Synthesis and Antimicrobial Activity of Novel Benzoxazoles

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A series of 2-(*p*-substituted-benzyl)-5-[[4-(*p*-chloro/fluoro-phenyl)piperazin-1-yl]acetamido]-benzoxazoles were synthesized in need of new compounds for the fight against microbial pathogens. Their structures were elucidated by spectral techniques. These new derivatives, along with previously synthesized 2-(*p*-substituted-benzyl)-5-substituted-benzoxazoles, were evaluated for their antibacterial and antifungal activities against standard strains and drug-resistant isolates in comparison with ampicillin, gentamicin sulfate, ofloxacin, vancomycin, fluconazole, and amphotericin B trihydrate. The minimum inhibitory concentration (MIC) of each compound was determined by a two-fold serial dilution technique. The compounds were found to possess a broad spectrum of antimicrobial activities with MIC values of 32–256 μg/ml. Although standard drugs were more active against the pathogenes employed in this study, the activities of the new benzoxazoles and reference drugs against drug-resistant isolates of the microorganisms were largely similar.

Key words: Benzoxazoles, Antibacterial Activity, Antifungal Activity

Introduction

The fight against bacterial infections has resulted in the development of a wide variety of antibiotics. After years of misuse of antibiotics, bacteria have become antibiotic-resistant, resulting in a potential global health crisis. Infectious diseases due to Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VREF), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) are the leading causes of morbidity and mortality today (Moustafa *et al.*, 2004). Besides, during the past 20 years an increase in invasive fungal infections, particularly in immunosuppressed patients, has been observed which are now considered to be causes of morbidity and mortality as well. Therefore, there is still need for new antifungal and antibacterial agents (Andriole, 1999). Benzoxazoles are the structural isosteres of natural nucleotides and interact easily with the biopolymers so that they constitute an important class of heterocyclic compounds with antimicrobial and antibiotic activity (Prudhomme *et al.*, 1986; Sarma *et al.*, 2003; Haansuu *et al.*, 2001; Temiz-Arpaci *et al.*, 2002, 2005; Yildiz-Oren *et al.*, 2004; Tekiner-Gulbas *et al.,* 2007). The 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 (Prudhomme *et al.*, 1986).

In the last seven years we have described the synthesis of different derivatives of some 2,5-disubstituted benzoxazoles and their *in vitro* antimicrobial activity against some Gram-positive and Gram-negative bacteria and the fungus *Candida albicans* (Temiz-Arpaci *et al.*, 2005, 2008; Oksuzoglu *et al.*, 2007, 2008; Arisoy *et al.*, 2008). In the present study, a new series of 2-(*p*-substituted-benzyl)-5-[[4-(*p*-chloro/fluoro-phenyl)-piperazin-1-yl]acetamido]-benzoxazoles, **3**–**12**, has been synthesized as target compounds and evaluated for their antibacterial and antifungal activities, along with previously synthesized benzoxazole derivatives, against standard strains and drug-resistant isolates in comparison with several reference drugs. Furthermore, structure-activity relationships (SAR) are discussed.

