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Original article

# Synthesis and biological activity of some new benzoxazoles

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**Abstract**

The synthesis and antimicrobial activity of a new series of 5-ethylsulphonyl-2-(substituted-phenyl/substituted-benzyl and/or phenylethyl)benzoxazole derivatives (**3a**–**3t**) except **3a**, **3g**, **3h**, **3k** [R.S. Pottorf, N.K. Chadha, M. Katkevies, V. Ozola, E. Suna, H. Ghane, T. Regberg, M.R. Player, Tetrahedron Lett. 44 (1) (2003) 175] were described. The in vitro antimicrobial activity of the compounds was determined against some Gram-positive, Gram-negative bacteria, a fungi *Candida albicans* and their drug-resistant isolates in comparison with standard drugs. Antimicrobial results indicated that the synthesized compounds possessed a broad spectrum of activity with MIC values 250–7.81 μg/ml. While all compounds are less potent than fluconazole against *C. albicans*, most of them are more potent than fluconazole against *C. albicans* isolate.


*Keywords:* Benzoxazoles; Antifungal activity; Antibacterial activity

## 1. Introduction

The rising prevalence of multi-drug-resistant microbial strains such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant *Enterococcus* is a problem of ever-increasing significance [2–5]. Although antimicrobial resistance was recognized soon after the deployment of sulfonamides and penicillins [2–7], it now appears that the emergence of antibiotic-resistant bacteria is inevitable to almost every new drug. As a consequence, new efforts to develop new antibacterial agents are highly needed.

Benzoxazole ring is one of the most common heterocycles in medicinal chemistry. Previous reports revealed that substituted benzoxazoles possess diverse chemotherapeutic activities including antibiotic [8], antimicrobial [9–13], antiviral [14], topoisomerase I and II inhibitors [15] and antitumor activities [16,17].

In previous studies, we synthesized some compounds which bearing a hydrogen, chlorine, methyl, nitro, an amine and amide substitution at the 5th position on the benzoxazole ring and examined their in vitro antimicrobial activity against some Gram-positive, Gram-negative bacteria and *Candida albicans* [18–23].

On the basis of these considerations, we designed and synthesized the series of antimicrobial agents (**3a**–**3t**) (Scheme 1) reported in this work, choosing an ethylsulphonyl fragment at the 5th position of benzoxazole with together a substituted-phenyl/benzyl/2-phenylethyl at the 2nd position of it. The strategy employed was to examine the effect of the position of 2 against some Gram-positive, Gram-negative bacteria and their drug-resistant isolates with the fungus *C. albicans* and its isolate in comparison with control drugs.

## 2. Experimental procedures

### 2.1. Chemistry

The chemicals were purchased from the commercial venders and were used without purification. The reactions

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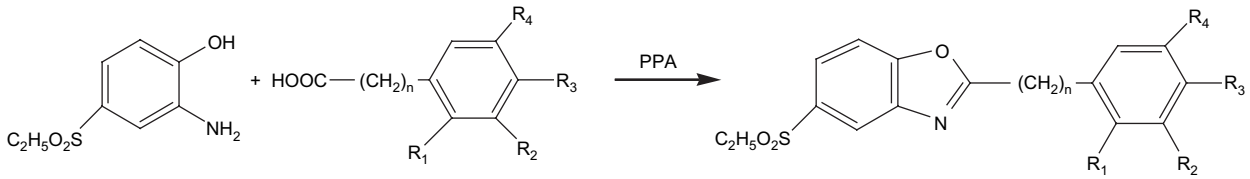



$R_1$ = H, F, Cl, Br, $CH_3$

$R_2$ = H, $CH_3$

$R_3$ = H, F, Cl, Br, $CH_3$, $C_2H_5$, $NO_2$, $C(CH_3)_3$

$R_4$ = H, Br

n = 0, 1, 2

Scheme 1. Synthesis of the target 5-ethylsulphonyl-2-substituted benzoxazoles (**3a–3t**).

were monitored and the purity of the products was checked by thin layer chromatography (TLC). Kieselgel HF 254 chromatoplates (0.3 mm) were used for TLC and the solvent systems were ethylacetate:*n*-hexane (5:5). All the melting points were taken on a Buchi SMP 20 capillary apparatus and are uncorrected. IR spectra were recorded on a Jasco FT/IR-420 spectrometer as KBr discs. $^1$H and $^{13}$C NMR spectra were obtained with a Varian 400 MHz spectrometer in *d*-chloroform ($CDCl_3$) or $d_6$-dimethylsulfoxide (DMSO-$d_6$) and tetramethylsilane (TMS) was used as an internal standard. Mass analysis was carried out with a Waters Micromass ZQ by using ESI (+) method.

