Experiments
Експерименти в природните науки

SYNTHESIS OF NEW [(3-NITRO-2-OXO-2H-CHROMEN-4-YLAMINO)-PHENYL]-PHENYL-TRIAZOLIDIN-4-ONES AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract. A series of reactions that result in the synthesis of novel thiazolidynones based on 4-chloro-3-nitrobenzopyran-2-one is presented in this study. During the condensation reaction of 4-chloro-3-nitrobenzopyran-2-one **2** and benzene-1,4-diamine, 4-(4-Amino-phenylamino)-3-nitro-chromen-2-one **3** is synthesized in high yield. By catalytic condensation of product **3** and benzaldehyde, salicylaldehyde and 3-nitrobenzaldehyde, novel derivatives of 4-[4-(Benzylidene-amino)-phenylamino]-3-nitrobenzopyran-2-one, **4(a-c)**, as condensation products are synthesized. In the following series of reactions, by cyclization of the products **4(a-c)** and 2-mercaptopropionic acid, corresponding substituted thiazolidinones **5(a-c)** are synthesized. Determination of structure of the synthesized products is done on the basis of spectrometric data. Compounds **4(a-c)** and **5(a-c)** are examined for their antibacterial activity against *S. aureus*, *E. coli* and *Klebsiella* by measuring the zones of inhibition around the disks impregnated with the corresponding solutions of compounds in N,N-DMF concentration 2 mg/mL, 4 mg/mL and 6 mg/mL. Compounds of series **4** have shown moderate antibacterial activity against these microorganisms, whereas compounds of series **5** have shown significant activity. The impact of substitutions in antmicrobial activity is also explored.

Keywords: thiazolidin-4-one, benzopyran-2-one, cyclization, inhibition zones

Introduction

Many derivatives on the basis of 2H[1]-benzopyran-2-one are heterocyclic compounds that play an important role in various life processes. Many such derivatives are found as ingredient of the plant world and exhibit various biological activities (Mohamed et al., 2012; Rajasekaran et al., 2011) such as antimicrobial (Desai et al., 2013; Reihman et al., 2013; Mayekar & Mulwad, 2008) and antimalarial (Sashidhara et al., 2012) and antifungal (De Araújo et al., 2013). Many of coumarinic analogues exhibited also cytotoxic

(Nawrot-Modranka et al., 2006), antioxidant (Tyagi et al., 2005; Osman et al., 2012; Vazquez-Rodriguez et al., 2013) and anti-tubercular activity (Yu et al., 2003). It was reported that a significant number of substituted derivatives of benzopyran-2-one also show anticoagulant, anti-HIV (Rao et al., 2002), sedative, analgesic and hepatoprotective activity (Atmaca et al., 2011; Ahmed et al., 2003; Okamoto et al., 2007). According to that, many of them have found widespread usage in pharmacies (Rajasekaran et al., 2011). On the other hand, thiazolidynone derivatives reported to have a wide range of pharmacological activities, including those antibacterial (Mayekar & Mulwad, 2008), antifungal activity (Mazei et al., 2008) and anti-convulsant (Siddiqui et al., 2007). The biological activity of these derivatives is conditioned by their structure, so 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 (Hoti et al., 2010; 2014). In this paper we had intended to synthesize some new amino-phenylamino)-3-nitro-chromen-2-ones and substituted thiazolidyne-4-ones with benzopyran-2-one moiety, which could serve as pro-pharmaceutical products.

Material and methods

The compounds are synthesized under reflux conditions, using commercial reagents of Aldrich company as precursors. Reactionsare monitoredbyTLC using Merck Kieselgel-60 (F-254) as the stationary phase anda mixture of benzene, toluene, glacial acetic acid (v/v/v, 80:10:10) as mobile phase. The synthesized products are purified by crystallization from methanol and ethanol. Melting points are determined used a paraffin oil bath with open capillary tube. IR spectra are recorded in KBr discs on Shimadzu 8400xFT-IR spectrometer with 4cm^{-1} resolution. ^{1}H-NMR and ^{13}C-NMR spectra are recorded in DMSO on UNITYplus-500"NMR 1" spectrometer. Chemical shifts were reported in ppm downfield from TMS as internal standard(δ0.00). Examination of the antibacterial activity of the synthesized compounds is done using standard discs (d=5.0 mm, maximum capacity10 pg) on the basis of Kirby-Bayer's method. Standard discs are previously saturated with 2 mg/mL, 4 mg/mL and 6 mg/mL solutions of compounds in N,N-DMF.

4-(4-Amino-phenylamino)-3-nitro-chromen-2-one, ***3***

2g (9.0mmol) 4-chloro-3-nitrobenzopyran-2-one are dissolved in 6 mL of ethanol, then in small portions was added the mixture containing 0.97g (9.0 mmol) benzene-1,2-diamine in 5 mL of ethanol and then 2-3 drops of triethylamine was added

into the mixture. The content is mixed for 10 min. at room temperature, then refluxed for about 90 minutes. After cooling, the mixture is concentrated in the rotary evaporator and the crystalline product is filtered off under vacuum and washed with 2 × 1 mL of ethanol. The crystalline product is dried and crystallized from methanol, giving 4-(4-Amino-phenylamino)-3-nitro-chromen-2-one, R = 79.68%. mp = 240-242 °C. **IR** (KBr disc, cm^{-1}): 3470.92, 3386.12, 3220.86, 3058.34, 2872.24, 2595.12, 1708.54, 1614.38, 1511.25, 1327.77, 1220.36, 1130.62, 1067.34, 905.94, 757.95.