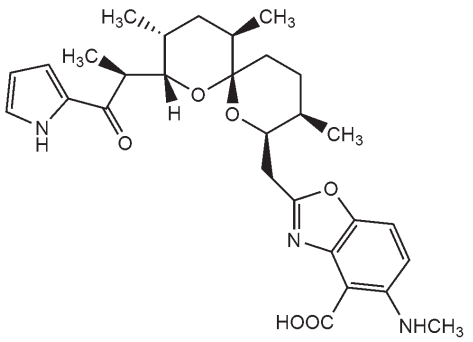


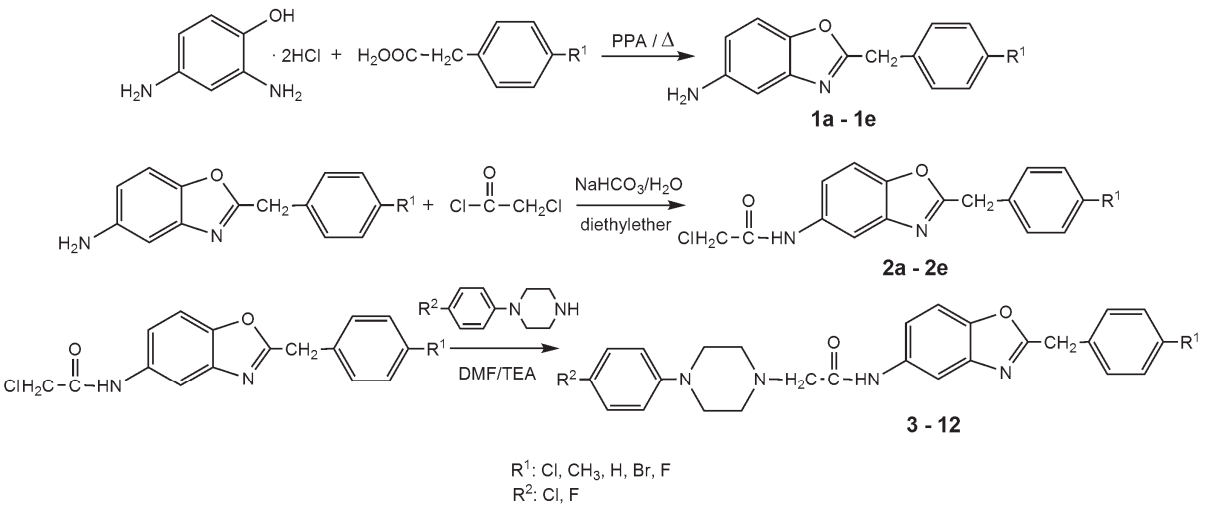
Fig. 1. Chemical structure of calcimycin.

Results and Discussion

We aimed at enhancing the antimicrobial activity of 2,5-disubstituted benzoxazoles by expanding the substituent at the C-5 position. To this end, some new 2-(*p*-substituted-benzyl)-5-[[4-(*p*-chloro/fluoro-phenyl)piperazin-1-yl]acetamido]-benzoxazoles, **3**–**12**, were synthesized. The synthetic route for preparation of the target compounds is shown in Scheme 1. First the 5-amino-2-(*p*-substituted-benzyl)-benzoxazoles **1a**–**1e** were obtained by heating appropriate acids with 2,4-diaminophenol in polyphosphoric acid (PPA). The amides **2a**–**2e** were then obtained through the reaction of 5-amino-2-(*p*-substituted-benzyl)-benzoxazoles with chloroacetyl chloride. In the last step the newly synthesized compounds **3**–**12** were prepared from the amides by treating them with 4-substituted piperazine derivatives. Their structures were elucidated by mass and NMR spectroscopy, respectively, and their purity was analysed through elemental analysis (Table I). The compounds were also evaluated for their antimicrobial activity along with previously synthesized benzoxazole derivatives (Table II).

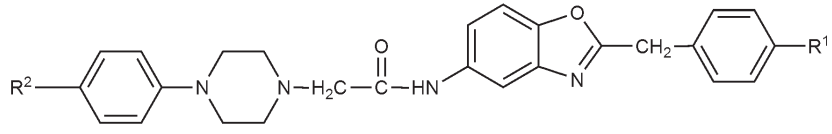
Compounds **3**–**12** had same but low antibacterial activity against the bacteria *S. aureus* and *E. faecalis* with minimum inhibitory concentration (MIC) values between 128–256 μg/ml which are higher than those of the standards ampicillin, gentamicin, ofloxacin, and vancomycin. Nevertheless, these compounds had enhanced activity against the drug-resistant isolates of these bacteria, in the range of a MIC value of 64 μg/ml of ampicillin against MRSA (*Staphylococcus aureus* isolate resistant to methicillin), except for compound **8** which was as potent as gentamicin and vancomycin against the isolate of *E. faecalis* with a MIC value of 32 μg/ml. The new benzoxazole derivatives possessed low antibacterial activity against *B. subtillis* and its drug-resistant isolate with MIC values of 128 μg/ml.

Compounds **3**–**12** provided moderate activity against *P. aeruginosa* and its drug-resistant isolate with MIC values of 64 μg/ml. They had the same activity like ofloxacin against the drug-resistant isolate of *P. aeruginosa*. All derivatives **3**–**32** possessed low activity against the standard strains of *E. coli* and *K. pneumoniae* in comparison with the standard drugs and, in general, the activities of the previously synthesized derivatives **13**–**32**



Scheme 1. Synthetic pathway of the target compounds **3**–**12**.

