Turk J Chem
35 (2011) , 561 – 571.

doi:10.3906/kim-1012-914

Synthesis and antimicrobial activities of some bridged bis-benzimidazole derivatives

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Received: 28.12.2010

Some bis-benzimidazoles derivatives bearing allyl, crotyl, cinammyl, furfuryl, and thenyl groups were synthesized and their in vitro antimicrobial activity determined against gram-positive and gram-negative bacteria, and fungi by disk-diffusion method. The structures of all new compounds were identified by ^{1}H-NMR, ^{13}C-NMR, and FT-IR spectroscopic techniques and elemental analysis. All the compounds synthesized in this work were examined for their in vitro antimicrobial activities against gram-positive (*Staphylococcus aureus* and *Bacillus megaterium*) and gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*), and the yeasts like fungi (*Candida globrata* and *Candida tropicalis*). Compared to the reference substances, Cefozine and nystatin, most of the compounds showed high antibacterial and antifungal activities against studied strains with inhibition zones between 8 and 28 mm.

Key Words: Bis-benzimidazole derivatives, antibacterial activity, antifungal activity

Introduction

Despite significant progress in antimicrobial therapy, infectious diseases caused by bacteria and fungi remain a major worldwide health problem due to the rapid development of resistance to the existing antimicrobial drugs (antibacterial and antifungal). In other words, the increasing use and misuse of existing antimicrobial drugs has resulted in the development of resistant pathogens.[1] In recent years, considerable attention has been given to

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the synthesis of benzimidazole/bis-benzimidazole derivatives because of their various pharmacological activities such as antitumour,[2–8] anti-ulcer,[9] anti-inflammatory,[10] antiviral,[11] anthelmintic,[12,13] antibacterial,[14–20] and antifungal[21–25] properties. Although there are different antibacterial and antifungal drugs used in the treatment of bacterial and fungal infections, some of them have undesirable side effects.[26] Therefore, many clinically effective antibacterial and antifungal drugs have become less effective due to the development of resistance to these drugs. Since benzimidazole compounds have been found to have a broad range of pharmacological activity, many research groups as well as our group have been interested in this type of heterocyclic compound.[2–25]

In the light of the general importance of these compounds, the study of bis-benzimidazole derivatives has remained an active area of research despite extensive investigation. Therefore, we synthesized a number of novel bis-benzimidazole-3-ium derivatives bearing allyl, crotyl, cinnamyl, cyclohexyl, furfuryl, and thenyl moieties in order to investigate their antibacterial and antifungal activities.

Materials and methods

Chemistry

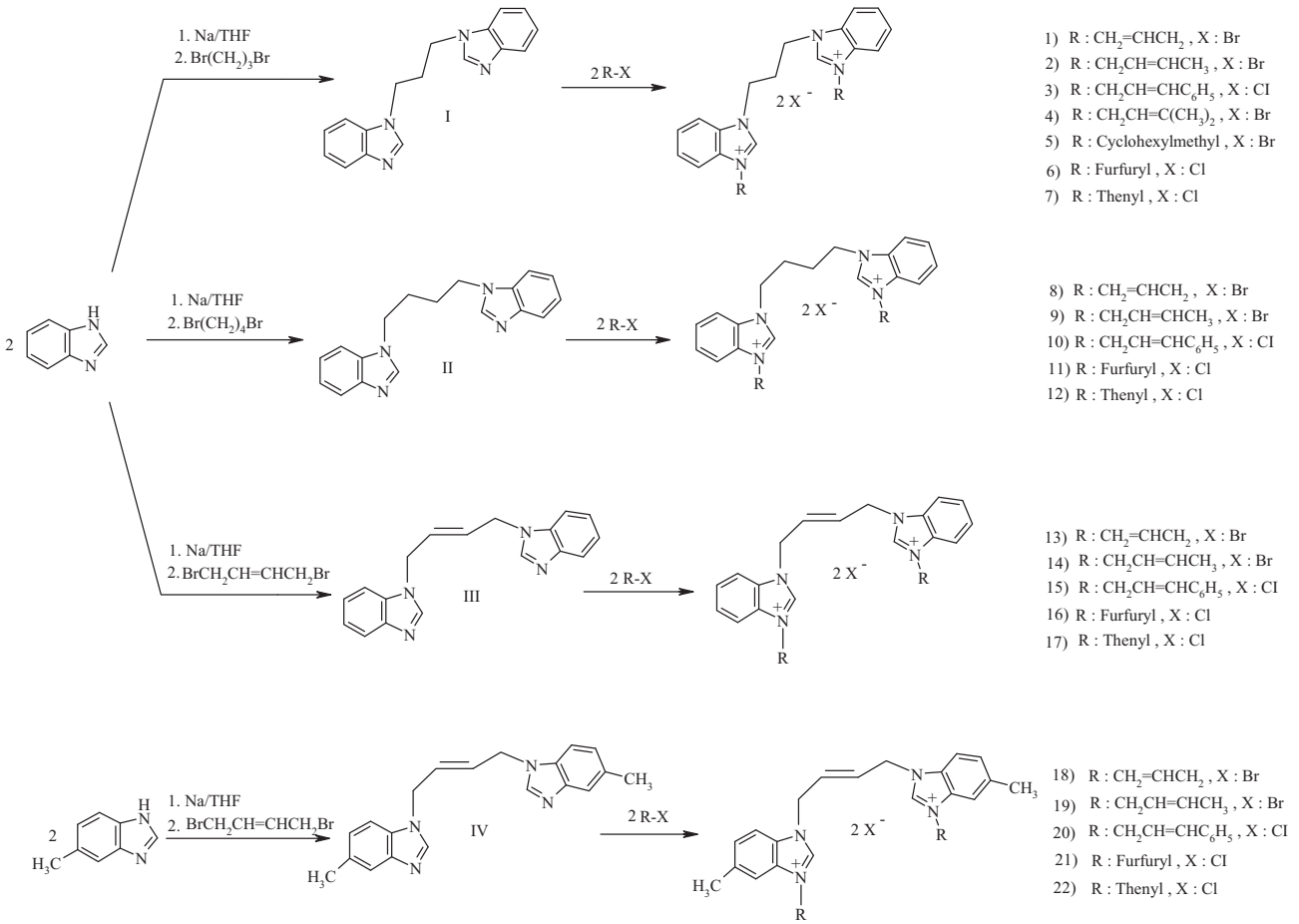
All preparations were carried out in an atmosphere of purified argon using standard Schlenk techniques. Starting materials and reagents used in the reactions were supplied commercially from Aldrich or Merck Chemical Co. Solvents were dried by standard methods and freshly distilled prior to use. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded using a Bruker DPX-300 high performance digital FT NMR spectrometer. Infrared spectra were recorded as KBr pellets in the range 4000-400 cm^{-1} on a Perkin-Elmer Spectrum one FT-IR spectrophotometer. Elemental analyses were performed using a LECO CHNS-932 elemental analyzer. MS analyses were performed on an Agilent Technologies 1100 series LC/MSD SL mass spectrometer. Melting points were recorded using an electrothermal-9200 melting point apparatus, and are uncorrected.

