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Synthesis and biological activity of 6-substituted 5-acetyl-4,7-dimethoxybenzofuran derivatives

Abstract: In the search for new antimicrobial and anticancer agents, a series of (aryl/heteroaryl-piperazino-alkyl)-substituted derivatives of benzo[*b*]furans were prepared. All compounds were characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS spectra and elemental analyses. Most of the investigated compounds had no antimicrobial activity (MIC > 512 mg/L) except for **2l**, **2m** and **2o**, which showed activity against *Candida albicans*. None of the tested compounds showed significant anticancer activity in K562 and HeLa cells.

Keywords: antifungal activity; antimicrobial activity; benzo[*b*]furans; cytotoxicity.

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Introduction

The benzofuran system, as an important pharmacophore, is present in numerous compounds isolated from natural sources as well as in synthetic products. These heterocyclic compounds show a variety biological activity, including antiarrhythmic, spazmolitic, antiviral, anticancer, antifungal and anti-inflammatory properties [1–10]. The most recognized derivatives of the benzofurans are Khellinone and Visnaginone isolated from *Ammi visnaga* (*Apiaceae*) [11].

Our research group obtained a large group of compounds which show antimicrobial, antiviral and cytotoxic activities [12–15]. This article describes our latest research results on the design and synthesis of new compounds with potential biological activity (Scheme 1).

Results and discussion

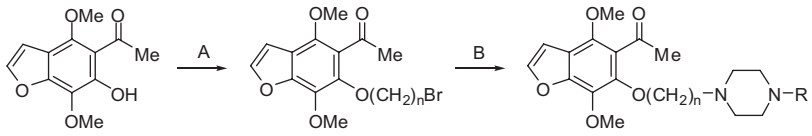
Synthesis

The synthesis of compounds **1a,b** and **2a–p** was accomplished as presented in Scheme 1. The starting compound was 5-acetyl-6-hydroxy-4,7-dimethoxybenzofuran which was alkylated with 1,2-dibromoethane or 1,3-dibromopropane to give the respective products **1a** or **1b**. The final products **2a–p** were prepared with good yields by condensation of the intermediate product **1a** or **1b** with the appropriate 1-aryl/heteroarylpiperazine in the presence of K_2CO_3 and KI in refluxing acetonitrile. The amino derivatives were converted into their hydrochlorides. All reported products showed ^{1}H NMR, ^{13}C NMR, ESI-MS spectra and elemental analyses in agreement with the assigned structures.

Antimicrobial activity

The obtained compounds **1a**,**b** and **2a–p** were tested for their antimicrobial activity against aerobic bacteria: *Staphylococcus aureus, Escherichia coli, Stenotrophomonas maltophilia* and yeast strain *Candida albicans* and some (**1a, 2a–e**) against selected anaerobes *Propionibacterium acnes, Bacteroides thetaiotaomicron* and *Bacteroides fragilis*.

Most of the investigated compounds showed no antimicrobial activity and did not inhibit growth even at the concentration of 512 mg/L. Some activity was observed for compounds **2l**, **2m** and **2o**. They were active against *C. albicans*. The compounds inhibited the growth of yeast at a concentration of 512 mg/L. The highest activity was observed for compound **2o**. For compounds **2l** and **2m**, the growth of fungi was observed on the second day.



1a-b 2a-p

1a: n = 2
1b: n = 3
2a: R = Ph; n = 2
2b: R = 2-OMe-Ph; n = 2
2c: R = 2-Cl-Ph; n = 2
2d: R = 2-pyridyl; n = 2
2e: R = 2-pyrimidinyl; n = 2
2f: R = 3-OMe-Ph; n = 2
2g: R = 3-Cl-Ph; n = 2
2h: R = 4-NO_2-Ph; n = 2
2i: R = Ph; n = 3
2j: R = 2-OMe-Ph; n = 3
2k: R = 2-Cl-Ph; n = 3
2l: R = 2-pyridyl; n = 3
2m: R = 2-pyrimidinyl; n = 3
2n: R = 3-OMe-Ph; n = 3
2o: R = 3-Cl-Ph; n = 3
2p: R = 4-NO_2-Ph; n = 3

Scheme 1 Synthesis of compounds **2a–p**; (A) $Br(CH_2)_2Br$ or $Br(CH_2)_3Br$, K_2CO_3, acetonitrile; (B) 1-aryl/heteroarylpiperazine, K_2CO_3, KI.

Cytotoxic properties

The selected derivatives **1b**, **2i**, **2j**, **2m**, **2o** and **2p** were tested for their cytotoxic properties in K562 and HeLa cells. On the basis of dose-response curves it was not possible to calculate IC_{50} values, which indicates that none of the tested compounds show significant anticancer activity.