### 2.2. General procedure for the synthesis of 5-ethylsulphonyl-2-(substituted-phenyl/substituted-benzyl and/or phenylethyl)benzoxazoles

5-Ethylsulphonyl-2-(substituted-phenyl/substituted-benzyl and/or phenylethyl)benzoxazole derivatives (**3a–3t**) were synthesized by heating 0.01 mol 4-ethylsulphonyl-2-aminophenol. HCl with 0.01 mol benzoic acid and *o*- and/or *p*- and/or *o*,*m*-di- and/or *o*,*p*-di-substituted benzoic acid and/or substituted-phenylacetic acid and/or 3-phenylpropionic acid in 24 g polyphosphoric acid was stirred for 2.5 h. At the end of the reaction period, the residue was poured into ice-water, mixtured and neutralized with excess of 10% NaOH solution extracted with benzene. The benzene solution was dried over anhydrous sodium sulphate and evaporated under diminished pressure. The residue was boiled with 200 mg charcoal in ethanol and filtered. After the evaporation of solvent in vacuo, the crude product was obtained and recrystallized from ethanol–water mixture and needles were dried in vacuo [9]. The chemical, physical and spectral data of the compounds are reported in Table 1.

### 2.3. Microbiology

#### 2.3.1. Materials

Mueller Hinton Agar (MHA) (Merck), Mueller Hinton Broth (MHB) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), RPMI-1640 medium with L-glutamine (Sigma), 3-[*N*-morpholino]-propansulfonic acid (MOPS) (Sigma), 96 well microplates (Falcon®), transfer pipette (Biohit), ampicillin trihydrate (Paninkret Chem. Pharm.), gentamicin sulphate (Deva İlaç Sanayii), ofloxacin (Zhejiang Huangyan East-Asia Chemical CO.), fluconazole (Nobel), amphotericin B (Bristol Myers Squibb), ethanol (Riedel de Haen®), dimethylsulphoxide (DMSO) (Riedel de Haen®), dimethylformamide (Riedel de Haen®).

## 3. Microorganisms

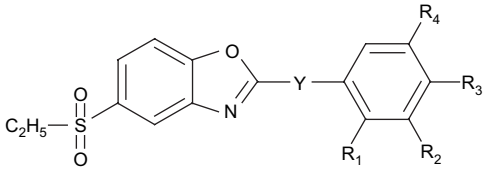
*Klebsiella pneumoniae* isolate (resistant to trimethoprim sulfamethoxazole, amoxicillin clavulonat, ceftriaxon, cephepim, aztreonam), *Escherichia coli* isolate (resistant to trimethoprim sulfamethoxazole, cephepim, tazobactam), *Bacillus subtilis* isolate (resistant to ceftriaxon), *S. aureus* isolate (resistant to oxacilin, gentamicin, aztreonam, trimethoprim sulfamethoxazole), *C. albicans* isolate (Biofilm positive), *K. pneumoniae* RSHM 574 (Refik Saydam Hıfzısıhha Merkezi Culture Collection), *Pseudomonas aeruginosa* ATCC 25853 (American Type Culture Collection), *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *C. albicans* ATCC 10231.

### 3.1. Methods

Standard strains of *K. pneumoniae* RSHM 574, *P. aeruginosa* ATCC 25853, *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *C. albicans* ATCC 10231 and clinical isolates of these microorganisms (except for *P. aeruginosa* ATCC 25853) that are known to be resistant to various antimicrobial agents were included in the study. Resistance was determined by Kirby Bauer Disk Diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [24] in the clinical isolates.

Standard powders of ampicillin trihydrate, gentamicin sulphate, ofloxacin, fluconazole and amphotericin B were obtained from the manufacturers. Stock solutions were dissolved in dimethylsulphoxide (ofloxacin), pH 8 phosphate

Table 1
Physical properties and spectral data of the compounds (**3a–3t**)