Table I. Physical and spectral data of the newly synthesized benzoxazole derivatives **3**–**12**.



Compound	R^1	R^2	M.p. [°C]	Yield (%)	^{1}H NMR (δ in ppm, J in Hz)	^{13}C NMR (δ in ppm)	*m/e* (%X) (M+H)	Formula Calculated Found
3	Cl	Cl	150–153	83	2.66–2.68 (4H, t), 3.21 (6H, s), 4.35 (2H, s), 6.94–6.97 (2H, d, J_o = 9.2), 7.22–7.24 (2H, d, J_o = 8.8), 7.42 (4H, s), 7.53–7.56 (H, dd, J_o = 8.8, J_m = 2.0), 7.59–7.61 (H, d, J_o = 8.4), 8.08–8.08 (H, d, J_m = 1.6), 9.92 (H, s)	34.07, 48.55, 53.21, 62.32, 110.91, 110.97, 117.51, 118.00, 122.96, 129.28, 131.68, 132.49, 134.84, 136.14, 141.61, 147.26, 150.48, 166.37, 168.81	495(100) 497(70) 499(15)	$C_{26}H_{24}Cl_2N_4O_2$ C 63.04, H 4.88, N 11.31 C 63.23, H 5.07, N 11.71
4	Cl	F	131–134	76	2.67–2.69 (4H, t), 3.15–3.17 (4H, t), 3.22 (2H, s), 4.35 (2H, s), 6.94–7.07 (4H, m), 7.42 (4H, s), 7.53–7.56 (H, dd, J_o = 8.8, J_m = 2.0), 7.59–7.62 (H, d, J_o = 8.8), 8.08–8.09 (H, d, J_m = 2.0), 9.92 (H, s)		479(100) 481(37)	$C_{26}H_{24}ClFN_4O_2$ C 65.20, H 5.05, N 11.70 C 65.18, H 5.23, N 11.81
5	CH_3	Cl	148–152	75	2.25 (3H, s), 2.63–2.65 (4H, t), 3.19 (6H, s), 4.24 (2H, s), 6.92–6.94 (2H, d, J_o = 8.8), 7.12–7.14 (2H, d, J_o = 8), 7.19–7.23 (4H, m), 7.49–7.52 (H, dd, J_o = 8.4, J_m = 2.0), 7.55–7.57 (H, d, J_o = 8.4), 8.04–8.04 (H, d, J_m = 1.6), 9.89 (H, s)		475(100) 477(37)	$C_{27}H_{27}ClN_4O_2$ C 68.27, H 5.73, N 11.80 C 68.00, H 5.68, N 11.99
6	CH_3	F	148–153	61	2.28 (3H, s), 2.66–2.69 (4H, t), 3.14–3.17 (4H, t), 3.21 (2H, s), 4.26 (2H, s), 6.94–6.97 (2H, m), 7.03–7.07 (2H, m), 7.15–7.16 (2H, d, J_o = 7.6), 7.23–7.25 (2H, d, J_o = 8), 7.51–7.54 (H, dd, J_o = 8.8, J_m = 2.0), 7.57–7.59 (H, d, J_o = 8.8), 8.07–8.07 (H, d, J_m = 2.0), 9.91 (H, s)	21.30, 34.49, 49.53, 53.39, 62.36, 110.91, 115.81, 116.03, 117.76, 117.83, 117.88, 129.54, 129.89, 132.75, 136.09, 136.85, 141.71, 147.29, 148.60, 155.49, 157.84, 166.87, 168.82	459(100)	$C_{27}H_{27}FN_4O_2$ C 70.72, H 5.94, N 12.22 C 71.12, H 5.67, N 12.26
7	H	Cl	118–122	85	2.66–2.68 (4H, t), 3.21 (6H, s), 4.32 (2H, s), 6.94–6.96 (2H, d, J_o = 8.8), 7.22–7.38 (7H, m), 7.52–7.55 (H, dd, J_o = 9.2, J_m = 2.0), 7.58–7.60 (H, d, J_o = 8.8), 8.08–8.08 (H, d, J_m = 2.0), 9.90 (H, s)		461(100) 463(37)	$C_{26}H_{25}ClN_4O_2$ C 67.75, H 5.47, N 12.15 C 67.50, H 5.15, N 11.97
8	H	F	95–100	73	2.67–2.69 (4H, t), 3.15–3.17 (4H, t), 3.22 (2H, s), 4.32 (2H, s), 6.94–7.07 (4H, m), 7.28–7.38 (5H, m), 7.53–7.56 (H, dd, J_o = 8.8, J_m = 1.6), 7.58–7.61 (H, d, J_o = 8.8), 8.09–8.10 (H, d, J_m = 1.2), 9.90 (H, s)	34.88, 49.53, 53.39, 62.37, 110.94, 115.81, 116.03, 117.76, 117.83, 117.93, 127.73, 129.34, 129.68, 135.84, 136.13, 141.71, 147.31, 148.58, 155.50, 157.84, 166.69, 168.83	444(100)	$C_{26}H_{25}FN_4O_2$ C 70.25, H 5.67, N 12.60 C 70.34, H 5.64, N 12.38
9	F	Cl	130–134	74	2.66–2.69 (4H, t), 3.22 (6H, s), 4.33 (2H, s), 6.94–6.97 (2H, d, J_o = 8.8), 7.17–7.25 (4H, m), 7.41–7.45 (2H, m), 7.54–7.57 (H, d, J_o = 9.2), 7.60–7.62 (H, d, J_o = 8.4), 8.10 (H, s), 9.93 (H, s)		479(100) 481(37)	$C_{26}H_{24}ClFN_4O_2$ C 65.20, H 5.05, N 11.70 C 65.29, H 4.71, N 11.53

