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Synthesis and preliminary evaluation of antimicrobial activity of selected derivatives of 2-benzofurancarboxylic acid

Synteza i wstępne badania aktywności mikrobiologicznej wybranych pochodnych kwasu 2-benzofuranokarboksylowego

INTRODUCTION

Benzofuran derivatives are an important class of heterocyclic compounds that are known to possess important biological properties [14, 22]. Numerous compounds containing a benzo[b] furan system isolated from natural sources as well as synthetic ones show biological activity, such as antiarrhytmic, spazmolitic, antiviral, anticancer, antifungal and antiinflammatory [1–3, 5, 7–13, 15, 20, 21, 23–27]. The most recognized of the benzofurans are *Amiodaron, Benzaron, Angelicin, Xanthotoxin* (Fig. 1). Our previous studies show that the halogen introduced to the benzofuran system increase its biological activity [16–18]. Taking this fact under consideration we have synthesized the new derivatives including bromine in a system (Scheme1).

The starting compound, 7-acetyl-6-hydroxy-3-methyl-1-benzofuran-2-carboxylic acid was synthesized according to a previously published paper [28]. Compound **I** was obtained in the reaction with excess of $(CH_3O)_2SO_2$. Next the bromo derivatives were prepared by reaction with Br_2 and / or N-Bromosuccinimide (NBSI). Three compounds **III–V** in the reaction with Br_2 with different bromine substitution were obtained. The main product **III** was used in reaction with NBSI to give compound **VI** and additional compound **VII**. Finally, derivative **VI** was condensed with amines.

The structures of all compounds were confirmed by ^{1}H NMR and elemental analysis. All halogen derivatives were tested for their antimicrobial activity against five microbial species: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Stenotrophomonas maltophilia and Candida albicans*. They belong to the three large group of microorganisms, which differ in their built, cell wall

structure, virulence potential, pathogenicity mechanism, ability to cause infections and susceptibility to various antimicrobial agents, but all of them can play an important role as the most common human pathogens.

Staphylococcus aureus is a Gram-positive, coccus shape bacteria, which can form multicellular sets as a grape. It can be a part of the natural skin and mucous membrane microflora, but it also can cause many site localized and systemic diseases both, in nosocomial and community-acquired treatment. *Escherichia coli, Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* are Gram-negative rods, which can be noticed as etiologic factors of many septic diseases, particularly *pneumoniae* in the hospital wards. They can produce a wide spectrum of enzymes which often make this species of bacteria multi drug resistant organisms [6]. *Candida albicans* differ from the previous ones, because it belongs to the fungus group of microorganisms, which means that it possesses the distinct cell and cell wall structure distinct as compared to bacteria. But similarly to the other bacteria strains, it is responsible for most of the *Candida* sp. infections in humans [19].

The chosen set of species provides a good model for screening new synthesized chemical compounds for antimicrobial activity. The above-mentioned screening test showed the antimicrobial activity of compound **VIII**.

