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Some new [(chromen-4-ylamino)-ethyl]-azetidin-2-ones and their antibacterial activity

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ABSTRACT

A series of new azetidin-2-ones, on the basis of 3-nitrobenzopyran-2-one were synthesized by cyclo-condensation of various Schiff bases of coumarin with acetyl chloride. 4-(2-Amino-ethylamino)-3-nitro-chromen-2-one (3) is synthesized by condensation of 4-chloro-3-nitrobenzopyran-2-one (2) and ethane-1,2-diamine. The catalytic condensation of compound 3 with benzaldehyde, salicylaldehyde or 3-nitrobenzaldehyde yielded corresponding 4-[4-(benzylidene-amino)-phenylamino]-3-nitrobenzopyran-2-ones, 4a-c. The cyclization reaction of compounds 4a-c with acetyl chloride yielded corresponding substituted azetidin-2-ones, 5a-c. The structures of the obtained compounds were established by FT-IR and NMR spectrometric data and their elemental analysis. Prepared compounds 4a-c and 5a-c were screened for their antibacterial activity against *S. aureus*, *E. coli* and *Klebsiella* by disc diffusion method. Compounds 4a-c exhibited moderate antibacterial activity, whereas compounds 5a-c displayed significant activity against these microorganisms. The impact of substitutions in antimicrobial activity was also explored.

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1. Introduction

Coumarins have been found in nature as oxygen heterocycles isolated from various plants. They are important class of compounds and are the well-known for their biological activity. Coumarin derivatives play an important role in various life processes and they are found as an ingredient of the plant world. Many of such derivatives exhibit various biological activities [1]. Novobiocin Coumaromycin and Chartesium are potent antibiotics which includes coumarin moiety. Many of coumarins exhibited anticoagulant [2], antimicrobial [3], antibacterial [4,5], antifungal [6] and antimalarial [7] activity. Some of coumarin analogues exhibited antioxidant [8,9] antitubercular [10] and anticonvulsant [11] activity as well. It was reported that a significant number of substituted coumarin derivatives showed, sedative [12], analgesic and anti-inflammatory [13], anti-HIV [14] and hepatoprotective [15] activity. It is indicative that many of naturally and synthetic coumarins have found widespread usage in pharmacies [16].

On the other hand, azetidin-2-ones also have great importance because of the use of β-lactames as antibacterial agents. The most widely used antibiotics such as penicillin, cephalosporin, aztreonam, nocardicins and others, contain β-lactam moiety. Azetidin-2-ones reported to have a wide range of biological activities [17], including those antimicrobial [18], anti-fungal [19], anti-convulsant [20] and anti HIV [21] activity. The biological activity 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 biological importance of derivatives on the basis of thiazolidine-4-one has generated a constant interest in their synthesis and research. In view of the considerable importance of these derivatives and in continuation of our previous studies [22,23], the present work is aimed at the design and synthesis of new amino-ethylamino)-3-nitro-chromen-2-ones and substituted azetidin-4-ones with coumarin moiety. Moreover, the study includes testing of target compounds for their antibacterial activity against *S. Aureus*, *E. Coli* and *Klebsiella*.

2. Experimental

All the chemicals used in the synthesis were of analytical grade as commercial reagents of Aldrich Company.

