Acta Poloniae Pharmaceutica – Drug Research, Vol. 70 No. 2 pp. 245–253, 2013 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS AND BIOLOGICAL ACTIVITY OF NOVEL SERIES OF 1,3-BENZOXAZOL-2(3*H*)-ONE DERIVATIVES

MARIOLA KRAWIECKA[1], BOŻENA KURAN[1*], JERZY KOSSAKOWSKI[1], MARTA KIERZKOWSKA[2], GRAŻYNA MŁYNARCZYK[2], MARCIN CIEŚLAK[3], JULIA KAŹMIERCZAK-BARAŃSKA[3], KAROLINA KRÓLEWSKA[3] and MICHAŁ A. DOBROWOLSKI[4]

[1]Department of Medical Chemistry, Medical University of Warsaw, 3 Oczki St., 02-007 Warszawa, Poland
[2]Department of Medical Microbiology, Medical University of Warsaw, 5 Chałubińskiego St., 02-004 Warszawa, Poland
[3]Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, 112 Sienkiewicza St., 90-363 Łódź, Poland
[4]Department of Chemistry, University of Warsaw, Pasteura 1 St., 02-093 Warszawa, Poland

Abstract: In the search for novel biological agents, a series of new derivatives N-substituted 1,3-benzoxazol-2(3*H*)-one, 5-chloro-1,3-benzoxazol-2(3*H*)-one, 6-bromo-1,3-benzoxazol-2(3*H*)-one were prepared. All of the compounds were characterized by ^{1}H NMR, ^{13}C NMR and ESI MS spectra. Moreover, for compound **1** an X-ray structure was determined. All derivatives were tested for antimicrobial activity against a selection of Gram-positive, Gram-negative bacteria and yeasts. The selected compounds (**2**-**8**, **10**) were tested for their cytotoxic properties in K562, HeLa and normal cells.

Keywords: antimicrobial activity, anticancer activity, 6-bromo-1,3-benzoxazol-2(3*H*)-one, 5-chloro-1,3-benzoxazol-2(3*H*)-one, N-substituted 1,3-benzoxazol-2(3*H*)-one, X-ray diffraction

Substituted benzoxazoles and their analogues such as benzothiazoles and benzoxazolones are an important class of heterocyclic compounds that are known to possess important biological properties. These compounds have been the aim of many researches for many years.

It was reported that the benzoxazoles exhibited substantial chemotherapeutic activities (1-12), antiviral activity (8, 9), multidrug-resistance cancer cell activities (12). In recent years there have been many reports on some benzoxazoles which exhibited antimicrobial activity (3-5, 13-15). These compounds possessed a broad spectrum of activity against the Gram-positive and Gram-negative microorganisms. Moreover, they showed significant antifungal activity (14).

New series of benzothiazoles have been synthesized as antitumor agents that showed potent inhibitory activity against human breast cancer cell lines (16).

Many derivatives of benzoxazolone have been described in as possessing a wide variety of pharmacological activities. In the French patents were reported 6-acylbenzoxazolones as analgesics, 6-(2-aminoethyl)benzoxazolones which are usable in therapy of sleep and behavioral disorders, and 6-(aminoacetyl)benzoxazolones which are used in the treatment of arterial hypertension as well as in that of painful syndromes (17-19). In the literature, we can also find reports of biologically active halogeno derivatives of benzoxazolone (20-22). Moreover, 5-chloro-2-benzoxazolone (chlorzoxazone) is a centrally-acting muscle relaxant used to treat muscle spasm and the resulting pain or discomfort (23).

Various anticancer and antimicrobial qualities of benzoxazole and benzoxazolone derivatives were descirbed also by Murty et al. (24, 25). Their studies indicate that benzoxazole and benzoxazolone derivatives present high or moderate activity in relation to various cancer cells (24). In other studies they described that some of benzoxazole and benzoxazolone compounds present also moderate antimicrobial activity (25).

* Corresponding author: e-mail: bozena.kuran@interia.pl

Considering the above suggestions and in continuation of our previous work in the synthesis of biologically active heterocycles, we planned to synthesize a novel series of 1,3-benzoxazol-2(3*H*)-one derivatives. Scheme 1 explains the method of their preparation.

In order to determine the antimicrobial activity of the synthesized compounds selected G-positive, G-negative bacteria and yeasts species were screened.

The chosen compounds (**2**-**8**, **10**) were also tested for their cytotoxic properties in cancer (K562, HeLa) and normal cells. The results are reported in Table 1.

EXPERIMENTAL

General

Melting points were determined in a capillary in Electrothermal 9100 apparatus and are uncorrected. Nuclear magnetic resonance spectra of protons (^{1}H NMR) were recorded in DMSO-d_6 or in $CDCl_3$ on a Bruker VMNRS300 instrument operating at 300 MHz but nuclear magnetic resonance spectra of carbons (^{13}C NMR) were recorded in DMSO-d_6 on a Bruker VMNRS300 apparatus operating at 75 MHz. Chemical shift values are expressed in parts per million (ppm) in relation to tetramethylsilane as an internal standard. Mass spectral ESI (Electrospray Ionization) measurements were carried out on a Mariner PE Biosystems instrument with TOF detector. The spectra were obtained in the positive ion mode with a declustering potential 140–300 V. Chromatographic columns were filled with Merck 0.05–0.2 mm (70–325 mesh ASTM) silica gel. Reactions were monitored by TLC on silica gel G (plates with fluorescent indicator 254 nm, layer thickness 0.2 mm, Merck), using chloroform-methanol 98 : 2 and 95 : 5, v/v as eluents. 6-Bromo-1,3-benzoxazol-2(3*H*)-one (**1**) was synthesized according to method described earlier (26).

