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Short communication

Synthesis and *in vitro* antimicrobial activity of new 2-[*p*-substituted-benzyl]-5-[substituted-carbonylamino]benzoxazoles

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Abstract

Some new 2-(benzyl/*p*-chlorobenzyl)-5-[(substituted-thienyl/phenyl/phenylthiomethyl/benzyl)carbonylamino]benzoxazole derivatives have been synthesized by reacting 5-amino-2-(benzyl/*p*-chlorobenzyl)benzoxazoles with appropriate carboxylic acid chlorides. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR and MASS spectral data. *In vitro* antimicrobial activities of the compounds were investigated using twofold serial dilution technique against different two Gram-positive, two Gram-negative bacteria and three *Candida* spp. in comparison with standard drugs. Microbiological results indicated that the newly synthesized 2-(benzyl/*p*-chlorobenzyl)-5-[(substituted-thienyl/phenyl/phenylthiomethyl/benzyl)carbonylamino]benzoxazole derivatives (**3**–**12**) possessed a broad spectrum of activity having MIC values of 6.25–100 μg/ml against the tested microorganisms. Especially, with an MIC value of 6.25 μg/ml, 2-(*p*-chlorophenyl)-5-[(2,5-dimethylphenyl)carbonylamino]benzoxazole **4** displayed the same activity against *Candida albicans* as the standard drug clotrimazole.

Keywords: 2-Benzylbenzoxazole; Antibacterial and antifungal activity

1. Introduction

Disease-causing microbes that have become resistant to drug therapy are an increasing public health problem. Tuberculosis, gonorrhea, malaria, and childhood ear infections are just a few of the diseases that have become hard to treat with antibiotic drugs. The hospital-acquired infections are resistant to the most powerful antibiotics available, methicillin and vancomycin. These drugs are reserved to treat only the most intractable infections in order to slow development of resistance to them [1]. So there is still need for the new classes of antimicrobial agents.

Benzoxazoles and benzimidazoles, which are the structural isosters of natural nucleotides and interact easily with the biopolymers, constitute an important class of heterocyclic compounds with antitumor, antiviral, antibacterial and antibiotic activities [2–19]. Therefore, these have been the aim of many researchers for many years.

A benzoxazole derivative, calcimycin (Fig. 1), is a carboxylic polyether antibiotic from a strain of *Streptomyces chartreusis* (NRRL 3882). It was found to be very active against Gram-positive bacteria including some *Bacillus* and *Micrococcus* strains [3,20,21]. Two calcimycin analogues, routiennocin and cezomycin (Fig. 1) which are 3-hydroxy-11,15-desmethyl and 3-demetylamino derivatives of it, respectively, were found to be highly active against *Bacillus cereus*, *Bacillus negaterium*, *Micrococcus luteus* and *Streptomyces rimosus* [22–24]. Additionally, frankamide, that is 11-demethyl cezomycin, showed some activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* and against several plant pathogenic fungal strains [25,26].

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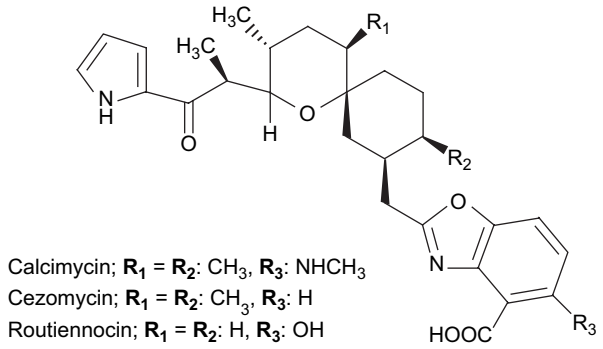


Fig. 1.

Recently, we reported the synthesis and the antimicrobial activity of 2-[*p*-substituted-phenyl/*p*-substituted-benzyl]-5-[substituted-aryl-carbonylamino]benzoxazole derivatives (Fig. 2) [27–29].

In this study, a new series of 2-(benzyl/*p*-chlorobenzyl)-5-[(substituted-thienyl/phenyl/phenylthiomethyl/benzyl)carbonylamino]benzoxazole derivatives (**3**–**12**) (Fig. 3) has been synthesized as the target compounds in order to examine their *in vitro* microbiological activity, and that of the previously synthesized 2-(*p*-substituted-benzyl)-5-[(substituted-aryl)carbonylamino]benzoxazoles derivatives [29], against various Gram-positive, Gram-negative bacteria and the different fungi in comparison with several control drugs, including structure–activity relationship (SAR) studies.