4-[4-(Benzylidene-amino)-phenylamino]-3-nitrobenzopyran-2-ones,4(a-c), general procedure

Product **3 (**0.3 g, 1.0 mmol) is dissolved in 20 mL of absolute ethanol and to this mixture was added in small portions 1.5 mmol of aromatic aldehyde (benzaldehyde, salicylaldehyde or 3-nitrobenzaldehyde respectively) dissolved in 10 mL of absolute ethanol. Then 2 drops of piperidine as a catalyst was added and the mixture was mixed for 15 min at room temperature and refluxed for 10 to 12 hours. After cooling, the mixture is concentrated and the crystals are filtered off under reduced pressure, then washed with 2 × 1 mL of ethanol and dried in the air. Crystallization of the products **4(a-c)** was conducted from ethanol or methanol.

4a; Mp=222-223^{o} C, R=92.78 %, **IR** (KBr disc, cm^{-1}): 3460.38, 3291.54, 3076.3, 2923.81, 1686.12, 1634.82, 1605.00, 1547.12, 1511.25, 1420.34, 1323.28, 1215.31, 1054.34, 900.02, 757.95. **^{1}H-NMR** (*δ, ppm)*; 8.37 (s, 1H, N=C-H), 7.4-7.6 (m, 4H, Ar), 7.2-7.4 (m, 5H, Ar), 7.0 (d, 2H, Ar), 6.4 (d, 2H, Ar),3.9 (1H,NH). **^{13}C-NMR** (*δ, ppm);* 164.2 (C=N), 161.4 (C=O), 160.5, 149.7, 142.3, 130.6, 130.1, 128.2, 127.4, 127.2, 126.9, 126.2, 124.8, 122.2, 115.6, 105.5.

4b; Mp=204-205^{o}C, R=34.68 %,IR (KBr disc, cm^{-1}): 3452.98, 330.24, 3076.28, 2923.81, 2870.54, 2372.68, 1686.12, 1610.98, 1551.61, 1511.25, 1430.52, 1321.47, 1280.58, 1210.94, 1196.56, 1058.37, 904.24, 762.43.**^{1}H-NMR** (*δ, ppm)*; 8.39 (s, 1H, N=C-H), 7.4-7.6 (m, 4H, Ar), 7.2-7.5 (m, 4H, Ar), 7.1 (d, 2H, Ar), 6.5 (d, 2H, Ar), 4.9 (1H, OH), 4.1 (1H, NH).**^{13}C-NMR** (*δ, ppm); 163.1 (C*=N), 161.6 (C=O), 160.4, 149.3, 142.8, 131.3, 129.8, 127.6, 125.9, 124.5, 121.4, 120.6, 118.0, 115.4, 115.0, 105.7.

4c; Mp=224-225^{o}C, R=65.74 %, **IR** (KBr disc, cm^{-1}):3450-3400, 3083.36, 3076.34,2950,86, 2363.74, 1686.12, 1614.38, 1551.61, 1349.83, 1204.76, 1062.86, 762,43.**^{1}H-NMR** (*δ, ppm)*; 8.4 (s, 1H, N=C-H), 8.3-8.0 (m, 3H, Ar), 7.4-7.8 (m, 5H, Ar), 7.0 (d, 2H, Ar), 6.6 (d, 2H, Ar), 4.1(1H, NH). **^{13}C-NMR** (*δ, ppm); 162.8 (C*=N), 161.4 (C=O), 160.4, 149.6, 142.2, 133.9,130.6, 128.9, 127.3, 126.8, 125.4, 125.1, 124.7, 123.3, 121.4, 115.7, 105.1.

3-[4-(3-Nitro-2-oxo-2H-chromen-4-ylamino)-phenyl]-2-phenyl-thiazolidin-4-ones, 5(a-c), general procedure

In the solution that containing 0.5 mmol of the corresponding product **4(a-c)** dissolved in 10 mL of benzene, 0.12 g (1.5 mmol) 2-mercaptopropionic acid was added. The mixture is stirred for 10 min at room temperature and then is refluxed for 12-13 hours. After cooling the product is concentrated and the remaining solid is dissolved in 5 mL of methanol, then heated to boiling and the excess of acetic acid is neutralized by adding 0.3 mmol of sodium bicarbonate $NaHCO_3$ (controlled with paper litmus until the solution undertake the blue color). The mixture is cooled in an ice bath and then filtered off under vacuum, then is washed with 2×1 mL of ether and dried in the air. The products are crystallized from methanol.