Analysis for 6-bromo-1,3-benzoxazol-2(3H)-one (**1**)

$C_7H_4BrNO_2$, M = 214.01. Yield: 72%; m.p. 196-197^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$ + TMS, δ, ppm): 6.96 (1H, d, J = 8.4 Hz, H arom.), 7.31 (1H, dd, J_1 = 9.9 Hz, J_2= 8.1 Hz, H arom.), 7.39 (1H, d, J = 1.5 Hz, H arom.), 8.89 (brs, 1H, NH). MS (m/z): 100% = 237.9 [L + Na^+]. ^{13}C NMR (DMSO-d_6, δ, ppm): 153.97 (1C), 143.98 (1C), 129.83 (1C), 126.41 (1C), 113.02 (1C), 112.73 (1C), 111.20 (1C).

General procedure of preparing amino derivatives 2-16

The appropriate starting material (1,3-benzoxazol-2(3*H*)-one, 6-bromo-1,3-benzoxazol-2(3*H*)-one (**1**), 5-chloro-1,3-benzoxazol-2(3*H*)-one) (0.01 mol) was dissolved in acetone (30 mL), then powdered anhydrous K_2CO_3 (0.01 mol) and catalytic amount of 98% 1,8-diazabicyclo[5.4.0]undec-7-ene and an appropriate amine (0.01 mol) were added. The reaction mixture was heated for 8-14 h, respectively. After the reaction was completed, the inorganic residue was filtered off and the solvent was evaporated. The obtained compound was purified by column chromatography (eluent: chloroform or chloroform/methanol 50 : 0.2, v/v).

Table 1. The IC_{50} values calculated from the dose-response curves.

Compound	HeLa IC_{50} 48 h	K562 IC_{50} 48 h	HUVEC IC_{50} 48 h
2	> 1 mM	> 1 mM	Nd
3	> 1 mM	> 1 mM	Nd
4	> 1 mM	> 1 mM	Nd
5	> 1 mM	> 1 mM	Nd
6	> 1 mM	> 1 mM	Nd
7	> 1 mM**	> 1 mM*	200 μM**
8	90 μM**	> 1 mM*	100 μM**
10	100 μM**	> 1 mM*	200 μM**

Nd – not determined. * These compounds interfere with MTT colorimetric assay. At 1 mM concentration strong purple color developed when MTT was added to cells (these results were not taken into account for IC_{50} calculation). ** to avoid non-specific color development in HeLa cell cultures, medium containing compounds at 1 mM concentration was replaced with fresh medium (without compounds) before addition of MTT.

All new derivatives were converted to their hydrochlorides and crystallized from methanol.

3-[2-(Dimethylamino)ethyl]-1,3-benzoxazol-2(3H)-one (2)

$C_{11}H_{14}N_2O_2 \times HCl$, M = 206.24. Yield: 70%; m.p. 162-165°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 2.81 (6H, s, -CH_3), 3.40 (2H, m, C1'-H), 4.23 (2H, t, *J* = 6.3 Hz, C2'-H), 7.19 (2H, m, H arom.), 7.38 (2H, m, H arom.), 10.40 (s, 1H, HCl). MS (m/z): 100% = 207.1 [L + H^+]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.81 (1C), 142.17 (1C), 130.55 (1C), 123.84 (1C), 122.49 (1C), 109.69 (1C), 109.46 (1C), 53.40 (1C), 42.45 (2C), 37.28 (1C).

3-[2-(Diethylamino)ethyl]-1,3-benzoxazol-2(3H)-one (3)

$C_{13}H_{18}N_2O_2 \times HCl$. M = 234.29. Yield: 74%; m.p. 166-167°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 1.22 (6H, t, *J* = 7.3 Hz, -CH_3), 3.22 (4H, m, -CH_2-), 3.42 (2H, m, C1'-H), 4.28 (2H, t, *J* = 6.7 Hz, C2'-H), 7.20 (2H, m, H arom.), 7.39 (2H, m, H arom.), 10.43 (s, 1H, HCl). MS (m/z): 100% = 235.1 [L + H^+]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.67 (1C), 142.15 (1C), 130.53 (1C), 123.92 (1C), 122.54 (1C), 109.75 (1C), 109.42 (1C), 53.40 (1C), 47.27 (1C), 46.04 (2C), 36.54 (1C), 8.20 (2C).

3-[2-(Morpholin-4-yl)ethyl]-1,3-benzoxazol-2(3H)-one (4)

$C_{13}H_{16}N_2O_3 \times HCl$, M = 248.27. Yield: 63%; m.p. 247-252°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 3.18 (2H, m, H morph.), 3.53 (4H, m, H morph.), 3.72 (2H, m, C1'-H), 3.98 (2H, m, H morph.), 4.29 (2H, m, C2'-H), 7.17 (2H, m, H arom.), 7.39 (2H, m, H arom.), 10.83 (s, 1H, HCl). MS (m/z): 100% = 271.1 [L + Na^+]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.82 (1C), 142.09 (1C), 130.87 (1C), 123.84 (1C), 122.37 (1C), 109.67 (1C), 109.47 (1C), 66.07 (1C), 63.14 (1C), 55.04 (1C), 53.08 (1C), 51.02 (1C), 36.10 (1C).

