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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 2 and corresponding arylamines 3(a-b), 4-(4'-carboxyphenylamino)-3-nitro-2H-[1]-benzopyran-2-one 4a and 4-(4'phenylsulphonylamino)-3-nitro-2H-[1]-benzopyran-2-one 4b are synthetized in high yield. Catalytic condensation of product 2 and pyridylamines 3(c-d), afforded novel substitued 4-pyridylamino-3-nitro-2H-[1]-benzopyran-2-ones 4(c-d). Alkali hydrolysis of 4(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; condensiation, 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 sush 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 derivates [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

(F-254) on a benzene: toluene: glac. acetic acid bath (ratio 80:10:10 by volume), visualization on a bromine bath). Purification of products was done by crystallization from various solvents.

Melting points were measured on a paraffin bath in open capillary tubes and are uncorrected.

IR spectra were recorded in KBr discs on a Perkin-Elmer 1725 x FT-IR Spectrometer with 2cm^{-1} resolution. ^{1}H-NMR spectra were obtained in DMSO on UNITYplus-500 "NMR 1" Spectrometer. Chemical shifts are reported in parts per million (ppm) down field from tetramethylsilane as an internal standard (δ0, 00). Microanalyses were preformed on a Perkin-Elmer 240 B CHN analyzer. 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 (2mg/mL, 4 mg/mL and 6 mg/mL).

2.1 Arylamino- and 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 arylamine 3(a-b) heteroaryalmine 3(c-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 recrystallization.

2.2 4-(4'-Carboxyphenylamino)-3-nitro-2H-[1]-benzopyran-2-one, 4a

To a solution of 4-aminobenzoic acide 3a (0,17g, 1 mmol) in acetonitrile (4 cm^3) three drops of triethylamine was added. After that a solution of 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one 2 (0,22g, 1 mmol) in 4 mL acetonitrile was added under vigorously stirring. The reaction mixture was heated under reflux for 18 h and the yellow crystalline product was formed (TLC monitoring). After that the product was filtered off under vacuum, and vashed with 0, 5 mL acetonitrile. Crystallization from ethanol gave 0,26g (75%) of product 4c. mp >210˚C. IR: 3610 cm^{-1}, 3535- 3250 cm^{-1}, 2988 cm^{-1}, 1726 cm^{-1}, 1709 cm^{-1}, 1614 cm^{-1}, 1595 cm^{-1}, 1548 cm^{-1}, 1381 cm^{-1}, 761 cm^{-1}. ^{1}H-NMR: δ11, 3 (s, 1H), δ8, 7 (s, 1H), δ7, 8 – 7, 3 (m, 8H),

2.3 4-(4'-phenylsulphonylamino)-3-nitro-2H-[1]-benzopyran-2-one 4b

4-sulphanilic acide 3b (0,17g, 1 mmol) was added to a 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one 2 (0,22g, 1mmol) in 10 mL of acetonitrile solution and 2 mL of triethylamine was added. The mixture was heated slightly and then was refluxed for 5 h and cooled for a long time. The crude product was filtered off under vacuum, washed with 0, 5 mL of acetonitrile and dried. Crystallization from ethanol gave 0,28g (80%) of yellow crystalline product 4b. mp = 258-260˚C.

IR: 3438 cm^{-1}, 3110 - 3005 cm^{-1}, 2890 cm^{-1}, 2359 cm^{-1}, 1715 cm^{-1}, 1612 cm^{-1}, 1446 cm^{-1}, 1425 cm^{-1} 1403 cm^{-1}, 1369 cm^{-1}, 1370 cm^{-1}, 1214 cm^{-1}, 1176 cm^{-1}, 1031 cm^{-1}, 1008 cm-1, 852 cm^{-1}, 757 cm^{-1}, 746 cm^{-1}, 701 cm^{-1}, 636 cm^{-1}, 581 cm^{-1}.