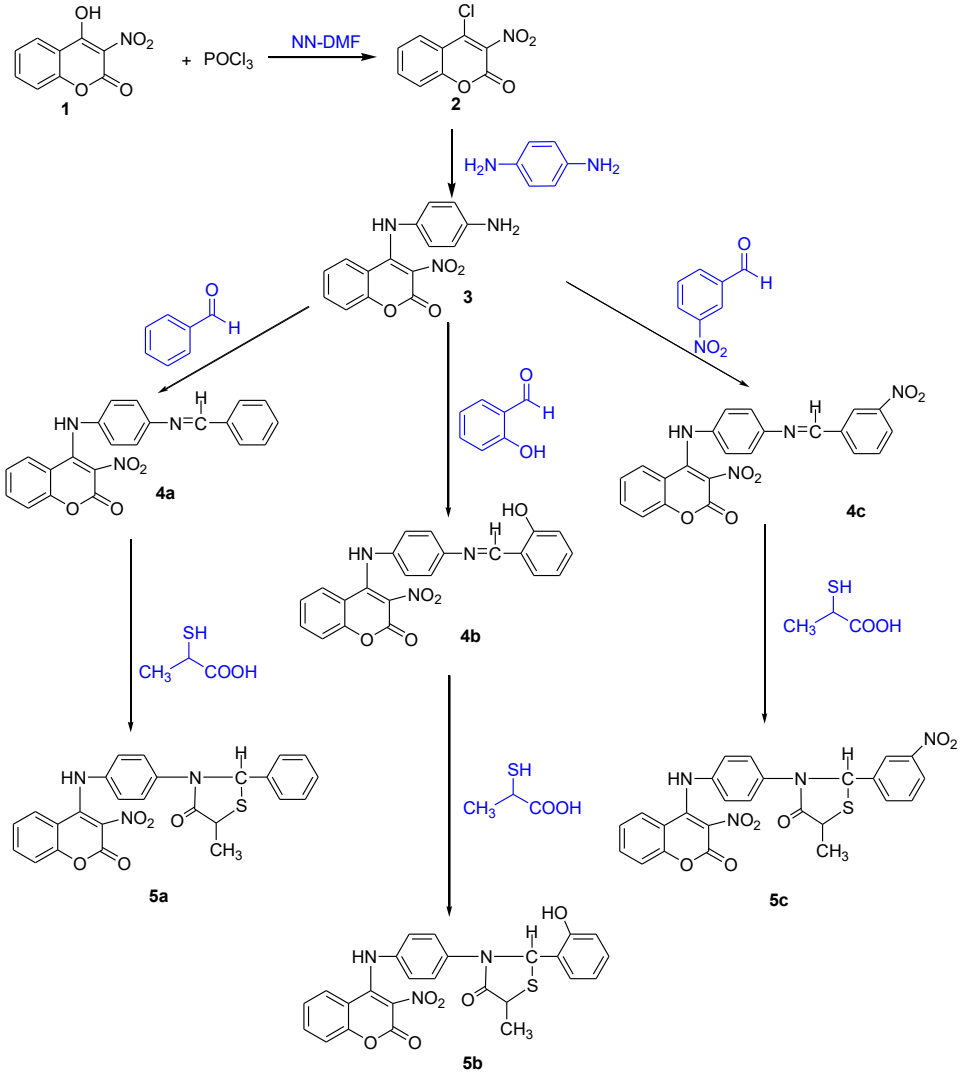
5a; Mp=239-240 °C, R=40.73 %, **IR** (KBr disc, cm^{-1}): 3490-3410, 3240.20, 3085.25, 2932.78, 2376.22, 1686.12, 1610.74, 1585.56, 1511.25, 1420.52, 1327.74, 1207.96, 1067.34, 856.20, 757.95, 583.079. **^{1}H-NMR** (*δ, ppm)*; 7.0-7.6 (m, 8H, Ar), 6.8 (d, 2H, Ar), 6.6 (d, 2H, Ar), 5.9 (s, 1H, N-C-H), 4.1 (1H, NH), 3.6 (q, 1H, CH) 1,6 (d, 3H, CH_3). **^{13}C-NMR** (*δ, ppm); 172.7 (C=O),* 162.5 (C=N), 161.3 (C=O), 149.4, 137.4, 130.2, 129.7, 128.5, 128.1, 127.3, 127.0, 126.7, 126.3, 125.8, 125.4, 124.6, 120.4, 114.7, 105.3, 54.5 (CH-N), 44.6 (CH-S), 17.2 ((CH_3).

5b; Mp=233-234°C, R=65.68 %, **IR** (KBr disc, cm^{-1}): 3550-3280, 3110.05, 2940.46, 2554.98, 2378.64, 1923.81, 1720.62, 1668.19, 1618.87, 1540.04, 1412.32, 1343.58, 1340.86, 1187.18, 1050.64, 1000.08, 889.86, 704.145, 659.32. **^{1}H-NMR**; (*δ, ppm)7.1-7.6 (m, 7H,* Ar), 6.9 (d, 2H, Ar), 6.7 (d, 2H, Ar), 5.8 (s, 1H, N-C-H), 5.1 (1H,OH), 4.0 (1H, NH), 3.5 (q, 1H, CH) 1,5 (d, 3H, CH_3). **^{13}C-NMR**(*δ, ppm)*; 170.5 (C=O), 162.8 (C=N), 161.8 (C=O), 160.2, 156.4, 149.6, 130.2, 129.8, 129.3, 128.1, 127.4, 126.9, 126.5, 126.2, 125.7, 124.4, 124.1, 120.3, 120.0, 114.5, 114.2, 105.4, 44.2 (CH-S), 43.5 (CH-N), 16.2 (CH_3).