3-[2-(Piperidin-1-yl)ethyl]-1,3-benzoxazol-2(3H)-one (5)

$C_{14}H_{18}N_2O_2 \times HCl$, M = 246.30. Yield: 69%; m.p. 183-184°C; ^{1}H NMR (300 MHz, CD_3OD, δ, ppm): 1.56 (2H, m, H piper.), 1.71 (4H, m, H piper.), 2.91 (4H, m, H piper.), 3.08 (2H, m, C1'-H), 4.16 (2H, t, *J* = 6.3 Hz, C2'-H), 7.18 (1H, m, H arom.), 7.27 (3H, m, H arom.), 10.73 (s, 1H, HCl). MS (m/z): 100% = 247.1 [L + H^+]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.83 (1C), 141.99 (1C), 131.00 (1C), 123.81 (1C), 122.19 (1C), 109.60 (1C), 109.45 (1C), 54.90 (1C), 53.62 (2C), 36.92 (1C), 25.32 (2C), 23.59 (1C).

3-[3-(Dimethylamino)propyl]-1,3-benzoxazol-2(3H)-one (6)

$C_{12}H_{16}N_2O_2 \times HCl$, M = 220.26. Yield: 62%; m.p. 160-161°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 1.93 (2H, m, C2'-H), 2.37 (6H, s, -CH_3), 2.62 (2H, m, C1'-H), 3.87 (2H, t, *J* = 6.7 Hz, C3'-H), 7.13 (1H, m, H arom.), 7.23 (1H, m, H arom.), 7.33 (2H, m, H arom.), 10.01 (s, 1H, HCl). MS (m/z): 100% = 221.1 [L + H^+]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.75 (1C), 141.98 (1C), 131.06 (1C), 123.85 (1C), 122.15 (1C), 109.60 (1C), 109.16 (1C), 54.96 (1C), 43.71 (2C), 39.60 (1C), 24.03 (1C).

5-Chloro-3-[2-(dimethylamino)ethyl]-1,3-benzoxazol-2(3H)-one (7)

$C_{11}H_{13}ClN_2O_2 \times HCl$, M = 240.68. Yield: 66%; m.p. 230-234°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 2.80 (6H, s, -CH_3), 3.80 (2H, m, C1'-H), 4.20 (2H, t, *J* = 6.0 Hz, C2'-H), 7.20 (1H, dd, J_1 = 10.8 Hz, J_2 = 8.7 Hz, H arom.), 7.38 (1H, d, *J* = 8.4 Hz, H arom.), 7.61 (1H, d, *J* = 2.1 Hz, H arom.), 10.17 (s, 1H, HCl). MS (m/z): 100% = 241 [L + H^+]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.84 (1C), 140.96 (1C), 131.91 (1C), 128.02 (1C), 122.05 (1C), 110.95 (1C), 109.85 (1C), 53.48 (1C), 42.53 (2C), 37.54 (1C).

5-Chloro-3-[2-(diethylamino)ethyl]-1,3-benzoxazol-2(3H)-one (8)

$C_{13}H_{17}ClN_2O_2 \times HCl$, M = 268.73. Yield: 64%; m.p. 181-183°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 1.21 (6H, t, *J* =7.2 Hz, -CH_3), 3.23 (4H, m, -CH_2-), 3.44 (2H, m, C1'-H), 4.24 (2H, t, *J* = 6.6 Hz, C2'-H), 7.21 (1H, dd, J_1 = 10.8 Hz, J_2 = 8.4 Hz, H arom.), 7.40 (1H, d, *J* = 8.7 Hz, H arom.), 7.67 (1H, d, *J* = 2.1 Hz, H arom.), 9.99 (s, 1H, HCl). MS (m/z): 100% = 269 [L + H^+]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.71 (1C), 140.95 (1C), 131.92 (1C), 128.08 (1C), 122.11 (1C), 111.01 (1C), 109.82 (1C), 47.44 (1C), 45.97 (2C), 36.75 (1C), 8.17 (2C).

5-Chloro-3-[2-(morpholin-4-yl)ethyl]-1,3-benzoxazol-2(3H)-one (9)

$C_{13}H_{15}ClN_2O_3 \times HCl$, M = 282.72. Yield: 68%; m.p. 230-231°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 3.17 (2H, m, H morph.), 3.61 (6H, m, C1'-H, H morph.), 4.00 (2H, m, H morph.), 4.26 (2H, m, C2'-H), 7.23 (1H, m, H arom.), 7.41 (1H, d, *J* = 8.7 Hz, H arom.) 7.63 (1H, m, H arom,), 10.34 (s, 1H, HCl). MS (m/z): 70% = 283.0 [L + H^+], 100% = 305.2 [L + Na^+]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.85 (1C), 141.00 (1C), 131.86 (1C), 128.05 (1C), 122.13 (1C), 111.03 (1C), 109.91 (1C), 63.08 (1C), 52.47 (1C), 51.00 (2C), 36.14 (1C).

5-Chloro-3-[2-(piperidin-1-yl)ethyl]-1,3-benzoxazol-2(3H)-one (10)

$C_{14}H_{17}ClN_2O_2 \times HCl$, M = 280.74. Yield: 71%; m.p. 263-267°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 1.37 (1H, m, H piper.), 1.73 (5H, m, H piper.), 2.93 (2H, m, C1'-H), 3.39 (2H, m, H piper.), 3.56 (2H, m, H piper.), 4.29 (2H, t, J = 6.1 Hz, C2'-H), 7.20 (1H, dd, J_1 = 10.5 Hz, J_2 = 8.4 Hz, H arom.), 7.38 (1H, d, J = 8.7 Hz, H arom.) 7.67 (1H, m, H arom.), 10.38 (s, 1H, HCl). MS (m/z): 100% = 281.1 [L + H$^+$]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.69 (1C), 140.96 (1C), 131.89 (1C), 128.04 (1C), 122.07 (1C), 110.98 (1C), 109.87 (1C), 52.14 (1C), 51.93 (2C), 36.49 (1C), 22.21 (2C), 21.30 (1C).