For the synthesis of compounds **3**–**12**, firstly, 5-amino-2-(benzyl/*p*-chlorobenzyl)benzoxazoles (**1**,**2**) were obtained by heating *p*-chlorophenyl acetic acid and/or phenylacetic acid with 2,4-diaminophenol in polyphosphoric acid (PPA) [27–29].

Compounds **3**–**12** were obtained from 5-amino-2-(benzyl/*p*-chlorobenzyl)benzoxazoles with substituted-benzoic acid/5-methyl-2-thienylcarboxylic acid/substituted-phenylacetic acid/phenylthioacetic acid chlorides obtained by treating appropriate carboxylic acids with thionyl chloride [30,31], as given in Scheme 1.

Compounds **3**–**12** are new and their structures were supported by spectral data.

2. Experimental procedures

2.1. Chemistry

Silicagel HF_{254} chromatoplates (0.3 mm) were used for TLC. The solvent systems were chloroform:methanol (15:0.5) for compounds **3**–**12**. Melting points were taken on

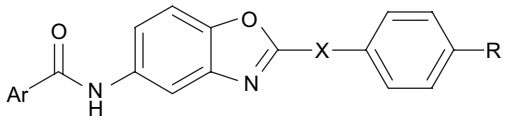


Fig. 2.

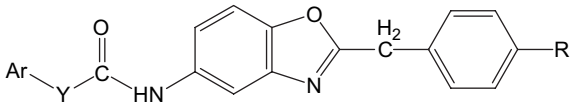


Fig. 3.

a Buchi SMP 20 capillary apparatus and are uncorrected. IR spectra were recorded by FT/IR-420 in KBr discs. ^{1}H NMR spectra were obtained with a Bruker GmbH D PX-400 MHz spectrometer in d_6-chloroform, tetramethylsilane (TMS) was used as an internal standard. Elemental analyses were carried out with a Perkin Elmer model 240-C apparatus. The results (C, H, N) were within ±0.4% of the calculated values. Mass analysis was obtained by Micromass VG Platform-II working with EI and Walters ZQ Micromass LC-MS working with ES^-, ES^+ apparatus.

2.2. General procedure for compounds *1* and *2* [29]

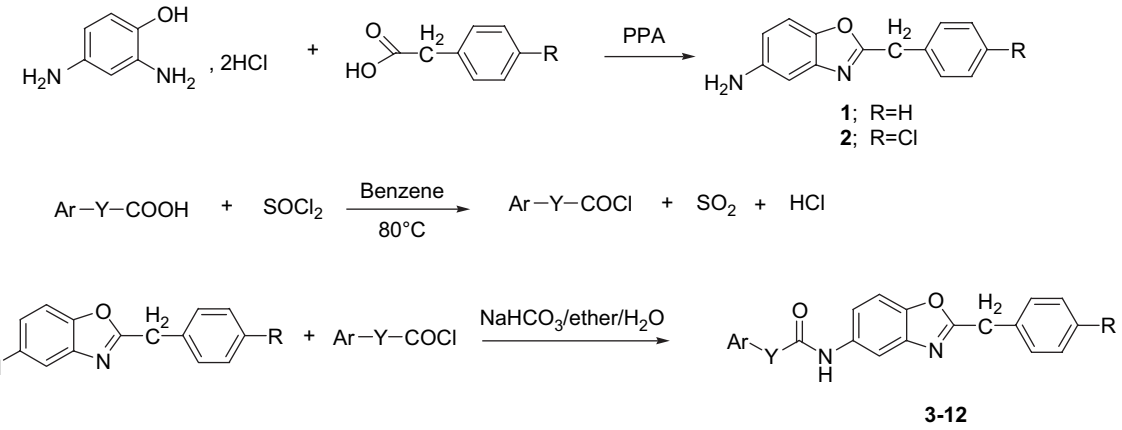
5-Amino-2-(benzyl/*p*-chlorobenzyl)benzoxazole was synthesized by heating 0.01 mol 2,4-diaminophenol·2HCl with 0.01 mol phenylacetic acid and/or *p*-chlorophenyl acetic acid in 12.5 g polyphosphoric acid (PPA) and stirring for 1.5–2.5 h. At the end of the reaction period, the residue was poured into ice–water mixture and neutralized with excess of 10 M NaOH solution extracted with benzene and then this 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.