2.4 4-(3-hydroxy-2-pyridinylamino)-3-nitro-2H-[1]-benzopyran-2-one, 4c

To a solution of 2- aminobenzothiazole **3c** (0, 18g; 1, 2mmol) in acetonitrile (10 mL), (0,27g 1, 2 mmol) of 4-Chloro-3-nitro-2H-[1]-benzopyran-2-one **2** was added. The reaction mixture was refluxed for 4 h under vigorously stirring and then monitored by TLC. After that the mixture was cooled in an ice bath and yellow-orange crystalline product was filtered under vacuum, then washed with a 1mL portion of ethanol. Crystallization of residue from methanol gave 0,29g (72%) 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}. ^{1}H-NMR (300MHz): δ8,72 (b, 1H), δ7,90 (t, 2H), δ7,86 (d, 1H), δ7,81 (d, 1H), δ7,34 (d, 1H), δ7,17 (d, 1H), δ7,27 (m, 2H). Anal: *Calculated for $C_{16}H_9N_3O_4S$:* (C, 56, 62%), (H, 2, 67%), (N, 12, 39%), (O, 18, 88%), (S, 9, 44%), *Found:* (C, 56, 80%), (H, 2, 70%), (N, 12, 27%), (S, 9, 71%).

2.5 4-(4-methoxy-2-benzothiazolylamino)-3-nitro-2H-1-benzopyran-2-one, 4d

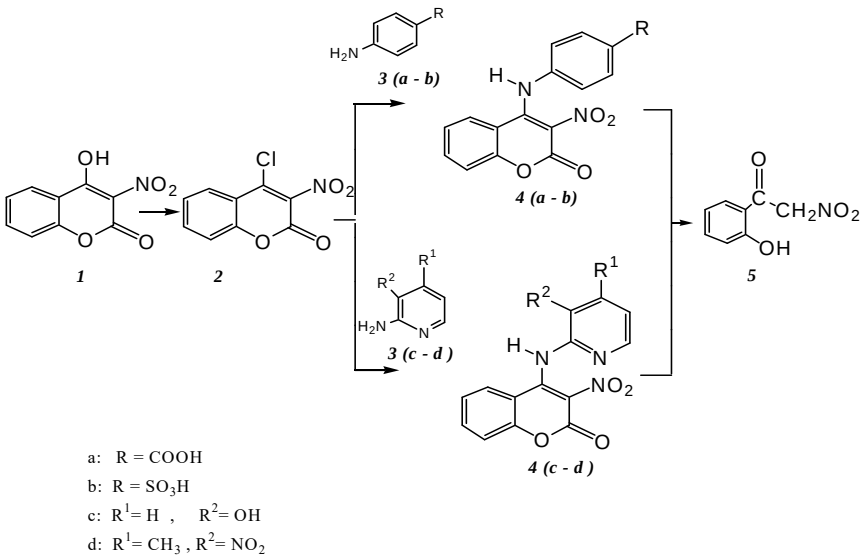
A mixture of 4-Chloro-3-nitro-2H-1-benzopyran-2-one 2 (0,221g, 1mmol) and 4-methoxy-2-aminobenzothiazole 3d (0,180g, 1mmol), containing triethylamine (three drops) in acetonitrile (5 mL) was refluxed on a water bath. A $CaCl_2$ guard tube was mounted and after 15 min., the yellow crystalline product 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 2x1mL portions of acetonitrile. Crystallization from methanol gave 0,32g (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}, 1367 cm^{-1}, 1281 cm^{-1}, 1041 cm^{-1}, 764 cm^{-1}. ^{1}H-NMR (300 MHz): δ8,65 (s, 1H), δ 7,92 (d, 1H) δ 7,84 (d, 1H), δ 7,74 (t, 1H), δ 7,28 (m, 2H), δ 7,16 (d, 1H), δ7,03 ppm (d, 1H) and δ1,90 ppm (s, 3H) ^{13}C-NMR: δ56,185; δ109,289; δ109,425; δ114,471; δ114,607; δ116,103; δ117,156; δ122,951; δ124,259; δ124,497; δ125,075; δ125,678; δ126,638; δ132,110; δ134,591; δ146,503; δ152,366 Anal: *Calculated for $C_{17}H_{11}N_3O_5S$:* (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-ω-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 3x2 mL of water. Crystallization from ethanol gave 0,3g, (84%) of product 5. mp=96˚C. 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}. ^{1}H-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 $C_8H_7NO_4$:* (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

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).