Bis-benzimidazoles **I,**[21] **II,**[27] and **IV**[22] used in this work as starting compounds were prepared according to the literature procedures. 1,1'-(*E*)-But-2-en-1,4-diylbis(1*H*-benzimidazole) **III** used as a starting compound was synthesized from benzimidazole and *trans*-1,4-dibromo-2-butene similar to the literature procedure[22] and full information concerning compound **III** is also provided in this report. The bis-benzimidazolium salts **2**,[28] **4**,[29] **5**,[30] **14**,[22] **6**, **7**, **11**, **12**, **16**, and **17**[31] were prepared according to our recently reported procedures. The synthesis of the bis-benzimidazoles (**I-IV**) and their salts (**1-22**) is summarized in the Scheme.

1,1'-(*E*)-But-2-en-1,4-diylbis(1*H*-benzimidazole) III

To a solution of benzimidazole (5.00 g, 42.37 mmol) in tetrahydrofuran (THF, 25 mL) was added metallic sodium (1.5 g, 65.22 mmol) and the mixture was stirred for 5 h at room temperature. Unreacted sodium was removed and *trans*-1,4-dibromo-2-butene (4.53 g, 21.18 mmol) was added to the solution, followed by stirring for 2 h at room temperature. All volatiles were removed in vacuo and water was added to precipitate the product. The crude product was filtered and crystallized from a toluene/*n*-hexane mixture (2:1) upon cooling to –20 °C. Yield. 8.38 g, 88%, m.p. 171-172 °C; $\upsilon_{(N=C)}$ = 1563 cm^{-1}, $\upsilon_{(C=C)}$ = 1644 cm^{-1}. Anal. found: C 74.59, H 5.59, N 19.18%. Calculated for $C_{18}H_{16}N_4$: C 74.98, H 5.59, N 19.43%. ^{1}H-NMR (δ, DMSO-d_6): 8.19 (s, 2H, NCHN), 7.19-7.71 (m, 8H, arH), 5.94-5.92 (m, 2H, $NCH_2C\underline{H}$ =), 4.95-4.93 (m, 4H, $NC\underline{H}_2CH$ =).

^{13}C-NMR (δ, DMSO-d_6): 145.5 (NCHN), 135.6, 130.4, 128.3, 124.0, 123.9 and 121.3, 112.3 ($NCH_2\underline{C}H$ =), 47.1 ($N\underline{C}H_2CH$ =).



Scheme. Synthesis pathways of the bis-benzimidazole derivatives.