Conclusion

In the present study, we obtained and tested for antimicrobial and anticancer activity new aryl/heteroaryl-piperazino-alkyl derivatives of benzo[*b*]furans. The results show that this class of benzofurans possess low antimicrobial activity and do not show significant anticancer activity.

Experimental

Melting points were determined by the capillary method using the Electrothermal 9100 apparatus and were uncorrected. Unless stated otherwise, nuclear magnetic resonance spectra were recorded in DMSO (dimethyl sulfoxide)-d_6 on a Bruker VMNRS300 operating at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). Mass spectral (electrospray ionization, ESI) measurements were carried out on a Mariner Perspective – Biosystem instrument with a TOF detector. The spectra were obtained in the positive ion mode with a declustering potential of 140–300 V. Elemental analyses were recorded using a CHN model 2400 Perkin-Elmer analyzer. Chromatographic columns were filled with Merck Kieselgel 0.05–0.2 mm (70–325 mesh ASTM) silica gel. Reactions were monitored by thin layer chromatography (TLC) on silica gel (plates with 254 nm fluorescent indicator, layer thickness 0.2 mm, Kieselgel G., Merck), eluting with 9.8:0.2 or 9.5:0.5 chloroform/methanol.

Synthesis of compounds 1a and 1b

A solution of 5-acetyl-1-(6-hydroxy)-4,7-dimethoxybenzofuran (0.01 mol) in acetonitrile (30 mL) was treated with anhydrous K_2CO_3 (0.01 mol) and 1,2-dibromoethane (0.03 mol) or 1,3-dibromopropane (0.03 mol). The mixture was heated under reflux for 7–15 h, then filtered and concentrated. The residue was purified by column chromatography eluting with chloroform.

5-Acetyl-6-(2-bromoethoxy)-4,7-dimethoxybenzofuran (1a) This compound was obtained in 84% yield as a colorless oil; ^{1}H NMR (DCl_3: δ 7.59 (d, 1H, C2-H, *J* = 2.1 Hz), 6.88 (d, 1H, C3-H, *J* = 2.1 Hz), 4.36 (t, 2H, C1′-H, *J* = 6.3 Hz), 4.09 (s, 3H, -OCH_3), 3.99 (s, 3H, -OCH_3), 3.58 (t, 2H, -C2′-H, *J* = 6.3 Hz), 2.55 (s, 3H, -$COCH_3$); ^{13}C NMR: δ 31.5, 32.6, 60.6, 61.0, 73.8, 105.4, 115.8, 123.3, 133.3, 143.0, 143.6, 145.8, 147.9, 200.4; ESI-MS: m/z 343.17 [M^+]. Anal. Calcd for $C_{14}H_{15}BrO_5$: C, 49.00; H, 4.41. Found: C, 48.98; H, 4.39.

5-Acetyl-6-(3-bromopropoxy)-4,7-dimethoxybenzofuran (1b) This compound was obtained in 80% yield as a colorless oil; ^{1}H NMR: δ 7.57 (d, 1H, C2-H, *J* = 2.1 Hz), 6.87 (d, 1H, C3-H, *J* = 2.1 Hz), 4.22–4.14 (m, 2H, C3′-H), 4.09 (s, 3H, -OCH_3), 3.99 (s, 3H, -OCH_3), 3.62–3.58 (m, 2H, C1′-H), 2.55 (s, 3H, -$COCH_3$), 2.29–2.21 (m, 2H, C2′-H); ^{13}C NMR: δ 30.1, 30.5, 33.3, 33.8, 61.6, 73.0, 105.5, 117.0, 124.7, 134.8, 144.5, 144.6, 145.1, 149.1, 202.4; ESI-MS: m/z 380.9 [M^+]. Anal. Calcd for $C_{15}H_{17}BrO_5$: C, 50.44; H, 4.80. Found: C, 50.30; H, 4.59.