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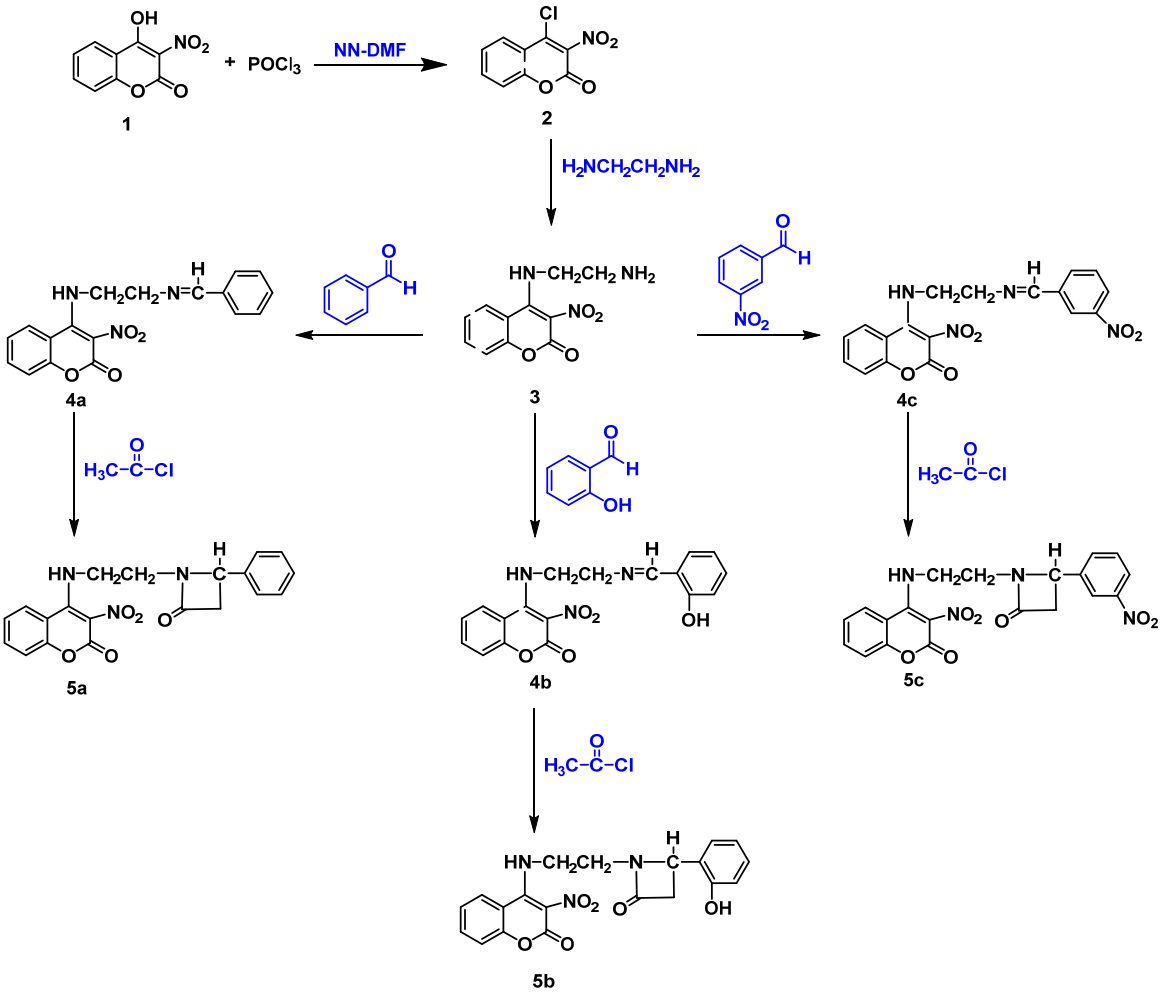

Scheme 1

Reactions were conducted by reflux under catalytic conditions. Reactions were monitored by TLC using Silica Gel $60F_{254}$ Merck as the stationary phase and a mixture of benzene, toluene, glacial acetic acid (*v*:*v*:*v*, 85:10:5) as the mobile phase. The synthesized products were purified by crystallization from methanol and ethanol. All melting points were determined on a paraffin oil bath with an open capillary tube.

Screening of the antibacterial activity of the synthesized compounds was done on the basis of Standardized Single Disk Method using standard disc [24]. The experiments were carried out at three different concentrations and standard discs were previously impregnated with 2, 4 and 6 mg/mL solutions of compounds in DMF.