5c; Mp=229-231°C, R=33.21 %, **IR** (KBr disc, cm^{-1}): 3435, 3220.28, 3076.28, 2914.84, 2550.38, 2387.46, 1913.44, 1686.12, 1627.35, 1614.38, 1560.57, 1507.48, 1325.77, 1213.32, 1050.20, 839.04, 704.14, 583.07, **^{1}H-NMR** (*δ, ppm)*; 7.1-7.8 (m, 7H, Ar), 6.8 (d, 2H, Ar), 6.5 (d, 2H, Ar), 5.7 (s, 1H, N-C-H), 3.9 (1H, NH), 3.5 (q, 1H, CH) 1,5 (d, 3H, CH_3).). **^{13}C-NMR** (*δ, ppm); 170.5 (C=O), 162.5 (C=N), 160.9 (C=O), 157.7, 150.1, 147.3, 138.4, 134.7, 130.3, 129.7, 128.8, 128.0, 127.2, 126.4, 125.5, 124.3, 123.6, 121.7, 120.6, 114.2, 105.2, 53.2 (CH-N), 44.6 (CH-S) 17.3 (CH_3).*

Results and discussion

During the condensation reactions of 4-chloro-3-nitrobenzopyran-2-one **2** and benzene-1,2-diamine, 4-(4-Amino-phenylamino)-3-nitro-chromen-2-one, **3** is synthesized in good yield. By condensation reaction of product **3** and benzal-



Scheme 1. Synthesis of thiazolidin-2-ones

dehyde, salicylaldehyde and 3-nitrobenzaldehyde, new derivatives of 4-[4-(Benzylidene-amino)-phenylamino]-3-nitrobenzopyran-2-ones, **4(ac)** are synthesized, as condensation products. In the last serie of reactions, by cyclization of the product **4(a-c)** and thioacetic acid, corresponding 3-[4-(3-nitro-2-oxo-2H-chromen-4-ylamino)-phenyl]-2-phenyl-thiazolidin-4-ones, **5(a-c)** are synthesized.

Structural characterization of the synthesized products is based on spectrometric IR and NMR data. In the IR spectrum of the product **3** appeared an absorption signal at 3470.92 cm^{-1} which is responsible for ν(NH_2) stretching vibrations. The absorption signal at 3055.16 cm^{-1} appeared due to ν(CH) stretching vibrations of the aromatic ring. The sharp peak at 1708.54 cm^{-1} region is responsible for ν(C=O) stretching vibrations, whereas the absorption peak at 1614.38 cm^{-1} region resulted from ν(C=C) stretching vibrations of the aromatic ring. The peak at 1511.25 cm^{-1} resulted from the absorptions of asymmetric ν(NO_2) stretching vibrations, while the peak at 1327.77 cm^{-1} resulted from symmetric ν(NO_2) stretching vibrations. On the other hand, the absorption peak at 1220.36 cm^{-1} is characteristic for vibrations of lactonic stretching system (C-O-C), while the sharp peak at 750.71 cm^{-1} resulted from characteristic bending vibrations δ(C-H) oop of the aromatic ring.

In the IR spectrum of **4a**, an absorption signal appeared in the 3460.98 cm^{-1} which is responsible for ν(NH) stretching vibrations, while absorption signal at 3076.30 cm^{-1} corresponds to ν(CH) of the aromatic ring. At 1686.12 cm^{-1}appeared the absorption signal which responds to ν(C=O) stretching vibrations, whereas the sharp peak in 1634.82 $cm^{-1}$and a signal at 1605.00 cm^{-1} correspond due to ν(C=N) and ν(C=C) stretching vibrations of the aromatic system. The characteristic absorption at 1547.12 resulted from asymmetric ν(NO_2) stretching vibrations whereas at 1323.28 cm^{-1} for symmetric ν(NO_2) stretching vibrations. Absorption at 1215.31 cm^{-1} is also characteristic for stretching (C-O-C) vibrations of the lactonic ring, whereas the sharp peak at 757.95 cm^{-1} is characteristic for bending δ(C-H) oop vibrations of the aromatic ring. On the other hand, signals from ^{1}H-NMR spectrum correspond to the absorption of respective protons. At 8.37 ppm displayed a proton singlet resulting from N=C-H. Also in the ^{13}C-NMR spectrum are displayed the signal at 164.2 ppm which corresponds to the C=N carbon.

In the IR spectrum of the product **4b**, the absorption signal appeared at 3452.98 cm^{-1} which is responsible for ν(NH) stretching vibrations, the broad band at 3300 cm-1 correspond to stretching ν(OH) absorption and signal at 3076.28 cm^{-1} resulted from ν(CH) stretching vibrations of aromatic ring. The sharp peak at 1686.12 cm^{-1} resulted from ν(C=O) stretching vibrations, whereas signals at 1610.98 and 1510.25 cm^{-1} results from ν(C=N) and ν(C=C) stretching vibrations. The peak at 1511.25 cm^{-1} correspond to absorptions of ν(NO_2) stretching asymmetric vibrations, while the one at 1321.47 cm^{-1} due to ν(NO_2) stretching symmetric vibrations. Absorption signal at 1196.56 cm^{-1} is characteristic for stretching vibrations of lactonic (C-O-C) system and the sharp peak at 762.43 cm^{-1} resulted from δ(C-H) bending oop vibrations of the aromatic ring. In the ^{1}H-NMR spectrum, besides multiplets of aromatic protons, a proton singlet at 8.39 ppm resulting from N=C-H is appeared. In the ^{13}C-NMR spectrum also displayed a signalat 163.1 ppm, that correspon to C=N carbon.