| Compound No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Y | Empirical formulas | MP (%C) | Yield (%) | IR($cm^{-1}$) | $^1$H NMR ($CDCl_3$ or $d_6$-DMSO) ($\delta$ ppm) $J$ = Hz | $^{13}$C NMR ($CDCl_3$ or $d_6$-DMSO) ($\delta$ ppm) | MS(ESI+) *m/z* (*X*%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **3a** | H | H | H | H | – | $C_{15}H_{13}SO_3N$ | 115 (Ref. [1][a]) | 42 | 3063, 2980, 1619, 1556, 1449, 1345–1312, 1238, 1172–1129, 1020, 815, 2929, 1613, 1561, 1455, 1349–1315, 1280, 1160–1123, 831 | 8.31–8.30 (1H, d, $J = 1.6$), 8.25–8.22 (2H, dd, $J = 1.2$, $J' = 8.0$), 8.08–8.06 (1H, d, $J = 8.4$), 7.96–7.93 (1H, dd, $J = 2.0$, $J' = 8.4$), 7.72–7.62 (3H, m), 3.36 (2H, q, $CH_2$ protons), 1.13 (3H, t, $CH_3$ protons) | 165.24, 153.84, 142.56, 136.10, 133.38, 130.13, 128.37, 126.35, 126.04, 120.69, 112.70, 50.16, 7.91 | 288(38%)(M + 1) |
| **3b** | H | H | F | H | – | $C_{15}H_{12}SO_3NF$ | 138 | 38 | 3098, 2924, 1618, 1557, 1454, 1347–1305, 1156–1133, 1045, 846 | 8.29–8.25 (3H, m), 8.06–8.04 (1H, d, $J = 8.4$), 7.95–7.93 (1H, d, $J = 8.4$), 7.50–7.46 (2H, dd, $J = 8.80$, $J' = 8.4$), 3.36 (2H, q, $CH_2$ protons), 1.10 (3H, t, $CH_3$ protons) | 166.56, 164.38, 164.06, 153.84, 142.51, 136.12, 131.11, 131.02, 126.02, 122.99, 122.97, 120.64, 117.47, 117.25, 112.66, 50.16, 7.88 | 306(21%)(M + 1) |
| **3c** | H | H | $C_2H_5$ | H | – | $C_{17}H_{17}SO_3N$ | 142 | 35 | 2969, 1620, 1553, 1455, 1306, 1259, 1132–1120, 1042, 808 | 8.29 (1H, s), 8.18–8.06 (2H, d, $J = 7.6$), 8.08–8.06 (1H, d, $J = 8.4$), 7.96–7.94 (1H, d, $J = 8.4$), 7.51–7.49 (2H, d, $J = 7.6$), 3.39 (2H, q, $CH_2$ protons), 2.73 (2H, q, $CH_2$ protons), 1.24 (3H, t, $CH_3$ protons), 1.13 (3H, t, $CH_3$ protons) | 165.41, 153.79, 149.80, 142.65, 136.02, 129.54, 128.47, 125.85, 123.84, 120.49, 112.58, 50.17, 28.89, 15.77, 7.91 | 316(16%)(M + 1) |
| **3d** | H | H | Cl | H | – | $C_{15}H_{13}SO_3NCl$ | 140 | 36 | 2924, 1617, 1452, 1308, 1273, 1130, 1047, 843 | 8.33 (1H, s), 8.27–8.25 (2H, d, $J = 8.4$), 8.11–8.09 (1H, d, $J = 8.4$), 7.98–7.96 (1H, d, $J = 8.0$), 7.75–7.73 (2H, d, $J = 8.0$), 3.36 (2H, q, $CH_2$ protons), 1.12 (3H, t, $CH_3$ protons) | 164.33, 153.86, 142.48, 138.20, 136.21, 130.31, 130.13, 126.22, 125.25, 120.79, 112.78, 50.14, 7.90 | 322.5(18%)(M+) |

(*continued on next page*)

Table 1 (*continued*)