Synthesis of 4-arylpiperazino derivatives 2a–p

A mixture of *N*-bromoalkyl derivative **1a** or **1b** (0.01 mol), a powdered anhydrous K_2CO_3 (0.01 mol), a catalytic amount of KI in acetone (30 mL) and a substituted piperazine (0.01 mol) was heated under reflux for 10–20 h, then filtered and concentrated. The residue was purified by column chromatography eluting with chloroform or chloroform/methanol, 50:0.2. All products were converted into their hydrochlorides and the salts were crystallized from methanol/ether.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[2-(4-phenylpiperazino)ethoxy]benzofuran (2a) The hydrochloride salt was obtained in 60% yield as a colorless oil; ^{1}H NMR: δ 10.92 (s, 1H, NH^+), 8.08 (d, 1H, C2-H, *J* = 2.4 Hz), 7.30–7.24 (m, 3H, CH, C3-H), 7.04–7.01 (m, 2H, CH), 6.87 (t, 1H, CH, *J* = 7.2 Hz), 4.40–4.37 (m, 2H, -CH_2-piperazine), 4.06 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 3.90–3.86 (m, 2H, C1′-H), 3.68–3.65 (m, 2H, -CH_2-piperazine), 3.58–3.50 (m, 5H, -$COCH_3$, -CH_2-piperazine), 3.30–3.13 (m, 4H, -CH_2-piperazine); ^{13}C NMR: δ 32.7, 45.3, 51.0, 54.9, 60.6, 61.1, 68.8, 105.5, 115.9, 116.2, 119.9, 123.0, 129.1, 133.4,