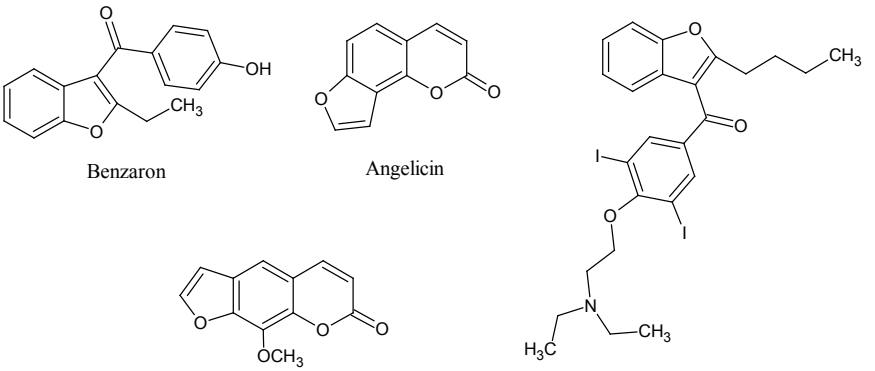
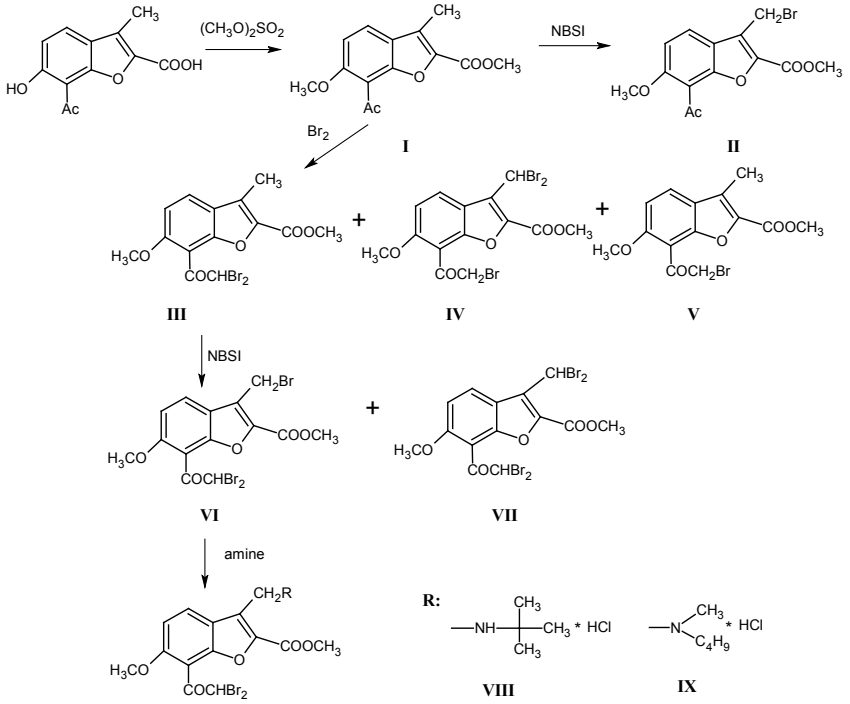


Fig. 1. The most recognized benzofurans

EXPERIMENTAL DESIGN

Melting points were determined in open capillary in Electrothermal 9100 apparatus and are uncorrected. The proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded in $CDCl_3$ or DMSO-d_6 + TMS on a Bruker AVANCE DMX400 operating at 400MHz or VMNRS300 operating at 300MHz. Chemical shift values are expressed in ppm (parts per million) in relation to tetramethylsilane as an internal standard and coupling constants *J* are given in Hz. Chromatography columns were filled with Merc Kieselgel 0.05–0.2 mm reinst (70–325 mesh ASTM) silica gel. Reactions described in the

experimental section were monitored by TLC on silica gel (plates with fluorescent indicator 254 nm, layer thickness 0,2 mm, Kieselgel G. Merck), using chloroform-methanol 9.8:0.2 and 9.5:05, respectively as eluents.



Scheme 1. Method of preparation of compounds **I – IX**

Synthesis of methyl 7-acetyl-6-methoxy-3-methyl-1-benzofuran-2-carboxylate (**I**)

A mixture of 7-acetyl-6-hydroxy-3-methyl-1-benzofuran-2-carboxylic acid (0.02 mol), K_2CO_3 (0.02 mol) and $(CH_3O)_2SO_2$ (0.04 mol) in acetone (30 ml) was refluxed for 5 h. When the reaction was completed, the mixture was filtered and the solvent was evaporated. The residue was purified by a column chromatography (eluent: chloroform).