General method for the synthesis of compounds 1, 3, 8-10, 13-15, and 18-22

Equivalent amount of the corresponding bis-benzimidazole (**I, II, III, IV**) and the appropriate alkyl halide was refluxed in dimethylformamide (5 mL) for 4 h. Then the mixture was cooled to room temperature and the volatiles were removed under reduced pressure. The residue was crystallized from dimethylformamide/ethanol (1:1) under argon atmosphere to avoid bonding crystal water, as in some of our previous works.[28–30]

1,1'-Propane-1,3-diylbis(3-ally-1*H*-benzimidazole-3-ium) dibromide (1). Yield 2.03 g, 78%, m.p. 225-227 °C; $\upsilon_{(C=N)}$ = 1557 cm^{-1}, $\upsilon_{(C=C)}$ = 1642 cm^{-1}. Anal. found: C 53.11, H 5.02, N 10.76%. Calculated for $C_{23}H_{26}N_4Br_2$: C 53.30, H 5.06, N 10.81%. ^{1}H-NMR (δ, DMSO-d_6): 10.09 (s, 2H, NCHN), 8.26-7.68 (m, 8H, arH), 6.15-6.13 (m, 2H, CH allyl), 5.51-5.39 (m, 4H, CH_2 allyl), 5.23 (d, 4H, CH_2 allyl, J = 5.7 Hz), 4.83-4.78 (m, 4H, $N\underline{C}H_2CH_2$-), 2.78-2.64 (m, 2H, $NCH_2C\underline{H}_2$-). ^{13}C-NMR (δ, DMSO-d_6): 143.0 (NCHN), 131.7, 131.5, 131.4, 127.1 and 127.0 (C_6H_4), 121.1 (CH allyl), 114.4 (CH_2 allyl), 49.4 (CH_2 allyl),

44.5 (N*C*H$_2$CH$_2$-) and 28.7 (CH$_2$CH$_2$-).

1,1'-Propane-1,3-diylbis(3-cinnamyl-1*H*-benzimidazole-3-ium) dibromide (3). Yield 2.21 g, 76%, m.p. 146-147 °C; $\upsilon_{(N=C)}$ = 1556 cm^{-1}, $\upsilon_{(C=C)}$ = 1640 cm^{-1}. Anal. found: C 72.09, H 5.80, N 9.57%. Calculated for $C_{35}H_{34}N_4Cl_2$: C 72.28, H 5.89, N 9.63%. m/z: 510 (15 [M-70]); 509 (100 M-71]); 393 (16 [M-188]. ^{1}H-NMR (δ, DMSO-d_6): 10.49 (s, 2H, NCHN), 8.31-7.47 (m, 8H, arH), 7.37-7.28 (m, 10H, arH, cinnamyl), 6.97 (d, 2H, CH cinnamyl, J = 15.9 Hz), 6.66-6.61(m, 2H, CH cinnamyl), 5.39 (d, 4H, CH$_2$ cinnamyl, J = 6.0 Hz), 4.85 (t, 4H, NC<u>H</u>$_2$CH$_2$-, J = 6.6 Hz), 2.71 (m, 2H, NCH$_2$C<u>H</u>$_2$-). ^{13}C-NMR (δ, DMSO-d_6): 142.8 (NCHN), 135.5, 135.0, 131.3, 131.0, 128.7 and 128.3 (C_6H_4), 127.2, 126.7, 126.5 and 126.4 (C_6H_5 cinnamyl), 122.04 and 113.9 (CHCH cinnamyl), 48.6 (CH$_2$ cinnamyl), 43.9 (N<u>C</u>H$_2$CH$_2$-), 33.8 (NCH$_2$<u>C</u>H$_2$-).

1,1'-Butane-1,4-diylbis(3-ally-1*H*-benzimidazole-3-ium) dibromide (8). Yield 2.19 g, 82%, m.p. 226-227 °C; $\upsilon_{(C=N)}$ = 1555 cm^{-1}. Anal. found: C 54.09, H 5.19, N 10.52%. Calculated for $C_{24}H_{28}N_4Br_2$: C 54.15, H 5.30, N 10.53%. ^{1}H-NMR (δ, DMSO-d_6): 10.04 (s, 2H, NCHN), 8.20-7.67 (m, 8H, arH), 6.15-6.09 (m, 2H, CH allyl), 5.47-5.38 (m, 4H, CH$_2$ allyl), 5.22 (d, 4H, CH$_2$ allyl, J = 6.0 Hz), 4.81 (t, 4H, NC<u>H</u>$_2$CH$_2$-, J = 6.9 Hz), 2.67 (quin, 4H, NCH$_2$C*H*$_2$-, J = 6.9 Hz). ^{13}C-NMR (δ, DMSO-d_6): 142.9 (NCHN), 131.6, 131.5, 131.4, 127.1 and 127.0 (C_6H_4), 121.0 (CH allyl), 114.4 (CH$_2$ allyl), 49.3 (NCH$_2$ allyl), 46.6 (N<u>C</u>H$_2$CH$_2$-) and 25.9 (CH$_2$ <u>C</u>H$_2$-).