142.6, 143.8, 146.2, 147.7, 149.5, 201.0; ESI-MS: m/z 425.2 [M+H$^+$]. Anal. Calcd for $C_{24}H_{28}N_2O_5$·HCl: C, 62.54; H, 6.34; N, 6.08. Found: C, 62.34; H, 6.14; N, 6.01.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[2-[4-(2-methoxyphenyl)piperazino]ethoxy]benzofuran (2b) The hydrochloride salt was obtained in 70% yield as a white powder; mp 174–176°C; ^{1}H NMR: δ 10.89 (s, 1H, NH$^+$), 8.08 (d, 1H, C2-H, *J* = 2.4 Hz), 7.26 (d, 1H, C-H, C3-H, *J* = 2.4 Hz), 7.06–6.88 (m, 4H, C-H), 4.39 (t, 2H, -CH_2-piperazine, *J* = 4.8 Hz), 4.07 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 3.80 (s, 3H, -OCH_3), 3.65–3.62 (m, 2H, C1′-H), 3.56–3.53 (m, 4H, -CH_2-piperazine, C2′-H), 3.39–3.28 (m, 2H, -CH_2-piperazine), 3.14–3.07 (m, 2H, -CH_2-piperazine), 2.50 (s, 3H, -$COCH_3$); ^{13}C NMR: δ 32.7, 46.8, 51.4, 54.9, 55.3, 60.6, 61.1, 68.6, 105.5, 111.9, 116.1, 118.3, 120.8, 123.0, 123.5, 133.3, 139.2, 142.5, 143.8, 146.2, 147.7, 151.8, 201.0; ESI-MS: m/z 455.2 [M+H$^+$]. Anal. Calcd for $C_{25}H_{30}N_2O_6$·HCl: C, 61.16; H, 6.36; N, 5.71. Found: C, 61.11; H, 6.12; N, 5.80.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[2-[4-(2-chlorophenyl)piperazino]ethoxy]benzofuran (2c) The hydrochloride salt was obtained in 72% yield as a white powder; mp 154–155°C; ^{1}H NMR: δ 11.04 (s, 1H, NH$^+$), 8.08 (d, 1H, C2-H, *J* = 2.4 Hz), 7.48–7.45 (m, 1H, C-H), 7.37–7.32 (m, 1H, C-H), 7.26–7.22 (m, 2H, C-H, C3-H), 7.15–7.09 (m, 1H, C-H), 4.40–4.38 (m, 2H, -CH_2-piperazine), 4.07 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 3.70–3.46 (m, 6H, -CH_2-piperazine, C1′-H), 3.30–3.19 (m, 4H, -CH_2-piperazine, C2′-H), 2.50 (s, 3H, -$COCH_3$); ^{13}C NMR: δ 32.6, 47.6, 51.5, 54.9, 60.6, 61.1, 68.6, 105.5, 116.2, 121.0, 123.0, 124.8, 127.5, 128.2, 130.4, 133.3, 142.5, 143.8, 146.2, 147.4, 147.7, 200.9; ESI-MS: m/z 459.1 [M+H$^+$]. Anal. Calcd for $C_{24}H_{27}ClN_2O_5$·HCl: C, 58.19; H, 5.70; N, 5.65. Found: C, 58.02; H, 5.80; N, 5.78.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[2-(4-(pyridin-2-yl) piperazino)ethoxy]benzofuran (2d) The hydrochloride salt was obtained in 86% yield as a white powder, mp 156–157°C; ^{1}H NMR: δ 10.88 (s, 1H, NH$^+$), 8.18–8.15 (m, 1H, C-H), 8.08 (d, 1H, C2-H, *J* = 2.4 Hz), 7.74–7.70 (m, 1H, C-H), 7.26 (d, 1H, C-H, C3-H, *J* = 2.4 Hz), 7.09–7.06 (m, 1H, C-H), 6.84–6.80 (m, 1H, C-H), 4.49–4.44 (m, 2H, -CH_2-piperazine), 4.38–4.35 (m, 2H, -CH_2-piperazine), 4.06 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 3.70–3.64 (m, 2H, C1′-H), 3.58–3.50 (m, 2H, -CH_2-piperazine,) 3.41–3.34 (m, 2H, -CH_2-piperazine), 3.23–3.16 (m, 2H, C2′-H), 2.50 (s, 3H, -$COCH_3$); ^{13}C NMR: δ 32.6, 42.3, 47.6, 50.5, 54.9, 60.6, 61.1, 68.6, 105.5, 109.6, 114.0, 116.2, 122.9, 130.4, 133.3, 140.3, 142.4, 143.8, 146.2, 147.7, 200.9; ESI-MS: m/z 426.2 [M+H$^+$]. Anal. Calcd for $C_{23}H_{27}N_3O_4$·HCl: C, 59.80; H, 6.11; N, 9.10. Found: C, 59.67; H, 6.01; N, 9.12.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[2-(4-(pyrimidin-2-yl)piperazino)ethoxy]benzofuran (2e) The hydrochloride salt was obtained in 76% yield as a white powder; mp 189–190°C; ^{1}H NMR: δ 10.49 (s, 1H, NH$^+$), 8.46 (d, 2H, C-H, *J* = 4.8 Hz), 8.08 (d, 1H, C2-H, *J* = 2.4 Hz), 7.26 (d, 1H, C-H, C3-H, *J* = 2.4 Hz), 6.79–6.76 (m, 1H, C-H), 4.77–4.73 (m, 2H, -CH_2-piperazine), 4.38–4.30 (m, 2H, -CH_2-piperazine), 4.05 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 3.68–3.64 (m, 2H, C1′-H), 3.56–3.48 (m, 2H, -CH_2-piperazine), 3.40–3.36 (m, 2H, -CH_2-piperazine), 3.31 (s, 3H, -$COCH_3$), 3.20–3.16 (m, 2H, C2′-H); ^{13}C NMR: δ 32.6, 47.6, 50.7, 55.0, 60.6, 61.0, 68.4, 105.5, 111.3, 116.1, 122.9, 133.3, 142.4, 143.8, 146.2, 147.7, 158.1, 160.7, 200.9; ESI-MS: m/z 427.2 [M+H$^+$]. Anal. Calcd for $C_{22}H_{26}N_4O_4$·HCl: C, 57.08; H, 5.88; N, 12.10. Found: C, 57.17; H, 6.00; N, 12.01.