2.1. Instrumentation

FT-IR spectra were recorded in KBr discs on Shimadzu 8400xFT-IR spectrometer with 4 cm^{-1} resolution. ^1H NMR and ^{13}C NMR spectra were recorded in DMSO on UNITYplus-300 NMR 1 spectrometer at 300 MHz and chemical shifts were reported in ppm downfield from TMS as internal standard (δ, 0.00).

2.2. Synthesis

2.2.1. Synthesis of 4-(2-amino-ethylamino)-3-nitro-chromen-2-one (3)

4-Chloro-3-nitrobenzopyran-2-one (2 g, 9.0 mmol) was dissolved in 6 mL of ethanol, then in small portions was added the mixture containing ethane-1,2-diamine (0.55 g, 9.0 mmol) in 5 mL of ethanol and 2-3 drops of triethylamine. The reaction mixture was stirred for 10 minutes at room temperature, then refluxed for about 90 minutes. After cooling, the mixture was concentrated in the rotary evaporator and the product was filtered off under vacuum and washed with 2 × 1 mL of ethanol. The crystalline product was dried and crystallized from methanol, giving 4-(2-amino-ethylamino)-3-nitro-chromen-2-one (Scheme 1). Color: White. Yield: 79.68 %. M.p.: 240-242 °C. FT-IR (KBr, ν, cm^{-1}): 3485, 3427, 3365, 3235, 3051, 2868, 2591, 1710, 1620, 1512, 1331, 1224, 1132, 1074, 906, 755.

2.2.2. Synthesis of 4-[4-(benzylidene-amino)-ethylamino]-3-nitrobenzopyran-2-ones (4a-c)

General procedure: Compound **3** (0.24 g, 1.0 mmol) was dissolved in 20 mL of absolute ethanol and 1.5 mmol of aromatic aldehyde (benzaldehyde, salicylaldehyde or 3-nitro benzaldehyde, respectively) dissolved in 10 mL of absolute ethanol was added in small portions to this mixture. Then, 2 drops of piperidine as a catalyst were added and the mixture

was mixed for 15 min at room temperature and refluxed for 10 to 12 hours. After cooling, the mixture was concentrated and the residue was filtered off under reduced pressure, then washed with 2×1 mL of ethanol and dried in the air. Crystallization of the products 4a-c was conducted using ethanol or methanol (Scheme 1).

4-[4-(Benzylidene-amino)-ethylamino]-3-nitro-chromen-2-one (**4a**): Color: White. Yield: 91.24%. M.p.: 212-214 °C. FT-IR (KBr, ν, cm^{-1}): 3475, 3294, 3073, 2953, 1691, 1640, 1605, 1554, 1513, 1426, 1329, 1219, 1053, 905, 754. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.35 (s, 1H, N=C-H), 7.4-7.6 (m, 4H, Ar-H), 7.2-7.4 (m, 5H, Ar-H), 3.7 (t, 2H, *J* = 6 Hz, CH_2), 3.2 (t, 2H, *J* = 6 Hz, CH_2), 2.5 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 165.4, 162.3, 160.2, 148.9, 141.5, 131.7, 130.6, 128.3, 127.6, 127.2, 126.8, 126.4, 123.8, 105.2, 48.4, 45.2. Anal. calcd. for $C_{18}H_{15}N_3O_4$: C, 64.02; H, 4.48; N, 12.45. Found: C, 64.09; H, 4.48; N, 12.46%.

4-{4-[(2-Hydroxy-benzylidene)-amino]-ethylamino}-3-nitro-chromen-2-one (**4b**): Color: White. Yield: 42.65%. M.p.: 202-203 °C. FT-IR (KBr, ν, cm^{-1}): 3476, 3303, 3045, 2945, 2894, 2375, 1679, 1609, 1546, 1512, 1436, 1325, 1283, 1209, 1194, 1052, 907, 766. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.38 (s, 1H, N=C-H), 7.4-7.6 (m, 4H, Ar-H), 7.2-7.5 (m, 4H, Ar-H), 3.8 (t, 2H, *J* = 6 Hz, CH_2), 3.2 (t, 2H, *J* = 6 Hz, CH_2), 2.6 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 163.8, 161.2, 160.6, 148.3, 141.6, 132.4, 130.2, 128.6, 126.8, 125.2, 124.4, 123.4, 121.2, 115.6, 115.2, 105.8, 49.2, 45.6. Anal. calcd. for $C_{18}H_{15}N_3O_5$: C, 61.11; H, 4.29; N, 11.86. Found: C, 61.13; H, 4.28; N, 11.89 %.