1,1'-Butane-1,4-diylbis[3-(2-buten-1-yl)-1*H*-benzimidazole-3-ium] dibromide (9). Yield 2.28 g, 81%, m.p. 145-146 °C; $\upsilon_{(C=N)}$ = 1559 cm^{-1}, $\upsilon_{(C=C)}$ = 1642 cm^{-1}. Anal. found: C 55.56, H 5.69, N 9.96%. Calculated for $C_{26}H_{32}N_4Br_2$: C 55.73, H 5.76, N 10.00%. ^{1}H-NMR (δ, DMSO-d_6): 9.99 (s, 2H, NCHN), 8.18-7.66 (m, 8H, arH), 6.06-6.01 (m, 4H, C<u>H</u>CHCH$_3$ crotyl), 5.79-5.74 (m, 4H, CHC<u>H</u>CH$_3$ crotyl), 5.12 (d, 4H, CH$_2$ crotyl, J = 6.3 Hz), 4.63-4.61 (m, 4H, NC<u>H</u>$_2$CH$_2$-), 2.05-2.03 (m, NCH$_2$C<u>H</u>$_2$-), 1.70 (d, 6H, CH$_3$ crotyl, J = 6.6 Hz). ^{13}C-NMR (δ, DMSO-d_6): 142.6 (NCHN), 133.2, 131.9, 131.4, 131.3, 127.0 and 123.9 (C_6H_4), 122.8 (<u>C</u>HCHCH$_3$ crotyl), 114.3 (CH<u>C</u>HCH$_3$ crotyl), 48.9 (CH$_2$ crotyl), 46.6 (N<u>C</u>H$_2$CH$_2$-), 25.9 (NCH$_2$<u>C</u>H$_2$-) and 18.0 (CH$_3$ crotyl).

1,1'-Butane-1,4-diylbis(3-cinnamyl-1*H*-benzimidazole-3-ium) dichloride (10). Yield 2.63 g, 88%, m.p. 166-168 °C; $\upsilon_{(C=N)}$ = 1560 cm^{-1}, $\upsilon_{(C=C)}$ = 1641 cm^{-1}. Anal. found: C 72.39, H 6.02, N 9.34%. Calculated for $C_{36}H_{36}N_4Cl_2$: C 72.60, H 6.09, N 9.41%. m/z: 560 (100 [M-35.5]). ^{1}H-NMR (δ, DMSO-d_6): 10.42 (s, 2H, NCHN), 8.22-7.66 (m, 8H, arH), 7.49-7.29 (m, 10H, arH, cinnamyl), 6.95 (d, 2H, CH cinnamyl, J = 15.9 Hz), 6.65-6.60 (m, 2H, CH cinnamyl), 5.39 (d, 4H, CH$_2$ cinnamyl, J = 6.3 Hz), 4.69-4.67 (m, 4H, NC<u>H</u>$_2$CH$_2$-), 2.11-2.09 (m, 4H, CH$_2$C<u>H</u>$_2$-). ^{13}C-NMR (δ, DMSO-d_6): 142.6 (NCHN), 135.5, 135.0, 131.2, 131.0, 128.7 and 128.3 (C_6H_4), 126.7, 126.5, 126.4 and 122.1 (C_6H_5 cinnamyl), 113.9 and 113.8 (CHCH cinnamyl), 48.5 (CH$_2$ cinnamyl), 46.0 (N<u>C</u>H$_2$CH$_2$-) and 25.3 (NCH$_2$<u>C</u>H$_2$-).

1,1'-(*E*)-But-2-en-1,4-diylbis(3-(prop-2-en-1-yl)-1*H*-benzimidazole-3-ium) dibromide (13). Yield 2.29 g, 86%. m.p. 233-235 °C; $\upsilon_{(C=N)}$ = 1553 cm^{-1}, $\upsilon_{(C=C)}$ = 1638 cm^{-1}. Anal. found: C 54.31, H 4.92, N 10.55%. Calculated for $C_{24}H_{26}N_4Br_2$: C 54.36, H 4.94, N 10.57%. m/z: 370 (20 [M-160]); 369 (65 [M-161]). ^{1}H-NMR (δ, DMSO-d_6): 10.00 (s, 2H, NCHN), 8.04-7.61 (m, 8H, arH), 6.27-6.26 (m, 2H, NCH$_2$C<u>H</u> =), 6.14-6.08 (m, 2H, CH, allyl), 5.47-5.38 (m, 4H, CH$_2$, allyl), 5.28 (d, 4H, NC<u>H</u>$_2$CH = , J = 6.3 Hz), 5.24 (d, 4H, CH$_2$ allyl, J = 6.0 Hz). ^{13}C-NMR (δ, DMSO-d_6): 141.9 (NCHN), 131.3, 131.2, 130.3, 128.8, 127.4

and 127.3 (C_6H_4), 121.6 ($NCH_2\underline{C}H$ =), 114.1 (CH allyl), 114.0 (CH_2 allyl), 49.4 ($N\underline{C}H_2CH$ =) and 48.1 (CH_2 allyl).