$C_{14}H_{14}O_5$ M=262.25; 88%; m.p.131-132°C, ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm): 7.62(d, 1H, H arom., J=8.7 Hz), 7.03(d, 1H, H arom., J=8.7 Hz), 3.97(s, 3H, -$COOCH_3$), 3.94(s, 3H, -OCH_3), 2.71(s, 3H, -$COCH_3$), 2.57(s, 3H, -CH_3); Analysis: cal. 64.12 % C, 5.38 % H, found 64.09 % C, 5.42 % H.

Synthesis of methyl 7-acetyl-3-(bromomethyl)-6-methoxy-1-benzofuran-2-carboxylate (**II**)

To a solution of **I** (0.02 mol) in dry carbon tetrachloride (50 mL) N-bromosuccinimide (NBSI) (0.02 mol) and catalytic amount of benzoyl peroxide were added. The reaction mixture was refluxed for 8 h. When the reaction was completed, the mixture was filtered and the solvent was evaporated. The residue was purified by a column chromatography (eluent: chloroform).

$C_{14}H_{13}BrO_5$ M= 341.15; 78%; m.p.155-157°C, ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm): 7.82(d, *J*=8.7, 1H, H arom.), 7.10(d, *J*=9, 1H, H arom.), 4.99(s, 2H, -CH_2Br), 3.98(s, 6H, -$COOCH_3$, -OCH_3), 2.71(s, 3H, -$COCH_3$); Analysis: cal. 49.28 % C, 3.84 % H, found 49.37 % C, 3.60 % H.

Synthesis of bromo derivatives of compound **I** using Br_2-derivatives **III**, **IV** and **V**

Compound **I** (0.02 mol) was dissolved in $CHCl_3$ (20 mL). Next, the solution of bromine (0.04 mol) in $CHCl_3$ (10 mL) was added dropwise with stirring for 0.5 h. Stirring was continued for 8 h at room temperature. When the reaction was completed, the mixture was filtered and the solvent was evaporated. The mixture of derivatives was purified by column chromatography (eluent: chloroform). Then the obtained compounds were crystallized from ethanol. Finally, three compounds **III**, **IV**, **V** were isolated.

methyl 7-(dibromoacetyl)-6-methoxy-3-methyl-1-benzofuran-2-carboxylate (**III**)

$C_{14}H_{12}Br_2O_5$ M=420.05; 55%; m.p.194-195°C, ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm): 7.72(d, *J*=9, 1H, H arom.), 7.10 (s, 1H, -$COCHBr_2$), 7.03(d, J=8.7, 1H, H arom.), 4.02(s, 3H, -$COOCH_3$), 3.95(s, 3H, -OCH_3), 2.57(s, 3H, -CH_3); Analysis: cal. 40.03 % C, 2.88 % H, found 40.30 % C, 3.09 % H.

methyl 7-(bromoacetyl)-3-(dibromomethyl)-6-methoxy-1-benzofuran-2-carboxylate (**IV**)

$C_{14}H_{11}Br_3O_5$ M= 498.94; 15%; m.p.186-189°C, ^{1}H NMR (400MHz, $CDCl_3$) δ (ppm): 7.91(d, *J*=8.8, 1H, H arom.), 7.10(d, *J*=6.6, 1H, H arom.), 7.05(s, 1H, -$CHBr_2$), 4.99(s, 2H, -$COCH_2$Br), 4.04(s, 3H, -$COOCH_3$), 3.99(s, 3H, -OCH_3); Analysis: cal. 33.71 % C, 2.18 % H, found 33.78 % C, 2.20 % H.

methyl 7-(bromoacetyl)-6-methoxy-3-methyl-1-benzofuran-2-carboxylate (**V**)

$C_{14}H_{13}BrO_5$ M=341.15; 35%; m.p.131-132°C, ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm): 7.72 (d, *J*=8.7, 1H, H arom.), 7.03(d, *J*=8.7, 1H, H arom.,), 4.60(s, 2H, -$COCH_2$Br), 4.00(s, 3H, -$COOCH_3$), 3.94(s, 3H, -OCH_3), 2.57(s, 3H, -CH_3); Analysis: cal. 49.28 % C, 3.84 % H, found 49.28 % C, 3.81 % H.

