Synthesis and Antimicrobial Evaluation of 2-(*p*-Substituted Phenyl)-5-[(4-substituted piperazin-1-yl)acetamido]-benzoxazoles

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A series of 2-(*p*-substituted phenyl)-5-(2-{4-[(*p*-chloro-fluorophenyl)/phenyl] piperazin-1-yl}-acetamido)-benzoxazoles were synthesized and tested for their antimicrobial activities. The structures of the new derivatives were elucidated by spectral techniques. The minimum inhibitory concentrations (MIC) of the new benzoxazoles, along with those of previously synthesized analogues, were determined against standard bacterial and fungal strains and drug-resistant isolates, and compared with those of several reference drugs. The new benzoxazole derivatives were found to possess a broad spectrum of antimicrobial activity with MIC values of 32 – 1024 μg/ml. Although the standard drugs were more active against the tested pathogens, the activities of the new benzoxazoles and the reference drugs were largely similar against the drug-resistant isolates.

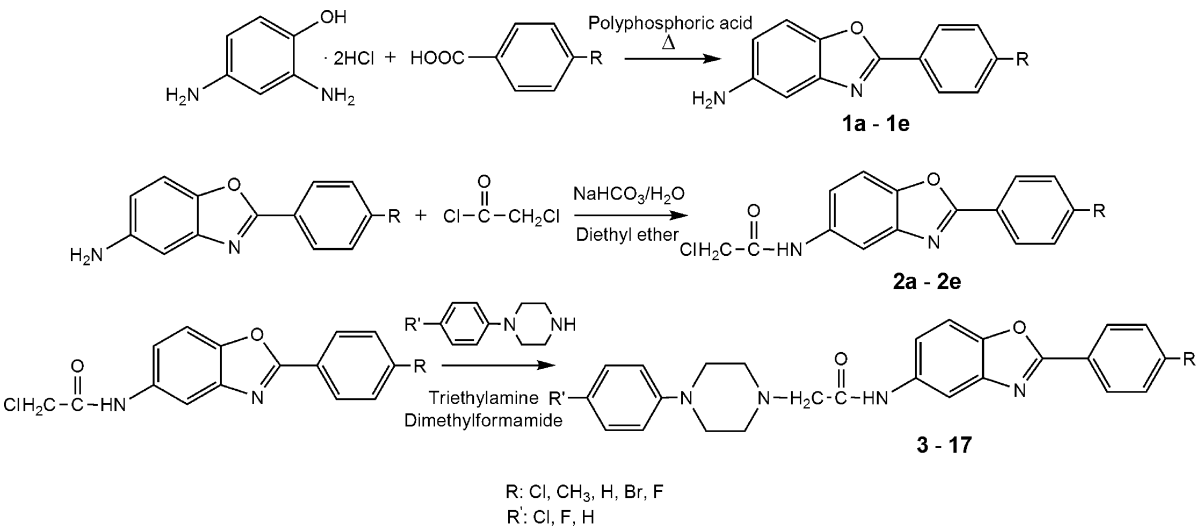
Key words: Benzoxazole, Piperazine, Antimicrobial Activity

Introduction

Microbial infectious diseases continue to be one of the leading causes of morbidity and mortality. It has been estimated that microbial species comprise about 60% of the Earth's biomass. This, together with the fact that their genetic, metabolic, and physiological diversities are extraordinary, makes them a major threat to health across the world (Radulovic *et al*., 2013). Antibiotics and antimicrobial agents are still the most potent weapons to fight bacterial infections, but the evolution of resistance has increasingly been becoming problematic both in hospitals and in agriculture (Betts *et al*., 2013). Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VREF), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) are important 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 the causes of morbidity and mortality as well. Thus, new drug classes are urgently needed (Chopra, 2013).

Benzoxazoles, structural isosteres of natural nucleotides that can interact with biopolymers, constitute an important class of heterocyclic compounds with antimicrobial and antifungal activity (Prudhomme *et al*., 1986; Haansuu *et al*., 2001; Sarma *et al*., 2003; Temiz-Arpaci *et al*., 2002, 2013). Recently, we have described the synthesis of some 2-(*p*-substituted benzyl/phenyl)-5-[2-(4-substituted piperazin-1-yl)acetamido]-benzoxazoles and their *in vitro* antimicrobial activity against some Gram-positive and Gram-negative bacteria as well as the fungus *Candida albicans* (Temiz-Arpaci *et al*., 2005; Arisoy *et al*., 2008, 2012).