3-Nitro-4-{4-[(3-nitro-benzylidene)-amino]-ethylamino}-chromen-2-one (**4c**): Color: White. Yield: 58.66%. M.p.: 219-221 °C. FT-IR (KBr, ν, cm^{-1}): 3468, 3087, 3046, 2962, 2360, 1689, 1627, 1612, 1556, 1342, 1208, 1069, 763. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.5 (s, 1H, Ar-H), 8.1-8.3 (m, 3H, Ar-H), 7.7-7.4 (m, 4H, Ar-H), 3.9 (t, 2H, *J* = 6 Hz, CH_2), 3.1 (t, 2H, *J* = 6 Hz, CH_2), 2.7 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 163.7, 162.6, 158.8, 148.4, 141.2, 134.6, 131.6, 128.5, 128.1, 127.4, 126.4, 125.2, 124.9, 124.6, 123.2, 103.2, 49.4, 46.1. Anal. calcd. for $C_{18}H_{14}N_4O_6$: C, 56.46; H, 3.71; N, 14.62. Found: C, 56.49; H, 3.69; N, 14.65%.

2.2.3. Synthesis of 3-[4-(3-nitro-2-oxo-2H-chromen-4-yl amino)-ethyl]-4-phenyl-azetidin-2-ones (5a-c)

General procedure: The corresponding product **4a-c** (0.5 mmol) was dissolved in 10 mL of benzene, and then 0.12 g (1.5 mmol) of thioacetic acid was added. The reaction mixture was stirred for 10 min at room temperature and then refluxed for 12-13 hours. After cooling the product was concentrated and the remaining solid was dissolved in 5 mL of methanol, then heated to the boiling point and the excess of acetic acid was neutralized by adding 0.3 mmol sodium bicarbonate (controlled paper litmus until the solution takes a blue colour). The mixture was cooled in an ice bath and filtered off under vacuum, then was washed with 2 × 1 mL of ether and dried in air. The products were crystallized using methanol (Scheme 1).

1-[4-(3-Nitro-2-oxo-2H-chromen-4-ylamino)-ethyl]-4-phenyl-azetidin-2-one (**5a**): Color: White. Yield: 54.35%. M.p.: 230-232 °C. FT-IR (KBr, ν, cm^{-1}): 3495, 3236, 3083, 2946, 2372, 1683, 1616, 1583, 1521, 1424, 1327, 1210, 1065, 860, 754, 586. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.0-7.5 (m, 9H, Ar-H), 5.6 (t, 1H, *J* = 8 Hz, N=C-H), 3.9 (t, 2H, *J* = 6 Hz, CH_2), 3.5 (d, 2H, *J* = 8 Hz, CH_2), 3.1 (t, 2H, *J* = 6 Hz, CH_2), 2.8 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 172.5, 162.6, 161.8, 149.4, 137.4, 130.2, 129.7, 128.1, 127.3, 127.0, 126.7, 125.8, 124.6, 105.3, 55.4, 49.6, 45.2, 36.4. Anal. calcd. for $C_{20}H_{17}N_3O_5$: C, 63.25; H, 4.49; N, 11.10. Found: C, 63.26; H, 4.52; N, 11.07%.