2.2.1. 2-Benzyl-5-aminobenzoxazole, *1*

Reaction time: 2.5 h. Reaction temperature: 150–160 °C. Yield: 66%. MW: 224. mp: 82–83 °C (82–83 °C) [29]. ^{1}H NMR ($CDCl_3$): 4.00–4.10 (s, 2H, CH_2), 6.40–6.60 (dd, 1H, C-6H $J_{6,7} = 8.56$ Hz, $J_{6,4} = 2.25$ Hz), 6.70–6.80 (d, 1H, C-4H, $J_{4,6} = 2.22$ Hz), 7.00–7.05 (d, 1H, C-7H, $J_{7,6} = 8.58$ Hz), 7.05–7.20 (m, 7H, C-2′, C-3′, C-4′, C-5′, C-6′, NH). IR (KBr disc): 3385, 1617, 1562, 1486, 1452, 1270, 1190.

2.2.2. 2-(*p*-Chlorobenzyl)-5-aminobenzoxazole, *2*

Reaction time: 1.5 h. Reaction temperature: 195–198 °C. Yield: 100%. MW: 224. mp: 85–87 °C (85–87 °C) [29]. ^{1}H NMR ($CDCl_3$): 4.10–4.30 (s, 2H, CH_2), 6.70–6.80 (dd, 1H, C-6H $J_{6,7} = 8.56$ Hz, $J_{6,4} = 2.66$ Hz), 6.95–7.00 (d, 1H, C-4H, $J_{4,6} = 2.21$ Hz), 7.20–7.25 (d, 1H, C-7H, $J_{7,6} = 8.6$ Hz), 7.25–7.40 (m, 6H, C-2′, C-3′, C-5′, C-6′, NH). IR (KBr disc): 3381, 2210, 1915, 1615, 1563, 1486, 1452, 1298, 1271, 1191.



Ar = 5-methy-2-thienyl, 2,5-dimethylphenyl, 3-nitro-4-chlorophenyl, 3,4-dimethylphenyl, phenyl, 4-nitrophenyl
Y = –, CH_2, SCH_2
R = H, Cl

Scheme 1.

2.3. General procedure for compounds 3–12

Appropriate carboxylic acid (0.5 mmol) and thionyl chloride (1.5 ml) were refluxed in benzene (5 ml) at 80 °C for 3 h. Excess thionyl chloride was removed *in vacuo*. The residue was dissolved in ether (10 ml) and this solution was added during 1 h to a stirred, ice-cold mixture of 5-amino-2-(*p*-substituted-benzyl)benzoxazoles **1**,**2** (0.5 mmol), sodium bicarbonate (0.5 mmol), diethyl ether (10 ml) and water (10 ml). The mixture was kept stirred overnight at room temperature and filtered. The precipitate was washed with water, 2 N HCl and water and finally with ether to give **3–12**. The products were recrystallized from ethanol–water as needles, which were dried *in vacuo*. The chemical, physical and spectral data of the compounds **3–12** are reported below.

2.3.1. Compound 3: 2-benzyl-5-[(5-methyl-2-thienyl)-carbonylamino]benzoxazole

$C_{20}H_{16}N_2O_2S$, yield: 53%, mp: 151–152 °C.

^{1}H NMR (ppm): 2.50–2.60 (CH_3, 3H, s); 4.30–4.40 (CH_2, 2H, s); 6.75–6.85 (C-4″, 1H, d, $J_{4'',5''} = 3.56$ Hz); 7.25–7.45 (C-7, 2′, 3′, 4′, 5′, 6′, 6H, m); 7.45–7.55 (C-3′, 1H, d, $J_{3'',4''} = 3.61$ Hz); 7.90–8.00 (C-4 and NH, 2H, d, $J_{4,6} = 1.63$ Hz).

IR (cm^{-1}): 3291, 3075, 2919, 1662, 1565–1539, 1487, 1277–1048, 970–675.

MS (70 eV) *m/z*: 350 (M + 2), 349 (M + 1), 348 (M+), 91 (100%).

2.3.2. Compound 4: 2-(p-chlorobenzyl)-5-[(2,5-dimethyl-phenyl)carbonylamino]benzoxazole

$C_{23}H_{19}ClN_2O_2$, yield: 47%, mp: 160 °C.