In this study, a new series of 2-(*p*-substituted phenyl)-5-(2-{4-[(*p*-chloro-fluorophenyl)/phenyl]piperazin-1-yl}acetamido)-benzoxazoles, **3** – **17**, has been synthesized using a three-step procedure as shown in



Scheme 1. Synthesis of compounds **3** – **17**.

Scheme 1. In comparison with several control drugs and previously synthesized benzoxazole compounds with a homologous structure, the newly synthesized compounds were evaluated for their antibacterial and antifungal activities against standard strains and drug-resistant isolates.

Materials and Methods

Chemicals and analytical methods

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

Materials for microbiology

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), ampicillin (Mustafa Nevzat Pharmaceuticals, Istanbul, Turkey), gentamicin sulfate (Paninkret Chem.-Pharm., Pinneberg, Germany), ofloxacin (Zhejiang Huangyan East Asia Chemical Co. Ltd., Huangyan, Zhejiang, China), vancomycin (Mayne Pharma, Salisbury South, SA, Australia), fluconazole (Sigma-Aldrich), amphotericin B trihydrate (Riedel de Haen, Seelze, Germany), dimethylsulfoxide (DMSO) (Riedel de Haen). Microorganisms used in the assay were; *Escherichia coli* isolate [has extended spectrum beta lactamase (ESBL) enzyme], *Enterococcus faecalis* isolate [resistant to vancomycin (VRE)], *Pseudomonas aeruginosa* isolate [resistant to gentamicin], and *Staphylococcus aureus* isolate [resistant to methicillin (MRSA)], *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 25853, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 10231. Clinical isolates and reference strains were procured from Gazi University Hospital Microbiology Laboratory (Ankara, Turkey) and from the culture collection

of Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, respectively.

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

5-Amino-2-(*p*-substituted phenyl)-benzoxazoles were synthesized by heating 0.02 mol 2,4-diaminophenol-hydrochloride with 0.02 mol *p*-substituted benzoic acid in 25 g polyphosphoric acid (PPA) and stirring for about 1 h at 160 – 200 °C. At the end of the reaction period, the residue was poured over ice, and the solution was neutralized with 10% NaOH. The resulting precipitate was filtered off, washed with distilled water, dissolved in boiling ethanol with 0.2 g charcoal, and again filtered off. Crystallization was achieved by dissolving the precipitate in ethanol and adding distilled water. The crude compounds **1a – 1e** were obtained by filtration and drying the filtrate under ambient conditions (Arisoy *et al.*, 2008).

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

Chloroacetyl chloride (0.02 mol) was added over a period of 1 h to a stirred, ice-cooled mixture of a 5-amino-2-(*p*-substituted phenyl)-benzoxazole derivative (0.02 mol), sodium bicarbonate (0.02 mol), diethyl ether (40 ml), and water (20 ml). The mixture was stirred overnight. The precipitate formed was filtered off, washed with water, and dissolved in ethanol. Crystallization was done by adding distilled water, and the crude product was obtained by drying the filtrate under ambient conditions (Arisoy *et al.*, 2008).

*General procedure for the preparation of 2-(p-substituted phenyl)-5-(2-{4-[(p-chloro-fluorophenyl)/phenyl]piperazin-1-yl}acetamido)-benzoxazoles **3 – 17***

0.002 mol 5-(2-chloroacetamido)-2-(*p*-substituted phenyl)-benzoxazole derivative was added to a mixture of 0.002 mol *N*-[(*p*-chloro-fluorophenyl)/phenyl] piperazine and 2 ml of triethylamine solution in 3 ml of *N*,*N*-dimethylformamide (DMF) and 2 ml of ethanol. The mixture was stirred at room temperature for 24 h. At the end of the reaction time, the mixture was poured over ice, an equal volume of 5% (w/v) of aqueous NaOH solution was added, and the resulting mixture extracted with chloroform. The solvent was evaporated under reduced pressure, and the resulting crude product was purified by column chromatography using chloroform as mobile phase. Finally, the chloroform fractions were collected, the solvent evaporated, and crystallization was achieved by dissolving the residue in chloroform and adding petroleum ether. The crystalline material was dried *in vacuo*.

Microbiological assays

Stock solutions of the test compounds were prepared in DMSO. Bacterial susceptibility tests were 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 10^6 CFU/ml by diluting fresh cultures at a McFarland density of 0.5. Suspensions of the bacteria at 10^6 CFU/ml were inoculated into the two-fold diluted solution of the respective test compound. A 10-μl bacterial inoculum was added to each well of the microplates. There were 10^5 CFU/ml bacteria in the wells after inoculation. Microplates were incubated at 37 °C overnight.