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% yeld. 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 respective. Alkaly hydrolysis of the products 4a, 4b, 4c and 4d, afford 2-Hydroxy-ω-nitroacetophenone 5. Formaton of product 5 folloved 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, ^{1}H-NMR and ^{13}C –NMR spectra and their elemental analysis.

IR spectrum of 4a exhibited a broad band at 3535-3250 cm^{-1} responsible for υOH stretching of carboxyl group whereas a sharp peak at 3610 cm^{-1} is responsible for υNH stretching vibrations. The characteristic mode at 2981 resulted from aromatic υCH stretching frequencies. The characteristic absorption bands at 1726 $cm^{-1}$and 1709 cm^{-1} are assigned from corresponding carbonyl functions of carboxyl group and lactonic system. The modes at 1614 cm^{-1} and 1595 cm^{-1} are responsible for C=N and C=C aromatic functions respectively. The typical modes for υNO_2 (as), υNO_2 (sym) and δCH out of plane were appeared at 1548 cm^{-1}, 1381 cm^{-1} and 761 cm^{-1} respectively.

In ^{1}H-NMR specter of compound 4a, was appeared a multiplet at δ7, 3–7,8 ppm responsible for aromatic protons,. Absorption signals at δ 8,7ppm 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 υNH stretching absorption. The absorption peaks at 3110-3005 cm^{-1} are responsible for aromatic υCH (as. and sym.). The OH stretching vibrations of SO_2-OH group are assigned at 2890 cm^{-1}.The characteristic band which may resulted from stretching carbonyl vibration were appeared at 1715 cm^{-1}. Absorption at 1612 cm^{-1} are responsible for υ C=C (ar) vibrations. The absorption modes at 1517 cm^{-1} and 1369 cm^{-1} my be assigned to stretching υNO_2 (as) and υNO_2 (sym). Characteristic. absorptions at 1299 cm^{-1} and 1122 cm^{-1} are responsible for SO_3H 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 δCH in plane and out of plane.

IR spectrum of 4c showed the absorption as a sharp peak at 3260 cm^{-1} responsible for υNH stretching, and a band at about 3390 cm^{-1} characteristic for υOH 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 in 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 υCO str., υC=N and υC=C (ar) were appeared. Two absorption at 1459 cm^{-1} and 1373 cm^{-1} attributable to υNO_2 (as) and υNO_2 (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 [24]

The formation of 4d is identified from ^{1}H-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 δ 2,1 ppm (s, 3H, assigned for CH_3), and a singlet at δ 9,2 ppm (s. 1H responsible for NH proton absorption). ^{13}C-NMR spectrum of 4d showed characteristic absorptions responsible for 15 carbon atoms.

In the IR spectrum of 4d 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 unsatured six-membered lactones was observed. 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 υNO_2 (as) and υNO_2 (sym), and at 766 cm^{-1} for bending δ CH (ar) was also appeared.

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 υCH 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 NO_2 (as), 1369 cm^{-1} for υNO_2 (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 adition to that, the elementary analysis of obtained products indicated in favor of described structures.

4. Antibacterial

Following this study, compounds 4 (a-c) are scrrened 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 concentracion causes heigh 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 espressed both bacteriostatic and bactericide activity against *S. Aureus*. Bacteriostatic activity is exhibited in large range, whereas bactericide activity showed in small diameter.