^{1}H NMR (ppm): 2.10–2.20 (CH_3, 3H, s); 2.20–2.35 (CH_3, 3H, s); 4.00–4.10 (CH_2, 2H, s); 6.90–7.45 (aromatic protons, 10H, m); 7.70–7.80 (NH, 1H, s).

IR (cm^{-1}): 3251, 3068, 2924, 1650, 1574–1532, 1491, 1267–1015, 992–669.

MS (70 eV) *m/z*: 392 (M + 2), 391 (M + 1), 390 (M+), 133 (100%).

2.3.3. Compound 5: 2-benzyl-5-[(2,5-dimethylphenyl)-carbonylamino]benzoxazole

$C_{23}H_{20}N_2O_2$, yield: 18%, mp: 147–148 °C.

^{1}H NMR (ppm): 2.30–2.40 (CH_3, 3H, s); 2.40–2.50 (CH_3, 3H, s); 4.25–4.35 (CH_2, 2H, s); 7.10–7.65 (aromatic protons, 11H, m); 7.9–7.95 (NH, 1H, s).

IR (cm^{-1}): 3261, 3031, 2922, 1649, 1570–1544, 1479–1426, 1270–1028, 970–680.

MS (70 eV) *m/z*: 358 (M + 2), 357 (M + 1), 356 (M+), 133 (100%).

2.3.4. Compound 6: 2-(p-chlorobenzyl)-5-[(3-nitro-4-chlorophenyl)carbonylamino]-benzoxazole

$C_{21}H_{13}Cl_2N_3O_4$, yield: 48%, mp: 165–166 °C.

^{1}H NMR (ppm): 4.20–4.30 (CH_2, 2H, s); 7.20–7.80 (C-7, 2′, 3′, 5′, 6′, 5″, 6″, 7H, m); 7.90–8.00 (C-4, 1H, s); 8.00–8.05 (C-6, 1H, dd, $J_{6,7} = 8.32$ Hz, $J_{6,4} = 1.50$ Hz); 8.10–8.25 (NH, 1H, s); 8.35–8.45 (C-2″, 1H, d, $J_{2'',6''} = 1.58$ Hz).

IR (cm^{-1}): 3273, 3060, 1648, 1600–1530, 1482, 1298–1016, 967–685.

MS (70 eV) *m/z*: 443 (M + 2), 441 (M+), 105 (100%).

2.3.5. Compound 7: 2-benzyl-5-[(3-nitro-4-chlorophenyl)-carbonylamino]benzoxazole

$C_{21}H_{14}ClN_3O_4$, yield: 38%, mp: 150–151 °C.

^{1}H NMR (ppm): 4.20–4.30 (CH_2, 2H, s); 7.20–7.70 (C-6, 7, 2′, 3′, 4′, 5′, 6′, 5″, 6″, 9H, m); 7.90–8.02 (C-4, 1H, s); 8.02–8.15 (NH, 1H, s); 8.40–8.50 (C-2″, 1H, m).

IR (cm^{-1}): 3347, 3077, 1644, 1482–1459, 1295–1016, 970–688.

MS (70 eV) *m/z*: 409 (M + 2), 407 (M+), 83 (100%).

*2.3.6. Compound **8**: 2-(p-chlorobenzyl)-5-[(3,4-dimethylphenyl)carbonylamino]benzoxazole*

$C_{23}H_{19}ClN_2O_2$, yield: 57, mp: 170–171 °C.

^{1}H NMR (ppm): 2.05–2.20 (CH_3, 6H, s); 4.00–4.10 (CH_2, 2H, s); 7.00–7.10 (5″, 1H, d, $J_{5'',6''} = 7.84$ Hz); 7.10–7.45 (C-6, 7, 2′, 3′, 5′, 6′, 6″, 7H, m); 7.45–7.55 (C-4, 2H, d, $J_{4,6} = 1.09$ Hz); 7.85–7.90 (NH, 1H, s); 7.90–8.00 (C-2″, 1H, d, $J_{2'',6''} = 2.40$ Hz).

IR (cm^{-1}): 3303, 3083, 2932, 1671, 1556, 1487, 1270–1015, 966–688.

MS (70 eV) *m/z*: 392 (M + 2), 391 (M + 1), 390 (M+), 133 (100%).