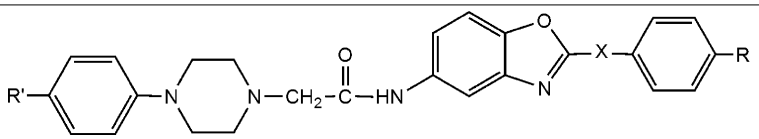
Fungal susceptibility tests were performed according to the guidelines of CLSI M27-A3 (CLSI, 2006). RPMI-1640 medium with L-glutamine, buffered to pH 7 with MOPS, was added to each well of a microplate. The colonies were suspended in sterile saline, and the resulting suspension was adjusted to McFarland 0.5 density (10^6 CFU/ml). A working suspension was prepared by appropriate dilution of the stock suspension. Ten μl of this suspension were inoculated into the two-fold diluted solution of the respective test compound resulting in $5 \cdot 10^2$ CFU/ml in the wells. Microplates were incubated at 35 °C for 24 – 48 h.

After incubation, the lowest concentration of the compounds that completely inhibited macroscopic growth was determined and reported as minimum inhibitory concentration (MIC). All solvents, pure microorganisms, and pure media were used in control wells. All experiments were done in three parallel series.

Results and Discussion

In the present investigation, a new series of 2-(*p*-substituted phenyl)-5-(2-{4-[(*p*-chloro-fluorophenyl)/phenyl]piperazin-1-yl}acetamido)-benzoxazoles were synthesized. Their structures were elucidated by mass and ^{1}H NMR spectroscopy, and their purity was controlled through elemental analysis (Table I). All newly

Table I. Physical and spectral data of the newly synthesized benzoxazole derivatives **3** – **17**.