1,1'-(*E*)-But-2-en-1,4-diylbis[3-crotyl-1*H*-benzimidazole-3-ium] dibromide (14). Yield 2.24 g, 80%, m.p. 150-152 °C; $\upsilon_{(C=N)}$ = 1556 cm^{-1}, $\upsilon_{(C=C)}$ = 1638 cm^{-1}. Anal. found: C 55.88, H 5.39, N 10.01%. Calculated for $C_{26}H_{30}N_4Br_2$: C 55.93, H 5.42, N 10.03%. ^{1}H-NMR (δ, DMSO-d_6): 10.02 (s, 2H, NCHN), 8.06-7.60 (m, 8H, arH), 6.29-6.22 (m, 2H, $NCH_2C\underline{H}$ =), 6.09-6.02 (m, 2H,NCH_2 $C\underline{H}$ = , crotyl), 5.79-5.74 (m, 2H, = $C\underline{H}CH_3$, crotyl), 5.29 (d, 4H, $NC\underline{H}_2CH$ = , J = 6.3 Hz), 5.14 (d, 4H, CH_2 allyl, J = 6.3 Hz), 1.71 (d, 6H, CH_3 crotyl, J = 6.3 Hz). ^{13}C-NMR (δ, DMSO-$\underline{d}_6$): 142.2 (NCHN), 132.9, 131.6, 131.0, 128.6, 126.5 and 123.4 (C_6H_4), 122.2 ($NCH_2\underline{C}H$ =), 113.9 and 113.8 (CHCH crotyl), 48.4 ($N\underline{C}H_2CH$ =), 47.6 (CH_2 crotyl) and 17.5 (CH_3 crotyl).

1,1'-(*E*)-But-2-en-1,4-diylbis[3-cinnamyl-1*H*-benzimidazole-3-ium] dichloride (15). Yield 2.47 g, 83%, m.p. 180-181 °C; $\upsilon_{(C=N)}$ = 1561 cm^{-1}, $\upsilon_{(C=C)}$ = 1643 cm^{-1}. Anal. found: C 72.74, H 5.69, N 9.40%. Calculated for $C_{36}H_{34}N_4Cl_2$: C 72.84, H 5.77, N 9.44%. m/z: 558 (65 [M-35.5]); 557 (100 [M-36.5]). ^{1}H-NMR (δ, DMSO-d_6): 10.39 (s, 2H, NCHN), 8.12-7.63 (m, 8H, arH), 7.49-7.29 (m, 10H, arH), 6.95 (d, 2H, CH cinnamyl, J = 15.9 Hz), 6.64-6.58 (m, 2H, CH cinnamyl), 6.33-6.6.28 (m, 2H, $NCH_2C\underline{H}$ =), 5.41 (d, 4H, CH_2 cinnamyl, J = 6.3 Hz), 5.32-5.30 (m, 4H, NCH_2). ^{13}C-NMR (δ, DMSO-d_6): 142.8 (NCHN), 135.5, 135.1, 131.1, 131.0 and 128.7 (C_6H_4), 128.4, 126.6, 126.5 and 126.4 (C_6H_5 cinnamyl), 122.0 and 114.0 (CHCH cinnamyl), 113.9 ($NCH_2\underline{C}H$ =), 48.5 (CH_2 cinnamyl) and 47.6 ($N\underline{C}H_2CH$ =).

1,1'-(2E)-but-2-en-1,4-diylbis[3-(prop-2-ene-1-yl)-1H-5-methylbenzimidazole-3-ium] dichloride (18). Yield 2.13 g, 76%. m.p. 162-163 °C; $\upsilon_{(C=N)}$ = 1558 cm^{-1}, $\upsilon_{(C=C)}$ = 1639 cm^{-1}. Anal. found: C 55.87, H 5.38, N 9.88%. Calculated for $C_{26}H_{30}N_4Br_2$: C 55.93, H 5.42, N 10.03%. ^{1}H-NMR (δ, DMSO-d_6): 9.97 (s, 2H, NCHN), 7.91-7.40 (m, 6H, arH), 6.28-6.24 (m, 2H, $NCH_2C\underline{H}$ =), 6.17-6.11 (m, 2H, CH allyl), 5.45-5.37 (m, 4H, CH_2 allyl), 5.27-5.25 (m, 4H, $NC\underline{H}_2CH$ =), 5.21 (d, 4H, CH_2 allyl, J = 6.0 Hz), 2.41 (s, 6H, CH_3 arH). ^{13}C-NMR (δ, DMSO-d_6): 142.0 (NCHN), 136.8, 131.3, 131.2, 128.4, 127.4 and 127.9 (C_6H_3), 120.4 ($NCH_2\underline{C}H$ =), 113.6 (CH allyl), 113.2 (CH_2 allyl), 48.8 ($N\underline{C}H_2CH$ =), 47.7 (CH_2 allyl) and 21.1 (CH_3 arH).