5-Chloro-3-[3-(dimethylamino)propyl]-1,3-benzoxazol-2(3H)-one (11)

$C_{12}H_{15}ClN_2O_2 \times HCl$, M = 254.71. Yield: 68%; m.p. 185-188°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 2.07 (2H, m, C2'-H), 2.73 (6H, s, -CH_3), 3.11 (2H, m, C1'-H), 3.90 (2H, t, J = 6.7 Hz, C3'-H), 7.18 (1H, dd, J_1 = 10.5 Hz, J_2 = 8.4 Hz, H arom.), 7.38 (1H, d, J = 8.4 Hz, H arom.), 7.56 (1H, d, J = 2.1 Hz, H arom.), 9.94 (s, 1H, HCl). MS (m/z): 100% = 255.1 [L + H$^+$]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.73 (1C), 140.80 (1C), 132.30 (1C), 128.12 (1C), 121.87 (1C), 110.93 (1C), 109.60 (1C), 53.63 (1C), 41.93 (2C), 38.67 (1C), 22.41 (2C).

6-Bromo-3-[2-(dimethylamino)ethyl]-1,3-benzoxazol-2(3H)-one (12)

$C_{11}H_{13}BrN_2O_2$, M = 285.13. Yield: 54%; m.p. 181-182°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 2.84 (6H, s, -CH_3), 3.43 (2H, m, C1'-H), 4.24 (2H, t, J = 6.1 Hz, C2'-H), 7.40 (1H, d, J = 8.4 Hz, H arom.), 7.47 (1H, dd, J_1 = 10.2 Hz, J_2 = 8.1 Hz, H arom.), 7.7 (1H, d, J = 1.8 Hz, H arom.), 10.32 (s, 1H, HCl). MS (m/z): 100% = 286.9 [L + H$^+$]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.52 (1C), 142.88 (1C), 130.09 (1C), 126.47 (1C), 113.79 (1C), 112.98 (1C), 110.97 (1C), 53.12 (1C), 42.15 (2C), 37.14 (1C).

6-Bromo-3-[2-(diethylamino)ethyl]-1,3-benzoxazol-2(3H)-one (13)

$C_{13}H_{17}BrN_2O_2$, M = 313.19. Yield: 44%; m.p. 261-262°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 3.23 (4H, m, -CH_2-), 3.31 (6H, s, -CH_3), 3.44 (2H, m, C1'-H), 4.24 (2H, t, J = 6.5 Hz, C2'-H), 7.46 (2H, m, H arom.), 7.71 (1H, d, J = 1.5 Hz, H arom.), 9.98 (s, 1H, HCl). MS (m/z): 100% = 315.0 [L + H$^+$]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.35 (1C), 142.82 (1C), 130.29 (1C), 126.52 (1C), 113.76 (1C), 113.00 (1C), 110.95 (1C), 47.19 (1C), 46.01 (2C), 36.68 (1C), 8.20 (2C).

6-Bromo-3-[2-(morpholin-4-yl)ethyl]-1,3-benzoxazol-2(3H)-one (14)

$C_{13}H_{15}BrN_2O_3$, M = 327.17. Yield: 52%; m.p. 175-176°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 3.16 (2H, m, H morph.), 3.52 (2H, m, H morph.), 3.71 (2H, m, H morph.), 3.99 (2H, m, C1'-H), 4.27 (2H, m, C2'-H), 7.44 (2H, m, H arom.), 7.71 (1H, d, J = 1.8 Hz, H arom.), 10.70 (s, 1H, HCl). MS (m/z): 100% = 329.0 [L + H$^+$]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.48 (1C), 142.88 (1C), 130.11 (1C), 126.50 (1C), 113.80 (1C), 113.03 (1C), 110.98 (1C), 63.04 (2C), 52.37 (1C), 50.97 (2C), 36.11 (1C).

6-Bromo-3-[2-(piperidin-1-yl)ethyl]-1,3-benzoxazol-2(3H)-one (15)

$C_{14}H_{17}BrN_2O_2$, M = 325.20. Yield: 30%; m.p. 175-177°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 1.68 (5H, m, H piper.) 2.94 (1H, m, H piper.), 3.43 (7H, m, C1'-H, C2'-H, H piper.), 4.27 (1H, m, C2'-H), 7.06 (1H, m, H arom.), 7.32 (1H, m, H arom.), 7.59 (1H, d, J = 1.8 Hz, H arom.), 11.81 (s, 1H, HCl). MS (m/z): 100% = 326.20 [L + H$^+$]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.41 (1C), 142.80 (1C), 130.10 (1C), 126.50 (1C), 113.79 (1C), 112.98 (1C), 110.97 (1C), 53.00 (1C), 52.12 (1C), 35.90 (1C), 22.32 (2C), 21.40 (1C).