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[2-[4-(3-methoxyphenyl)piperazino]ethoxy]benzofuran (2f) The hydrochloride salt was obtained in 76% yield as an oil; ^{1}H NMR: δ 10.94 (s, 1H, NH$^+$), 8.08 (d, 1H, C2-H, *J* = 2.4 Hz), 7.25 (d, 1H, C3-H, *J* = 2.4 Hz), 7.16 (t, 1H, C-H, *J* = 8.2 Hz), 6.62–6.54 (m, 2H, C-H), 6.47–6.43 (m, 1H, C-H), 4.80–4.70 (m, 2H, -CH_2-piperazine), 4.38 (t, 2H, -CH_2-piperazine, *J* = 4.8 Hz), 4.06 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 3.90–3.86 (m, 2H, -CH_2-piperazine), 3.73 (s, 3H, -OCH_3), 3.66–3.63 (m, 2H, C1′-H), 3.54–3.53 (m, 2H, -CH_2-piperazine), 3.28–3.21 (m, 2H, C2′-H), 2.50 (s, 3H, -$COCH_3$); ^{13}C NMR: δ 32.6, 45.2, 50.9, 54.9, 54.9, 60.6, 61.1, 68.6, 102.1, 105.2, 105.5, 108.3, 116.2, 119.1, 123.0, 129.8, 133.4, 142.4, 143.9, 146.2, 147.7, 150.8, 160.2, 200.9; ESI-MS: m/z 455.3 [M+H$^+$]. Anal. Calcd for $C_{25}H_{30}N_2O_6$·HCl: C, 61.16; H, 6.36; N, 5.71. Found: C, 61.20; H, 6.30; N, 5.68.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[2-[4-(3-chlorophenyl)piperazino]ethoxy]benzofuran (2g) The hydrochloride salt was obtained in 76% yield as a white powder; mp 140–142°C; ^{1}H NMR: δ 10.35 (s, 1H, NH$^+$), 8.08 (d, 1H, C2-H, *J* = 2.1 Hz), 7.30–7.24 (m, 2H, C-H), 7.11–7.08 (m, 1H, C3-H), 7.01–6.98 (m, 1H, C-H), 6.89–6.87 (m, 1H, C-H), 4.36–4.30 (m, 2H, -CH_2-piperazine), 4.06 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 3.69–3.65 (m, 2H, -CH_2-piperazine) 3.58–3.54 (m, 2H, C1′-H), 3.43–3.12 (m, 5H, -$COCH_3$, -CH_2-piperazine), 2.50–2.48 (m, 4H, -CH_2-piperazine, C2′-H); ^{13}C NMR: δ 32.6, 44.7, 50.7, 54.8, 60.6, 61.1, 68.6, 105.5, 114.1, 115.2, 116.2, 119.1, 123.0, 130.6, 133.4, 133.9, 142.4, 143.8, 146.2, 147.7, 150.7, 200.9; ESI-MS: m/z 459.2 [M+H$^+$]. Anal. Calcd for $C_{24}H_{27}ClN_2O_5$·HCl: C, 58.19; H, 5.70; N, 5.65. Found: C, 58.01; H, 5.71; N, 5.69.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[2-[4-(4-nitrophenyl) piperazino]ethoxy]benzofuran (2h) The hydrochloride salt was obtained in 54% yield as a white powder; mp 206–208°C; ^{1}H NMR: δ 11.14 (s, 1H, NH$^+$), 8.14–8.10 (m, 2H, C-H), 8.08 (d, 1H, C2-H, *J* = 2.4 Hz), 7.26 (d, 1H, C3-H, *J* = 2.4 Hz), 7.17–7.14 (m, 2H, C-H), 4.39–4.36 (m, 2H, -CH_2-piperazine), 4.28–4.24 (m, 2H, -CH_2-piperazine), 4.06 (s, 3H, -OCH_3), 4.00 (s, 3H, -OCH_3), 3.70–3.66 (m, 2H, -CH_2-piperazine), 3.62–3.50 (m, 2H, C1′-H), 3.47–3.40 (m, 4H, -CH_2-piperazine, C2′-H), 3.28 (s, 3H, -$COCH_3$); ^{13}C NMR: δ 32.6, 43.5, 50.5, 54.8, 60.6, 61.1, 68.6, 105.5, 113.5, 116.2, 123.0, 125.6, 133.4, 138.0, 142.4, 143.9, 146.2, 147.7, 153.7, 200.9; ESI-MS: m/z 492.2 [M+Na$^+$]. Anal. Calcd for $C_{24}H_{27}N_3O_7$·HCl: C, 56.97; H, 5.58; N, 8.31. Found: C, 56.91; H, 5.70; N, 8.21.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[3-(4-phenylpiperazino)propoxy]benzofuran (2i) The hydrochloride salt was obtained in 60% yield as a white powder; mp 180–182°C (lit. mp 175°C [16]); ^{1}H NMR: δ 10.84 (s, 1H, NH$^+$), 8.04 (d, 1H, C2-H, *J* = 2.1 Hz), 7.29–7.22 (m, 1H, C3-H), 7.02–7.00 (m, 2H, C-H), 6.89–6.84 (m, 1H, C-H), 4.06 (t, 2H, C3′-H, *J* = 8.1 Hz), 4.01 (s, 3H, -OCH_3), 3.98 (s, 3H, -OCH_3), 3.84–3.81 (m, 2H, -CH_2-piperazine), 3.61–3.58 (m, 2H, C1′-H), 3.30–3.25 (m, 2H, -CH_2-piperazine), 3.19–3.09 (m, 4H, -CH_2-piperazine), 2.46 (s, 3H, -$COCH_3$), 2.19–2.14 (m, 2H, C2′-H); ^{13}C NMR: δ 24.0, 32.6, 45.4, 50.6, 52.9, 60.5, 60.9, 71.8, 105.4, 115.7, 115.9, 120.0, 123.2, 129.1, 133.4, 143.3, 143.6, 145.9, 147.9, 149.5, 200.8; ESI-MS: m/z 439.1 [M+H$^+$]. Anal. Calcd for $C_{25}H_{30}N_2O_5$·HCl: C, 63.22; H, 6.58; N, 5.90. Found: C, 63.11; H, 6.56; N, 5.89.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[3-[4-(2-methoxyphenyl)piperazino]propoxy]benzofuran (2j) The hydrochloride salt was obtained in 80% yield as a white powder; mp 181–184°C (lit.:

