Synthesis and Antimicrobial Activity of a Number of 3-substituted-8-allylbenzoxazines and their Hydrobromic Salts

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Summary: In this study, novel 1,3-benzoxazines **3a–d** were synthesized starting from 2-allylphenol (**2**), formaldehyde and primary amines under Mannich reaction conditions, as well as their ammonium salts **4a–d,** which were prepared by reaction of compounds **3a–d** with HBr. As primary amines aniline, propylamine, n-hexylamine and n-octylamine were used. Synthesized heterocyclic compounds and their ammonium salts (**3a–d** and **4a–d**) were characterized by elemental analysis, IR and NMR spectra. The antimicrobial activity of the synthesized compounds **3a–d** and **4a–d** at concentration 15 and 30 mg×L^{-1} against microorganisms (*Staphylococcus aureus* NCTC6571, *Staphylococcus aureus* ATCC®25923, *Escherichia coli* ATCC®25922, *Candida albicans* NSTC - 3255/ATCC2091, *Shigella flexneri* ATCC®12022, *Salmonella enterica* ATCC®13076, and *Aspergillus niger* [isolate obtained from water]) was studied by the disk-diffuse method. It was found that compounds **3a–c** are very effective against bacteria *Staphylococcus aureus* - NCTC 6571, *Staphylococcus aureus* ATCC®25923, *Escherichia coli* ATCC®25922, *Shigella flexneri* ATCC®12022, and *Salmonella enterica* ATCC®13076, but slightly effective against *Candida albicans* and *Aspergillus niger*. However, analysis revealed that the surfactants **4a–d** have antimicrobial activity against all the above microorganisms, i.e., **4a–d** have both bactericidal and fungicidal activity, which is explained by the present in its structure of the bromine anion, an ammonium fragment and a multiple bond. For comparison, as references at the identical concentration (30 mg×L^{-1}) and conditions amoxicillin and fluconazole were used. It was revealed, that the substances **4a–d,** in comparison with the references used, are much higher in bactericidal and fungicidal effectiveness. Bactericidal efficacy was also confirmed by electron microscopy studies of *Escherichia coli* ATCC®25922 cells in the present of heterocyclic compound **4c** (contain in the structure N-hexyl and allyl fragments) and amoxicillin (reference). It was found that, in the present of the synthesized compound **4c** complete destruction of *Escherichia coli* ATCC®25922 cells was carried out (unlike to the presence of amoxicillin, used as reference).

Keywords: Allylphenol, 1,3-benzoxazines, Ammonium salts, Bactericides, Fungicides.

Introduction

Functionally substituted aromatic and other organic compounds, which especially contain multiple bonds in their structures, have a number of valuable properties [1–12].

A huge number of heterocyclic products with valuable properties have been synthesized based on phenols, including benzoxazines obtained by triple condensation of phenol, bis-phenol, and its substituted derivatives with formaldehyde and different types of primary amines (Mannich) [13–18]. Even though this reaction has been studied for a long time [19, 20], interest in it does not weaken, as evidenced by numerous articles published in recent years [18, 21–26].

It should be noted that benzoxazines and their derivatives have a wide range of interesting applications in several areas [13, 27–36] and are used as precursors in different important reactions, such as polymerization reactions, due to the opening of 1,3-benzoxazine rings [37–39].

Moreover, benzoxazines which contain double bonds in their structure were also obtained and their thermal properties, transformations and application as monomers were studied [23, 24, 38, 39–41]. In addition, the study [42] presents the mechanochemical synthesis of benzoxazines based on 2-allylphenol, paraform, and some aromatic amines of various structures. However, synthesis of benzoxazines based on 2-allylphenol and aryl, alkyl substituted primary amines by known method [15] with higher yields [42], and study of their antimicrobial properties are not explored.

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It is important to note that in recent work [18], synthesis and study of the antimicrobial properties of tert-alkyl substituted benzoxazines was shown, and it was revealed that introduction of the bulky substituent in the ortho-position of aryl ring of benzoxazines caused a loss of their antibacterial efficacy. Also, it is known that the allyl substituted compound showed antimicrobial efficacy [43, 44].

Therefore, we synthesized benzoxazines and their derivatives based on 2-allylphenol, contained in the simultaneous structures of a multiple bond and a 1,3-benzoxazine ring and other fragments while studying their antimicrobial activity depending on their structure.

This study aimed to discover and characterize new types of 1,3-benzoxazines based on 2-allylphenol (using the Mannich reaction), formaldehyde and several primary amines (aniline, propylamine, n-hexylamine and n-octylamine), as well as ammonium salts based on the synthesized 8-allylbenzoxazines and study their antimicrobial activity against microorganisms (*Staphylococcus aureus, Escherichia coli, Candida albicans, Shigella flexneri, Salmonella enterica* and *Aspergillus niger*).

Experimental

Measurements

The structures of the prepared compounds **3a–d** were confirmed and characterized by ^{1}H, ^{13}C NMR spectroscopy using BRUKER FT NMR spectrometer AVANCE 300 (300 MHz) with a BVT 3200 variable temperature unit, with 5-mm sample tubes and Bruker Standard software. The solvent used was $CDCl_3$. IR spectra of were recorded in a Varian 640 FT-IR spectrometer in the form of thin films. For this purpose, KBr substrates were used. The spectra were taken in the range of 4000–400 cm^{-1}. spectrometer. Elemental analysis was performed with a Carlo Erba device, model EA 1108. The refractive index (n_D^{20}) was determined with an ABBEMAT 350/500 refractometer, and the density (*d*), with a DMA 4500 M device.