1,1'-(2E)-but-2-en-1,4-diylbis[3-crotyl-1H-5-methylbenzimidazole-3-ium] dibromide (19). Yield 2.09 g, 71%, m.p. 163-166 °C; $\upsilon_{(CN)}$ = 1559 cm^{-1}, $\upsilon_{(C=C)}$ = 1645 cm^{-1}. Anal. found: C 57.28, H 5.82, N 9.48%. Calculated for $C_{28}H_{34}N_4Br_2$: C 57.35, H 5.84, N 9.55%. m/z: 586 (98 [M-35.5]); 371 (20 [M-215]); 213 (75 [M-373]). ^{1}H-NMR (δ, DMSO-d_6): 9.87 (s, 2H, NCHN), 7.92-7.43 (m, 6H, arH), 6.24-6.20 (m, 2H, $NCH_2C\underline{H}$ =), 6.06-5.99 (m, 4H, $C\underline{H}$ = $CHCH_3$ crotyl), 5.77-5.75 (m, 4H, $NC\underline{H}_2CH$ =), 5.08 (d, 4H, CH_2 crotyl, J = 6.0 Hz), 2.41 (s, 6H, CH_3 arH), 1.71 (d, 6H, CH_3 crotyl, J = 6.6 Hz). ^{13}C-NMR (δ, DMSO-d_6): 141.7 (NCHN), 136.8, 132.7, 131.5, 128.6, 127.9 and 123.4 (C_6H_4), 122.3 ($NCH_2\underline{C}H$ =), 113.5 and 113.2 (CHCH crotyl), 48.3 ($N\underline{C}H_2CH$ =), 47.6 (CH_2 crotyl), 21.1 (CH_3 arH) and 17.5 (CH_3 crotyl).

1,1'-(2*E*)-But-2-en-1,4-diylbis[3-cinnamyl-1*H*-5-methylbenzimidazole-3-ium] dichloride (20). Yield 2.68 g, 86%, m.p. 142-143 °C; $\upsilon_{(C=N)}$ = 1557 cm^{-1}, $\upsilon_{(C=C)}$ = 1638 cm^{-1}. Anal. found: C 73.32, H 6.13, N 8.93%. Calculated for $C_{38}H_{38}Cl_2$ N_4: C 73.42, H 6.16, N 9.01%. m/z: 587 (62 [M-35.5]); 433 (19 [M-188]); 275 (54 [M-346]); 117 (10 [M-504]).^{1}H-NMR (δ, DMSO-d_6): 10.35 (s, 2H, NCHN), 7.99-7.46 (m, 6H, C_6H_3), 7.42-7.28 (m, 10H, C_6H_5), 6.93 (d, 2H, CH cinnamyl, J = 15.9 Hz), 6.64-6.57 (m, 2H, CH cinnamyl),

6.30-6.26 (m, 2H, $NCH_2C\underline{H}=$), 5.38 (d, 4H, CH_2 cinnamyl, $J = 6.3$ Hz), 5.29-5.27 (m, 4H, $NC\underline{H}_2CH=$), 2.41 (s, 6H, CH_3). ^{13}C-NMR (δ, DMSO-d_6): 142.8 (NCHN), 137.2, 136.0, 135.5, 135.4, 131.8 and 129.7 (C_6H_3), 129.1, 128.8, 128.4 and 127.1 (C_6H_5 cinnamyl), 122.5 and 114.0 (CH = CH cinnamyl), 113.8 ($NCH_2\underline{C}H=$), 48.9 (CH_2 cinnamyl), 48.0 ($N\underline{C}H_2CH=$) and 21.6 (CH_3).

1,1'-(2*E*)-But-2-en-1,4-diylbis[3-furfuryl-1*H*-5-methylbenzimidazole-3-ium] dichloride (21). Yield 2.04 g, 74%, m.p. 97-98 °C; $\upsilon_{(CN)} = 1553$ cm^{-1}, $\upsilon_{(C=C)} = 1643$ cm^{-1}. Anal. found: C 65.37, H 5.42, N 10.08%. Calculated for $C_{30}H_{30}Cl_2N_4O_2$: C 65.57, H 5.50, N 10.20%. ^{1}H-NMR (δ, DMSO-d_6): 10.24 (s, 2H, NCHN), 8.17-6.64 (m, 6H, $2C_6H_3$ and m, 4H, C_4H_3O), 6.60-6.57 (m, 2H, C_4H_3O), 6.32-6.31 (m, 2H, $NCH_2C\underline{H}=$), 6.09 (s, 4H, $\underline{CH}_2C_4H_3O$), 5.31-5.29 (m , 4H, $NC\underline{H}_2CH=$), 2.34 (s, 6H, $2CH_3$). ^{13}C-NMR (δ, DMSO-d_6): 142.6 (NCHN), 137.6, 136.3, 134.7, 131.6, 131.5 and 129.9 (C_6H_3), 129.5, 128.7, 128.4 and 127.9 (C_4H_3O), 113.8 ($NCH_2\underline{C}H=$), 45.1 (CH_2-furan), 44.6 ($N\underline{C}H_2CH=$), 21.5 (CH_3).

1,1'-(2*E*)-But-2-en-1,4-diylbis[3-thenyl-1*H*-5-methylbenzimidazole-3-ium] dichloride (22). Yield 2.10 g, 72%, m.p. 89-91 °C; $\upsilon_{(CN)} = 1558$ cm^{-1}, $\upsilon_{(C=C)} = 1643$ cm^{-1}. Anal. found: C 61.83, H 5.17, N 9.58, S 10.76%. Calculated for $C_{30}H_{30}Cl_2N_4S_2$: C 61.95, H 5.20, N 9.63, S 11.03%. ^{1}H-NMR (δ, DMSO-d_6): 10.47 (s, 2H, NCHN), 8.02-7.42 (m, 6H, C_6H_3 and m, 4H, C_4H_3S), 7.05 (m, 2H, C_4H_3S), 6.31-6.19 (m, 2H, $NCH_2C\underline{H}=$), 6.11 (s, 4H, $\underline{CH}_2$-C_4H_3S), 5.32-5.20 (m, 4H, $NC\underline{H}_2CH=$), 2.37 (s, 6H, CH_3). ^{13}C-NMR (δ, DMSO-d_6): 142.5 (NCHN), 137.5, 136.2, 131.8, 131.4, 129.9 and 129.7 (C_6H_3), 129.2, 128.6, 128.4 and 127.8 (C_4H_3S), 113.9 ($NCH_2\underline{C}H=$), 48.1 ($CH_2C_4H_3S$), 44.9 ($N\underline{C}H_2CH=$), 21.6 (CH_3).