IR spectrum of the product **4c** showed an absorption peakat 34500 cm^{-1} which is responsible for ν(NH) stretching vibrations, while the absorption signal at 3083.36 cm^{-1} resulted from ν(CH) aromatic vibrations. The peak at 1686.12 cm^{-1} is responsible for absorbing the ν(C=O) stretching vibrations whereas two signals at 1627.35 and 1614.86 cm^{-1} results from ν(C=N) and ν(C=C) stretching vibrations of the aromatic ring. The sharp peak in the wavelength of 1551.61 cm^{-1} resulted due to ν(NO_2) asymmetric stretching vibrations, while absorption signal at 1349.83 cm^{-1} reflects ν(NO_2) symmetric stretching vibrations. A signal at 1204.76 cm^{-1} is characteristic for (C-O-C) stretching vibrations of lactonic system, while the sharp peak at 762.43 cm^{-1}appeared from δ(C-H) bending oop vibrations of the aromatic ring. In the ^{1}H-NMR spectrum, the multiplet signals of aromatic protons appeared at 8.0-8.3 ppm and 7.4-7.8 ppm. A proton singlet resulting from N=C-H is appeared at 8.4 ppm. In the ^{13}C-NMR spectrum also displayed a signalat 162.8 ppm, that correspond to C=N and a signal at 161.4 ppm resulted from C=O carbon.

In the IR spectrum of the compound **5a** a sharp absorption signal appeared at 3490 cm^{-1}, which is responsible for ν(NH) stretching vibrations whereas the absorption peak at 3085.25 cm^{-1} resulted from ν(CH) vibrations of aromatic system. The medium band at 2932.78 cm^{-1} resulted from the absorptions of ν(CH) stretching vibrations of aliphatic protons, whereas the sharp peak at 1686.12 cm^{-1} from ν(C=O) stretching vibrations. The absorption signal at 1610.74 cm^{-1} resulted from ν(C=C) stretching aromatic vibrations. The characteristic signals of nitro group appeared at 1511.25 cm^{-1} due to asymmetric stretching, whereas at 1327.74 cm^{-1} for symmetric ν(NO_2) stretching vibrations. The absorption peak at 1207.96 cm^{-1} resulted from lactonic ν(C-O-C) stretching vibrations, whereas at 757.95 cm^{-1} appeared the absorption signal resulted from δ(CH) oop vibrations of aromatic system. In the ^{1}H-NMR spectrum of **5a**, a proton singlet at 5.9 ppm resulted from N-C-H, while two doublets at 6.6 and 6.8 ppm and a multiplet at 7.0-7.6 ppm correspond to aromatic protons. A quartet at 3.6 ppm and doublet at 1.6 ppm results from aliphatic CH and CH_3 protons of thiazolidinone system. ^{13}C-NMR spectrum, appeared three peaks at 172.7, 162.5 and 161.3 ppm, which results from C=O and C-N carbons, whereas a signal at 54.5 ppm resulted from the C-N of thiazolidinone ring. An absorption signal at 17.2 ppm appeared due to methyl carbon.

Table 1. Physical properties of compounds 4(a-c) and 5(a-c) and their elemental analysis

Nr.	Molecular formulas	Molecular Mass	Elemental analysis (%), calc / found	mp/ °C	Yield (%)
4a	$C_{22}H_{15}N_3O_4$	385,37	(C-68.55; H-3.93; N-10.90; O-16.62) (C-67.89; H-3.75; N-10.46)	222-223	92.78
4b	$C_{22}H_{15}N_3O_5$	401,37	(C-68.82; H-3.77; N-10.47; O-19.94) (C-68.03; H-3.34; N-10.18)	204-205	34.68

4c	$C_{22}H_{14}N_4O_6$	430.37	(C-61.38; H-3.28; N-13.02; O-22.31) (C-61.02; H-3.12; N-12.89)	224-225	65.74
5a	$C_{25}H_{19}N_3O_5S$	473,50	(C-63.40; H-4.05; N-8.88; O-16.90; S-6.76) (C-63.16; H-4.62; N-8.32; S-6.34)	239-240	40.73
5b	$C_{25}H_{19}N_3O_6S$	489,50	(C-61.33; H-3.91; N-8.59; O-19.62; S-6.54) (C-1.04; H-3.46; N-8.24; S- 6.02)	233-234	65.68
5c	$C_{25}H_{18}N_4O_7S$	518,50	(C-57.90; H-3.50; N-10.80; O-21.62; S-6.18) (C-57.53; H-3.12; N-10.34; S-5.98)	229-231	33.21

In the IR spectrum of the product **5b,** a broad absorption signal appeared at 3500-3280 cm^{-1} which is responsible for ν(OH) stretching vibrations and the absorption signal at 3110.05 cm^{-1} for ν(CH) stretching vibrations of the aromatic ring. The peak at 2940.46 cm^{-1} resulted from the absorptions ν(CH) stretching vibrations of methyl group, while at the peak of 1720.62 cm^{-1} correspond to ν(C=O) stretching vibrations. The characteristic peak at 1668.19 cm^{-1} resulted from ν(C=C) stretching vibrations of aromatic moety. Signals at 1540.04 and 1343.58 cm^{-1} appeared due to ν(NO_2) asymmetric and symmetric stretching vibrations. The characteristic signal at 1187.18 cm^{-1} is responsible for lactonic ν(C-O-C) vibrations and the sharp peak at 704.14 cm^{-1} is characteristic for bending vibrations δ (C-S) of the aromatic ring. In the **^{1}H-NMR** spectrum of **5b** are shown characteristic signals at 1.5 ppm (d, 3H, CH_3), and 3.5 ppm (q, 1H, CH). **^{13}C-NMR** spectrum also showed a signa at 162.8 (C=N) and absorptions at 44.2m, 43.5 and 16.2 ppm, which correspond to CH-S, CH-N, CH_3 carbons.