Synthesis of **3a-d**

Synthesis of 8-allyl-3-phenyl-3,4-dihydro-2H-benzo[e][1,3]oxazine **(3a)**

8-Allyl-3-phenyl-3,4-dihydro-2H-benzo[e][1,3]oxazine (**3a**) was obtained by interaction of 13.4 g (0.1 mol) 2-allylphenol (Aldrich) (**2**) with the mixture of 16.2 ml formalin (37% aqueous solution of formaldehyde (0.2 mol)) (Aldrich) and 9.3 g (0.1 mol) aniline (Karmalab) [45]. The reaction was carried out at 95°C for 1.5 h (Scheme 1). Yield of **3a** was 62%. n_D^{20} = 1.5650, d_4^{20} = 1.020 g×cm^{-3}. Found, %: C 81.15; H 6.80; N 5.52. Calculated for $C_{17}H_{17}NO$, %: C 81.24; H 6.82; N 5.57. ^{1}H NMR-spectra ($CDCl_3$; δ, ppm): 3.64 (2H, d, J=6 Hz, CH_2=CH-C<u>H</u>$_2$); 4.81 (2H, s, N-CH_2-Ar); 5.31-5.36 (2H, m, CH_2=); 5.57 (2H, s, O-CH_2-N-); 6.21-6.35 (1H, m, Ar-CH_2-C<u>H</u>=); 7.10-7.51 (8H, m, arom). ^{13}C NMR-spectra ($CDCl_3$; δ, ppm): 33.96; 50.78; 79.55; 115.75; 117.91; 118.37; 120.55; 120.77; 121.30; 125.05; 128.21; 129.56; 136.99; 148.49; 152.24. IR (cm^{-1}): 694 (mono substituted aromatic ring); 750 (three substituted aromatic ring); 3026 (C-H-arom.), 1497, 1463, 1369, 2892 (CH_2); 2848, 942 (C-H of double bond of allyl fragment); 1637 (C=C-allyl); 1599 (C=C-arom); 1221 (C-O); 1156 (C-N).

Synthesis of 8-allyl-3-propyl-3,4-dihydro-2H-benzo[e][1,3]oxazine **(3b)**

8-Allyl-3-propyl-3,4-dihydro-2H-benzo[e][1,3]oxazine (**3b**) was prepared by reacting 13.4 g (0.1 mol) 2-allylphenol (Aldrich) (**2**), 16.2 ml formalin (37% aqueous solution of formaldehyde (0.2 mol)) (Aldrich) and 5.9 g (0.1 mol) propylamine (Karmalab). Firstly, the mixture containing formalin and propylamine was stirred at temperatures of up to 10°C for 0.5 h. The resultant mixture was stirred with 2-allylphenol at 95°C for 1.5 h (Scheme 1). Yield of **3b** was 68%. bp=134-136°C/3-4mm. n_D^{20} = 1.5291, d_4^{20} = 1.0223 g×cm^{-3}. Found, %: C 77.33; H 8.78; N 6.42. Calculated for $C_{14}H_{19}NO$, %: C 77.38; H 8.81; N 6.45. ^{1}H NMR-spectra ($CDCl_3$; δ, ppm): 1.07 (3H, t, J=6 Hz, CH_2-C<u>H</u>$_3$); 1.65-1.78 (2H, m, -C<u>H</u>$_2$-CH_3); 2.82 (2H, t, J=7 Hz, N-C<u>H</u>$_2$-CH_2); 3.48 (2H, d, J=6 Hz, =CH-C<u>H</u>$_2$-Ar); 4.09 (2H, s, N-CH_2-Ar); 4.99 (2H, s, O-CH_2-N); 5.15-5.25 (2H, m, C<u>H</u>$_2$=CH-); 6.06-6.15 (1H, m, CH_2=C<u>H</u>-); 6.93-7.12 (3H, m, arom). ^{13}C NMR-spectra ($CDCl_3$; δ, ppm): 11.86; 21.46; 33.81; 50.33; 53.42; 82.69; 115.45; 120.03; 125.59; 127.61; 127.97; 136.97; 152.04. IR (cm^{-1}): 749 (three substituted aromatic ring); 3072 (C-H-arom), 1464, 1335, 2959, 2931 (CH_2,CH_3); 1637 (C=C-allyl); 1593 (C=C-arom); 2878, 934 (C-H of double bond of allyl fragment); 1216 (C-O); 1139 (C-N).

Synthesis of 8-allyl-3-hexyl-3,4-dihydro-2H-benzo[e][1,3]oxazine **(3c)**

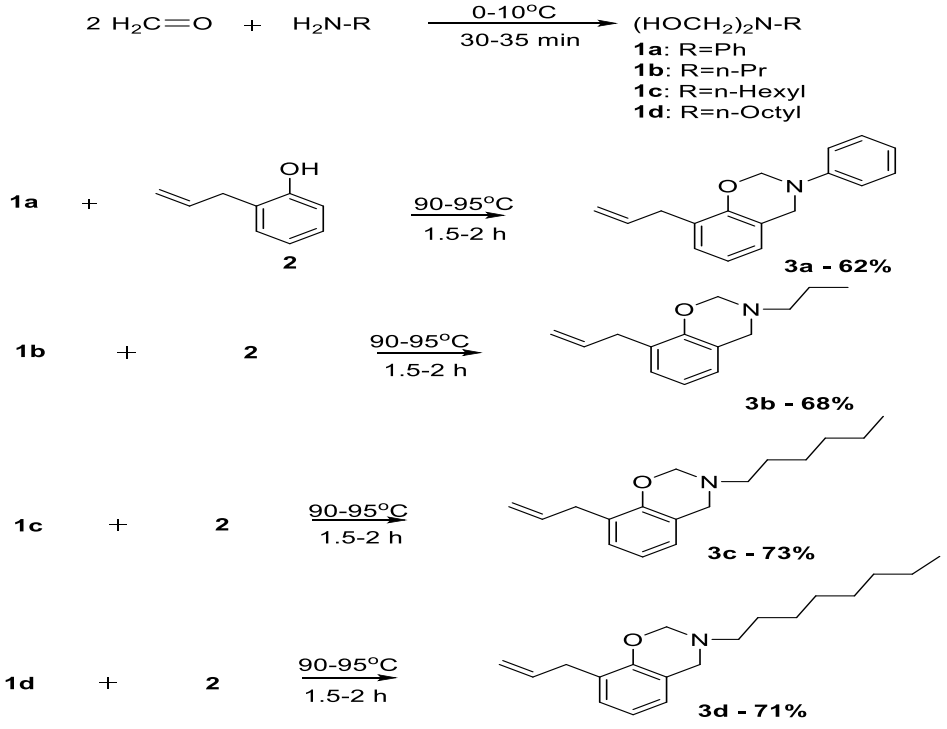
8-Allyl-3-hexyl-3,4-dihydro-2H-benzo[e][1,3]oxazine (**3c**) was obtained by the same method as above – by interaction of 13.4 g (0.1 mol)