4-(2-Hydroxy-phenyl)-1-[4-(3-nitro-2-oxo-2H-chromen-4-ylamino)-ethyl]-azetidin-2-one (**5b**): Color: White. Yield: 69.76%. M.p.: 233-234 °C. FT-IR (KBr, ν, cm^{-1}): 3550-3300, 3105, 2937, 2554, 2381, 1922, 1720, 1673, 1612, 1540, 1414, 1348, 1340, 1176, 1057, 1006, 882, 708, 657. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 7.1-7.4 (m, 6H, Ar-H), 6.8 (d, 1H, Ar-H), 6.6 (d, 1H, Ar-H), 5.7 (t, 1H, *J* = 8 Hz, N-CH), 3.6 (d, 2H, *J* = 8 Hz, CH_2), 3.7 (t, 2H, *J* = 6 Hz, CH_2), 3.3 (t, 2H, *J* = 6 Hz, CH_2), 2.4 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 170.5, 162.8, 161.8, 160.2, 156.4, 149.6, 130.2, 128.1, 126.9, 126.2, 125.7, 124.9, 124.1, 122.2, 120.3, 105.4, 56.5, 49.2, 46.4, 37.2. Anal. calcd. for $C_{20}H_{17}N_3O_6$: C, 60.67; H, 4.31; N, 10.59. Found: C, 60.70; H, 4.33; N, 10.62%.

1-[4-(3-Nitro-2-oxo-2H-chromen-4-ylamino)-ethyl]-4-(3-nitro-phenyl)-azetidin-2-one (**5c**): Color: White. Yield: 44.73%. M.p.: 221-223 °C. FT-IR (KBr, ν, cm^{-1}): 3465, 3235, 3067, 2922, 2548, 2382, 1918, 1678, 1624, 1617, 1566, 1509, 1334, 1213, 1050, 837, 709, 584. ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 8.1 (s, 1H, Ar-H), 7.1-7.7 (m, 7H, Ar-H), 5.8 (t, 1H, N-CH), 3.9 (t, 2H *J* = 6 Hz, CH_2) 3.7 (d, 2H, CH_2), 3.0 (t, 2H, *J* = 6 Hz, CH_2), 2.8 (1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, δ, ppm): 171.6, 162.8, 160.6, 156.9, 152.4, 148.4, 137.4, 131.6, 129.2, 128.6, 127.1, 126.2, 125.3, 123.8, 121.2, 105.4, 54.6, 48.8, 45.4, 38.6. Anal. calcd. for $C_{20}H_{16}N_4O_7$: C, 56.57; H, 3.77; N, 13.21. Found: C, 56.55; H, 3.80; N, 13.19%.

3. Results and discussion

3.1. Synthesis

By condensation reaction of 4-chloro-3-nitrobenzopyran-2-one (**2**) with ethane-1,2-diamine, 4-(4-amino-ethylamino)-3-nitro-chromen-2-one (**3**) was synthesized in good yield. Corresponding Schiff bases are synthesized by condensation reaction of compound **3** with benzaldehyde, salicylaldehyde and/or 3-nitrobenzaldehyde. Novel 4-[4-(benzylidene-amino)-ethylamino]-3-nitrobenzopyran-2-one derivatives (**4a-c**) as condensation products are synthesized in good yield. In the last step, by cyclization of compounds **4a-c** with acetyl chloride, corresponding 1-[4-(3-nitro-2-oxo-2*H*-chromen-4-ylamino)-ethyl]-azetidin-2-ones (**5a-c**) are synthesized. Synthesis of Schiff bases and related azetidine-2-ones are summarized in Scheme 1.

Structural characterization of the synthesized products is based on spectrometric FT-IR and NMR data. The IR spectrum of compound **3** showed absorption bands at 3485 and 3427 cm^{-1} confirming the presence of NH_2 group. The absorption signal at 3051 cm^{-1} appears due to aromatic ν(CH) stretching vibrations. The sharp peak at 1710 cm^{-1} is responsible for ν(C=O) stretching vibrations, whereas the absorption peak at 1620 cm^{-1} coming from aromatic ν(C=C) stretching vibrations. The peak at 1512 cm^{-1} results from the absorptions of asymmetric ν(NO_2), while the peak at 1331 cm^{-1} coming from symmetric ν(NO_2) stretching vibrations. On the other hand, the absorption peak at 1224 cm^{-1} is characteristic for lactonic system (C-O-C), while the sharp peak at 755 cm^{-1} coming from characteristic aromatic δ(C-H) oop bending vibrations.