Table I continued.

Compound	R^1	R^2	M.p. [°C]	Yield (%)	^{1}H NMR (δ in ppm, J in Hz)	^{13}C NMR (δ in ppm)	*m/e* (%X) (M+H)	Formula Calculated Found
10	F	F	96–99	72	2.67–2.69 (4H, t), 3.14–3.17 (4H, t), 3.21 (2H, s), 4.33 (2H, s), 6.94–6.98 (2H, m), 7.03–7.07 (2H, m), 7.17–7.21 (2H, m), 7.41–7.44 (2H, m), 7.53–7.56 (H, dd, J_o = 9.2, J_m = 2.0), 7.59–7.61 (H, d, J_o = 8.4), 8.08–8.08 (H, d, J_m = 2.0), 9.91 (H, s)		463(100)	$C_{26}H_{24}F_2N_4O_2$ C 67.52, H 5.23, N 12.11 C 67.24, H 5.01, N 11.83
11	Br	Cl	158–160	78	2.66–2.68 (4H, t), 3.22 (6H, s), 4.33 (2H, s), 6.94–6.96 (2H, d, J_o = 8.8), 7.22–7.24 (2H, d, J_o = 8.8), 7.34–7.36 (2H, d, J_o = 8.4), 7.54–7.62 (4H, m), 8.09–8.10 (H, d, J_m = 2.0), 9.93 (H, s)	33.38, 47.80, 52.46, 61.55, 110.19, 116.73, 117.25, 120.23, 122.21, 128.51, 131.26, 131.44, 134.48, 135.38, 140.86, 146.51, 149.71, 165.51, 168.04	539(77) 541(100) 543(27)	$C_{26}H_{24}BrClN_4O_2$ C 57.85, H 4.48, N 10.38 C 57.90, H 4.32, N 10.33
12	Br	F	136–139	80	2.67–2.69 (4H, t), 3.15–3.17 (4H, t), 3.22 (2H, s), 4.33 (2H, s), 6.94–6.98 (2H, m), 7.03–7.07 (2H, m), 7.34–7.36 (2H, d, J_o = 8), 7.54–7.61 (4H, m), 8.08–8.09 (H, d, J_m = 1.6), 9.92 (H, s)		523(100) 525(100)	$C_{26}H_{24}BrFN_4O_2$ C 59.66, H 4.62, N 10.70 C 59.38, H 4.43, N 10.47

were higher than those of the new benzoxazoles. The activities of all compounds against the isolates of these bacteria were as high as those of ampicillin and better than those of gentamicin.

The comparison of the activities of the new benzoxazoles with those of the antifungal drugs fluconazole and amphothericin B showed that the newly synthesized compounds were less inhibitory against *C. albicans* and *C. krusei* with MIC values between 64 μg/ml and 256 μg/ml.