2-allylphenol (Aldrich) (**2**), 16.2 ml formalin (37% aqueous solution of formaldehyde (0.2 mol)) (Aldrich) and 8.4 g (0.1 mol) hexylamine (Karmalab). Reaction was carried out in the same conditions – at 95°C for 2 h (Scheme 1). Yield of **3c** was 73%. bp=154-156°C/3-4mm. n_D^{20}= 1.5157, d_4^{20} = 1.0301 g×cm^{-3}. Found,%: C 78.68; H 9.69; N 5.38. Calculated for $C_{17}H_{25}NO$, %: C 78.72; H 9.71; N 5.4. ^{1}H NMR-spectra ($CDCl_3$; δ, ppm): 0.95 (3H, t, J=6 Hz, CH_3); 1.34-1.62 (8H, $(CH_2)_4$); 2.79 (2H, t, J=6 Hz, N-C<u>H</u>$_2$-CH_2); 3.4 (2H, d, J=7 Hz, =CH-C<u>H</u>$_2$-); 4.05 (2H, s, N-CH_2-Ar); 4.95 (2H, s, O-CH_2-N); 5.09-5.15 (2H, m, CH_2=); 5.99-6.12 (1H, m, =CH-); 6.83-7.05 (3H, m, arom). ^{13}C NMR-spectra ($CDCl_3$; δ, ppm): 14.13; 22.92; 27.05; 28.01; 31.83; 33.77; 51.47; 52.50; 82.68; 115.6; 120.00; 125.53; 127.49; 128.29; 136.89; 137.15; 151.86. IR (cm^{-1}): 748 (three substituted aromatic ring); 3073 (C-H-arom.), 1462, 1375, 1335, 2951, 2929 (CH_2,CH_3); 1638 (C=C-allyl); 1593 (C=C-arom); 2855, 931 (C-H of double bond of allyl fragment); 1220 (C-O); 1138 (C-N).

Synthesis of 8-allyl-3-octyl-3,4-dihydro-2H-benzo[e][1,3]oxazine **(3d)**

3d was obtained by reacting 13.4 g (0.1 mol) **2**, 16.2 ml formalin (0.2 mol formaldehyde) and 12.9 g (0.1 mol) n-octylamine (Alfa Aesar) at 95°C for 2 h (Scheme 1). Yield of **3d** was 71%. bp=179-180°C/1-2mm. n_D^{20}= 1.5085, d_4^{20} = 1.0379 g×cm^{-3}. Found,%: C 79.68; H 10.13; N 4.67. Calculated for $C_{19}H_{29}NO$, %: C 79.39; H 10.17; N 4.88. ^{1}H NMR-spectra ($CDCl_3$; δ, ppm): 0.95 (3H, t, J=6 Hz, CH_3); 1.34-1.62 (12H, $(CH_2)_6$); 2.78 (2H, t, J=9 Hz, N-C<u>H</u>$_2$-CH_2); 3.4 (2H, d, J=6 Hz, =CH-C<u>H</u>$_2$-); 4.04 (2H, s, N-CH_2-Ar); 4.93 (2H, s, O-CH_2-N); 5.08-5.13 (2H, m, CH_2=); 5.99-6.12 (1H, m, =CH-); 6.83-7.05 (3H, m, arom). ^{13}C NMR-spectra ($CDCl_3$; δ, ppm): 14.17; 22.73; 27.34; 28.22; 29.35; 31.91; 33.71; 50.44; 51.48; 52.50; 82.56; 115.37; 119.94; 125.52; 127.46; 136.87; 151.86. IR (cm^{-1}): 748 (three substituted aromatic ring); 3075 (C-H-arom.), 1461, 1376, 1333, 2924, 2952 (CH_2,CH_3); 1638 (C=C-allyl); 1593 (C=C-arom); 2853, 926 (C-H of double bond of allyl fragment); 1221 (C-O); 1137 (C-N).

Obtained **3a-d** are liquids with orange and yellow coloured and with characteristic odor, insoluble in water but readily soluble in organic solvents (acetone, benzene, n-hexane, CCl_4, $CHCl_3$, etc.). **3a-d** were extracted from the reactants' mixture by treatment with an aqueous solution of potassium hydroxide, and then extracted in benzene and dried with Na_2SO_4, evaporated and distilled.