| Compound No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Y | Empirical formulas | MP (%C) | Yield (%) | IR($cm^{-1}$) | $^1$H NMR ($CDCl_3$ or $d_6$-DMSO) ($\delta$ ppm) $J$ = Hz | $^{13}$C NMR ($CDCl_3$ or $d_6$-DMSO) ($\delta$ ppm) | MS(ESI+) *m/z* (*X*%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **3e** | H | H | $C(CH_3)_3$ | H | – | $C_{19}H_{21}SO_3N$ | 120 | 27 | 2965, 1617, 1551, 1456, 1363–1313, 1136–1120, 1057, 841 | 8.31–8.32 (1H, d, $J = 1.6$), 8.19–8.22 (2H, d, $J = 8.8$), 7.91–7.94 (1H, dd, $J = 1.6$, $J' = 8.6$), 7.74–7.76 (1H, d, $J = 8.0$), 7.54–7.60 (2H, d, $J = 8.8$), 3.16–3.22 (2H, q, $CH_2$ protons), 1.39 (9H, s, *tert*-butyl protons), 1.29–1.31 (3H, t, $CH_3$ protons) | 165.80, 156.51, 153.97, 143.08, 135.36, 128.11, 126.35, 125.30, 123.53, 121.02, 111.48, 51.25, 35.41, 31.32, 7.83 | 344(18%)(M + 1) |
| **3f** | H | H | $CH_3$ | H | – | $C_{16}H_{15}SO_3N$ | 160 | 37 | 2923, 1621, 1557, 1455, 1307, 1260, 1132–1046, 807 | 8.29 (1H, s), 8.16–8.14 (2H, d, $J = 8.0$), 8.06–7.96 (2H, m), 7.49–7.47 (2H, d, $J = 7.6$), 3.38 (2H, q, $CH_2$ protons), 2.44 (3H, s, aromatic $CH_3$ protons), 1.12 (3H, t, $CH_3$ protons) | 165.42, 153.77, 143.76, 142.65, 136.02, 130.70, 128.34, 125.85, 123.59, 120.49, 112.57, 50.20, 21.90, 7.92 | 301(20%)(M+) |
| **3g** | H | H | Br | H | – | $C_{15}H_{12}SO_3BrN$ | 152 (Ref. [1][a]) | 32 | 2923, 1616, 1590, 1456, 1307, 1259, 1133, 1043, 841 | 8.33 (1H, s), 8.18–8.16 (2H, d, $J = 8.4$), 8.08–7.99 (2H, m), 7.89–7.87 (2H, d, $J = 8.4$), 3.39 (2H, q, $CH_2$ protons), 1.12 (3H, t, $CH_3$ protons) | 164.45, 153.85, 142.48, 136.22, 133.25, 130.22, 127.19, 126.25, 125.59, 120.80, 112.79, 50.14, 7.90 | 366(16%)(M+) |
| **3h** | H | H | $NO_2$ | H | – | $C_{15}H_{12}SO_5N_2$ | 212 (Ref. [1][a]) | 11 | 2925, 1636, 1553, 1425, 1349–1310, 1140–1120, 1049, 865 | 8.50–8.41 (5H, m), 8.03–8.01(1H, dd, $J = 2.0$, $J' = 7.4$), 7.84–7.82 (1H, d, $J = 8.4$), 3.20 (2H, q, $CH_2$ protons), 1.33 (3H, t, $CH_3$ protons) | 163.39, 154.08, 150.26, 142.37, 136.51, 131.98, 129.74, 126.85, 125.26, 121.35, 113.13, 50.11, 7.89 | 333(12%)(M + 1) |
| **3ı** | F | H | H | H | – | $C_{15}H_{12}SO_3NF$ | 124 | 38 | 2925, 1621, 1556, 1455, 1349–1309, 1276, 1136, 1052, 833 | 8.41–8.40 (1H, d, $J = 1.6$), 8.29–8.24 (1H, dd, $J = 1.6$, $J' = 8.0$), 7.99–7.96 (1H, dd, $J = 2.0$, $J' = 7.9$), 7.81–7.79 (1H, d, $J = 8.8$), 7.63–7.57 (1H, m), 7.38–7.29 (2H, m), 3.19 (2H, q, $CH_2$ protons), 1.31 (3H, t, $CH_3$ protons) | 162.52, 162.14, 162.09, 159.94, 153.69, 142.50, 135.65, 134.42, 134.34, 131.00, 125.82, 124.97, 124.94, 121.59, 117.59, 117.38, 114.82, 114.71, 111.79, 51.23, 7.81 | 306(20%)(M + 1) |
| **3k** | Br | H | H | H | – | $C_{15}H_{12}SO_3NBr$ | 130 (Ref. [1][a]) | 35 | 3082, 2974, 1614, 1565, 1458, 1346–1301, 1262, 1137–1121, 1039, 816 | 8.44–8.43 (1H, d, $J = 1.6$), 8.14–8.11(1H, dd, $J = 1.6$, $J' = 7.8$), 8.0–7.98 (1H, dd, $J = 1.6$, $J' = 8.2$), 7.83–7.79 (2H, m), 7.54–7.41(2H, m), 3.20 (2H, q, $CH_2$ protons), 1.32 (3H, t, $CH_3$ protons) | 164.04, 153.73, 142.33, 135.63, 135.15, 133.02, 132.56, 127.88, 127.49, 125.94, 122.33, 121.80, 111.88, 51.23, 7.82 | 366(54%)(M+), 368(56%)(M + 2) |
| **3l** | Cl | H | H | H | – | $C_{15}H_{12}SO_3NCl$ | 108 | 34 | 2934, 1615, 1556, 1472, 1347–1307, 1258, 1140–1122, | 8.41(1H, s), 8.22–8.20 (1H, d, $J = 7.2$), 8.15–8.13 (1H, d, $J = 8.8$), 8.03–8.01 | 163.08, 153.56, 142.06, 136.21, 134.15, 133.12, 132.83, 132.05, 128.49, | 322(60%)(M+) |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 1024, 817 | (1H, d, $J = 8.8$), 7.78–7.61 (3H, m), 3.39 (2H, q, $CH_2$ protons), 1.14 (3H, t, $CH_3$ protons) | 126.47, 125.37, 121.18, 112.84, 50.19, 7.91 | |
| **3m** | $CH_3$ | $CH_3$ | H | H | – | $C_{17}H_{17}SO_3N$ | 125 | 21 | 2924, 1608, 1543, 1455, 1311, 1136, 1053, 781 | 8.34–8.33 (1H, d, $J = 2.0$), 8.07–8.05 (1H, d, $J = 8.8$), 7.96–7.94 (1H, dd, $J = 2.0$, $J' = 8.4$), 7.91–7.89 (1H, d, $J = 7.2$), 7.47–7.45 (1H, d, $J = 7.2$), 7.35–7.31(1H, dd, $J = 7.6$; $J' = 7.6$), 3.36 (2H, q, $CH_2$ protons), 2.63 (3H, s, aromatic $CH_3$ protons), 2.36 (3H, s, aromatic $CH_3$ protons), 1.11 (3H, t, $CH_3$ protons) | 165.94, 153.34, 142.47, 139.04, 137.93, 135.89, 133.97, 128.69, 126.61, 125.95, 125.93, 120.72, 112.52, 50.16, 20.94, 17.47, 7.93 | 316(50%)(M + 1) |
| **3n** | Cl | H | H | Br | – | $C_{15}H_{11}SO_3NClBr$ | 134 | 29 | 2975, 1605, 1558, 1452, 1343–1301, 1134–1124, 1042, 819 | 8.44–8.43 (1H, d, $J = 2.0$), 8.36–8.35 (1H, d, $J = 2.0$), 8.03–7.99 (1H, dd, $J = 2.0$, $J' = 8.0$), 7.83–7.80 (1H, d, $J = 8.8$), 7.64–7.61 (1H, dd, $J = 2.4$, $J' = 8.8$), 7.49–7.47 (1H, d, $J = 8.4$), 3.19 (2H, q, $CH_2$ protons), 1.32 (3H, t, $CH_3$ protons) | 162.06, 153.63, 142.20, 135.92, 135.79, 134.71, 133.24, 132.99, 126.93, 126.30, 122.00, 120.92, 111.97, 51.23, 7.82 | 400(35%)(M+), 402(50%)(M + 2) |
| **3o** | H | H | Br | H | $CH_2$ | $C_{16}H_{14}SO_3NBr$ | 141 | 22 | 3098, 2965, 1609, 1561, 1455, 1348–1313, 1164–1122, 1052, 837 | 8.26 (1H, s), 7.90–7.89 (1H, m), 7.66–7.63 (1H, m), 7.50–7.49 (2H, d, $J = 5.6$), 7.28–7.26 (2H, d, $J = 5.6$), 4.28 (2H, s, benzylic $CH_2$ protons), 3.16 (2H, q, $CH_2$ protons), 1.28 (3H, t, $CH_3$ protons) | 167.35, 154.22, 142.07, 135.32, 133.09, 132.34, 131.02, 125.49, 121.99, 121.23, 111.59, 51.23, 34.88, 7.81 | 380(54%)(M+), 382(56%)(M + 2) |
| **3q** | H | H | F | H | $CH_2$ | $C_{16}H_{14}SO_3NF$ | 82 | 21 | 2929, 1613, 1561, 1455, 1349–1315, 1280, 1160–1123, 831 | 8.26 (1H, s), 7.91–7.89 (1H, dd, $J = 1.2$; $J' = 7.2$), 7.66–7.64 (1H, d, $J = 8.4$), 7.39–7.35 (2H, m), 7.09–7.04 (2H, m), 4.3 (2H, s, benzylic $CH_2$ protons), 3.1 (2H, q, $CH_2$ protons), 1.28 (3H, t, $CH_3$ protons) | 167.75, 163.73, 161.28, 154.24, 142.11, 135.31, 130.95, 130.86, 129.86, 129.83, 125.45, 121.22, 116.23, 116.02, 111.55, 51.23, 34.67, 7.79 | 320(60%)(M + 1) |
| **3p** | Cl | H | H | H | $CH_2$ | $C_{16}H_{14}SO_3NCl$ | 120 | 18 | 3097, 2922, 1617, 1596, 1458, 1370–1304, 1266, 1138–1117, 1052, 823 | 8.18–8.17 (1H, d, $j = 1.2$), 7.97–7.95 (1H, dd, $J = 0.8$, $J' = 8.4$), 7.88–7.85 (1H, dd, $J = 2.0$, $J' = 8.4$), 7.55–7.48 (2H, m), 7.37–7.35 (2H, m), 4.53 (2H, s, benzylic $CH_2$ protons), 3.31(2H, q, $CH_2$ protons), 1.05 (3H, t, $CH_3$ protons) | 167.65, 153.89, 141.89, 135.78, 134.22, 133.21, 132.75, 130.19, 128.30, 125.69, 120.47, 112.44, 50.14, 33.12, 7.89 | 336(62%)(M+) |