Table 1: The diameters of the inhibition zones (mm) of the discs wetered with various concentracion of the synthesised compounds

	S. aureus			***E. coli***			***Klebsiella***		
	2mg/mL	**4mg/mL**	**6mg/mL**	**2mg/mL**	**4mg/mL**	**6mg/mL**	**2mg/mL**	**4mg/mL**	**6mg/mL**
4a	9,4	9,6	9,9	8,7	8,9	9,5	7,9	8,2	9,0
4b	(15,2) 8,7	9,7	10,1	9,6	9,9	10,7	6,8	10,3	9,9
4c	(17,6) 11,7	18,1	18,6	10,1	11,2	11,8	(10,5) 7,9	8,0	9,5
4d	7,8	7,7	8,3	11,3	10,9	11,8	8,0	8,3	8,7

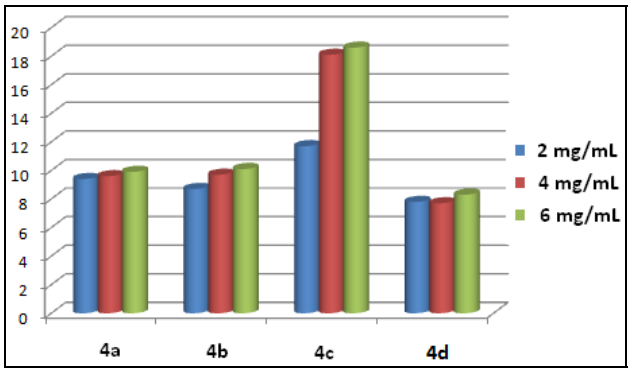


Fig 1: Graphical presentation of inhibition zone diameter (mm) against *S. aureus*

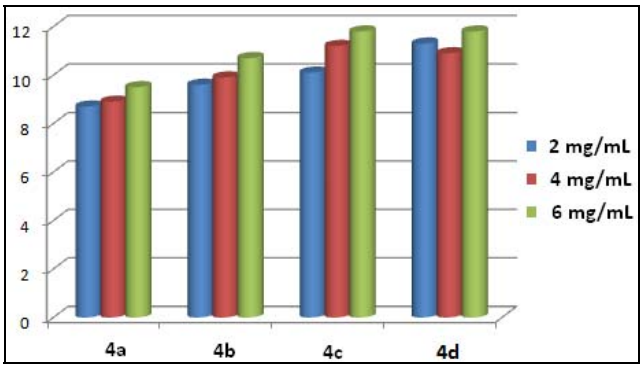


Fig 2: Graphical presentation of inhibition zone diameter (mm) against *E. coli*

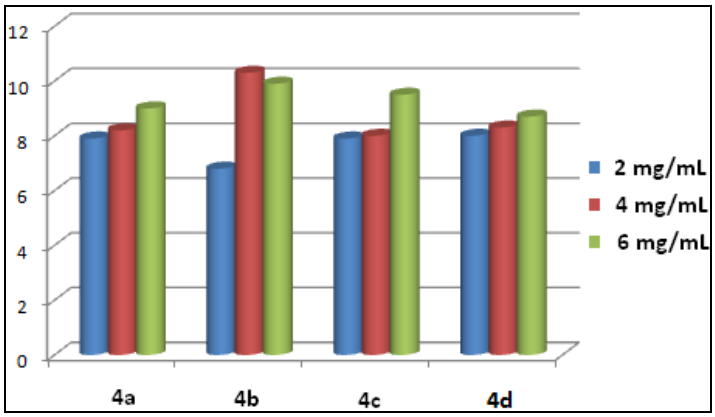


Fig 3: Graphical presentation of inhibition zone diameter (mm) against *Klebsiella*

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 espresed considerable activity against *Klebsiella*. In general increasing of concentracion causes heigh activity against these microorganisms. Antibacterial activity is shown to be proportional to the concentration of these compounds.

6. References

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