IR spectrum of the product **5c** appeared the absorption signal at 3435 cm^{-1} which is responsible for ν(NH) stretching vibrations and the absorption signal at 3076.28 cm^{-1}, which resulted from ν(CH) aromatic stretching vibrations. The peak at 2914.84 cm^{-1} resulted from the absorptions of vibrations stretching ν(CH) of methyl group, while the signal at 1686.12 cm^{-1} absorption reflects the vibrations ν(C=O) stretching. The peak at the wavelength of 1627.35 cm^{-1} resulted from vibrations ν(C=C) stretching mode. The absorption peak at 1507.48 cm^{-1} resulted from ν(NO_2) asymmetric stretching vibrations, while symmetric ν(NO_2) stretching vibrations resulted by absorption peak at 1325.77 cm^{-1}. The absorption signal at 1213.32 cm^{-1} is characteristic for (C-O-C) stretching vibrations of lactonic system, while the sharp peak at 704.14 cm^{-1} is characteristic for δ(C-H) oop bending vibrations of the aromatic ring. On the other hand, characteristic signals from ^{1}H-NMR spectrum, a singlet at 5.7 ppm, a quartet 3.5 ppm and a doublet at 1.5 ppm correspond to proton absorption of N- C-H, CH and CH_3 of thiazolidinone ring. Also in the ^{13}C-NMR spectrum the thiazole carbon signals are appeared at 53.2 and 44.6 ppm, whereas a signal at 17.3 ppm resulted due to methyl carbon.

Antibacterial activity of the products 4(a-c) and 5(a-c)

Following this study, products **4(a-c)** and 5(a-c) are investigated for their antibacterial activity. Our research is oriented to test the activity against bacteria *S. aureus*, *E. coli* and *Klebsiella*, on the basis of Standard Disc Method (Bauer et al., 1966) by measuring the inhibition zones around the standard discs. The discs have previously been impregnated with solutions of the products in N,N-DMF with concentrations of 2 mg/mL, 4 mg/mL and 6mg/mL. Results are shown in Table 2 and Figs. 1-3.

Table 2. Diameter of zones of inhibition (mm) of the discs impregnated with various concentration of synthetised 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	7.0	7.5	9.0	6.5	6.5	8.0	6.0	7.0	6.5
4b	7.0	8.0	9.5	7.0	8.0	9.5	6.5	7.5	9.0
4c	7.5	8.5	8.0	7.0	8.5	9.0	7.0	8.5	9.0
5a	7,5	8.0	9.0	8.0	8.5	9.0	10.0	10.5	12.0
5b	10.5	14.5	16	9.0	10.5	11.0	8.5	10.0	10.5
5c	9.5	12.0	14.5	9.5	12.0	12.5	7.0	7.5	9.0

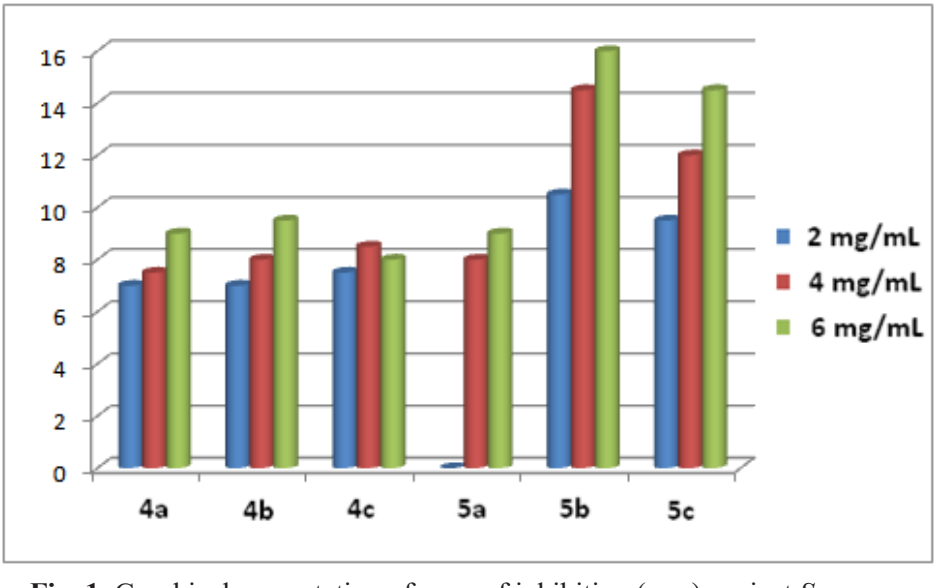


Fig. 1. Graphical presentation of zone of inhibition (mm) against *S. aureus*

Compounds of series **4** showed moderate antimicrobial activity against these microorganisms, whereas those of series **5** exhibited significant activity. Products **5b** and **5c** were most active against *S. aureus*, while **5c** and **5b** showed the most activity against *E. Coli*. Compoundes **5a** and **5b** were more active against *Klebsiella*. Antibacterial activity against *E. Coli* and *Klebsiella* appeared as bactericide activity is displayed in large-scale. Furthermore, these compounds expressed both bacteriostatic and bactericide activity against *S. Aureus*. Bacteriostatic activity is exhibited in large range (+2.5 mm), whereas bactericide activity showed in small diameter. Thiazolidin-2-one moiety showed significant impact on antimicrobial activity, whereas the impact of polar groups also was distinctly. It is particularly noted the impact of the hydroxy group of **5c**, which has affected the increase of antibacterial activity. Moreowever nitro group of **5b** has shown significant impact on the range of inhibition of *E. coli* and *S. aureus*. Assumption is that antibacterial activity may result as a consequence of the involvement of these products in enzymatic reactions. These products can cause enzyme inhibition, by inhibiting cell wall construction of the microorganisms, however, mechanism of enzymatic inhibition is not yet fully studied. In general, by increasing the concentration of solvents, their antimicrobial activity increased.