*2.3.7. Compound **9**: 2-benzyl-5-[(3,4-dimethylphenyl)-carbonylamino]benzoxazole*

$C_{23}H_{20}N_2O_2$, yield: 55, mp: 177–178 °C.

^{1}H NMR (ppm): 2.30–2.40 (CH_3, 6H, s); 4.20–4.30 (CH_2, 2H, s); 7.20–7.75 (C-6, 7, 2′, 3′, 4′, 5′, 6′, 2″, 5″, 6″, 10H, m); 7.85–7.90 (NH, 1H, s); 7.90–8.00 (C-4, 1H, d, $J_{4,6} = 1.98$ Hz).

IR (cm^{-1}): 3273, 3067, 2969, 1638, 1552, 1496–1420, 1298–1022, 948–670.

MS (70 eV) *m/z*: 358 (M + 2), 357 (M + 1), 356 (M+), 133 (100%).

*2.3.8. Compound **10**: 2-(p-chlorobenzyl)-5-[(phenylthiomethyl)carbonylamino]benzoxazole*

$C_{22}H_{17}ClN_2O_2S$, yield: 6, mp: 133–134.

^{1}H NMR (ppm): 3.50–3.70 (CH_2, 2H, s); 4.00–4.10 (S-CH_2, 2H, s); 7.00–7.70 (aromatic protons, 11H, m); 7.60–7.65 (C-4, 1H, d, $J_{4,6} = 2.60$ Hz); 8.40–8.50 (NH, 1H, s).

IR (cm^{-1}): 3258, 3056, 2360, 1660, 1575–1534, 1485, 1263–1015, 977–690.

MS (70 eV) *m/z*: 410 (M + 2), 409 (M + 1), 408 (M+), 407 (100%).

*2.3.9. Compound **11**: 2-benzyl-5-[(phenylthiomethyl)-carbonylamino]benzoxazole*

$C_{21}H_{18}N_2O_2S$, yield: 19%, mp: 171–172 °C.

^{1}H NMR (ppm): 3.80–3.90 (CH_2, 2H, s); 3.90–4.00 (S-CH_2, 2H, s); 7.00–7.70 (aromatic protons, 13H, m); 8.50–8.60 (NH, 1H, s).

IR (cm^{-1}): 3278, 3061, 2958–2911, 1648, 1549, 1497–1480, 1244–1024, 991–636.

MS (70 eV) *m/z*: 373 (M − 1), 372 (M − 2), 43 (100%).

*2.3.10. Compound **12**: 2-benzyl-5-[(4-nitrophenylmethyl)-carbonylamino]benzoxazole*

$C_{20}H_{17}N_3O_4$, yield: 35, mp: 204–206 °C.

^{1}H NMR (ppm): 4.00–4.10 (CH_2, 2H, s); 4.40–4.50 (CH_2, 2H, s); 7.50–7.70 (C-4, 6, 7, 2′, 3′, 4′, 5′, 6′, 2″, 6″, 10H, m); 7.90–8.00 (NH, 1H, s); 8.30–8.45 (C-3″, 5″, 2H, dd, $J_{3'',2''} = 8.73$ Hz, $J_{5'',6''} = 8.63$ Hz).

IR (cm^{-1}): 3320, 3050–3090, 1650, 1510–1560, 1480, 1050–1230, 960–690.

MS (70 eV) *m/z*: 389 (M + 2), 388 (M + 1), 387 (M+), 91 (100%).

2.3. Microbiology

For the antibacterial and antimycotic assays, the compounds were dissolved in absolute ethanol (0.8 mg/ml). Further dilutions of the compounds and standard drugs in the test medium were prepared at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 μg/ml concentrations with Mueller–Hinton broth and Sabouraud dextrose broth. The minimum inhibitory concentrations (MIC) were determined using the twofold serial dilution technique [32,33]. A control test was also performed containing inoculated broth supplemented with only ethanol at the same dilutions used in our experiments and found inactive in the culture medium. All the compounds were tested for their *in vitro* growth inhibitory activity against different bacteria and the yeasts *Candida albicans ATCC 10145*, *Candida krusei ATCC 6258*, and *Candida glabrata* (*isolated*). Origins of bacterial strains are *Staphylococcus aureus ATCC 25923*, *Bacillus subtilis ATCC 6633* as Gram-positive and *Escherichia coli ATCC 23556* and *Pseudomonas aeruginosa ATCC 10145* as Gram-negative bacteria. ATCC strains of the microorganisms used in this study were obtained from the culture collection of Refik Saydam Health Institution of Health Ministry, Ankara, and maintained at the Microbiology Department of Faculty of Pharmacy of Ankara University.