Scheme-1: Synthesis of **3a-d.**

Synthesis of **4a-d**

8-Allyl-3-benzyl-3,4-dihydro-2H-benzo[e][1,3]oxazin-3-ium bromide (**4a**), 8-allyl-3-propyl-3,4-dihydro-2H-benzo[e][1,3]oxazin-3-ium bromide (**4b**), 8-allyl-3-hexyl-3,4-dihydro-2H-benzo[e][1,3]oxazin-3-ium bromide (**4c**) and 8-allyl-3-octyl-3,4-dihydro-2H-benzo[e][1,3]oxazin-3-ium bromide (**4d**) were prepared with a reaction of equimolar amounts 8-allylbenzoxazines (**3a-d**) with hydrogen bromide at room temperature for a reaction period 0.5 h (Scheme 2) in benzene (solvent). The obtained **4a-d** were purified several times in benzene from initial benzoxazines (**3a-d**) and then dried under vacuum. Ammonium salts **4a-d** are powders with yellow and pink colour, soluble in water and ethanol.

4a prepared by the reaction of 5 g (0.02 mol) **3a** with HBr (gas) (hydrogen bromide was obtained by the reaction of 3.6 g (0.03 mol) KBr (Karmalab) with sulfuric acid (98%) (Karmalab)). Yield was 69%, mp = 169°C. Found,%: C 61.36; H 5.41; N 4.15. Calculated for $C_{17}H_{18}NOBr$, %: C 61.46; H 5.46; N 4.22. IR-spectra (cm^{-1}): 692 (mono substituted aromatic ring); 751 (three substituted aromatic ring); 3282 (N^+-H); 1464, 2892 (CH_2); 1650 (C=C-allyl); 1594 (C=C-arom); 2847, 993, 913 (C-H of double bond allyl fragment); 1196 (C-O); 1072 (C-N).

4b prepared by the reaction of 5 g (0.023 mol) **3b** with HBr, which obtained by the reaction of 4.1 g (0.035 mol) KBr with 98% H_2SO_4. Yields was 76%, mp = 145°C. Found,%: C 56.34; H 6.71; N 4.69. Calculated for $C_{14}H_{20}NOBr$, %: C 56.43; H 6.75; N 4.71. IR-spectra (cm^{-1}): 751 (three substituted aromatic ring); 3355 (N^+-H); C-H-arom.), 1471, 1318, 2937, 2779 (CH_2, CH_3); 1642 (C=C-allyl); 1597 (C=C-arom); 2838, 914 (C-H of double bond of allyl fragment); 1267 (C-O); 1194 (C-N).

4c prepared by the reaction of 5 g (0.019 mol) **3c** with HBr, which obtained by the reaction of 3.5 g (0.028 mol) KBr with 98% H_2SO_4. Yield was 78%, mp = 149°C. Found,%: C 59.81; H 7.71; N 4.11. Calculated for $C_{17}H_{26}NOBr$, %: C 59.99; H 7.70; N 4.13. IR (cm^{-1}): 748 (three substituted aromatic ring); 3330 (N^+-H); 1467, 1376, 1335, 2924, 2557, 2400 (CH_2,CH_3); 1650 (C=C-allyl); 1595 (C=C-arom); 2855, 979 (C-H of double bond of allyl fragment); 1242 (C-O); 1213 (C-N).

4d prepared by the reaction of 5 g (0.017 mol) **3c** with HBr, which obtained by the reaction of 3.1 g (0.025 mol) KBr with 98% H_2SO_4. Yield was 75%, mp = 135°C. Found,%: C 61.88; H 8.18; N 3.77. Calculated for $C_{19}H_{30}NOBr$, %: C 61.95; H 8.21; N 3.80. IR-spectra (cm^{-1}): 748 (three substituted aromatic ring); 3235 (N^+-H); 1466, 1376, 1422, 2926, 2854 (CH_2,CH_3); 1639 (C=C-allyl); 1593 (C=C-arom); 2854, 979 (C-H of double bond of allyl fragment); 1263 (C-O); 1202 (C-N).

Antimicrobial activity tests

The antimicrobial activity of the synthesized compounds (**3a–d, 4a–d**) was tested against microorganisms (*Staphylococcus aureus* NCTC 6571, *Staphylococcus aureus* ATCC ®25923, *Escherichia coli* ATCC®25922, *Candida albicans* NSTC-3255/ATCC2091, *Shigella flexneri* ATCC®12022, *Salmonella enterica* ATCC®13076 and *Aspergillus niger* (isolate obtained from water)) by the disk-diffuse method [46]. The bacterial strains were taken from the museum culture collection of the Republican Centre of Sanitary-Quarantine (Ministry of Health, Republic of Azerbaijan). Equal 1 ml volumes of microbial flora were layered on Petri dishes with selective medium for each pathogen using a standard inoculum (Muller Hinton medium "Liofilchem") corresponding to 0.5 density according to McFarland standard and containing approximately $1.5x10^6$ $CFU \times mL^{-1}$. Antifungal activity was determined in Sabouraud liquid nutrient medium and on Sabouraud Dextrose Agar medium (Himedia).

Agar dishes were dried in a box at room temperature for 15 minutes. Then, under laboratory conditions, disks were prepared with solutions of the tested compounds (in DMSO for **3a–d**, in water for **4a–d**) at concentrations 15 and 30 $mg \times L^{-1}$ and added to agar culture media with sterile forceps. Petri dishes were incubated for 24 hours (for bacteria at 37°C and fungi at 28°C) using thermostat TC1/80 CİTY (Russia). The degree of activity of the tested compounds **3a–d**, **4a–d** were determined by the diameter of the zone of inhibition of the growth of microorganisms (in mm). For comparison, we used as references at the identical concentration (30 $mg \times L^{-1}$) and conditions amoxicillin (for bacteria) and fluconazole (for fungi).