Compound	R′	R	X	M.p. [°C]	Yield (%)	^{1}H NMR [δ in ppm, J in Hz]	MS (m/z) (%)	Formula Elemental analysis (calculated/found)
3	Cl	Cl	–	217 – 220	37	2.811 – 2.836 (4H, t), 3.249 – 3.272 (6H, t), 6.862 – 6.884 (2H, d, $J_o = 8.8$), 7.228 – 7.250 (2H, d, $J_o = 8.8$), 7.495 – 7.526 (4H, m), 8.065 (H, s), 8.169 – 8.190 (2H, d, $J_o = 8.4$), 9.234 (H, s)	481 [$M^+ + H$] (100), 483 (65), 485 (12)	$C_{25}H_{22}Cl_2N_4O_2 \cdot 0.2H_2O$ C 61.91, H 4.66, N 11.55/ C 61.84, H 4.52, N 11.52
4	Cl	CH_3	–	227 – 230	34	2.439 (3H, s), 2.799 – 2.823 (4H, t), 3.236 – 3.260 (6H, t), 6.852 – 6.874 (2H, d, $J_o = 8.8$), 7.220 – 7.242 (2H, d, $J_o = 8.8$), 7.317 – 7.337 (2H, d, $J_o = 8.0$), 7.507 – 7.510 (2H, m), 8.026 – 8.033 (H, m), 8.117 – 8.138 (2H, d, $J_o = 8.4$), 9.211 (H, s)	461 [$M^+ + H$] (100), 463 (40)	$C_{26}H_{25}ClN_4O_2$ C 67.75, H 5.47, N 12.15/ C 68.15, H 5.44, N 12.34
5	Cl	H	–	226 – 228	47	2.803 – 2.828 (4H, t), 3.241 – 3.265 (6H, t), 6.856 – 6.878 (2H, d, $J_o = 8.8$), 7.223 – 7.245 (2H, d, $J_o = 8.8$), 7.523 – 7.541 (5H, m), 8.057 – 8.060 (H, d, $J_m = 1.2$), 8.234 – 8.259 (2H, m), 9.226 (H, s)	447 [$M^+ + H$] (100), 449 (37)	$C_{25}H_{23}ClN_4O_2$ C 67.18, H 5.19, N 12.54/ C 67.17, H 5.13, N 12.44
6	Cl	F	–	218 – 220	63	2.803 – 2.828 (4H, t), 3.242 – 3.265 (6H, t), 6.855 – 6.877 (2H, d, $J_o = 8.8$), 7.188 – 7.263 (4H, m), 7.516 (2H, s), 8.056 (H, s), 8.222 – 8.257 (2H, m), 9.228 (H, s)	465 [$M^+ + H$] (100), 467 (33)	$C_{25}H_{22}ClFN_4O_2 \cdot 0.2H_2O$ C 64.09, H 4.82, N 11.96/ C 64.16, H 4.87, N 11.89
7	Cl	Br	–	224 – 225	65	2.801 – 2.826 (4H, t), 3.238 – 3.262 (6H, t), 6.851 – 6.874 (2H, d, $J_o = 9.2$), 7.219 – 7.241 (2H, d, $J_o = 8.8$), 7.516 – 7.519 (2H, m), 7.647 – 7.669 (2H, d, $J_o = 8.8$), 8.061 – 8.064 (H, d, $J_m = 1.2$), 8.085 – 8.106 (2H, d, $J_o = 8.4$), 9.227 (H, s)	525 [$M^+ + H$] (83), 527 (100), 529 (26)	$C_{25}H_{22}BrClN_4O_2 \cdot 0.3H_2O$ C 56.52, H 4.29, N 10.55/ C 56.50, H 4.33, N 10.50
8	F	Cl	–	206 – 207	40	2.815 – 2.840 (4H, t), 3.202 – 3.226 (4H, t), 3.256 (2H, s), 6.891 – 6.925 (2H, m), 6.974 – 7.018 (2H, m), 7.486 – 7.525 (4H, m), 8.068 (H, s), 8.158 – 8.181 (2H, d, $J_o = 9.2$), 9.262 (H, s)	465 [$M^+ + H$] (100), 467 (39)	$C_{25}H_{22}ClFN_4O_2$ C 64.58, H 4.77, N 12.05/ C 64.82, H 5.00, N 12.06
9	F	CH_3	–	201 – 203	36	2.444 (3H, s), 2.816 – 2.840 (4H, t), 3.202 – 3.226 (4H, t), 3.255 (2H, s), 6.893 – 6.927 (2H, m), 6.975 – 7.018 (2H, m), 7.322 – 7.342 (2H, d, $J_o = 8.0$), 7.516 – 7.519 (2H, m), 8.027 (H, s), 8.121 – 8.142 (2H, d, $J_o = 8.4$), 9.240 (H, s)	445 [$M^+ + H$] (100)	$C_{26}H_{25}FN_4O_2$ C 70.25, H 5.67, N 12.60/ C 70.60, H 5.89, N 12.68
10	F	H	–	198 – 200	72	2.813 – 2.838 (4H, t), 3.199 – 3.223 (4H, t), 3.255 (2H, s), 6.889 – 6.923 (2H, m), 6.972 – 7.016 (2H, m), 7.523 – 7.547 (5H, m), 8.065 (H, s), 8.233 – 8.258 (2H, m), 9.255 (H, s)	431 [$M^+ + H$] (100)	$C_{25}H_{23}FN_4O_2$ C 69.75, H 5.39, N 13.02/ C 69.56, H 5.18, N 13.11
11	F	F	–	215 – 219	67	2.820 – 2.844 (4H, t), 3.205 – 3.229 (4H, t), 3.260 (2H, s), 6.895 – 6.929 (2H, m), 6.977 – 7.020 (2H, m), 7.194 – 7.237 (2H, m), 7.522 – 7.526 (2H, m), 8.056 (H, s), 8.228 – 8.264 (2H, m), 9.258 (H, s)	449 [$M^+ + H$] (100)	$C_{25}H_{22}F_2N_4O_2$ C 66.95, H 4.94, N 12.49/ C 67.07, H 5.24, N 12.52

Table I. Continued.