It can be concluded that different substituents at the C-5 position of the 2-(*p*-substituted-benzyl)-benzoxazole nucleus provided similar antimicrobial activity. The activities of standard drugs against standard strains were higher than those of the benzoxazole derivatives, whereas in most cases the MIC values of standard drugs against the isolates of the bacteria were quite similar to those of the benzoxazoles.

Experimental

Materials and methods

Chemicals and solvents were purchased from Sigma-Aldrich (Taufkirchen, Germany) and Fisher Scientific (Pittsburgh, PA, USA) and were used without further purification. Silica gel HF_{254} chromatoplates (0.3 mm) were used for thin layer chromatography (TLC), and the mobile phase was chloroform/methanol (10:0.5, v/v) for compounds **3**–**12**. Melting points (M.p.) were recorded on a Stuart Scientific SMP 1 (Bibby Scientific Limited, Staffordshire, UK) instrument and are uncorrected. NMR spectra were recorded on a Varian (Palo Alto, CA, USA) Mercury 400 MHz NMR spectrometer in $CDCl_3$ or dimethylsulfoxide (DMSO-d_6); tetramethylsilane (TMS) was used as an internal standard. The mass spectra were recorded on a Waters (Milford, MA, USA) ZQ Micromass LC-MS spectrometer using the ESI(+) method. Elemental analyses were performed on an LECO 932 CHNS (St. Joseph, MI, USA) instrument and were within ± 0.4% of theoretical values.

Materials used in the microbiology study were; Mueller Hinton agar (MHA) (Merck, Darmstadt, Germany), Mueller Hinton broth (MHB) (Merck), Sabouraud dextrose agar (SDA) (Merck), RPMI-1640 medium with L-glutamine (Sigma-Aldrich), 3-(*N*-morpholino)-propane-sulfonic acid (MOPS) (Sigma-Aldrich), 96-well microplates (BD, Franklin Lakes, NJ, USA), transfer pipette (Eppendorf, Hamburg, Germany), am-

Table II. *In vitro* antimicrobial activities of newly and previously synthesized benzoxazole derivatives in comparison with reference drugs.

Compound	R^1	R^2	MIC [μg/ml]													
			Gram-negative bacteria						Gram-positive bacteria						Fungi	
			E.c.	E.c.*	K.p.	K.p.*	P.a.	P.a.*	S.a.	S.a.*	E.f.	E.f.*	B.s.	B.s.*	C.a.	C.k.
3	(*p*-Cl)Ph-N	Cl	128	128	128	128	64	64	128	64	128	64	128	128	64	64
4	(*p*-F)Ph-N	Cl	128	128	128	128	64	64	128	64	128	64	128	128	64	64
5	(*p*-Cl)Ph-N	CH_3	128	128	128	128	64	64	128	64	128	64	128	128	128	64
6	(*p*-F)Ph-N	CH_3	128	128	128	128	64	64	256	64	128	64	128	128	64	64
7	(*p*-Cl)Ph-N	H	128	128	128	128	64	64	256	64	128	64	128	128	128	64
8	(*p*-F)Ph-N	H	128	128	128	128	64	64	128	64	128	32	128	128	128	64
9	(*p*-Cl)Ph-N	F	128	128	128	128	64	64	256	64	128	64	128	128	256	64
10	(*p*-F)Ph-N	F	128	128	128	128	64	64	128	64	128	64	128	128	64	64
11	(*p*-Cl)Ph-N	Br	128	128	128	128	64	64	128	64	128	64	128	128	128	256
12	(*p*-F)Ph-N	Br	128	128	128	128	64	64	128	64	128	64	128	128	128	64
13	O	Cl	128	128	128	128	64	64	128	64	128	64	128	128	64	64
14	CH_2	Cl	64	128	64	128	64	64	32	64	128	64	128	128	128	64
15	CH_3-N	Cl	64	128	128	128	64	64	128	64	128	64	128	128	64	64
16	Ph-N	Cl	64	128	64	128	64	64	128	64	128	64	128	256	64	64
17	O	CH_3	128	128	128	128	64	64	128	64	128	64	128	128	32	64
18	CH_2	CH_3	64	128	64	128	64	64	64	64	64	64	128	128	64	64
19	CH_3-N	CH_3	64	128	64	128	64	64	64	64	128	64	128	256	64	64
20	Ph-N	CH_3	64	128	64	128	64	64	128	64	128	64	128	256	64	64
21	O	H	128	128	128	128	64	64	128	64	128	64	128	128	128	64
22	CH_2	H	64	128	64	128	64	128	128	64	128	64	128	128	128	64
23	CH_3-N	H	128	128	128	128	64	128	256	64	128	64	128	128	128	64
24	Ph-N	H	32	128	64	128	64	128	128	64	128	64	128	128	64	64
25	O	Br	64	128	128	128	64	128	128	64	128	64	128	128	128	64
26	CH_2	Br	64	128	128	128	64	128	128	64	128	64	128	128	128	64
27	CH_3-N	Br	64	128	64	128	64	128	128	64	128	64	128	128	128	64
28	Ph-N	Br	64	128	128	128	64	128	256	64	128	64	128	128	128	64
29	O	F	32	128	64	128	64	64	128	64	128	64	128	128	128	64
30	CH_2	F	128	128	128	128	64	64	128	64	128	64	128	128	128	64
31	CH_3-N	F	32	128	128	128	64	64	128	64	128	64	128	128	128	64
32	Ph-N	F	64	128	64	128	64	128	128	64	128	64	128	128	64	64
Ampicillin			2	128	2	128	n.d.	n.d.	2	64	2	2	0.5	0.5	n.d.	n.d.
Gentamicin			0.5	>512	0.5	256	0.5	>512	0.125	32	4	32	0.25	0.125	n.d.	n.d.
Ofloxacin			<0.0625	64	0.125	0.5	8	64	0.25	0.25	1	4	0.125	0.25	n.d.	n.d.
Vancomycin			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	1	1	32	n.d.	n.d.	n.d.	n.d.
Fluconazole			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1	32
Amphothericin B			n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.25	2