Electron microscopy (EM)

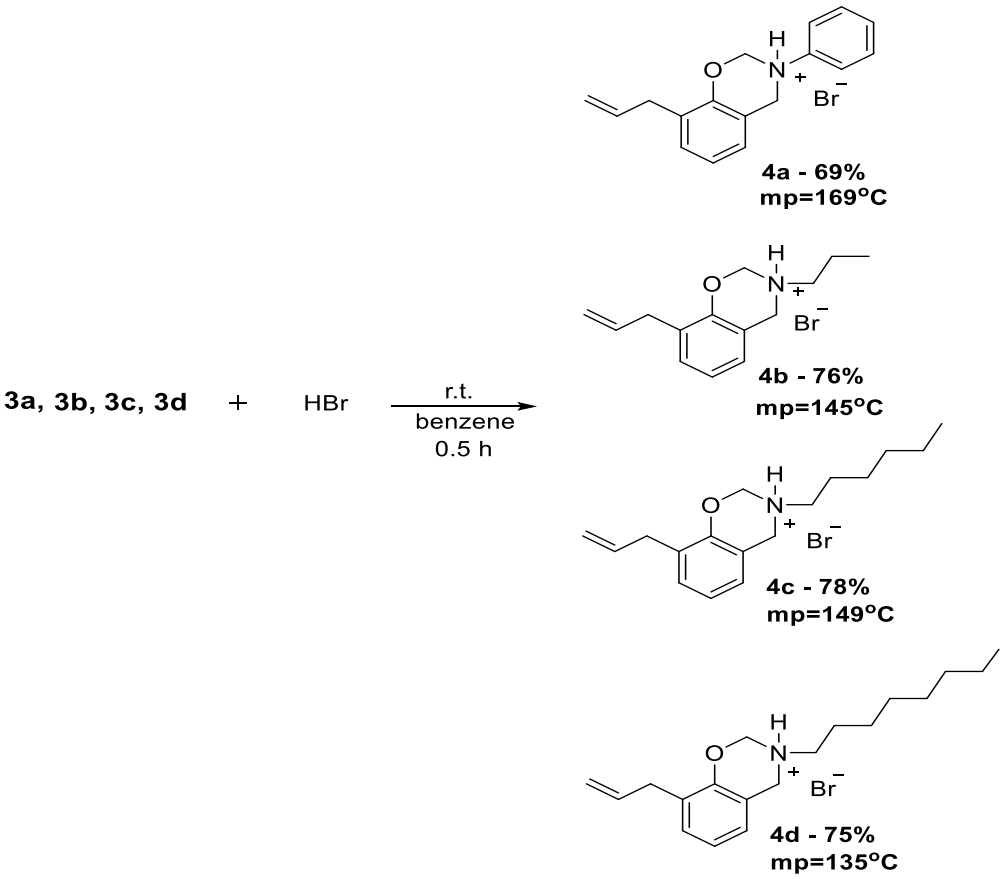
The antimicrobial efficacy of compound **4c** against the bacteria E.coli was also confirmed using EM. *E.coli* cells treated with 30 $mg \times L^{-1}$ **4c**, amoxicillin and untreated control of *E.coli* were incubated using thermostat TC1/80 CİTY (Russia) for 24 h at 37°C. After that incubation, samples were centrifuged at 4500 rpm for 10 min. Prepared samples for EM were fixed by immersion for a day with a

mixture of 2.5% glutaraldehyde solution, 2.5% paraformaldehyde solution and 0.1% picric acid solution in phosphate buffer (pH = 7.4). The subsequent post-fixation was carried out in a 1% solution of osmium tetroxide and in a 1.5% solution of potassium ferricyanide in 0.1 M phosphate buffer (pH = 7.4) for 1.5–2 hours. Further processing of the material (dehydration and pouring into Araldite-Epon) was carried out according to the generally accepted EM method [47]. Semi- and ultrathin sections were obtained using an EM UC7 (Leica, Germany) ultramicrotome. Semithin sections (1–2 μm) were stained with methylene blue, azure II and toluidine blue [48], and examined under a Primo Star (Zeiss, Germany) light microscope. The images were photographed with an EOS D650 (Canon, China) digital camera. Ultrathin sections (50–70 nm) were stained with a 2% saturated aqueous solution of uranyl acetate, then with a 0.4% solution of pure lead citrate (Electron Microscopy Science, USA) in a 0.1 M NaOH solution. Viewing and photographing of stained ultrathin sections was carried out on an EM JEM-1400 (Joel, Japan) at an accelerating voltage of 80–120 kV.

Results and Discussion

Characterization of the synthesized benzoxazines 3a–d and their ammonium salts **4a–d**

Heterocyclic compounds **3a–d** derived from 2-allylphenol **(2)** were obtained by the reaction of **2** with formaldehyde (37% aqueous solution) and different primary amines (aniline, propylamine, n-hexylamine and n-octylamine) (Scheme 1). It was revealed that the yields (62%, 68%, 73%, 71%) of the compounds **3a–d** vary depending on the nature of the primary amine used. First, for preparing benzoxazines **3a–d** with the required structures, the reaction was carried out with formaldehyde using primary amines (aniline, propylamine, n-hexylamine and n-octylamine) (Scheme 1), and then compound **2** was added to the obtained **1a–d,** and the reactants were stirred at 95°C and for 2 h.