For compound **4a**, the IR spectrum showed an absorption signal at 3475 cm^{-1} which is responsible for ν(NH) stretching vibrations, while signal at 3073 and 2953 cm^{-1} corresponds to aromatic and ethylene ν(CH) vibrations. At 1691 cm^{-1} appears the absorption signal which responds to ν(C=O) stretching vibrations, whereas the sharp peak at 1640 cm^{-1} and a signal at 1605 cm^{-1} correspond due to aromatic ν(C=N) and ν(C=C) stretching vibrations. The characteristic peak at 1554 cm^{-1} results from asymmetric ν(NO_2), while at 1329 cm^{-1} due to symmetric ν(NO_2) stretching vibrations. A signal at 1219 cm^{-1} is also characteristic for lactonic stretching (C-O-C) vibrations, whereas the sharp peak at 754 cm^{-1} is charac-teristic for aromatic bending δ(C-H) oop vibrations. On the other hand, the ^{1}H NMR spectrum correspond to the absorp-tion of respective protons, at δ 8.35 ppm a proton singlet resulting from N=C-H is displayed.

Table 1. Zone of inhibition (mm) of the discs impregnated with various concentration of synthesized compounds.

Compound	*S. aureus*			*E. coli*			*Klebsiella*		
	2 mg/mL	4 mg/mL	6 mg/mL	2 mg/mL	4 mg/mL	6 mg/mL	2 mg/mL	4 mg/mL	6 mg/mL
4a	6.0	7.0	8.0	6.5	6.5	8.0	6.0	6.5	6.5
4b	6.5	7.0	7.5	6.5	7.0	7.5	7.5	8.0	8.0
4c	7.0	7.0	8.5	7.0	8.5	9.0	6.5	7.5	8.0
5a	7.5	8.8	9.5	9.0	10.5	11.0	10.5	11.5	13.0
5b	12.5	13.5	15.0	9.5	11.0	12.0	9.5	10.0	11.0
5c	9.0	11.0	13.5	9.0	8.5	9.5	7.5	8.5	9.0

Furthermore two triplets at δ 3.7 and 3.2 ppm appear due to ethylene protons. Also, in the ^{13}C NMR spectrum the signal at δ 165.4 ppm which corresponds to the C=N carbon is displayed.

The IR spectrum of compound **4b** show a signal at 3476 cm^{-1} responsible for ν(NH) stretching vibrations and a broad band at 3303 cm^{-1} due to stretching ν(OH) absorption. Absorption signals appeared at 3045 and 2945 cm^{-1} results from aromatic and ethylene ν(CH) stretching vibrations. The sharp peak at 1679 cm^{-1} results from ν(C=O) stretching vibrations, whereas signals at 1609 and 1546 cm^{-1} results from ν(C=N) and ν(C=C) stretching vibrations. The peak at 1512 cm^{-1} corresponds to absorptions of ν(NO_2) stretching asymmetric, while the one at 1325 cm^{-1} to ν(NO_2) stretching symmetric vibrations. A signal at 1209 cm^{-1} is characteristic for stretching vibrations of lactonic (C-O-C) system and the sharp peak at 766 cm^{-1} results from aromatic δ(C-H) bending oop vibrations. In the ^{1}H NMR spectrum, besides multiplets of aromatic protons, a proton singlet resulting from N=C-H is appears at δ 8.38 ppm and triplets at δ 3.8 and 3.2 ppm correspond to ethylene protons. In the ^{13}C NMR spectrum a signal at δ 163.8 ppm for C=N carbon and signals at δ 49.2 and 45.6 ppm due to ethylene group are displayed as well.