6-Bromo-3-[3-(dimethylamino)propyl]-1,3-benzoxazol-2(3H)-one (16)

$C_{12}H_{15}BrN_2O_2$, M = 299.16. Yield: 58%; m.p. 132-134°C; ^{1}H NMR (300 MHz, DMSO-d_6 + TMS, δ, ppm): 2.07 (2H, m, C2'-H), 2.71 (6H, s, -CH_3), 3.12 (2H, m, C1'-H), 3.91 (2H, t, J = 6.6 Hz, C3'-H), 7.35 (1H, d, J = 8.4 Hz, H arom.), 7.46 (1H, dd, J_1 = 10.2 Hz, J_2 = 8.4 Hz, H arom.), 7.69 (1H, d, J = 1.8 Hz, H arom.), 10.25 (s, 1H, HCl). MS (m/z): 100% = 300.03 [L + H$^+$]; ^{13}C NMR (DMSO-d_6, δ, ppm): 153.40 (1C), 142.69 (1C), 130.52 (1C), 126.54 (1C), 113.55 (1C), 112.93 (1C), 110.76 (1C), 53.66 (1C), 41.93 (2C), 39.35 (1C), 22.41 (2C).

Crystallography

The crystal of 6-bromo-1,3-benzoxazol-2(3*H*)-one (**1**) was obtained by crystallization from ethanol. The X-ray measurement of (**1**) was performed at 100(2) K on a KUMA CCD k-axis diffractometer with graphite-monochromated Mo Ka radiation (0.71073 Å). The crystal of (**1**) was positioned at 62.25 mm from the KM4CCD camera. The data were corrected for Lorentz and polarization

effects, additionally, absorption correction was applied. Data reduction and analysis were carried out with the Kuma Diffraction (Wrocław, Poland) programs (27). The structure was solved by direct methods (28) and refined by using SHELXL (29). The refinement was based on F^2 for all reflections except for those with very negative F^2. The weighted R factor, wR and all goodness-of-fit S values are based on F^2. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were located from a difference map and were refined isotropically. The atomic scattering factors were taken from the International Tables (30). Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 845184 (**1**). Copy of the data can be obtained on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk).

Microbiology

Organisms

The standard strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 14053 and one clinical isolate *Stenotrophomonas maltophilia* CO2275 were used.

Screening for the antimicrobial activity

The method according to CLSI (Clinical and Laboratory Standards Institute) directives was applied (31). The compounds **2–16** were tested for their bacteriostatic activity at the high concentrations (512 mg/L).

The tested substances were dissolved in DMSO and then the solutions were added to brain heart infusion broth (BHI-B) medium to the final concentration 512 mg/L.

The bacteria were cultured on the plates with BHI agar (BHI-A) medium supplemented with 7% horse blood, at temperature 35–37°C, in an aerobic atmosphere, for 18–24 h. The fungal strain was cultured in the Sabouraud agar (SA), at the same temperature and atmosphere, but for at least for 24 h. The cultures which were in mid-logarithmic phase of growth were suspended in 0.9% NaCl solution to obtain 0.5 McFarland's optical density. Cells ($1.0–9.0 \times 10^5$ - 0.1 mL of the prepared suspension) were added to sample tubes with 2 mL of BHI-B broth medium containing the tested substances. Samples were incubated at temperature 35–37°C for 24-48 h. If after 48 h the growth was absent, the substance was considered as potentially possessing antimicrobial activity.

In all experiments, strains vitality controls and DMSO antimicrobial activity controls in the applied concentrations were performed.

Cells and cytotoxicity assay

Human umbilical vein endothelial cells (HUVEC) were isolated from freshly collected umbilical cords and cultured in plastic dishes coated with gelatin, in RPMI 1640 medium supplemented with 20% FBS, 90 U/mL heparin, 150 µg/mL ECGF (Roche Diagnostics, Mannheim, Germany) and antibiotics (100 µg/mL streptomycin and 100 U/mL penicillin). Cells (10^4) were seeded on each well on 96-well plate (Nunc).

The HeLa (human cervix carcinoma) and K562 (leukemia) cells were cultured in RPMI 1640 medium supplemented with antibiotics and 10% fetal calf serum, in a 5% CO_2 - 95% air atmosphere. Cells (7×10^3) were seeded on each well on 96-well plate (Nunc). Twenty four hours later, cells were exposed to the test compounds. Stock solutions (100 mM) of test compounds were freshly prepared in DMSO. The final concentrations of the compounds tested in the cell cultures were: 1 mM, 1×10^{-2} mM, 1×10^{-4} mM and 1×10^{-6} mM. The concentration of DMSO in the cell culture medium was 1%. After addition of compounds, the cells were grown for another 48 h The cytotoxicity was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, St. Louis, MO] assay as described in (32). Briefly, after 48 h of incubation with drugs, the cells were treated with the MTT reagent and incubation was continued for 2 h. MTT-formazan crystals were dissolved in 20% SDS and 50% DMF at pH 4.7 and absorbance was read at 570 and 650 nm on an ELISA-PLATE READER (FLUOstar Omega). As a control (100% viability), we used cells grown in the presence of vehicle (1% DMSO) only.

The values of IC_{50} (the concentration of test compound required to reduce the cell survival fraction to 50% of the control) were calculated from dose-response curves and used as a measure of cellular sensitivity to a given treatment.

RESULTS AND DISCUSSION

Chemistry

In this study, a series of 16 derivatives of 1,3-benzoxazol-2(3*H*)-one has been synthesized. The starting materials were converted into alkylamine derivatives by using appropriate amines (Scheme 1). All of the derivatives (**1**-**16**) were supported by spectral data. ESI MS, ^{1}H NMR and ^{13}CNMR spec-

tra are in agreement with the proposed structures. Additionally, for compound **1** an X-ray crystal structure was obtained.