Synthesis of compounds **VI** and **VII**

To a solution of **III** (0.02 mol) in dry carbon tetrachloride (50 mL) NBSI (0.02 mol) and catalytic amount of benzoyl peroxide were added. The reaction mixture was refluxed for 16 h. When the reaction was completed, the mixture was filtered and the solvent was evaporated. The residue was purified by column chromatography (eluent: chloroform). The obtained compounds were crystallized from ethanol. Finally, two compounds **VI**, **VII** were isolated.

methyl 3-(bromomethyl)-7-(dibromoacetyl)-6-methoxy-1-benzofuran-2-carboxylate (**VI**)

$C_{14}H_{11}Br_3O_5$ M= 498.94; 55%; m.p.189-191°C, ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm): 7.92 (d, *J*=9, 1H, H arom.,), 7.10(d, *J*=8.7, 1H, H arom.,), 7.05(s, 1H, -$COCHBr_2$), 4.99(s, 2H, -CH_2Br), 4.04(s, 3H, -$COOCH_3$), 3.99(s, 3H, -OCH_3); Analysis: cal. 33.71 % C, 2.18 % H, found 33.83 % C, 2.26 % H.

methyl 7-(dibromoacetyl)-3-(dibromomethyl)-6-methoxy-1-benzofuran-2-carboxylate(**VII**)

$C_{14}H_{10}Br_4O_5$ M= 577.84; 15%; m.p.133-134°C, ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm): 8.38 (d, *J*=9, 1H, H arom.), 7.73(s, 1H, -$CHBr_2$), 7.19(d, *J*=9, 1H, H arom.), 7.03(s, 1H, -$COCHBr_2$), 4.07(s, 3H, -$COOCH_3$), 4.00(s, 3H, -OCH_3); Analysis: cal. 29.74% C, 1.74% H, found 29.38 % C, 1.63 % H.

Synthesis of derivatives **VIII** and **IX**

Compound **VI** (0.02 mol) was dissolved in acetone (20 mL). Next, an appropriate amine (0.02 mol) and powdered anhydrous K_2CO_3 (0.02 mol) were added. The reaction mixture was refluxed for 16–24 h. When the reaction was completed, the mixture was filtered and the solvent was evaporated. The residue was purified by column chromatography (eluent: chloroform). In the next step, compounds were crystallized from ethanol. The amino derivatives were converted to their hydrochlorides and recrystallized from methanol.

methyl 3-[(*tert*-butylamino)methyl]-7-(dibromoacetyl)-6-methoxy-1-benzofuran-2-carboxylate (**VIII**)

$C_{18}H_{21}Br_2NO_5$ * HCl M=491.17*HCl; 35%; m.p.201-202°C, ^{1}H NMR (300MHz, DMSO-d_6 + TMS) δ (ppm): 8.91(br.s, 2H, NH_2^+), 8.37 (d, *J*=9, 1H, H arom.), 7.46(d, *J*=9 , 1H, H arom.), 7.37(s, 1H, -$COCHBr_2$), 4.56(m, 2H, -CH_2-), 4.04(s, 3H, -$COOCH_3$), 3.99(s, 3H, -OCH_3), 3.31(s, 9H, -$(CH_3)_3$); ESI MS(m/z): 100%= 491.9; 70%=489.9; 60%=493.9 [L+H^+]

methyl 3-{[butyl(methyl)amino]methyl}-7-(dibromoacetyl)-6-methoxy-1-benzofuran-2-carboxylate (**IX**)