The IR spectra of compound **4c** show an absorption peak at 3468 cm^{-1} which is responsible for ν(NH) stretching vibrations, while the signals at 3087 and 2962 cm^{-1} appear due to aromatic and ethylene ν(CH) stretching vibrations. The peak at 1689 cm^{-1} is responsible for absorbing the ν(C=O) stretching vibrations while signals at 1627 and 1612 cm^{-1} result from aromatic ν(C=N) and ν(C=C) stretching vibrations. The sharp peak at 1556 cm^{-1} results from ν(NO_2) asymmetric stretching vibrations, while absorption signal at 1342 cm^{-1} reflects ν(NO_2) symmetric stretching vibrations. A signal at 1208 cm^{-1} is characteristic for lactonic (C-O-C) stretching vibrations, while the sharp peak at 763 cm^{-1} appears from aromatic δ(C-H) bending oop vibrations. The ^{1}H NMR spectra show the multiplet signals of aromatic protons at δ 8.1-8.3 and 7.7-7.4 ppm. A proton singlet resulting from N=C-H at δ 8.5 ppm appears as well. In the ^{13}C NMR spectra a signal at δ 163.7 ppm, that correspond to C=N and signals at δ 162.6, 49.4 and 46.1 ppm resulting from C=O and respective CH_2 carbons are displayed.

In the IR spectra of the compound **5a** a sharp absorption signal appears at 3495 cm^{-1} which is responsible for ν(NH) stretching vibrations whereas the absorption peak at 3083 cm^{-1} results from aromatic ν(CH) vibrations. A medium band at 2946 cm^{-1} resulted from the absorptions of ν(CH) stretching vibrations of aliphatic protons, whereas a sharp peak at 1683 cm^{-1} results from ν(C=O) stretching vibrations. The absorption signal at 1616 cm^{-1} resulted from ν(C=C) stretching aromatic vibrations. The characteristic signals due to ν(NO_2) stretching vibrations appeared at 1521 and 1327 cm^{-1}. The absorption peak at 1210 cm^{-1} results from lactonic ν(C-O-C) stretching vibrations, whereas at 754 cm^{-1} from aromatic δ(CH) oop vibrations. The ^{1}H NMR spectra, a proton triplet at δ 5.6 ppm results from N-C-H, while two triplets at δ 3.9 and 3.1 ppm appear due to ethylene protons. Moreover a multiplet at δ 7.5-7.0 ppm corresponds to aromatic protons, while a doublet at δ 3.5 ppm results from aliphatic azetidinone CH_2 protons. The ^{13}C NMR spectra exhibit three peaks at δ 172.5, 162.6 and 161.8 which results from C=O and C-N carbons, whereas a signal at δ 55.4 ppm results due to CH-N of azetidiidinone ring. An absorption signal at δ 36.4 ppm appears due to methylene carbon and signals at δ 49.6 and 45.2 results from ethylene carbons.

In the IR spectra of compound **5b**, a broad absorption signal appears at 3550-3300 cm^{-1} which is responsible for ν(OH) stretching vibrations and the absorption signal at 3105 cm^{-1} for aromatic ν(CH) stretching vibrations. The peak at 2937 cm^{-1} results from methylene ν(CH) stretching vibrations, while at the peak of 1720 cm^{-1} correspond to ν(C=O) stretching vibrations. The characteristic peak at 1673 cm^{-1} results from ν(C=C) stretching vibrations of aromatic moiety. Signals at 1540 and 1340 cm^{-1} appear due to ν(NO_2) asymmetric and symmetric stretching vibrations, whereas the characteristic signal at 1176 cm^{-1} is responsible for lactonic ν(C-O-C) vibrations. In the ^{1}H NMR spectra are shown characteristic triplets at δ 3.7 and 3.3 ppm due to ethylene protons. A doublet at δ 3.6 ppm for azetidine CH_2 and a triplet at δ 5.7 ppm for N-C-H appear as well. The ^{13}C NMR spectrum also shows a signal at δ 162.8 (C=N) and absorptions at δ 56.5 ppm (CH-N) and 37.2 ppm (CH_2). Characteristic signals at δ 49.2 and 46.4 ppm correspond to ethylene carbons.