X-ray structure analysis

6-Bromo-1,3-benzoxazol-2(3*H*)-one (**1**, Fig. 1) crystallizes in $P2_1/c$ monoclinic space group where an asymmetric part of the unit cell consists of three molecules. Their geometries do not differ within the experimental error (the largest difference is 0.014Å). There are hydrogen bond donors (N-H) and acceptors (O) present, therefore, the structure is stabilized by a set of N-H…O hydrogen bonds. Additionally, C-H…O interactions could be found in the crystal lattice with distances of 2.509 and 2.717Å (see Fig. 2). Moreover, there are short contacts between the bromine atoms of adjacent molecules (Fig. 3). The Br…Br distances in range 3.2-3.9Å can be considered as attractive interactions (33, 34).

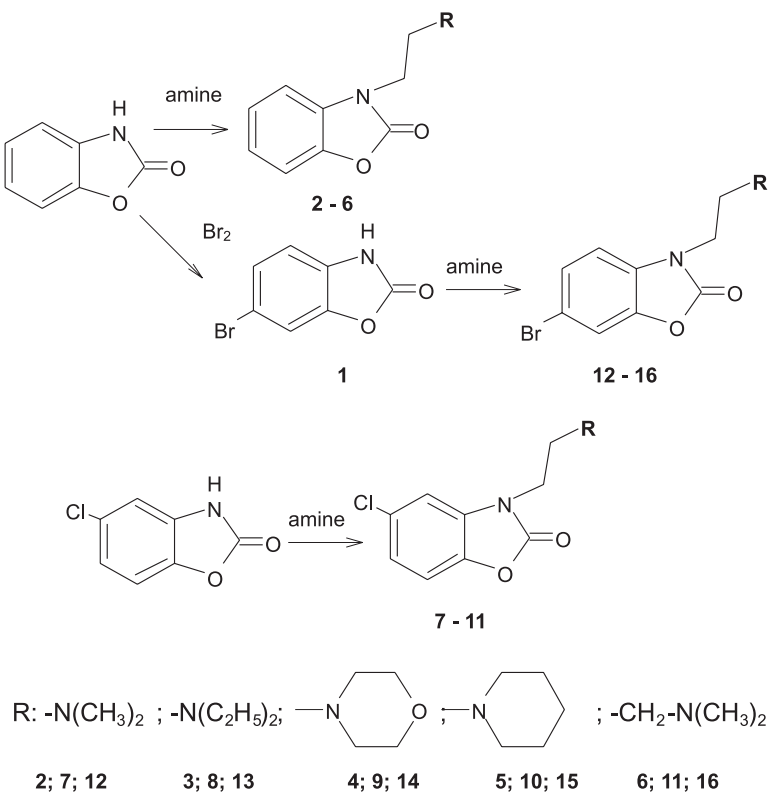
Antimicrobial activity

All derivatives were tested for their antimicrobial activity against four microbial species: *Staphylococcus aureus, Escherichia coli, Stenotrophomonas maltophilia and Candida albicans*.

The chosen set of species provides a good model for screening of newly synthesized chemical compounds for antimicrobial activity. They differ in cell wall structure, mechanism of the patogenicity and susceptibility to antimicrobial drugs. These microorganisms are the cause of many hospital infections.

Staphylococcus aureus is a Gram-positive coccus, it is found in the nose and skin of humans and animals. *Staphylococcus aureus* infections usually cause purulent skin and food poisoning through the production of toxins. *Escherichia coli* is a Gram-negative bacillus, which is the part of the physiological bacterial flora of the colon. It may cause urinary tract infections, abscesses, nosocomial infections and food poisoning. *S. aureus* and *E. coli* are resistant to many antibiotics, because they synthesize of enzymes that degrade drugs. In the case of *S. aureus* also a modification of the antibiotic-binding proteins (MRSA) occurs. *Stenotrophomonas maltophilia* is a Gram-negative bacillus, which causes opportunistic infections. It is responsible for infection in immunosuppressed patients. It is characterized by a natural resistance to many antibiotics (35).

Candida albicans is a fungus (a form of yeast). It is causal agent of opportunistic infections oral



Scheme 1. Method of preparation of compounds **1–16**

cavity and genitals in humans. Systemic fungal infections (fungemias) have emerged as important causes of morbidity in immunocompromised patients. Under normal circumstances, *C. albicans* lives in 80% of the human population with no harmful effects, although overgrowth results in candidiasis (36).

From all tested compounds, two (**1** and **14**) displayed some antimicrobial activity. Derivative **1** was active against Gram-positive cocci represented by *E. coli*. The compound inhibited the growth of bacteria at a concentration 512 mg/L.

Introduction of aminoalkyl fragment decreased microbial activity of compounds containing bromine in the structure. Only compound **14** was active against *C. albicans*. The compound inhibited the growth of fungus at a concentration 512 mg/L.

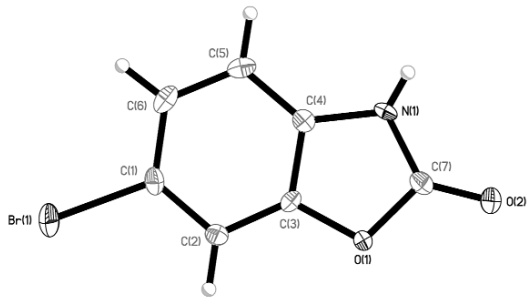


Figure 1. The molecular structure of 6-bromo-1,3-benzoxazol-2(3*H*)-one showing displacement ellipsoids at the 50% probability level.

Similarly, no microbial activity showed aminoalkyl derivatives containing chlorine or not having a halogen.