Scheme-2: Synthesis of **4a-d.**

Table-Antimicrobial activity of **3a-d, 4a-d**

Compounds	Concentration, mg×L^{-1}	Diameter of the inhibition zone of the growth of microorganisms, mm					
		S.aureus	E.coli	S.flexneri	S.enterica	C.albicans	A.niger
3a	**15**	**13**	**22**	**10**	**10**	**4**	**5**
	30	**25**	**38**	**20**	**21**	**11**	**13**
3b	**15**	**15**	**19**	**17**	**14**	**6**	**7**
	30	**29**	**39**	**33**	**26**	**15**	**15**
3c	**15**	**11**	**24**	**14**	**12**	**6**	**5**
	30	**24**	**41**	**29**	**25**	**14**	**11**
3d	**15**	**10**	**22**	**13**	**10**	**9**	**12**
	30	**26**	**27**	**22**	**19**	**17**	**21**
4a	**15**	**19**	**27**	**20**	**14**	**12**	**10**
	30	**34**	**45**	**36**	**30**	**25**	**22**
4b	**15**	**16**	**24**	**14**	**10**	**15**	**10**
	30	**31**	**42**	**27**	**21**	**29**	**23**
4c	**15**	**22**	**28**	**18**	**15**	**13**	**12**
	30	**43**	**45**	**38**	**31**	**28**	**25**
4d	**15**	**16**	**23**	**19**	**13**	**18**	**16**
	30	**31**	**38**	**32**	**29**	**33**	**32**
Amoxicillin	**30**	**14**	**9**	**10**	**9**	**-**	**-**
Fluconazole	**30**	**-**	**-**	**-**	**-**	**29**	**22**

It is noteworthy that the reaction of formaldehyde with aniline, propylamine, n-hexylamine and n-octylamine was very rapid. Therefore, the reaction was carried out by cooling the reactants to 10°C for half an hour, then **2** was added, and the reaction was carried out at 95°C.

Antimicrobial activity of **3a–d** *and* **4a–d**

The results of the antimicrobial activity of new original nitrogen-containing heterocyclic compounds **3a–d** and **4a–d** are shown in Table.

As Table shows, the studied compounds **3a–d** and **4a–d** at a concentration of 15 and 30 mg×L^{-1} exhibit antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Candida albicans, Shigella flexneri, Salmonella enterica* and *Aspergillus niger.* At a concentration of 15 mg×L^{-1}, compounds **3a–d** were effective against bacteria *Staphylococcus aureus, Escherichia Coli, Shigella flexneri, Salmonella enterica* (especially against *E.coli*), but were practically ineffective against *Candida albicans* and *Aspergillus niger.* At a concentration of 30 mg×L^{-1}, compounds **3a–d** also demonstrated fungicidal activity. Of the compounds **3a–d** and **4a–d**, **4a–d** have the highest antimicrobial efficiency. At a concentration of 15 mg×L^{-1}, they were approximately twice as effective as compounds **3a–d**. Compounds **4a** and **4c** have the highest antimicrobial effect against bacteria, especially against *Staphylococcus aureus, E.coli* and *Shigella flexneri.* Compounds **3a–d** showed low efficiency against *Candida albicans* and *Aspergillus niger*. However, compounds **4a–d** exhibited antimicrobial activity against all types of bacteria and fungi. Compounds **3a–d** and water-soluble surfactants **4a–d** were more effective against *E.coli* where, at a concentration of 30 mg×L^{-1}, the diameter of the inhibition zone of its growth reached 45 mm.

Thus, among the studied compounds, compound **4c** has the highest bactericidal efficiency, and **4d** has the highest fungicidal activity. This result shows that with an increase in the alkyl radical, bactericidal properties worsen and fungicidal properties improve, which is in accordance with the literature data [18].

The greatest efficiency of substances **4a–d** is apparently associated with their structure – the simultaneous presence in their structures of an organic cation – which contained the 1,3-benzoxazine ring and allyl, alkyl and other fragments. Moreover, the anion in bromine makes it possible to improve their antimicrobial properties.

To compare the antimicrobial efficacy of all compounds in identical concentrations (30 mg×L^{-1}) and conditions, amoxicillin (for bacteria) and fluconazole (for fungi) were studied (Table). The results of the study show that the efficiencies of compounds **3a–d** and the references taken (amoxicillin and fluconazole) are different in concentration (30 mg×L^{-1}). However, the substances **4a–d,** in comparison with the references used, are much higher in bactericidal and fungicidal effectiveness. Thus, the research results showed that the effectiveness of compounds **3a–d**, especially **4a–d,** is much higher than the references taken in identical concentrations (30 mg×L^{-1}).