Compound	R′	R	X	M.p. [°C]	Yield (%)	^{1}H NMR [δ in ppm, J in Hz]	MS (m/z) (%)	Formula Elemental analysis (calculated/found)
12	F	Br	–	220–223	31	2.820–2.844 (4H, t), 3.205–3.229 (4H, t), 3.260 (2H, s), 6.895–6.929 (2H, m), 6.977–7.021 (2H, m), 7.529–7.533 (2H, m), 7.656–7.678 (2H, d, $J_o = 8.8$), 8.069 (H, s), 8.095–8.116 (2H, d, $J_o = 8.4$), 9.264 (H, s)	509 [$M^+ + H$] (98), 511 (100)	$C_{25}H_{22}BrFN_4O_2$ C 58.95, H 4.35, N 11.00/ C 58.64, H 4.31, N 11.02
13	H	Cl	–	206–207	55	2.822–2.846 (4H, t), 3.260 (2H, s), 3.288–3.312 (4H, t), 6.890–6.926 (H, t), 6.954–6.974 (2H, d, $J_o = 8.0$), 7.281–7.321 (2H, t), 7.492–7.528 (4H, m), 8.073 (H, s), 8.168–8.189 (2H, d, $J_o = 8.4$), 9.280 (H, s)	447 [$M^+ + H$] (100), 449 (37)	$C_{25}H_{23}ClN_4O_2 \cdot 0.3H_2O$ C 66.38, H 5.26, N 12.39/ C 66.48, H 5.10, N 12.42
14	H	CH_3	–	197–199	55	2.441 (3H, s), 2.815–2.840 (4H, t), 3.253 (2H, s), 3.282–3.306 (4H, t), 6.886–6.923 (H, t), 6.950–6.972 (2H, d, $J_o = 8.8$), 7.259–7.339 (4H, m), 7.511–7.514 (2H, m), 8.036 (H, s), 8.121–8.142 (2H, d, $J_o = 8.4$), 9.255 (H, s)	427 [$M^+ + H$] (100)	$C_{26}H_{26}N_4O_2$ C 73.22, H 6.14, N 13.14/ C 73.11, H 5.85, N 13.05
15	H	H	–	202–205	41	2.822–2.846 (4H, t), 3.259 (2H, s), 3.288–3.312 (4H, t), 6.889–6.926 (H, t), 6.955–6.975 (2H, d, $J_o = 8.0$), 7.281–7.321 (2H, t), 7.513–7.555 (5H, m), 8.065 (H, s), 8.238–8.263 (2H, m), 9.270 (H, s)	413 [$M^+ + H$] (100)	$C_{25}H_{24}N_4O_2$ C 72.80, H 5.86, N 13.58/ C 72.61, H 5.60, N 13.46
16	H	F	–	207–210	80	2.818–2.843 (4H, t), 3.256 (2H, s), 3.285–3.309 (4H, t), 6.889–6.925 (H, t), 6.953–6.975 (2H, d, $J_o = 8.8$), 7.190–7.320 (4H, m), 7.520 (2H, s), 8.063 (H, s), 8.227–8.269 (2H, m), 9.273 (H, s)	431 [$M^+ + H$] (100)	$C_{25}H_{23}FN_4O_2$ C 69.75, H 5.39, N 13.02/ C 69.80, H 5.35, N 13.00
17	H	Br	–	212–214	69	2.822–2.847 (4H, t), 3.260 (2H, s), 3.288–3.312 (4H, t), 6.891–6.927 (H, t), 6.955–6.975 (2H, d, $J_o = 8.0$), 7.282–7.322 (2H, t), 7.529 (2H, s), 7.657–7.679 (2H, d, $J_o = 8.8$), 8.076 (H, s), 8.098–8.119 (2H, d, $J_o = 8.4$), 9.280 (H, s)	491 [$M^+ + H$] (88), 493 (100)	$C_{25}H_{23}BrN_4O_2$ C 61.11, H 4.72, N 11.40/ C 61.03, H 4.63, N 11.36

synthesized compounds **3**–**17** were evaluated for their antimicrobial activity in comparison with standard drugs and previously synthesized benzoxazole having homologous structures and the results are presented in Table II.

Compounds **3**–**17** exhibited broad antibacterial activity with MIC values of 128–256 μg/ml against *S. aureus* and the MRSA isolate, and derivative **9** had a MIC value of 64 μg/ml. All derivatives had lower antibacterial activity against the standard strain of this pathogen, whereas they possessed the same or similar MIC values as ampicillin (64 μg/ml) and gentamicin (32 μg/ml) against its drug-resistant isolate, MRSA. The previously synthesized benzoxazole derivatives **18**–**27** were more potent than those synthesized in this study. The newly synthesized compounds exhibited antibacterial activities with MIC values between 32 and 512 μ g/ml against *E. faecalis* and the VRE isolate. The activities of the new benzoxazoles were similar to those of the previously synthesized derivatives. Among the new compounds, **3**, **5**, and **12**–**14** were found as the most potent derivatives with a MIC value of 32 μg/ml against the vancomycin-resistant isolate of *E. faecalis*, having the same potency as the standard drugs gentamicin and vancomycin. All newly synthesized benzoxazole derivatives exhibited antibacterial activity against the Gram-negative bacteria *E. coli* and *P. aeruginosa* and their respective drug-resistant isolates, with MIC values between 32 and 512 μg/ml, except for derivative **17**, which was the least effective compound against gentamicin-resistant *P. aeruginosa* with a MIC value of 1024 μg/ml. On the other hand, many of the new compounds, **3**, **5**, **6**, **12**–**14**, possessed higher activity than the previously synthesized

Table II. *In vitro* antimicrobial activities of the newly synthesized benzoxazole derivatives in comparison with control drugs (MIC in μg/ml). See Table I for the structures of compounds **3**–**17**.