The obtained results in comparison with those described by Murty et al. (25) seem to be quite interesting. Murty et al. described that chlorobenzoxazolone derivatives containing different amine with propyl linker have moderate activity against both Gram-negative and Gram-positive bacteria. In our study, we obtained several derivatives with ethyl linker but none of them showed any activity. These results suggest that the alkyl chain may contribute to the activity of this compounds. On the other hand, our study shows that compounds containing propyl chain but connected with other amine (dimethylamine) are also inactive. Therefore, we can suppose that not only the length of linker chain but also a type of amine can decide about a antimicrobial activity of benzoxazolone derivatives.

Cytotoxic properties of compounds towards cancer HeLa and K562 cell lines and towards noncancerous HUVEC cells

In these screening studies 8 compounds were tested for their cytotoxic properties in K562 (leukemia), HeLa (cervix carcinoma) and normal (HUVEC) cells. As the control (100% viability in the MTT assay) cells treated with DMSO (1%) were used. The viability of cells was determined at four different drug concentrations: 1 mM, 1×10^{-2} mM, 1×10^{-4} mM and 1×10^{-6} mM. Cells grown in the presence of 1 μM staurosporine for 48 h were used as the control for MTT assay.

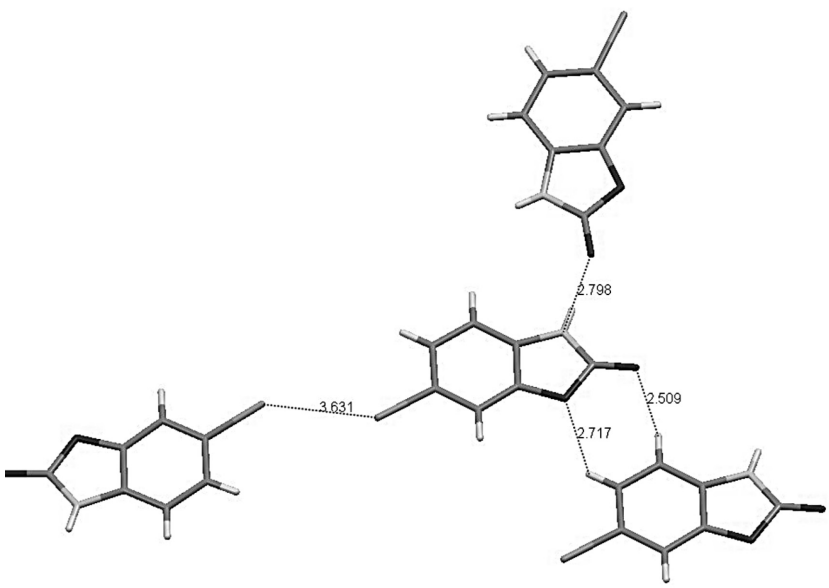


Figure 2. Hydrogen bonds and short contacts in 6-bromo-1,3-benzoxazol-2(3*H*)-one.

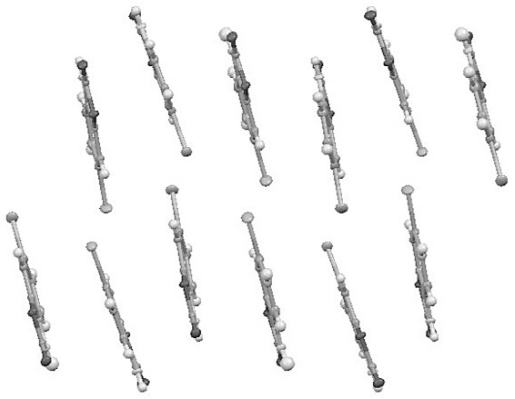


Figure 3. Short contacts between bromine atoms, packing in the crystal lattice of 6-bromo-1,3-benzoxazol-2(3*H*)-one viewed along [010] direction.

Based on dose-response curves we calculated IC_{50} values (the concentration of compound that reduces the cell growth by 50%), which are summarized in Table 1. The results obtained indicate that only compounds **8** and **10** showed limited toxicity toward human cervical carcinoma cells (HeLa), with IC_{50} values of 90 and 100 μM, respectively. Interestingly, these compounds seem to be non-toxic for human leukemia cells (K562), as evidenced by IC_{50} values (> 1 mM). This observation suggests that compounds **8** and **10** may selectively kill HeLa (adherent) over K562 (suspension) cells. The toxicity of these compounds was also assessed for normal, human umbilical vein endothelial cells (HUVEC). As shown in Table 1, after 48 h incubation, compounds **8** and **10** were slightly less toxic for normal cells (IC_{50} of 100 and 200 μM, respectively) than for cancer HeLa cells (IC_{50} of 90 and 100 μM). None of the screened compounds **2–7** showed significant toxicity toward both HeLa and K562 cancer cells in our experimental settings. This is evidenced by IC_{50} values (Table 1) of > 1 mM after 48 h incubation, which is characteristic for rather low toxicity compounds. Since compounds **2–7** were not toxic to human cancer cells, analogous experiments in human normal (HUVEC) cells were not performed (indicated as "Nd" in Table 1).

Acknowledgment

The cytotoxicity studies were performed in the Screening Laboratory, Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies of the Polish Academy of Sciences and financially supported by the Ministry of Science and Higher Education, project PBZ-MNiSW-07/I/2007 (2008–2010).