an oil for the free base [17]); ^{1}H NMR: δ 10.81 (s, 1H, NH$^+$), 8.04 (d, 1H, C2-H, *J* = 2.4 Hz), 7.22 (d, 1H, C3-H, *J* = 2.4 Hz), 7.06–6.89 (m, 4H, C-H), 4.06 (t, 2H, C3′-H, *J* = 6 Hz), 4.01 (s, 3H, -OCH$_3$), 3.98 (s, 3H, -OCH$_3$), 3.80 (s, 3H, -OCH$_3$), 3.60–3.49 (m, 4H, -CH$_2$-piperazine), 3.30–3.23 (m, 2H, C1′-H), 3.19–3.03 (m, 4H, -CH$_2$-piperazine), 2.47 (s, 3H, -COCH$_3$), 2.21–2.11 (m, 2H, C2′-H); ^{13}C NMR: δ 24.1, 32.6, 46.8, 51.1, 53.0, 55.3, 60.5, 60.9, 71.8, 105.5, 111.9, 115.7, 118.2, 120.8, 123.2, 123.5, 133.4, 139.3, 143.3, 143.6, 145.9, 147.9, 151.8, 200.9; ESI-MS: m/z 469.2 [M+H$^+$]. Anal. Calcd for $C_{26}H_{32}N_2O_6$·HCl: C, 61.84; H, 6.59; N, 5.55. Found: C, 61.80; H, 6.57; N, 5.61.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[3-[4-(2-chlorophenyl)piperazino]propoxy]-1-benzofuran (2k) The hydrochloride salt was obtained in 70% yield as a white powder; mp 206–207°C; ^{1}H NMR: δ 10.44 (s, 1H, NH$^+$), 8.04 (d, 1H, C2-H, *J* = 2.1 Hz), 7.48–7.44 (m, 1H, C-H), 7.38–7.32 (m, 1H, C-H), 7.23–7.21 (m, 2H, C3-H), 7.14–7.09 (m, 1H, C-H), 4.07 (t, 2H, C3′-H, *J* = 6 Hz), 4.01 (s, 3H, -OCH$_3$), 3.98 (s, 3H, -OCH$_3$), 3.65–3.61 (m, 2H, -CH$_2$-piperazine), 3.47–3.43 (m, 2H, -CH$_2$-piperazine), 3.34–3.30 (m, 2H, C1′-H), 3.24–3.09 (m, 4H, -CH$_2$-piperazine), 2.47 (s, 3H, -COCH$_3$), 2.17–2.12 (m, 2H, C2′-H); ^{13}C NMR: δ 24.1, 32.6, 47.6, 51.2, 53.0, 60.5, 60.9, 71.8, 105.4, 115.7, 120.9, 123.2, 124.7, 127.5, 128.2, 130.4, 133.4, 143.3, 143.6, 145.9, 147.3, 147.9, 200.8; ESI-MS: m/z 473.2 [M+H$^+$]. Anal. Calcd for $C_{25}H_{29}ClN_2O_5$·HCl: C, 58.94; H, 5.94; N, 5.50. Found: C, 58.80; H, 5.91; N, 5.59.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[3-(4-(pyridin-2-yl) piperazino)propoxy]benzofuran (2l) The hydrochloride salt was obtained in 75% yield as a white powder; mp 188–189°C; ^{1}H NMR: δ 10.67 (s, 1H, NH$^+$), 8.17–8.15 (m, 1H, C-H), 8.03 (d, 1H, C2-H, *J* = 2.4 Hz), 7.76–7.71 (m, 1H, C-H), 7.22 (d, 1H, C3-H, *J* = 2.4 Hz), 7.10–7.07 (m, 1H, C-H), 6.85–6.81 (m, 1H, C-H), 4.50–4.40 (m, 2H, -CH$_2$-piperazine), 4.08–4.06 (m, 2H, C3′-H), 4.00 (s, 3H, -OCH$_3$), 3.98 (s, 3H, -OCH$_3$), 3.64–3.60 (m, 2H, -CH$_2$-piperazine), 3.34–3.24 (m, 4H, -CH$_2$-piperazine, C1′-H), 3.16–3.11 (m, 2H, -CH$_2$-piperazine), 2.46 (s, 3H, -COCH$_3$), 2.17–2.12 (m, 2H, C2′-H); ^{13}C NMR: δ 24.0, 32.6, 42.7, 50.0, 53.0, 54.9, 60.5, 60.9, 71.8, 105.4, 110.6, 113.9, 115.7, 123.2, 129.8, 133.4, 141.4, 143.3, 143.6, 145.9, 147.9, 200.8; ESI-MS: m/z 440.2 [M+H$^+$]. Anal. Calcd for $C_{24}H_{29}N_3O_5$·HCl: C, 60.56; H, 6.35; N, 8.83. Found: C, 60.51; H, 6.50; N, 8.81.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[3-(4-(pyrimidin-2-yl)piperazino)propoxy]benzofuran (2m) The hydrochloride salt was obtained in 70% yield as a white powder; mp 172–173°C; ^{1}H NMR: δ 11.09 (s, 1H, NH$^+$), 8.45 (d, 2H, Harom., *J* = 4.8 Hz), 8.04 (d, 1H, C2-H, *J* = 2.4 Hz), 7.23 (d, 1H, C3-H, *J* = 2.4 Hz), 6.78–6.75 (m, 1H, C-H), 4.73–4.69 (m, 2H, -CH$_2$-piperazine), 4.07–4.04 (m, 2H, C3′-H), 4.00 (s, 3H, -OCH$_3$), 3.97 (s, 3H, -OCH$_3$), 3.60–3.57 (m, 2H, -CH$_2$-piperazine), 3.42–3.38 (m, 2H, C1′-H), 3.25–3.21 (m, 2H, -CH$_2$-piperazine), 3.09–3.05 (m, 2H, -CH$_2$-piperazine), 2.45 (s, 3H, -COCH$_3$), 2.19–2.15 (m, 2H, C2′-H); ^{13}C NMR: δ 24.1, 32.6, 47.6, 50.4, 53.1, 60.5, 60.9, 71.7, 105.4, 111.3, 115.7, 123.2, 133.4, 143.3, 143.6, 145.9, 147.9, 158.1, 160.6, 200.8; ESI-MS: m/z 441.3 [M+H$^+$]. Anal. Calcd for $C_{23}H_{28}N_4O_5$·HCl: C, 57.92; H, 6.13; N, 11.75. Found: C, 57.89; H, 6.16; N, 11.78.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[3-[4-(3-methoxyphenyl)piperazino]propoxy]benzofuran (2n) The hydrochloride salt was obtained in 70% yield as a white powder; mp 172–174°C; ^{1}H NMR: δ 10.42 (s, 1H, NH$^+$), 8.04 (d, 1H, C2-H, *J* = 2.1 Hz), 7.23 (d, 1H, C3-H, *J* = 2.1 Hz), 7.16 (t, 1H, Harom., *J* = 8.1 Hz), 6.60–6.57 (m, 2H, C-H), 6.47–6.43 (m, 1H, C-H), 4.06–4.08 (m, 2H, C3′-H), 4.00 (s, 3H, -OCH$_3$), 3.98 (s, 3H, -OCH$_3$), 3.86–3.82 (m, 2H, -CH$_2$-piperazine), 3.73 (s, 3H, -OCH$_3$), 3.60–3.57 (m, 2H, -CH$_2$-piperazine), 3.18–3.04 (m, 6H, C1′-H, -CH$_2$-piperazine), 2.46 (s, 3H, -COCH$_3$), 2.20–2.10 (m, 2H, C2′-H); ^{13}C NMR: δ 24.0, 32.6, 45.3, 50.6, 52.9, 54.9, 60.5, 60.9, 71.8, 102.1, 105.2, 105.4, 108.3, 115.7, 123.2, 129.8, 133.4, 143.3, 143.6, 145.9, 147.9, 150.9, 160.2, 200.8; ESI-MS: m/z 469.2 [M+H$^+$]. Anal. Calcd for $C_{26}H_{32}N_2O_6$·HCl: C, 61.84; H, 6.59; N, 5.55. Found: C, 61.80; H, 6.57; N, 5.59.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[3-[4-(3-chlorophenyl)piperazino]propoxy]-1-benzofuran (2o) The hydrochloride salt was obtained in 70% yield as a white powder; mp 189–192°C; ^{1}H NMR: δ 10.52 (s, 1H, NH$^+$), 8.04 (d, 1H, C2-H, *J* = 2.1 Hz), 7.28–7.26 (m, 1H, C-H), 7.24–7.22 (m, 1H, C3-H), 7.08–7.05 (m, 1H, C-H), 6.99–6.95 (m, 1H, C-H), 6.89–6.86 (m, 1H, C-H), 4.07–4.05 (m, 2H, C3′-H), 4.00 (s, 3H, -OCH$_3$), 3.98 (s, 3H, -OCH$_3$), 3.92–3.89 (m, 2H, -CH$_2$-piperazine), 3.62–3.56 (m, 2H, C1′-H), 3.30–3.25 (m, 2H, -CH$_2$-piperazine), 3.16–3.13 (m, 4H, -CH$_2$-piperazine), 2.46 (s, 3H, -COCH$_3$), 2.20–2.10 (m, 2H, C2′-H); ^{13}C NMR: δ 24.0, 32.6, 44.8, 50.4, 52.9, 60.5, 60.9, 71.8, 105.4, 114.1, 115.2, 115.7, 119.1, 123.2, 130.6, 133.4, 133.9, 143.3, 143.6, 145.9, 147.9, 150.8, 200.8; ESI-MS: m/z 473.2 [M+H$^+$]. Anal. Calcd for $C_{25}H_{29}ClN_2O_5$·HCl: C, 58.94; H, 5.94; N, 5.50. Found: C, 58.96; H, 5.95; N, 5.49.