In vitro antimicrobial assay

Microorganisms were obtained from the culture collection of the Microbiology Laboratory of the Biology Department, Faculty of Arts and Sciences, Fırat University, Turkey. In this work *Staphylococcus aureus* COWAN I, *Bacillus megaterium* DSM, *Klebsiella pneumoniae* FMCS, and *Escherichia coli* ATCC 25922 bacteria, and *Candida globrata* ATCC 66032 and *Candida tropicalis* ATCC 13803 yeasts were used to investigate the antimicrobial activity of compounds. In order to prepare the discs, compounds were dissolved in DMSO and absorbed onto discs (500 μg).

The bacterial strains were inoculated into nutrient broth and the yeast strains inoculated into malt extract broth for 24 and 48 h, respectively. In the disc-diffusion method, sterile Mueller-Hinton agar for bacteria and malt extract agar for yeast were separately inoculated with the test bacteria and yeasts (10^5 bacteria per mL, 10^4 yeast per mL). Discs were applied to the sold agar medium by pressing slightly. All the inoculated plates were incubated at 35 ± 0.1 °C and the results were evaluated after 24 h of incubation for bacteria and they were incubated at 25 ± 0.1 °C and the results were evaluated after 72 h of incubation for yeasts as inhibition zones in millimeters.[32]

Results and discussion

The general synthetic route chosen for preparation of bis-benzimidazolium salts included 2 steps. In the first step, the imino proton of the benzimidazole was removed by metallic sodium, then the nucleophilic benzimidazole species obtained were reacted with appropriate propylene, butylenes, or but-2-enylene dihalides by nucleophilic

substitution reaction. In the second step, the reactions involved quaternization of bis-benzimidazoles with 2 equivalent alkyl halides. The benzimidazolium salts are air- and moisture-stable both in the solid state and in solution. The structures of the new bis-benzimidazole, **III**, and bis-benzimidazole salts, **1**, **3**, **8**, **13-15**, and **18-22**, were identified by spectroscopic methods and elemental analysis. Mass spectral analysis was also performed for the compounds **3**, **10**, **13**, **15**, **19**, and **20**. From evaluations of mass spectra and elemental analysis it is clear that the compounds were crystallized without crystal water.

The chemical shift value of NCHN carbon in benzimidazole-3-ium salts in ^{13}C-NMR is usually around 144 ± 2.[31] These values for the benzimidazolium salts, **1**, **3**, **8**, **13-15**, and **18-22** ranged between 141.7 and 143.0 ppm. These values are in good agreement with the previously reported results.[31,33–36] In the ^{1}H-NMR spectrum of benzimidazolium salts for NC*H*N protons ranged between 9.87 and 10.49 ppm as singlets. These chemical shift values are also typical for NC*H*N protons of benzimidazolium salts for increasing the acidity of the NC*H*N proton.[31,33–36] The chemical shift value for compound **III** is 8.19 ppm and this value smaller than those for corresponding benzimidazolium salts as expected. The cinnamyl substituent on the **3**, **10**, **15**, and **20** compounds display a *trans* arrangement, which is also reflected in the proton NMR spectra, where the coupling constants of the protons connected to the C = C double bond are found to be both 15.9 Hz, being a typical value for protons in a *trans* arrangement.[37,38] The benzimidazolium salts, **1**, **3**, **8**, **13-15**, and **18-22**, showed IR absorption about at 1557 ± 4 cm^{-1}, which was assigned to ν(C = N). These IR absorption values are also in good agreement with the previously reported values for the benzimidazolium salts.[24] These values are slightly smaller than normal ν(C = N) values because of π-electron delocalization on the imidazolium ring. For example, the ν(C = N) value of compound **3** was found to be 1563 cm^{-1}. The benzimidazole compounds, **III, 1**, **3**, **8**, **13-15,** and **18-22**, showed IR absorption about between 1638 and 1644 cm^{-1}, which was assigned to ν(C = C).

The antimicrobial activities of all synthesized compounds (**1-22**) were studied and antimicrobial inhibition zones (mm) were determined using the disc-diffusion method. Cefozine was used as a control drug and tested under the same conditions with the microorganisms in the experiment. The antibacterial and antifungal inhibition zones (mm) of the tested compounds are given in Tables 1 and 2, respectively.