REFERENCES

1. Temiz-Arpaci O., Oren I., Altanlar N.: Farmaco, 57, 175 (2002).
2. Temiz-Arpaci O., Aki-Sener E., Yalcin I., Altanlar N.: Farmaco, 57, 771 (2002).
3. Yildiz-Oren I., Yalcin I., Aki-Sener E., Ucarturk N.: Eur. J. Med. Chem. 39, 291 (2004).
4. Yildiz-Oren I., Tekiner-Gulbas B., Yalcin I., Temiz-Arpaci O., Aki-Sener E., Altanlar N.: Arch. Pharm. 337, 402 (2004).
5. Temiz-Arpaci O., Ozdemir A., Yalcin I., Yildiz I., Aki-Sener E., Altanlar N.: Arch. Pharm. 338, 105 (2005).
6. Turan-Zitoni G., Demirayak S,. Ozdemir A., Kaplancikli Z.A., Yildiz M.T.: Eur. J. Med. Chem. 39, 267 (2004).
7. Vinsova J., Horak V., Buchta V., Kaustova J.: Molecules 10, 783 (2005).
8. Akbay A., Oren I, Temiz-Arpaci O., Aki-Sener E., Yalcin I.: Arzneim. Forsch. 53, 266 (2003).
9. Plemper R.K., Erlandson K.J., Lakdawala A.S., Sun A., Prussia A., Boonsombat J., Aki-Sener E. Eal.: Proc. Natl. Acad. Sci. USA 101, 5628 (2004).
10. Temiz-Arpaci O., Tekiner-Gulbas B., Yildiz I., Aki-Sener E., Yalcin I.: Bioorg. Med. Chem. 13, 6354 (2005).
11. Tekiner-Gulbas B., Temiz-Arpaci O., Yildiz I., Aki-Sener E., Yalcin I.: Environ. Res. 17, 121 (2006).
12. Lage H., Aki-Sener E., Yalcin I.: Int. J. Cancer 119, 213 (2006).
13. Temiz-Arpaci O., Aki-Sener E., Yalçin I., Altanlar N.: Arch. Pharm. 335, 283 (2002).
14. Yildiz-Oren I., Tekiner-Gulbas B., Yalcin I., Temiz-Arpaci O., Aki-Sener E., Altanlar N.: Arch. Pharm. 337, 402 (2004).
15. Yalçin I., Ören I., Şener E., Akin A., Uçartürk N.: Eur. J. Med. Chem. 27, 401 (1992).
16. Shi D. F., Bradshaw T.D., Wrigley S., McCall C.J., Lelieveld P., Fichtner I., Stevens M.F.G.: J. Med. Chem. 39, 3375 (1996).
17. Lespagnol C., Lesieur D., Bonte J.P.: FR19730023280, May 08 (1978).
18. Lesieur D., Lespagnol C., Vaccher M.P., Bonte J.P., Debaert M., Busch N.: FR19800020861, April 15 (1982).
19. Caignard D.H., Lespagnol C., Lesieur D., Busch N.: FR19820019812, January 25, (1991).

20. Shankaran K., Donnelly K.L., Shah S.K., Humes J.L., Pacholok S.G., Grant S.K., Green B.G., MacCoss M.: Bioorg. Med. Chem. Lett. 7, 2887 (1997).
21. Kalcheva V., Mincheva Z., Andreeva P.: Arzneim. Forsch. 40, 1030 (1990).
22. Gershon H., Clarke D.D., Gershon M.: Monatsh. Chem. 124, 367 (1993).
23. Wan J., Ernstgard L., Song B., Shoaf S.: J. Pharm. Pharmacol. 58, 51 (2006).
24. Murty M.S. R., Ram K.R., Venkateswara Rao R., Yadav J.S., Venkateswara Rao J., Cheriyan V.T., Anto R.J.: Med. Chem. Res. 20, 576 (2011).
25. Murty M.S.R., Ram K.R., Venkateswara Rao R., Yadav J.S., Murty U.S.N., Kumar K.P.: Med. Chem. Res. 20, 626 (2011).
26. Emmons G.T., Kongsamut S., Karson C.N., Legoff C.M.: US2008/138413 A1, June 12, (2008).
27. Oxford Diffraction CrysAlis CCD and CrysAlis RED; Oxford Diffraction Poland: Wrocław, Poland, 2001.
28. Sheldrick G.M. Acta Cryst. A46**,** 467 (1990).
29. Sheldrick G.M. SHELXL93. Program for the Refinement of Crystal Structures, University of Göttingen, Germany.
30. International Tables for Crystallography, Vol. C., Wilson A.J.C. Ed., Kluwer, Dordrecht 1992.
31. Clinical and Laboratory Standards Institute. Antimicrobial Susceptibility testing (M100-S16). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard 7th edn. (M7-A7). Performance standards for antimicrobial disc susceptibility test approved standard 9th edn. (M2-A9). Clinical and Laboratory Standards Institute, Wayne, Pa 2006.
32. Maszewska M., Leclaire J., Cieślak M., Nawrot B., Okruszek A., Caminade A.-M., Majoral J.-P.: Oligonucleotides 13, 193 (2003).
33. Tanaka K., Fujimoto D., Altreuther A., Oeser T., Irngartinger H., Tode F.: J. Chem. Soc., Perkin Trans. 2, 2115 (2000).
34. Broder C.K., Howard J.A.K., Keen D.A., Wilson C.C., Allen F.H., Jetti R.K.R., Nangia A., Desiraju G.R.: Acta Cryst. B56, 1080 (2000).
35. Murray P.R., Rosenthal K.S., Pfaller M.A.: Microbiology, Elsevier Urban & Partner, Wrocław 2011.
36. Baran E.: Mycology – what is new. Cornetis, Wrocław 2008.

Received: 29. 03. 2012