(*continued on next page*)

Table 1 (*continued*)

| Compound No. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Y | Empirical formulas | MP (%C) | Yield (%) | IR($cm^{-1}$) | $^1$H NMR ($CDCl_3$ or $d_6$-DMSO) ($\delta$ ppm) $J$ = Hz | $^{13}$C NMR ($CDCl_3$ or $d_6$-DMSO) ($\delta$ ppm) | MS(ESI+) *m/z* (*X*%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **3r** | H | H | Cl | H | $CH_2$ | $C_{16}H_{14}SO_3NCl$ | 130 | 19 | 3098, 2966, 1611, 1562, 1455, 1348–1313, 1280, 1164–1122, 1019, 839 | 8.26–8.25 (1H, d, $J = 1.2$), 7.91–7.88 (1H, dd, $J = 1.6$, $J' = 8.6$), 7.65–7.63 (1H, d, $J = 8.0$), 7.38–7.30 (4H, m), 4.29 (2H, s, benzylic $CH_2$ protons), 3.15 (2H, q, $CH_2$ protons), 1.27 (3H, t, $CH_3$ protons) | 167.46, 154.18, 142.04, 135.25, 133.81, 132.61, 130.67, 129.31, 125.43, 121.14, 111.59, 51.19, 34.75, 7.83 | 336(56%)(M+) |
| **3s** | H | H | $CH_3$ | H | $CH_2$ | $C_{17}H_{17}SO_3N$ | 114 | 20 | 2925, 1606, 1558, 1454, 1348–1315, 1162–1121, 1050, 828 | 8.25–8.24 (1H, d, $J = 2.0$), 7.89–7.86 (1H, dd, $J = 2.0$; $J' = 8.2$), 7.64–7.61 (1H, d, $J = 8.8$), 7.29–7.26 (3H, m), 7.19–7.17 (1H, d, $J = 8.0$), 4.3 (2H, s, benzylic $CH_2$ protons), 3.14 (2H, q, $CH_2$ protons), 2.34 (3H, s, aromatic $CH_3$ protons), 1.27 (3H, t, $CH_3$, protons) | 168.27, 154.28, 142.20, 137.61, 135.12, 131.07, 129.88, 129.14, 125.28, 121.13, 111.49, 51.23, 35.09, 21.30, 7.79 | 316(55%)(M + 1) |
| **3t** | H | H | H | H | $CH_2CH_2$ | $C_{17}H_{17}SO_3N$ | 105 | 12 | 3092, 2981, 1614, 1563, 1458, 1301, 1154–1119, 1047, 831 | 8.17–8.16 (1H, d, $J = 1.2$), 7.95–7.93 (1H, d, $J = 8.4$), 7.87–7.85 (1H, dd, $J = 8.0$, $J' = 2.0$), 7.27–7.18 (5H, m), 3.36–3.12 (6H, m), 1.07 (3H, t, $CH_3$ protons) | 169.57, 153.80, 141.90, 140.63, 135.58, 129.10, 128.96, 126.99, 125.50, 120.20, 112.25, 50.14, 32.31, 30.16, 7.88 | 316(60%)(M + 1) |