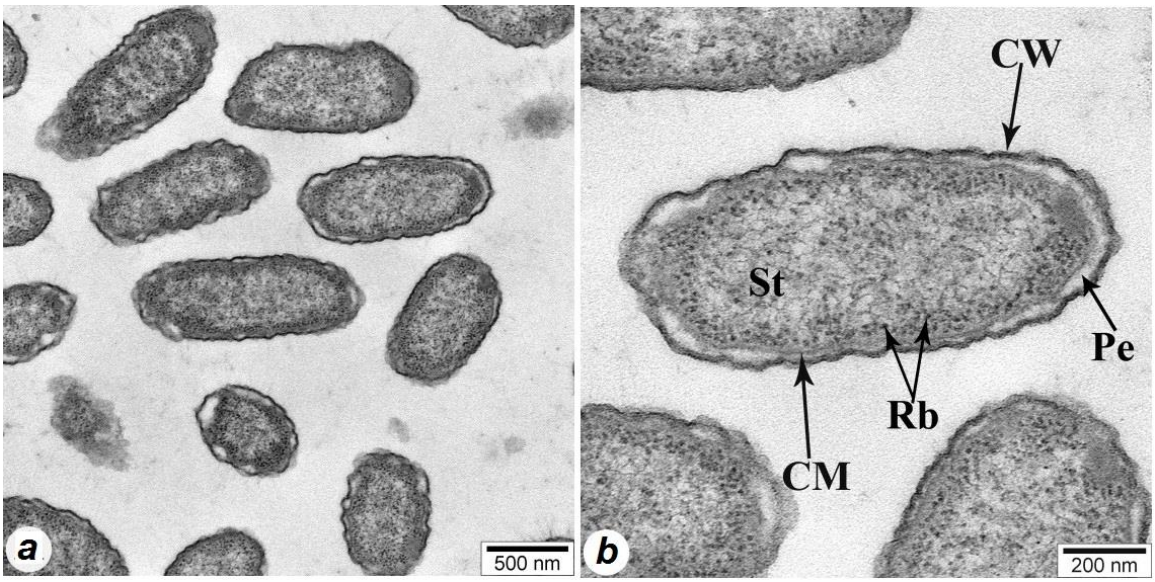


Fig. 1: TEM micrographs of untreated *E. coli* bacteria. A-overview, B- ultrastructure of bacteria. Staining: uranyl acetate and Pb-citrate. St-cytoplasm, CM-cell membrane or plasma membrane, CW-cell wall, Rb- ribosome, Pe-peptidoglycan.

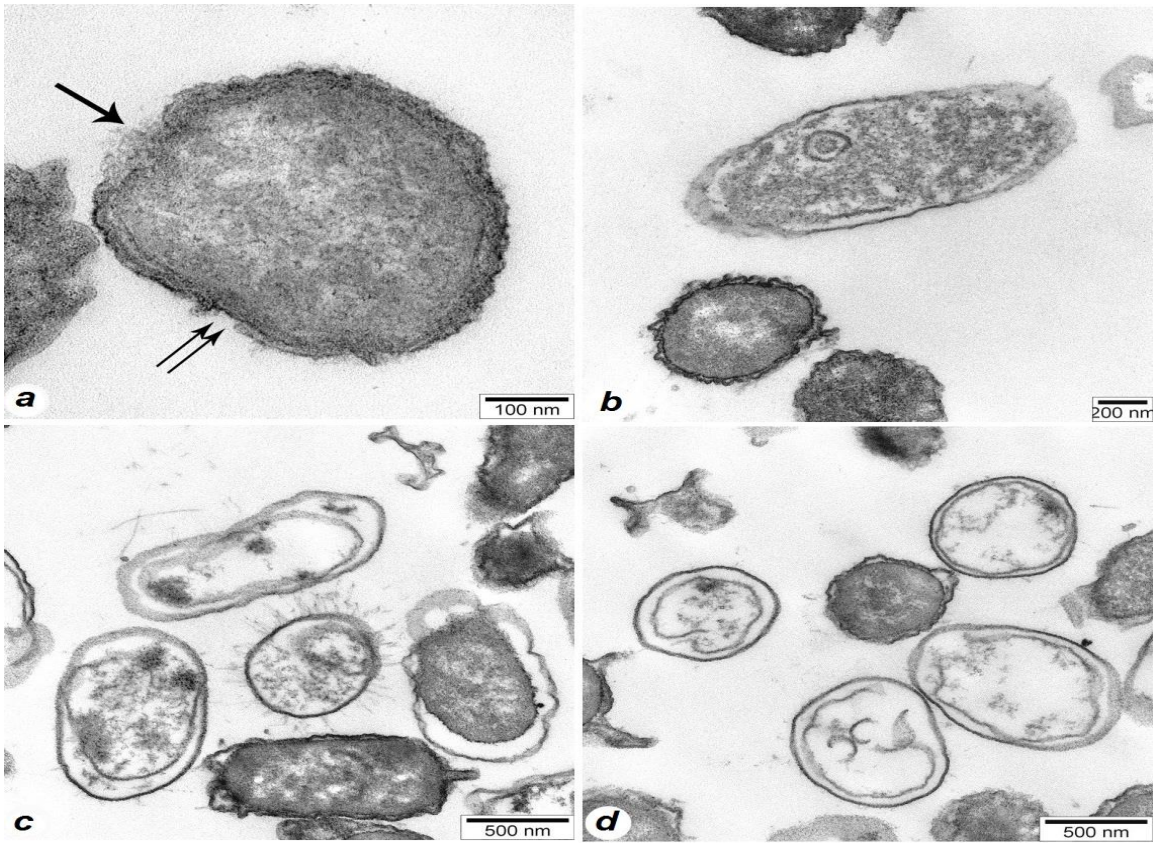


Fig. 2: TEM micrographs of damaged *E. coli* bacteria treated with amoxicillin. Staining: uranyl acetate and Pb-citrate.