Ampicillin, amoxycillin, tetracycline, streptomycin, ciprofloxacine, gentamicin, miconazol, clotrimazole, and haloprogin were used as control drugs. The data on the antimicrobial activity of the compounds and the control drugs as MIC (μg/ml) values are given in Table 1.

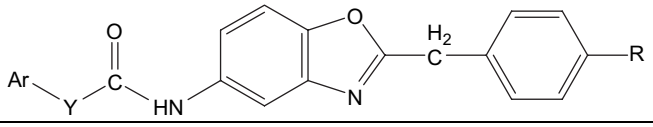
2.4. Antibacterial and antifungal assay

The cultures were obtained from Mueller–Hinton broth (Difco) for all the bacterial strains after 24 h of incubation at 37 ± 1 °C. *C. albicans*, *C. krusei* and *C. glabrata* were maintained in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25 ± 1 °C. Testing was carried out in Mueller–Hinton broth and Sabouraud dextrose broth (Difco) at pH 7.4 and the twofold serial dilution technique was applied. The final inoculum size was 10^5 CFU/ml for the antibacterial assay and 10^4 CFU/ml for the antifungal assay. A set of tubes containing only inoculated broth was used as controls. For the antibacterial assay after incubation for 24 h at 37 ± 1 °C and after incubation for 48 h at 25 ± 1 °C for the antifungal assay, the last tube with no growth of microorganism and/or yeast was recorded to represent the MIC expressed in μg/ml. Every experiment in the antibacterial and antifungal assays was replicated twice.

3. Results and discussion

Some of the new 2-(benzyl/*p*-chlorobenzyl)-5-[(substituted-thienyl/phenyl/phenyl-thiomethyl/benzyl)carbonylamino]benzoxazole derivatives (**3–12**) have been synthesized by using three-step procedure as shown in Scheme 1. All the derivatives (**3–12**) were supported by spectral data. The IR, ^{1}H NMR and

Table 1
Antimicrobial activity of all the new compounds and the standard drugs (MIC, μg/ml)



Com. No	Ar	Y	R	Microorganisms[a]						
				Gram-positive		Gram-negative		Fungi		
				Sa	*Bs*	*Ec*	*Pa*	*Ca*	*Ck*	*Cg*
3	5-Methyl-2-thienyl	–	H	25	25	25	25	12.5	6.25	6.25
4	2,5-Dimethylphenyl	–	Cl	50	12.5	12.5	50	6.25	12.5	25
5	2,5-Dimethylphenyl	–	H	50	25	50	50	25	25	25
6	3-Nitro-4-chlorophenyl	–	Cl	50	50	50	50	25	25	25
7	3-Nitro-4-chlorophenyl	–	H	50	25	50	25	12.5	12.5	12.5
8	3,4-Dimethylphenyl	–	Cl	12.5	25	50	50	100	12.5	50
9	3,4-Dimethylphenyl	–	H	50	50	50	25	25	50	50
10	Phenyl	S CH_2	Cl	100	100	100	100	50	25	50
11	Phenyl	S CH_2	H	100	50	50	25	25	25	25
12	4-Nitrophenyl	CH_2	H	50	50	50	25	25	25	25
13 [29]	Phenyl	–	H	200	50	100	100	50	12.5	25
14 [29]	4-Ethylphenyl	–	H	50	50	50	50	25	25	12.5
15 [29]	4-Nitrophenyl	–	H	50	25	100	25	12.5	12.5	12.5
16 [29]	4-*tert*-Butylphenyl	–	H	200	50	25	25	50	25	25
17 [29]	4-Bromophenyl	–	H	50	50	50	25	25	25	25
18 [29]	4-Fluorophenyl	–	H	50	25	50	25	12.5	50	25
19 [29]	4-Bromophenyl	CH_2	H	25	25	25	25	25	12.5	3.12
20 [29]	4-Fluorophenyl	CH_2	H	50	25	25	25	50	12.5	12.5
21 [29]	Phenyl	CH_2	H	25	25	25	50	12.5	12.5	25
22 [29]	4-Chlorophenyl	CH_2	H	100	100	50	50	25	25	25
23 [29]	4-Methylphenyl	CH_2	H	25	25	25	25	25	6.25	12.5
24 [29]	Phenyl	–	Cl	200	50	100	100	50	25	25
25 [29]	4-Ethylphenyl	–	Cl	50	50	50	50	25	25	25
26 [29]	4-Nitrophenyl	–	Cl	100	50	25	25	50	25	25
27 [29]	4-*tert*-Butylphenyl	–	Cl	25	50	50	50	50	50	50
28 [29]	4-Bromophenyl	–	Cl	50	50	50	50	50	100	100
29 [29]	4-Fluorophenyl	–	Cl	50	25	25	50	12.5	12.5	12.5
30 [29]	4-Bromophenyl	CH_2	Cl	50	25	50	50	12.5	25	50
31 [29]	4-Fluorophenyl	CH_2	Cl	12.5	100	50	25	50	25	50
32 [29]	Phenyl	CH_2	Cl	50	25	50	25	25	25	25
33 [29]	4-Chlorophenyl	CH_2	Cl	50	25	50	25	50	6.25	12.5
34 [29]	4-Nitrophenyl	CH_2	Cl	50	50	50	25	12.5	25	25
35 [29]	4-Methylphenyl	CH_2	Cl	100	25	50	25	12.5	12.5	12.5
Ampicillin				1.56	1.56	12.5	>200	–	–	–
Amoxycillin				1.56	1.56	3.12	>200	–	–	–
Tetracycline				1.56	1.56	3.12	50	–	–	–
Streptomycin				3.12	50	1.56	100	–	–	–13
Ciprofloxacine				3.12	1.56	3.13	0.78	–	–	–
Gentamicin				3.12	1.56	12.5	12.5	–	–	–
Miconazol				–	–	–	–	3.12	1.56	3.12
Clotrimazole				–	–	–	–	6.25	–	–
Haloprogin				–	–	–	–	3.12	–	–