Hydrochloride of 5-acetyl-4,7-dimethoxy-6-[3-[4-(4-nitrophenyl)piperazino]propan-1-oxy]benzofuran (2p) The hydrochloride salt was obtained in 80% yield as a white powder; mp 177–179°C; ^{1}H NMR: δ 10.86 (s, 1H, NH$^+$), 8.11–8.09 (m, 2H, C-H), 8.03 (d, 1H, C2-H, *J* = 2.1 Hz), 7.22 (d, 1H, C3-H, *J* = 2.1 Hz), 7.16–7.10 (m, 2H, C-H), 4.08–4.04 (m, 2H, C3′-H), 4.00 (s, 3H, -OCH$_3$), 3.97 (s, 3H, -OCH$_3$), 3.70–3.60 (m, 2H, -CH$_2$-piperazine), 3.50–3.40 (m, 2H, C1′-H), 3.34–3.28 (m, 4H, -CH$_2$-piperazine), 3.18–3.16 (m, 2H, -CH$_2$-piperazine), 2.45 (s, 3H, -COCH$_3$), 2.22–2.00 (m, 2H, C2′-H); ^{13}C NMR: δ 24.1, 32.6, 43.6, 50.3, 53.0, 60.5, 60.9, 71.8, 105.4, 113.4, 115.7, 123.2, 125.6, 133.4, 137.9, 143.4, 143.6, 145.9, 147.9, 153.8, 200.8; ESI-MS: m/z 484.3 [M+H$^+$]. Anal. Calcd for $C_{25}H_{29}N_3O_7$·HCl: C, 57.75; H, 5.82; N, 8.08. Found: C, 57.81; H, 5.83; N, 8.06.