n.d., not determined (microbiological assays were not performed due to following reasons: *P. aeruginosa* is naturally resistant to ampicillin; Gram-negative bacteria employed in the study are naturally resistant to vancomycin; vancomycin is not used in the therapy against *B. subtilis*; antibacterial drugs were not assayed against fungi; antifungal drugs were not assayed against bacteria).

E.c., *E. coli* ATCC 25922; E.c.*, *E. coli* isolate (ESBL); K.p., *K. pneumoniae* RSKK 574; K.p.*, *K. pneumoniae* isolate (ESBL); P.a., *P. aeruginosa* ATCC 25853; P.a.*, *P. aeruginosa* isolate (resistant to gentamicin); S.a., *S. aureus* ATCC 29213; S.a.*, *S. aureus* isolate (MRSA); E.f., *E. faecalis* ATCC 29212; E.f.*, *E. faecalis* isolate (VRE); B.s., *B. subtillis* ATCC 6633; B.s.*, *B. subtillis* isolate (resistant to ceftriaxon); C.a., *C. albicans* ATCC 10231; C.k., *C. krusei* ATCC 6258.

picillin (Mustafa Nevzat Pharmaceuticals, Istanbul, Turkey), gentamicin sulfate (Paninkret, Pinneberg, Germany), ofloxacin (Zhejiang Huangyan East Asia Chemical, Huangyan, Zhejiang, China), vancomycin (Mayne Pharma, Salisbury South, SA, Australia), fluconazole (Sigma-Aldrich), amphotericin B trihydrate (Riedel de Haen, Seelze, Germany), DMSO (Riedel de Haen).

Microorganisms used in the assay were; *Klebsiella pneumoniae* isolate [extended *β*-lactamase spectrum (ESBL)], *Escherichia coli* isolate (ESBL), *Enterococcus faecalis* isolate [resistant to vancomycin (VRE)], *Bacillus subtillis* isolate (resistant to ceftriaxon), *Pseudomonas aeruginosa* isolate (resistant to gentamicin), and *Staphylococcus aureus* isolate [resistant to methicillin (MRSA)], *K. pneumoniae* RSKK 574, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 25853, *B. subtillis* ATCC 6633, *S. aureus* ATCC 29213, *Candida albicans* ATCC 10231, and *Candida krusei* ATCC 6258. Reference strains and clinical isolates were obtained from Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Culture Collection (Ankara, Turkey) and Gazi University Hospital, Microbiology Laboratory (Ankara, Turkey), respectively.