[a] *Sa*: *Staphylococcus aureus ATCC 25923*; *Bs*: *Bacillus subtilis ATCC 6633*; *Ec*: *Escherichia coli ATCC 23556*; *Pa*: *Pseudomonas aeruginosa ATCC 10145*; *Ca*: *Candida albicans*; *Ck*: *Candida krusei ATCC 6258*; *Cg*: *Candida glabrata* (*isolate*).

mass spectra are in agreement with the proposed structures. Physical and spectral data of the compounds are reported in Section 2.

The synthesized compounds **3–12** were tested *in vitro* against two Gram-positive bacteria, two Gram-negative bacteria and three *Candida* spp. by using twofold serial dilution technique [32,33], and compared to their antimicrobial activity with several control drugs. All the biological results of the compounds are given in Table 1. The combined data reported that the newly synthesized compounds showing MIC values between 100 and 6.25 μg/ml were able to inhibit the *in vitro* growth of the microorganisms screened.

According to our previous study, we pointed out that the 2-benzylbenzoxazole derivatives showed significant activities against Gram-positive and Gram-negative bacteria with *C. albicans*, *C. krusei* and *C. glabrata* than 2-phenylbenzoxazole

derivatives [29]. Even if we concluded that the alternates of the substituents attached at 5-carbonylamino of benzoxazoles made no important difference for the antimicrobial activity, our goal here was to keep on investigating the role of the five position of the benzoxazole ring for antimicrobial activity. Therefore, we put different substituents such as 5-methylthienyl, disubstituted-phenyl or phenylthiomethyl on the 5-carbonylamino of 2-(*p*-substituted-benzyl)benzoxazoles. Consequently, the newly presented compounds **3**–**12** were compared with previously prepared compounds **13**–**35** [29] with regard to their antibacterial and antifungal activity (Table 1).

Table 1 indicated that all the new compounds **3**–**12** showed lower antibacterial activity against the screened Gram-positive bacteria either *S. aureus* or *B. subtilis* than the compared control drugs except streptomycin possessing MIC values between 100–12.5 μg/ml. The compounds **3**–**5**, **7** and **8** were found to be more active than standard drugs streptomycin with one or two dilutions better against *B. subtilis*. Only compounds **8** and **4** showed the best activity against *S. aureus* and *B. subtilis* with an MIC value of 12.5 μg/ml, respectively.