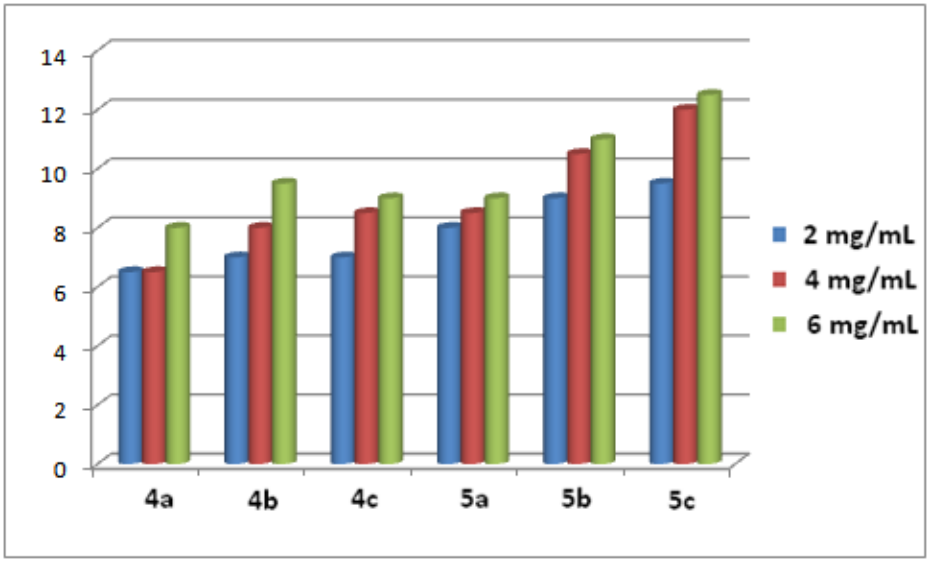


Fig. 2. Graphical presentation of zone of inhibition (mm) against *E. coli*

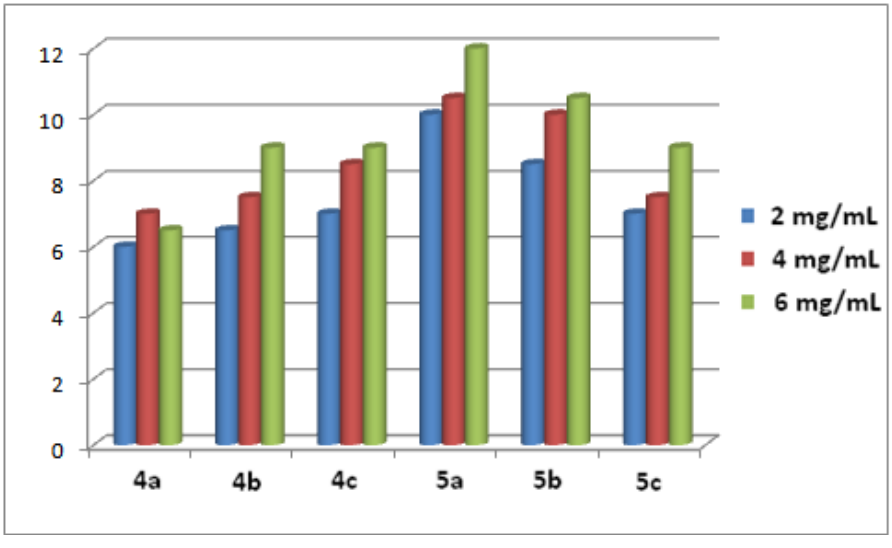


Fig. 3. Graphical presentation of zone of inhibition (mm) against *Klebsiella*

Conclusions

New derivatives of 4-[4-(Benzylidene-amino)-phenylamino]-3-nitrobenzopyran-2-ones, **4(a-c)** and respective thiazolidin-4-ones 5(a-c) are synthesized in the moderate and high yield. It is concluded that compounds **5b** and **5c** were most active against *S. aureus* and *E. Coli*, while **5a** and **5b** was more active against *Klebsiella* bacteria. The impact of polar groups in antibacterial activity was significant. Antibacterial activity is shown to be proportional to the concentration of these compounds.

REFERENCES

Ahmed, B., Khan, S.A. & Alam, T. (2003). Synthesis and antihepatotoxic activity of some heterocyclic compounds containing the 1,4-dioxane ring system. *Pharmazie, 58*, 173 – 176.

Atmaca, M., Bilgin, H.M., Obay, B.D., Diken, H., Kelle, M. & Kale, E. (2011). The hepatoprotective effect of coumarin and coumarin-derivates on carbon tetrachloride-induced hepatic injury by antioxidative activities in rats. *J. Physiol. Biochem., 67,* 569 – 576.

Bauer, A.W., Kirby, A.W., Sherris, J.C. &Turck, M. (1966). Antibiotic susceptibility testing by standardized single disc method. *Amer. J. Clin. Path., 44*, 493 – 496.