*General procedure for the preparation of 5-amino-2-(p-substituted-benzyl)-benzoxazoles (**1a–1e**)*

The 5-amino-2-(*p*-substituted-benzyl)-benzoxazoles were synthesized by heating 0.02 mol 2,4-diaminophenol hydrochloride with 0.02 mol *p*-substituted phenyl acetic acid in 25 g polyphosphoric acid (PPA) and stirring for 1–2 h. At the end of the reaction period, the residue was poured into an ice/water mixture, and the solution was neutralized with 10% NaOH. The resulting precipitate was filtered, washed with distilled water, dissolved in boiling ethanol with 0.2 g charcoal, and filtered off. Then distilled water was added slowly to the filtrate in order to stimulate crystallization. The crude compounds **1a**–**1e** were obtained by filtering and drying the crystalline material (Yildiz-Oren *et al.*, 2004; Oksuzoglu *et al.*, 2007, 2008; Temiz-Arpaci *et al.*, 2008; Arisoy *et al.*, 2008).

*General procedure for the preparation of 5-(2-chloroacetamido)-2-(p-substituted-benzyl)-benzoxazoles (**2a–2e**)*

Chloroacetyl chloride (0.02 mol) was added over a period of 1 h to a stirred, ice-cooled mixture of 5-amino-2-(*p*-substituted-benzyl)-benzoxazole (0.02 mol), sodium bicarbonate (0.02 mol), diethyl ether (40 ml), and water (20 ml). The mixture was continuously stirred overnight at room temperature and filtered. The precipitate was washed with water, 2 M HCl, and water, respectively, and the crude product was obtained by drying needles *in vacuo* (Arisoy *et al.*, 2008).

*General procedure for the preparation of 2-(p-substituted-benzyl)-5-[[4-(p-chloro/fluoro-phenyl)piperazin-1-yl]acetamido]-benzoxazoles (**3–12**)*

0.002 mol 5-(2-chloroacetamido)-2-(*p*-substituted-benzyl)-benzoxazole were added to 0.002 mol *N*-(*p*-chloro/fluoro-phenyl)piperazine and 0.006 mol triethylamine (TEA) solution in 3.5 ml *N*,*N*-dimethylformamide (DMF). The mixture was stirred at room temperature for 24 h. At the end of the reaction time, 5 ml toluene were added, and the reaction medium was evaporated under reduced pressure. The residue was dissolved in chloroform and washed three times with 5% NaOH and then once with distilled water. The solution was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure. The residue was dissolved in ethyl acetate and precipitated by adding *n*-hexane. If necessary, recrystallization was performed. Crystalline material was dried *in vacuo*. All the compounds **3**–**12** were prepared as original products. Their structures were supported by spectral data. The mass and ^{1}H NMR spectra, respectively, and the results of the elemental analyses agree with those of the proposed structures. ^{13}C NMR spectra were obtained only for compounds **3**, **6**, **8**, **11**. Physical and spectral data of the compounds are reported in Table I.

Microbiological assays

For microbiological assays, ampicillin, gentamicin sulfate, ofloxacin, vancomycin, fluconazole, and amphotericin B trihydrate were dissolved in appropriate solvents recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2006, 2008). Stock solutions of the test compounds were prepared in DMSO.

Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S18 (CLSI, 2008). MHB was added to each well of the microplates. The bacterial suspensions used for inoculation were prepared at 10^5 CFU/ml by

diluting fresh cultures at McFarland 0.5 density (10^7 CFU/ml). Two-fold diluted solutions of the compounds were inoculated with bacterial suspensions of 10^5 CFU/ml (10 μl inoculum per well to give 10^4 CFU/ml bacteria in the wells), and the microplates were incubated overnight at 37 °C.

Fungal susceptibility testing was performed according to the guidelines of CLSI M27-A3 (CLSI, 2006). RPMI-1640 medium with L-glutamine buffered to pH 7 with MOPS was added to each well of the microplates. The colonies were suspended in sterile saline, and the resulting suspension was adjusted to McFarland 0.5 density (10^6 CFU/ml). A working suspension was prepared by an 1:100 dilution followed by an 1:20 dilution of the stock suspension. 10 μl of this suspension at 10^3 CFU/ml were added to the two-fold diluted solution of the compounds, and microplates were incubated for 24–48 h at 35 °C.

After incubation, the lowest concentration of the compounds that completely inhibited visible growth was determined and reported as minimum inhibitory concentration (MIC). Control wells contained all components, except the tested compounds, and all experiments were done in triplicate.

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