$C_{19}H_{23}Br_2NO_5$ *HCl M= 505.19*HCl; 35%; m.p.151-153°C, ^{1}H NMR (300MHz, DMSO-d_6 + TMS) δ (ppm): 9.91(br.s, 1H, NH^+), 8.33 (d, *J*=9, 1H, H arom.,), 7.46(d, *J*=9, 1H, H arom.), 7.36(s, 1H, -$COCHBr_2$), 4.70(m, 2H, -CH_2-), 4.04(s, 3H, -$COOCH_3$), 3.94(s, 3H, -OCH_3), 2.75(m, 3H, N-CH_3), 1.77(m, 2H, N-CH_2-), 1.33(m, 4H, -$(CH_2)_2$-), 0.92(m, 3H, -CH_3); ESI MS(m/z): 100%= 505.9; 40%=519.9; 30%=503.9 [L+H^+].

MICROBIOLOGY

Organisms. The standard strains of *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 14053 and one clinical isolate *S. maltophilia* CO2275 were used.

Screening for the antimicrobial activity. The method according to CLSI (Clinical and Laboratory Standards Institute) directives was applied [4]. Compounds II–IX were tested to their bacteriostatic activity at 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 the temperature 35–37 °C, in an aerobic atmosphere, for 18–

24 hours. The fungal strain was cultured in the Sabouraud agar (SA), at the same temperature and atmosphere, but for at least 24 hours. The cultures which were in mid-logarithmic phase of growth were suspended in 0.9% NaCl solution to obtain 0.5 Mac Farland's optical density. 1.0–9.0 x 10^5 cells (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 the temperature 35–37 °C for 24–48 hours. If after 48 h the growth was absent, the substance was noticed as potentially possessing antimicrobial activity. In all experiments strains vitality controls and a DMSO antimicrobial activity controls in the applied concentrations were performed.

RESULTS

The antimicrobial activities of the examined compounds were presented in the table 1.

It appeared that all examined *P. aeruginosa* strains (the standard strain *P. aeruginosa* ATCC 27853 and twenty other clinical isolates, data non shown) were susceptible to DMSO in the concentration used in the experiment. Because of that testing the activity of the compounds against *P. aeruginosa* was not possible in the applied conditions. The other strains displayed full growth in the presence of DMSO in the used concentration.

Among the examined compounds only one (number VIII) displayed some bacteriostatic activity against both Gram-positive and Gram-negative bacteria as well as it inhibited the growth of *Candida albicans*. No inhibition of growth in the presence of other compounds was observed.

Basing on these findings, as well as on results from our earlier investigations, we can confirm that the search for biologically active compounds in the area of halogen derivatives of benzofurans remains an interesting field from the biological point of view.

Table 1. Microbiological activity compounds number II–IX against bacterial and fungus strains

Chemical compound (512 µg/ml)	Microbiological activity against strains				
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*S. maltophilia* CO2275	*C. albicans* ATCC 14053
DMSO control	-	-	+	-	-
II	-	-	ND	-	-
III	-	-	ND	-	-
IV	-	-	ND	-	-
V	-	-	ND	-	-
VI	-	-	ND	-	-
VII	-	-	ND	-	-
VIII	+	+	ND	+	+
IX	-	-	ND	-	-

ND – no data, strain susceptible to used concentration of DMSO

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SUMMARY

This study is the continuation of our research regarding benzo[b]furan-2-carboxylic acid with an expected biological activity. In our work we obtained eight new halogen derivatives which were tested for antimicrobial activity.

Keywords: 2-benzofurancarboxylic acid, antimicrobial activity

STRESZCZENIE

Praca ta jest kontynuacją naszych dotychczasowych badań nad poszukiwaniem nowych pochodnych kwasu benzo[b]furano-2-karboksylowego o potencjalnej aktywności biologicznej. W opisanej pracy otrzymaliśmy nowe halogenopochodne, które zostały poddane wstępnym badaniom mikrobiologicznym.

Słowa kluczowe: kwas 2-benzofuranokarboksylowy, aktywność mikrobiologiczna