De Araújo, R.S., Guerra, F.Q., De O Lima, E., *De* Simone, C.A., Tavares, J.F., Scotti, L., Scotti, M.T., *De* Aquino, T.M., *De* Moura, R.O., Mendonça, F.J. & Barbosa-Filho, J.M. (2013). Synthesis, structure-activity relationships (SAR) and in silico studies of coumarin derivatives with antifungal activity. *Int. J. Mol. Sci., 14*, 1293 – 1309.

Desai, N.C., Satodiya, H.M., Rajpara, K.M., Joshi, V.V. & Vaghani, H.V. (2013). Microwave assisted synthesis of new coumarin based 3-cyanopyridine scaffolds bearing sulfonamide group having antimicrobial activity. *Indian J. Chem., 52B,* 904 – 914.

Hoti, R., Kalaj, V., Vehapi, I., Ismaili, H.**,** Thaçi, V. & Bicaj, M. (2010). Novel pyrimidin-2-yl-benzylidene imines and 2-[(pyrimidin-ylimino)-methyl]-phenoles and their antibacterial activity. *FASEB J., 24*, lb487.

Hoti, R., Nura-Lama, A., Mulliqi-Osmani, G., Troni, N., Gashi, F., Ismaili, H. & Thaçi, V. (2014). Synthesis of 4-triazolylamino- and 4-benzothiazolylamino-3- nitro-2H-[1]-benzopyran-2-ones and their antimicrobial activity. *Orbital, 6,*184 – 190.

Mayekar, S.A. & Mulwad, V.V. (2008). Synthesis and antibacterial activity of 6-(5phenyl- {1,3,4}thiadiazol-2-ylimino)-benzopyran-2-ones. *Indian J. Chem., 47B,*1438 – 1442.

Mazzei, M., Nieddu, E., Miele, M., Balbi, A., Ferrone, M., Fermeglia, M., Mazei, M.T., Pricle, S., La Colla, P., Marongiu, F., Ibba, C. & Loddo, R. (2008). Synthesis of Mannich bases of 7-hydroxycoumarin and screened against Fla- viviridae. *Bioorg. & Med. Chem., 16,* 2591 – 2605.

Mohamed, H.M., Abd El-Wahab, A.H.F., Ahmed,,K.A., El-Agrody, A.M., Bedair, A.H., Eid, F.A. & Khafagy, M.M. (2012). Synthesis, reactions and antimicrobial activities of 8-ethoxycoumarin derivatives. *Molecules, 17*, 971 – 988.

Nawrot-Modranka, J., Nawrot, E. & Graczyk, J. (2006). 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.

Okamoto, T., Kobayashi, T.& Yoshida, S. (2007). Synthetic derivatives of osthole for the prevention of hepatitis. *Med. Chem., 3,* 35 – 44.

Osman, H., Arshad, A., Lam, C.K. & Bagley, M.C. (2012). Microwave-assisted synthesis and antioxidant properties of hydrazinylthiazolylcoumarin derivatives. *Chem. Central J.***,** *6,* art. 32.

Rajasekaran, S., Rao, G.K., Pai, S. & Rajan, A. (2011). Design, synthesis, antibacterial and in vitro antioxidant activity of substituted 2H-benzopyran-2-one derivatives. *Intern. J. ChemTech Res., 3*, 555 – 559.

Rao, A., Carbone, A. , Chimirri, A., De Clercq, E., Monforte, A.M., Monforte, P., Pannecouque, C. & Zappalà, M. (2002). Synthesis and anti-HIV activity of 2,3-diaryl-1,3-thiazolidin-4-(thi)one derivatives. *Farmaco, 57*, 747 – 751.

Rehman, S., Ikram, M., Baker, R.J., Zubair, M., Azad, E., Min, S., Riaz, K., Mok, K. & Rehman,S.-U. (2013).Synthesis, characterization, in vitro antimicrobial, and U2OS tumoricidal activities of different coumarin derivatives. *Chem. Central J., 7,* 1 – 12.

Sashidhara, K.V., Kumar, A., Dodda, R.P., Krishna, N.N., Agarwal, P., Srivastava, K. & Puri, S.K. (2012), Coumarin-trioxane hybrids: synthesis and evaluation as a new class of antimalarial scaffolds. *Bioorg.& Med. Chem. Lett., 22*, 3926 – 3930.

Siddiqui, N., Deepanjali, Arshad, M.F. & Rana, A. (2007). Synthesis and anticonvulsant screening of 2-(substituted aryl)-3(4H 1,2,4-triazol-4-yl), 1,3-thiazolidin-4-ones. *Indian J. Heterocyclic Chem., 16,* 403 – 404.

Tyagi, Y.K., Kumar, A., Raj, H.G., Vohra, P., Gupta, G., Kumari, R., Kumar, P. & Gupta, R.K. (2005). Synthesis of novel amino and acetyl amino-4-methylcoumarins and evaluation of their antioxidant activity. *Eur. J. Med. Chem. 40*, 413 – 420.

Vazquez-Rodriguez, S., FigueroaGuinez, R.F., Matos, M.J., Santana, L., Uriarte, E., Lapier, M., Maya, J.D. & Olea-Azar, C. (2013). Synthesis of coumarin–chalcone hybrids and evaluation of their antioxidant and trypanocidal properties. *Med. Chem. Commun.***,** *4*, 993 – 1000.

Yu, D., Suzuki, M., Xie, L., Morris-Natschke, S.L. & Lee, K.H. (2003). Recent progress in the development of coumarin derivatives as potent anti-HIV agents. *Med. Res. Rev., 23,* 322 – 345.

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