Microbiology

Organisms

Standard strains of *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *C. albicans* ATCC 14053, *P. acnes* ATCC 6919, *B. thetaiotaomicron* ATCC 29741, *B. fragilis* ATCC 25285 and one clinical isolate *S. maltophilia* CO 2275 were used.

Screening for antimicrobial activity

Compounds were tested for bacteriostatic activity at high concentrations (512 mg/L) and if the bacteriostatic effect was observed, the concentration was reduced. A method according to CLSI (Clinical and Laboratory Standards Institute) [18] directives was applied. The tested substances were dissolved in DMSO and then the solutions were added to brain heart infusion broth (BHI-B) medium to a final concentration of 512 mg/L. The aerobic bacteria were cultured on

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. Anaerobes were cultured in Schaedler agar with 5% of sheep blood at 35–37°C for 48 h, in anaerobic atmosphere. The fungal strain was cultured in the Sabouraud agar (SA), at the same temperature and atmosphere, but for at least 24 h. The cultures which were in mid-logarithmic phase of growth were suspended in 0.9% NaCl to obtain 0.5 MacFarland's optical density and in the case of anaerobes 1.0 in the same scale. 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 medium containing the tested substances. For anaerobes, all media were pre-reduced. Samples were incubated at temperature 35–37°C for 24–48 h and in the case of anaerobes for 48–60 h. If after 48 h (60 h for anaerobes), growth was absent, the substance was noted as potentially possessing antimicrobial activity. In all experiments, strain vitality controls and a DMSO antimicrobial activity control were performed at the used concentrations.

Anticancer activity

The HeLa (human cervix carcinoma) and K562 (leukemia) cells were cultured in RPMI1640 medium supplemented with antibiotics and 10% fetal calf serum, in a 5% CO_2–95% air atmosphere. The cells (7×10^3) were seeded on each well on a 96-well plate (Nunc). After 48 h 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%.

The values of IC_{50} (the concentrations 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.

The cytotoxicity of all compounds was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, St. Louis, MO, USA] assay as previously described [19]. Briefly, after 24 h or 48 h of incubation with drugs, the cells were treated with the MTT reagent and incubation was continued for 2 h. The 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). Cells grown in the presence of vehicle (1% DMSO) only were used as a control (100% viability).

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