Tables 1 and 2 also contain the results of the Cefozine and nystatin reference compounds, respectively, for all microorganisms used in this work to compare and to check the reliability of the method used. As can be seen in Table 1, nearly all compounds (except compounds **1**, **7**, **12**, **14**, **20**, and **21**) showed some antibacterial activity against gram-positive *Staphylococcus aureus* with an inhibition zone between 8 and 23 mm. The most potent activity was observed in compounds **6**, **17**, and **23** against gram-positive *Staphylococcus aureus* when compared to the respective standard drug Cefozine. Similarly, nearly all compounds (except compounds **6**, **12**, **17**, **21**, and **22**) also showed high antibacterial activity against gram-positive *Bacillus megaterium.* Comparison of antibacterial activities of the compounds showed that bis-benzimidazolium salts studied in this work are more active than the reference compound Cefozine. Compounds **8**, **10**, and **15** showed high activity against *Bacillus megaterium*, whereas compound **9** showed significant and the most profitable activity (33 mm). Compounds **1**, **3**, **5**, **8**, **9**, **10**, **13**, **15**, **16**, **18**, **19**, and **22** showed some activity against gram-negative *Klebsiella pneumoniae* with inhibition zones between 8 and 23 mm. In particular, compounds **1**, **8**, **10**, and **18** exhibited more activity against *Klebsiella pneumonia* among the tested compounds with inhibition zones of 20, 23, 21, and 20 mm, respectively.

Table 1. The antibacterial inhibition zone (mm) of the tested compounds.

Compound no.	Tested microorganisms[a)]			
	A	B	C	D
Cefozine	23	–	25	23
1	–	20	20	15
2	10	15	–	–
3	8	10	8	–
4	8	12	–	–
5	11	8	10	15
6	20	–	–	–
7	–	8	–	8
8	13	23	23	21
9	15	33	17	30
10	17	30	21	30
11	8	11	–	–
12	–	–	–	–
13	8	8	8	–
14	–	8	–	–
15	13	21	15	17
16	19	8	13	11
17	23	–	–	13
18	15	17	20	20
19	8	15	8	8
20	–	8	–	–
21	–	–	–	–
22	23	–	8	12

A = *Staphylococcus aureus*, B = *Bacillus megaterium*, C = *Klebsiella pneumoniae*, D = *Escherichia coli,* (-) the compounds have no activity against the microorganisms.

Compounds **1**, **5**, **7**, **8**, **15**, **16**, **17**, **18**, **19**, and **22** showed also some activity against gram-negative *Escherichia coli* with inhibition zones between 8 and 21 mm. These inhibition zone values for compounds **9** and **10** are also better than that for Cefozine. As shown in Table 2, compounds **2**, **3**, **4**, **7**, **8**, **9**, **10**, **11**, **13**, **15**, **16**, **18**, **19**, and **22** showed antifungal activity against *Candida globrata* with inhibition zones between 8 and 28 mm. The most potent activity was observed in compounds **9**, **10**, and **15** against *Candida globrata.* In particular, compound **9** showed the same antifungal activity when compared with nystatin. Compounds **2**, **4**, **6**, **7**, **8**, **9**, **10**, **12**, **13**, **15**, **16**, **18**, **19**, and **21** also showed antifungal activity against *Candida tropicalis* with inhibition zones between 8 and 18 mm. These inhibition zone values are smaller than that for nystatin. Considering the structural formulae of the compounds that exhibited antibacterial and antifungal activity, butylenes, but-2-enylene bridge, and unsaturated alkyl groups such as ally, crotyl, and cinnamyl generally may play a role in the

Table 2. The antifungal inhibition zones (mm) of the tested compounds.

Compound no.	Tested microorganisms	
	C. globrata	*C. tropicalis*
Nystatin	28	30
1	–	–
2	13	8
3	8	–
4	8	9
5	–	–
6	–	9
7	10	8
8	14	18
9	28	10
10	20	16
11	8	–
12	–	8
13	16	11
14	–	–
15	24	14
16	9	10
17	–	–
18	8	9
19	11	9
20	–	–
21	–	12
22	8	–

(-) The compounds have no activity against the microorganisms.

activity. Combination of unsaturated alkyl groups with furfuryl and thenyl moieties on the benzimidazolium salts such as **6**, **16**, **17**, and **22** may make an extra contribution to the activities. The compounds tested here generally show more activity against gram-positive bacteria than against gram-negative bacteria. This finding is also in good agreement with the difference between the cell structures of gram-positive and gram-negative bacteria.

Conclusion

Twenty-two bis-benzimidazole-3-ium compounds were synthesized and their structures were identified by ^{1}H-NMR, ^{13}C-NMR, and FT-IR spectroscopic techniques and elemental analysis. All compounds studied in this work were screened for their in vitro antimicrobial activities against gram-positive (*Staphylococcus aureus* and

Bacillus megaterium) and gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*) and the yeasts like fungi (*Candida globrata* and *Candida tropicalis*). Compared to the reference substances, Cefozine and nystatin, most of the compounds showed high antibacterial and antifungal activities against the studied strains.

Acknowledgments

This work was financially supported by the İnönü University Research Fund (I.Ü. B.A.P. 2008/05 and 2008/59).

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