[a] Melting point was not shown in the literature.

buffer saline (PBS) (ampicilin trihydrate) and distilled water (gentamicin sulfate, fluconazole and amphotericin B).

All bacterial isolates were subcultured in MHA plates and incubated over night at 37 °C and all *Candida* isolates were subcultured in SDA plates at 35 °C for 24–48 h. The microorganisms were passaged at least twice to ensure purity and viability.

The solution of the newly synthesized compounds (**3a–3t**) and standard drugs were prepared at 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8, 3.9, 1.95, 0.98 μg/ml concentrations, at 4096, 2048, 1024, 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 μg/ml concentrations in the wells of microplates by diluting in MHB, respectively.

Bacterial susceptibility testing was performed according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) M100-S16 [25]. The bacterial suspensions used for inoculation were prepared at $10^5$ cfu/ml by diluting fresh cultures at MacFarland 0.5 density ($10^7$ cfu/ml). Suspensions of the bacteria at $10^5$ cfu/ml concentration were inoculated to the twofold diluted solution of the compounds. There were $10^4$ cfu/ml bacteria in the wells after inoculations. MHB was used for diluting the bacterial suspension and for twofold dilution of the compound. 80% DMSO, 20% EtOH, methanol, DMSO, PBS, pure microorganisms and pure media were used as control wells. A 10 μl bacteria inoculum was added to each well of the microdilution trays. The trays were incubated at 37 °C in a humid chamber and MIC endpoints were read after 24 h of incubation. All organisms were tested in triplicate in each run of the experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported.

All *Candida* isolates were subcultured in SDA plates, and incubated at 35 °C for 24–48 h prior to antifungal susceptibility testing, and passaged at least twice to ensure purity and viability. Susceptibility testing was performed in RPMI-1640 medium with L-glutamine buffered pH 7 with MOPS and culture suspensions were prepared through the guideline of CLSI M27-A [26]. The yeast suspensions used for inoculation were prepared at $10^4$ cfu/ml by diluting fresh cultures at MacFarland 0.5 density ($10^6$ cfu/ml). Suspensions of the yeast at $10^4$ cfu/ml concentration were inoculated to the twofold diluted solution of the compounds. There were $10^3$ cfu/ml bacteria in the wells after inoculations. A 10 μl yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35 °C in a humid chamber and MIC endpoints were read after 48 h of incubation. All organisms were tested in triplicate in each run of the experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported in Table 2.

## 4. Results and discussion

The synthesis of the 5-ethylsulphonyl-2-(substituted-phenyl/substituted-benzyl and/or 2-phenylethyl)benzoxazole derivatives (**3a–3t**) was obtained by heating appropriate carboxylic acids (**2a–2t**) with 4-ethylsulphonyl-2-aminophenol (**1**) in PPA (polyphosphoric acid) as the cyclodehydration reagent in a one step procedure [9]. All of these syntheses are shown in Scheme 1.

All the compounds **3a–3t** were prepared as new products except **3a**, **3g**, **3h**, **3k** [1]. Their structures were supported by spectral data. The IR, $^1$H, $^{13}$C NMR and Mass spectra are in agreement with the proposed structures. Physical and spectral data of the compounds are reported in Table 1.