IR spectrum of compound **5c** exhibit the absorption signal at 3465 cm^{-1} responsible for ν(NH) stretching vibrations and a signal at 3067 cm^{-1} which resulted from aromatic ν(CH) stretching vibrations. The peak at 2922 cm^{-1} results from the absorptions of methylene stretching vibrations, while the signal at 1678 cm^{-1} reflects the ν(C=O) stretching vibrations. The peak at 1624 cm^{-1} results from ν(C=C) stretching mode. The absorption peak at 1509 cm^{-1} results from ν(NO_2) asymmetric, while symmetric ν(NO_2) stretching vibrations result in absorption peak at 1334 cm^{-1}. A signal at 1213 cm^{-1} is characteristic for lactonic (C-O-C) stretching vibrations, while the sharp peak at 709 cm^{-1} is characteristic for aromatic δ(C-H) oop bending vibrations. On the other hand, characteristic signals of ^{1}H NMR spectra, a triplet at δ 5.8 ppm and a doublet at δ 3.7 pm correspond to proton absorptions of N-C-H and CH_2 of azetidinone ring. Also, in the ^{1}H NMR spectrum the ethylene carbon signals are appears as triplets at δ 3.9 and 3.0 ppm. In the ^{13}C NMR spectrum were appear signals at δ 171.6, 162.8 and 160.6 due to (C=O) and (C=N) carbons, while absorption modes at δ 54.6 and 38.6 ppm resulted from (CH-N) and (CH_2) of azetidinone moiety. Ethylene carbons were exhibit signals at δ 48.8 and 38.6 ppm.

3.2. Antibacterial activity of the products 4a-c and 5a-c

Following this study, products **4a-c** and **5a-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 Standardized Single Disk Method [24]. The discs have previously been impregnated with solutions of the compounds in DMF with concentrations of 2, 4 and 6 mg/mL. Results are expressed in mm and were summarized in Table 1.

Compounds of series **4** show moderate antimicrobial activity against these microorganisms, whereas compounds of series **5** exhibit significant activity. Compounds **5b** and **5c** were most active against *S. aureus*, compounds **5b** and **5a**

show the most activity against *E. coli* whereas compounds **5a** and **5b** were more active against *Klebsiella*. Antibacterial activity against *E. coli* and *Klebsiella* shown as bactericide activity is displayed in large-scale. Furthermore, these compounds express both bacteriostatic and bactericide activity against *S. aureus*. Bacteriostatic activity is exhibited in large range (+2.0 mm), whereas bactericide activity shows smaller zone of inhibition. Azetidin-2-one moiety shows significant impact on antimicrobial activity. Likewise, the impact of polar groups is distinctive. The impact of the hydroxy group of compound **5b**, which has affected the increase of antibacterial activity, has been particularly noted. Moreover, nitro group of compound **5b** has shown significant impact on the range of inhibition of *E. coli*.

It has been assumed that antibacterial activity may result as a consequence of the involvement of these compounds in enzymatic reactions. They may cause enzymatic inhibition cell wall construction of the microorganisms. However, the mechanism of enzymatic inhibition has not been fully studied yet. In general, by increasing the concentration of solvents, their antimicrobial activity increases.

4. Conclusion

New derivatives of 4-[4-(benzylidene-amino)-ethylamino]-3-nitrobenzopyran-2-ones, **4a-c** and respective azetidin-2-ones **5a-c** are synthesized in the high to moderate yield. It has been concluded that compounds **5b** and **5c** show significant activity against *S. aureus,* compounds **5b** and **5a** display more activity against *E. coli*, whereas compounds **5a** and **5b** were 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.

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