force constant and the bond order\(^{[24]}\).

The formation of 4d is identified from \(^1\)H-NMR (DMSO) spectrum where are appeared the absorption as a multiplet at \(\delta 7.2–7.6\) ppm (responsible for aromatic protons ). The spectrum also displayed a singlet at \(\delta 2.1\) ppm (s, 3H, assigned for \(\text{CH}_3\)). A sharp peak at 1696 cm\(^{-1}\) and peaks at 1612 cm\(^{-1}\) and 1539 cm\(^{-1}\) responsible for \(\nu_{\text{C–C (ar)}}\) vibrations. The absorption for \(\nu_{\text{CH stretching}}\) of aromatic ring and \(\nu_{\text{CH}}\) out of plane were appeared at 1548 cm\(^{-1}\), 1381 cm\(^{-1}\) and 761 cm\(^{-1}\) respectively.

In \(^1\)H-NMR spectra of compound 4a, was appeared a multiplet at \(\delta 7.3–7.8\) ppm responsible for aromatic protons, Absorption signals at \(\delta 8.7\) ppm and 11.3 ppm correspond to NH and COOH proton vibrations.

The IR spectrum of 4b showed a characteristic mode at 3438 cm\(^{-1}\) as a result of \(\nu_{\text{NH}}\) stretching absorption. The absorption peaks at 3110-3005 cm\(^{-1}\) are responsible for aromatic \(\nu_{\text{CH}}\) (as. and sym.). The OH stretching vibrations of SO\(_2\)-OH group are assigned at 2890 cm\(^{-1}\). The characteristic band which may result from stretching carbonyl vibration were appeared at 1715 cm\(^{-1}\). Absorption at 1612 cm\(^{-1}\) are responsible for \(\nu_{\text{C–C (ar)}}\) (ar) vibrations. The absorption modes at 1517 cm\(^{-1}\) and 1369 cm\(^{-1}\) may be assigned to stretching \(\nu_{\text{NO2}}\) (as) and \(\nu_{\text{NO2}}\) (sym). Characteristic. absorptions at 1299 cm\(^{-1}\) and 1122 cm\(^{-1}\) are responsible for SO\(_2\)H as. and sym. vibrations whereas absorptions for C–O–C of lactonic system appears at 1214 cm\(^{-1}\). At 1176 cm\(^{-1}\) and 701 cm\(^{-1}\) were shown absorption modes for aromatic \(\delta_{\text{CH}}\) in plane and out of plane.

IR spectrum of 4c showed the absorption as a sharp peak at 3260 cm\(^{-1}\) responsible for \(\nu_{\text{NH}}\) stretching, and a band at about 3390 cm\(^{-1}\) characteristic for \(\nu_{\text{OH}}\) group of pyridine system. We may suppose that appearance of this absorption mode as an inflexive form may be resulted as a consequence of possibility of intramolecular hydrogen bonding association of this group. The CH stretching vibration of 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 \(\nu_{\text{C–C (ar)}}\) and \(\nu_{\text{C–C (ar)}}\) vibrations whereas absorptions for C–O–C of lactonic system were appeared at 3071 cm\(^{-1}\), 1696 cm\(^{-1}\), and 1539 cm\(^{-1}\). Two absorption at 1459 cm\(^{-1}\) and 1373 cm\(^{-1}\) attributable to \(\nu_{\text{NO2}}\) (as) and \(\nu_{\text{NO2}}\) (sym), and the \(\delta_{\text{CH (out of plane)}}\) at 762 cm\(^{-1}\) were also observed. The \(\nu_{\text{C–O}}\) of six-membered lactonic system was assigned at 1296 cm\(^{-1}\), whereas the mode at 1024 cm\(^{-1}\) resulted from \(\nu_{\text{C–O}}\) of hydroxy group.

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The characteristic modes of product 5 appeared at 3080 – 3400 cm⁻¹ (broad band) and 2950 cm⁻¹ which are responsible for ν OH stretching, ν OH (helat), aromatic ν CH and methylene νCH absorptions. The characteristic peak derived from lactonic carbonyl as a result of intramolecular hydrogen bond is moved down at 1637 cm⁻¹. IR spectrum of the hydrolysis product 5 also showed bands at 1560 cm⁻¹ for ν C=C (ar), 1449 cm⁻¹ for ν NO₂ (as), 1369 cm⁻¹ for νNO₂ (sym) and 754 cm⁻¹ aromatic δ CH out of plane. These values are compared with that ones from literature and shown to be similar. In addition to that, the elementary analysis of obtained products indicated in favor of described structures.

4. Antibacterial
Following this study, compounds 4 (a-c) are screened for their antibacterial activity. Our research is oriented to test the activity against bacteria *S. aureus*, *E. coli* and *Klebsiella*, on the basis of Kirby-Bayer’s method [23]. Antibacterial activity is investigated by measuring the inhibition zones around the standard discs that have previously been marked with solutions of the products in N,N-DMF with concentrations of 2 mg/mL, 4 mg/mL and 6mg/mL (table 1). Results are shown in fig. 1, 2 and 3. From these measurements resulted that these derivates were shown moderate to height activity against *S. aureus*.*E. coli* and *Klebsiella*. Compounds 4c and 4a were more active against *S. aureus*. Compounds 4b and 4c in low concentration except bactericide activity also showed bacteriostatic activity against *S. aureus* in a large zones. Emphatic activity against *E. coli* exhibited compound 4d and 4c, whereas 4b and 4c are more active against *Klebsiella*. In general increasing of concentration causes height activity against these microorganisms. Antibacterial activity against *E. Coli* and *Klebsiella* appeared as bactericide activity in large range is displayed in large-scale. Furthermore, these compounds expressed both bacteriostatic and bactericide activity against *S. Aureus*. Bacteriostatic activity is exhibited in large range, whereas bactericide activity showed in small diameter.

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<th>Table 1: The diameters of the inhibition zones (mm) of the discs wetered with various concentration of the synthesised compounds</th>
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<td><strong>S. aureus</strong></td>
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<td>2mg/mL</td>
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5. Conclusions
New substituted derivatives of 3-nitro-2H-[1]-benzopyran-2-one, 4(a-c) are synthesized in the moderate and high yield. Compounds 4c and 4a showed significant bactericide and bacteriostatic activity against *S. aureus*. Compounds 4d and 4c are more active against *E. coli* exhibited, whereas 4b expressed considerable activity against *Klebsiella*. In general increasing of concentration causes height activity against these microorganisms. Antibacterial activity is shown to be proportional to the concentration of these compounds.
6. References
6. Desai NC, Satodiya HM, Rajpara KM, Joshi VV, Vaghani HV, Microwave assisted synthesis of new coumarin based 3-cyanopyridine scaffolds bearing sulfonamide group having antimicrobial activity, Indian Journal of Chemistry. 2013, B 52B.
19. Siddiqui N, Deepanjali M, Arshad F, Rana A. Synthesis and anticonvulsant screening of 2-(substituted ary)-3(4H 1,2,4-triazol-4-YL), 1,3-thiazolidin-4-ones. Indian Journal of Heterocyclic Chemistry. 2007; 16(4):403-404.