All the synthesized derivatives (**3a–3t**) were assayed in vitro for antibacterial activity against *K. pneumoniae* RSHM 574, *P. aeruginosa* ATCC 25853, *E. coli* ATCC 25922, *K. pneumoniae* isolate (resistant to trimethoprim sulfamethoxazole, amoxicillin clavulonat, ceftriaxon, cephepim, aztreonam), *E. coli* isolate (resistant to trimethoprim sulfamethoxazole, cephepim, tazobactam) as Gram-negative bacteria, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *B. subtilis* isolate (resistant to ceftriaxon), *S. aureus* isolate (resistant to oxacilin, gentamicin, aztreonam, trimethoprim sulfamethoxazole) as Gram-positive bacteria and the antifungal activity was evaluated against *C. albicans* ATCC 10231 and its isolate. The MIC values were determined by twofold serial dilution technique in Mueller Hinton Broth and Sabouraud Dextrose Agar for the antibacterial and antifungal assay, respectively. For comparison of the antimicrobial activity, rifampicin, ampicillin trihydrate, gentamicin sulphate, ofloxacin were used as the reference antibacterial agents and fluconazole, amphotericin B were employed as the reference antifungal agents. All the biological results of the tested compounds are given in Table 2.

In this study, our goal was to investigate the role of efficient substitution on the position 2 of 5-ethylsulphonyl-benzoxazole ring for antimicrobial activity. Therefore, we put a methylene, an ethylene groups as a bridge between benzoxazole moiety and phenyl ring or did not place any component between them.

According to Table 2, the synthesized compounds showed a broad spectrum of activity with MIC values 125–7.81 μg/ml against some Gram-positive bacteria such as *S. aureus*, *B. subtilis* and their isolates. The compounds **3a–3t** displayed lower antibacterial activity against *S. aureus* and its isolate than the compared control drugs, ampicillin trihydrate, and ofloxacin. It can be considered that structurally differences on the substitution at position 2 of benzoxazole nucleus did not change their activity against *S. aureus* and its isolate.

Although derivatives **3d** and **3e** showed only significant activity with MIC values of 7.81 μg/ml but less active than tested standard drug ampicillin trihydrate against *B. subtilis*. They indicated two- or onefold less potency against drug-resistant *B. subtilis*, respectively. On the other hand, compounds **3e**, **3h**, **3o** and **3r** possessed the highest activity with MIC values of 15.625 μg/ml. Structure-activity relationships revealed that compounds possessing a substituent such as –Cl, –*t*-$C_4H_9$ of *para* position of phenyl improved the potency against *B. subtilis*.

All of the new compounds **3a–3t** showed a broad antibacterial activity against some *E. coli*, *K. pneumoniae*, *P. aeruginosa* as Gram-negative bacteria possessing MIC values between 125 and 31.25 μg/ml and they showed with MIC values 250–31.25 μg/ml against their isolates. All compounds

Table 2
Antimicrobial activity results (MIC, μg/ml) of old and newly synthesized compounds with the standard drugs

| Compound | S.a. | S.a.* | B.s. | B.s.* | E.c. | E.c.* | K.p. | K.p.* | P.a. | C.a. | C.a.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **3a** | 125 | 125 | 62.5 | 31.25 | 62.5 | 62.5 | 125 | 125 | 62.5 | 125 | 31.25 |
| **3b** | 125 | 125 | 31.25 | 31.25 | 62.5 | 62.5 | 31.25 | 125 | 62.5 | 125 | 31.25 |
| **3c** | 125 | 125 | 31.25 | 31.25 | 62.5 | 62.5 | 62.5 | 125 | 62.5 | 125 | 15.625 |
| **3d** | 125 | 125 | 7.8125 | 31.25 | 62.5 | 62.5 | 62.5 | 125 | 62.5 | 125 | 15.625 |
| **3e** | 125 | 125 | 7.8125 | 15.625 | 62.5 | 62.5 | 62.5 | 250 | 62.5 | 125 | 31.25 |
| **3f** | 125 | 125 | 31.25 | 31.25 | 62.5 | 62.5 | 62.5 | 31.25 | 62.5 | 125 | 31.25 |
| **3g** | 125 | 125 | 15.625 | 31.25 | 62.5 | 62.5 | 62.5 | 125 | 62.5 | 125 | 31.25 |
| **3h** | 125 | 125 | 31.25 | 15.625 | 62.5 | 62.5 | 125 | 125 | 62.5 | 125 | 62.5 |
| **3ı** | 125 | 125 | 62.5 | 31.25 | 62.5 | 250 | 31.25 | 125 | 62.5 | 62.5 | 62.5 |
| **3k** | 125 | 125 | 15.625 | 31.25 | 62.5 | 250 | 31.25 | 125 | 62.5 | 62.5 | 62.5 |
| **3l** | 125 | 125 | 15.625 | 31.25 | 62.5 | 125 | 31.25 | 125 | 62.5 | 31.25 | 62.5 |
| **3m** | 125 | 125 | 15.625 | 31.25 | 62.5 | 250 | 31.25 | 125 | 62.5 | 31.25 | 62.5 |
| **3n** | 125 | 125 | 62.5 | 31.25 | 62.5 | 125 | 62.5 | 125 | 62.5 | 62.5 | 62.5 |
| **3o** | 125 | 125 | 62.5 | 15.625 | 62.5 | 125 | 62.5 | 125 | 62.5 | 62.5 | 62.5 |
| **3q** | 125 | 125 | 62.5 | 31.25 | 62.5 | 125 | 31.25 | 125 | 62.5 | 62.5 | 31.25 |
| **3p** | 125 | 125 | 62.5 | 31.25 | 62.5 | 125 | 31.25 | 125 | 62.5 | 62.5 | 62.5 |
| **3r** | 125 | 125 | 31.25 | 15.625 | 62.5 | 125 | 62.5 | 125 | 62.5 | 31.25 | 62.5 |
| **3s** | 125 | 125 | 31.25 | 31.25 | 62.5 | 125 | 62.5 | 125 | 62.5 | 62.5 | 62.5 |
| **3t** | 125 | 125 | 62.5 | 31.25 | 62.5 | 125 | 31.25 | 125 | 62.5 | 62.5 | 62.5 |
| Ampicillin trihydrate | 0.03 | 2 | 0.25 | 8 | 8 | 256 | 2 | 256 | 4096 | – | – |
| Gentamicin sulphate | 0.06 | 1024 | 1 | 512 | 0.5 | 1 | 8 | 64 | 2 | – | – |
| Ofloxacin | 0.25 | 2 | 0.125 | 32 | 0.125 | 32 | 0.25 | 64 | 8 | – | – |
| Fluconazol | – | – | – | – | – | – | – | – | – | 1 | 64 |
| Amphotericin B | – | – | – | – | – | – | – | – | – | 1 | 1 |