Furthermore, the antibacterial activity of compounds **3**–**12** against *E. coli* and *P. aeruginosa* as Gram-negative bacteria possess MIC values between 12.5 and 100 μg/ml. Compound **4** showed the same activity with standard drug ampicillin and gentamicin against *E. coli*, some of the compounds (**3**, **7**, **9**, **11**, **12**) were found to be more active against *P. aeruginosa* with an MIC value of 25 μg/ml than the standard drug tetracycline.

Moreover, compounds **3**–**12** were also tested against *C. albicans*, *C. krusei* and *C. glabrata* for their antimycotic activity and they indicated significant antimycotic activity with MIC values between 6.25 and 100 μg/ml. Compound **4**, 2-(*p*-chlorobenzyl)-5-[(2,5-dimethylphenyl)carbonylamino]benzoxazole, was more active than the other tested compounds having an MIC value of 6.25 μg/ml and showed the same activity with control drug clotrimazole against *C. albicans*. Compound **8**, 2-benzyl-5-[(5-methyl-2-thienyl)carbonylamino]benzoxazole, was found to be more active than the other newly synthesized compounds having an MIC value of 6.25 μg/ml against *C. krusei* and *C. glabrata*.

Table 1 indicated that substitution at position 5 of the benzoxazole ring with 3,4-dimethylbenzamido/4-fluorophenyl-acetamido having a chlorine at the *para* position of 2-phenylbenzoxazole (compounds **8** and **31**, respectively) caused an increase in the activity against *S. aureus* resulting in MIC values of 12.5 μg/ml. However, attaching thiomethyl group between Ar and 5-carbonylamino of benzoxazole ring decreased the activity. Attaching 2,5-dimethylbenzamide group at 5 position of 2-(*p*-chlorobenzyl)benzoxazole enhanced the activity against *B. subtilis*.

Furthermore, the antibacterial activity of compounds **3**–**35** showed lower activity (MIC values of 25–100 μg/ml) than the standard drugs against *E. coli* except compound **4** which exhibited significant activity with MIC value of 12.5 μg/ml. Compounds **3**, **7**, **9**, **11**, **12**, **15**–**20**, **23**, **26**, and **31**–**35** were found to be more active against *P. aeruginosa* (MIC values of 25 μg/ml) than the standard drugs tetracycline and streptomycin. The substitution for position Ar with thienyl or *para*-substituted-phenyl instead of unsubstituted-phenyl generally causes an increase in the activity against *P. aeruginosa*.

Furthermore, with MIC values of 6.25–50 μg/ml, all the compounds indicated notable activity against *C. albicans*, except for **8** (MIC value of 100 μg/ml). Only compound **4** was more active than the other tested compounds bearing an MIC value of 6.25 μg/ml and showed the same activity with control drug clotrimazole against *C. albicans*.

The SAR of the synthesized compounds revealed that compounds possessing 5-methyl-2-thienyl/4-methylbenzyl-carbonylamino groups at position 5 of 2-phenylbenzoxazole or 4-chlorobenzyl-carbonylamino group at position 5 of the fused heterocyclic system and a chlorine substituent at the *para* position of the 2-benzyl moiety of the benzoxazole enhanced antifungal activity against *C. krusei*. On the other side, all the compounds showed very significant activity with MIC values of 3.12–50 μg/ml against *C. glabrata*, except compound **28**. The compound **19**, 2-phenyl-5-[(4-bromobenzyl)carbonylamino]benzoxazole, was found to have the same activity with standard drug, miconazol (MIC value of 3.12 μg/ml). Besides, newly synthesized compound **3** undeniably showed the activity with an MIC value of 6.25 μg/ml, as well.

In conclusion, in an effort to discover new 5-carbonylaminobenzoxazole analogues with antibacterial activity we found that compounds **4**, **8**, and **31** showed notable antibacterial activity. The compounds **3**, **4**, **19**, **23**, and **33** were found to be very significantly active against *Candida* spp. Specially, compounds **4** for *C. albicans* and **19** for *C. glabrata* showed the same antifungal activity with control drugs clotrimazole and miconazol, respectively. The results of the present study indicated that compounds **3**, **4**, **8**, **19**, **23**, **31**, and **33** might be of interest for the identification of new antimicrobial molecules.

Especially, it could be noticed that most of the compounds were more active as antifungals than as antibacterials, which could guide us to design further new lead antifungal compounds.

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