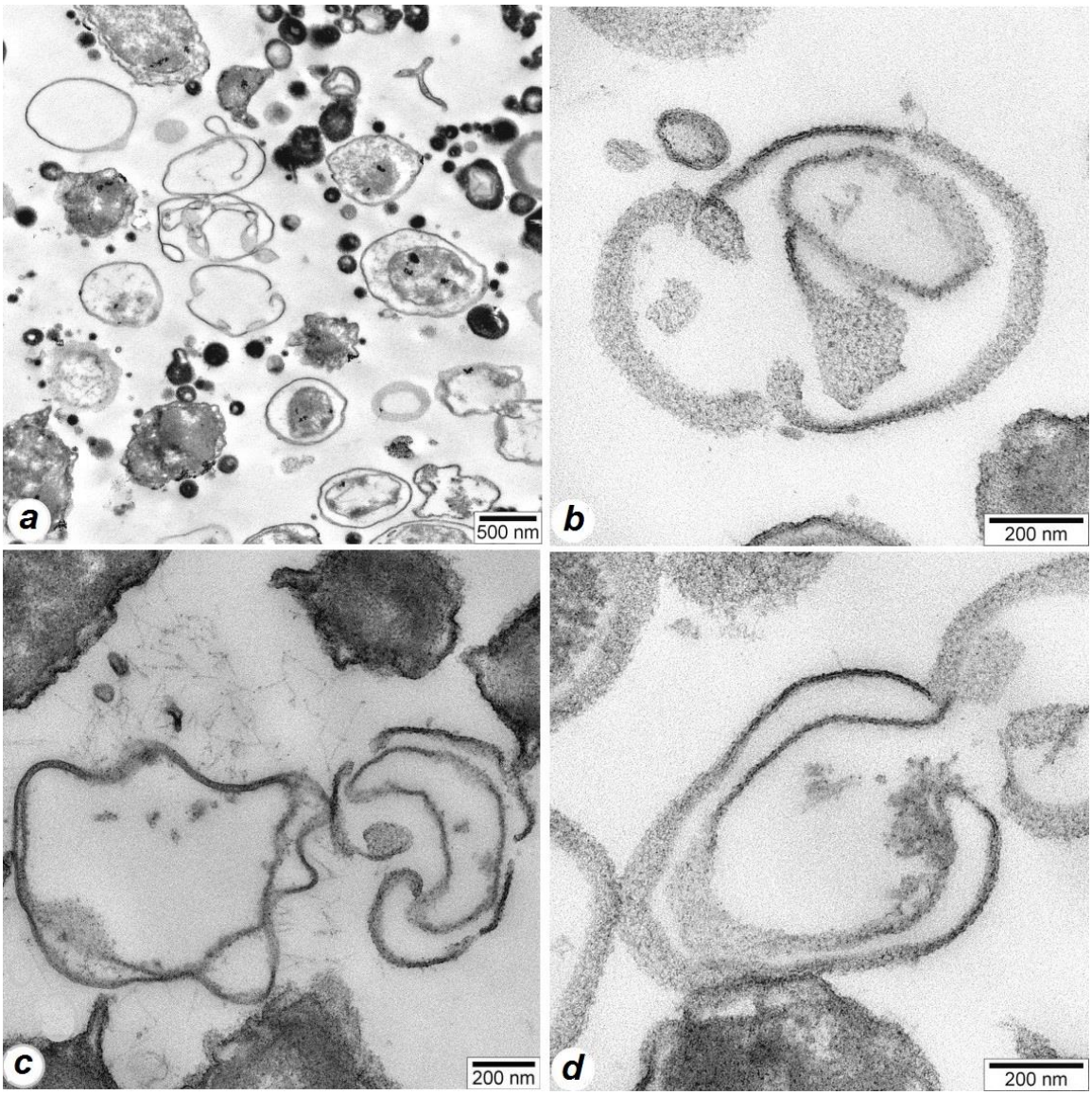


Fig. 3: TEM micrographs of destroyed *E. coli* bacteria treated with 4c. Staining: uranyl acetate and Pb-citrate

As a result of the TEM study, it can be assumed that compound **4c** performs its action by passing through the cell walls of the bacteria (Fig 1–3). Fig 1 shows the *E.coli* cell without treatment with **4c** and amoxicillin; more precisely, in Fig 1a, the general appearance of the bacterium in EM, and in Fig 1b the ultrastructural structure – the cell wall, cell membrane, peptidoglycan, ribosomes, cytoplasm, etc. (Fig 1b is marked with symbols on the Fig). Fig 2 shows the ultrastructural characteristics of changes in *E.coli* after the use of a reference (amoxicillin). Thus, in Fig 2a, it was observed that both the cell wall and the cell membrane (shown by a single black arrow) were damaged, and in the other part of the bacterium, only the cell wall (shown by a double black arrow) was damaged. In Fig 2b, Fig 2c and Fig 2d, it is shown that a) amoxicillin causes the cell wall to swell several times more than normal, b) causes the cell membrane integrity of many cells to be disrupted, and c) causes the cytoplasm to vacuolate. In this case, the bacteria's growth stops, and they lose their ability to reproduce.

Fig 3 shows the pathomorphological changes in the ultrastructure of *E.coli* bacteria in the presence of **4c**. Fig 3a, taken under a small microscope magnifier, clearly shows that all the bacteria have destroyed both the inner and outer shell layers, and that the intracellular cytoplasm and the organelles in it have spread out of the cell. In Fig 3b, Fig 3c and Fig 3d, it is observed that *E.coli* bacteria are completely

destroyed by **4c**. Experiments and electrographs have shown that the compound destroys almost all cells, creating more destructive changes in *E.coli* bacteria than amoxicillin **4c**.

These results once again confirm the bactericidal efficacy of compound **4c**, which is in agreement with the experimental data and literature [13,41]. The presence of an allyl group [43,44], nitrogen atom in the heterocyclic fragment and the simultaneous arrangement in the cationic part of **4c,** the multiple bond and the hexyl fragment, as well as the bromine anion [4,5,7], significantly improves their antimicrobial efficiency in comparison with **3a–d** and standards. Changes in the ultrastructure of the *E.coli* bacterium are almost consistent with other studies [49–54].

Conclusion

Benzoxazines **3a-d** and **4a-d** with a multiple bond and other groups in their structures were synthesized by the reaction of 2-allylphenol, formaldehyde and primary amines and characterized by NMR, IR and elemental analyze methods.

The benzoxazines showed antimicrobial activity against microorganisms (Staphylococcus aureus NCTC6571, Staphylococcus aureus ATCC®25923, Escherichia coli ATCC®25922, Candida albicans NSTC-3255/ATCC2091, Shigella flexneri ATCC®12022, Salmonella enterica ATCC®13076 and Aspergillus niger (isolate obtained from water)).

It was found that compounds **3a-d** at a low concentration (15 $mg \times L^{-1}$) are effective against bacteria and fungi. Among the synthesized compounds, the highest efficiency is possessed by **4a-d**. These compounds exhibit both bactericidal and fungicidal activity. The results obtained were also confirmed by EM method.

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