S.a.: *S. aureus* ATCC 25923, B.s.: *B. subtilis* ATCC 6633, E.c.: *E. coli* ATCC 25922, K.p.: *K. pneumoniae* RSHM 574, P.a.: *P. aeruginosa* ATCC 25853, C.a.: *C. albicans* ATCC 10231.
S.a.*: *S. aureus* isolate, B.s.*: *B. subtilis* isolate, E.c.*: *E. coli* isolate, K.p.*: *K. pneumoniae* isolate, C.a.*: *C. albicans* isolate.

were less active with MIC values of 62.5 μg/ml than compared standard drugs against *E. coli*. Structurally differences on the substitution at position 2 of benzoxazole ring did not change their activity against *E. coli*. It could be pointed out that without any bridge groups between phenyl and heterocyclic nucleus with together attaching some substituent such as $CH_3$, Cl, $C_2H_5$, Br, $NO_2$, *t*-$C_4H_9$ on position $R_3$ were performed better activity against drug-resistant *E. coli*.

Besides, the synthesized compounds showed lower antibacterial activity against *K. pneumoniae* and its isolate than compared control drug ofloxacin and gentamicin. Among the tested series the derivatives **3b**, **3k**–**3m**, **3q**–**3p**, **3t** were found to be more potent with MIC values of 31.25 μg/ml. Furthermore, all compounds showed more inhibitory effect with the MIC value of 62.5 μg/ml against *P. aeruginosa* than ampicillin.

In the past 10 years there has been a major expansion in the development of antifungal drugs, but there are still weaknesses in the range and scope of current antifungal chemotherapy [27]. New developments have included the modification of existing drug molecules to eliminate toxicity and improve activity.

The tested compounds possessed lower activity with MIC values of between 125 and 31.25 μg/ml against *C. albicans* than compared standard drugs as fluconazole and amphotericin B. Most of the compounds showed higher activity than fluconazole against *C. albicans* isolate. Substitution at the position 2 of benzoxazole ring with *p*-chloro-, *p*-ethyl-phenyl groups played an important role for enhancing activity.

In conclusion, we have discovered a novel series of benzoxazoles as antimicrobial agents. According to this study, it could be pointed out that structurally differences on the substitution at position 2 of benzoxazole nucleus did not change their activity against *S. aureus* and its isolate. On the other hand, compounds **3e**, **3h**, **3o** and **3r** possessed higher activity with MIC values of 15.625 μg/ml against drug-resistance *B. subtilis* than gentamicin and ofloxacin. Structure-activity relationships revealed that compounds possessing *p*-chloro-, *p*-bromo-, *p*-*tert*-butyl-, *o*-chloro-, *o*-bromo-phenyl groups of 2-benzoxazole played a significant role for improving the potency against *B. subtilis*.

If there were methyl, chloro, ethyl, bromo, nitro, *tert*-butyl, on position $R_3$ of phenyl with together without any bridge groups between phenyl and heterocyclic nucleus, better activity against drug-resistant *E. coli* was performed. Substitution at the position 2 of benzoxazole ring with *o*-chloro, *o*-bromo, *o*-fluorophenyl and/or *p*-fluoro, *o*-chlorobenzyl and/or phenylethyl groups caused higher inhibition effect against *K. pneumoniae* than ofloxacin.

Additionally, substitution at the position 2 of benzoxazole ring with *p*-chloro-, *p*-ethyl-phenyl groups played an important role for enhancing activity against *C. albicans* isolate. In conclusion, the newly synthesized benzoxazole derivatives showed significant activities against *C. albicans* isolate. It could be hopefully for drug-resistance on antifungal therapy. These observations provide some predictions in order to design further antimicrobial active compounds prior to their synthesis following with QSAR and molecular modelling studies.

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