Compound	R′	R	X	Gram-negative bacteria				Gram-positive bacteria				Fungus
				E.c.	E.c.*	P.a.	P.a.*	S.a.	S.a.*	E.f.	E.f.*	C.a.
3	Cl	Cl	–	32	64	64	128	128	128	64	32	128
4	Cl	CH_3	–	256	256	128	128	128	256	512	256	128
5	Cl	H	–	32	256	128	128	256	256	256	32	128
6	Cl	F	–	32	256	128	128	256	256	512	256	128
7	Cl	Br	–	128	256	128	128	256	256	128	64	128
8	F	Cl	–	128	256	128	128	256	256	128	64	128
9	F	CH_3	–	128	128	256	256	64	128	256	64	128
10	F	H	–	128	256	128	256	256	256	512	64	128
11	F	F	–	64	256	128	128	256	256	256	64	128
12	F	Br	–	32	256	128	128	256	256	128	32	128
13	H	Cl	–	32	128	128	128	128	128	128	32	128
14	H	CH_3	–	32	256	256	512	256	256	256	32	128
15	H	H	–	128	256	128	128	256	256	128	64	128
16	H	F	–	128	256	128	128	256	256	256	64	128
17	H	Br	–	128	256	256	1024	128	128	128	64	128
18[a]	Cl	Cl	CH_2	128	128	64	64	128	64	128	64	64
19[a]	F	Cl	CH_2	128	128	64	64	128	64	128	64	64
20[a]	Cl	CH_3	CH_2	128	128	64	64	128	64	128	64	128
21[a]	F	CH_3	CH_2	128	128	64	64	256	64	128	64	64
22[a]	Cl	H	CH_2	128	128	64	64	256	64	128	64	128
23[a]	F	H	CH_2	128	128	64	64	128	64	128	32	128
24[a]	Cl	F	CH_2	128	128	64	64	256	64	128	64	256
25[a]	F	F	CH_2	128	128	64	64	128	64	128	64	64
26[a]	Cl	Br	CH_2	128	128	64	64	128	64	128	64	128
27[a]	F	Br	CH_2	128	128	64	64	128	64	128	64	128
Ampicillin[a]				2	128	n.d.	n.d.	2	64	2	2	n.d.
Gentamicin[a]				0.5	>512	0.5	>512	0.125	32	4	32	n.d.
Ofloxacin[a]				<0.0625	64	8	64	0.25	0.25	1	4	n.d.
Vancomycin[a]				n.d.	n.d.	n.d.	n.d.	1	1	1	32	n.d.
Fluconazole[a]				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1
Amphotericin B[a]				n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.25

E.c., *Escherichia coli* ATCC 25922; E.c.*, *Escherichia coli* isolate (ESBL); P.a., *Pseudomonas aeruginosa* ATCC 25853; P.a.*, *Pseudomonas aeruginosa* isolate (resistant to gentamicin); S.a., *Staphylococcus aureus* ATCC 29213; S.a.*, *Staphylococcus aureus* isolate (MRSA); E.f., *Enterococcus faecalis* ATCC 29212; E.f.*, *Enterococcus faecalis* isolate (VRE); C.a., *Candida albicans* ATCC 10231.

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; antibacterial drugs were not assayed against fungi; antifungal drugs were not assayed against bacteria).

[a] Arisoy *et al.* (2012).

derivatives with a MIC value of 32 μg/ml against *E. coli*. Thus, compounds having a *p*-substituted phenyl group at position 2 of the benzoxazole ring mostly performed better against *E. coli* than those with a *p*-substituted benzyl group at the same position. Among the newly synthesized benzoxazoles, compound **3** had the same activity as ofloxacin, and compounds **9** and **13** had the same potency as ampicillin against the *E. coli* ESBL isolate, like all the previously synthesized derivatives. While many of the new benzoxazoles were more effective than gentamicin against the *P. aeruginosa* isolate, the previously synthesized derivatives **18**–**27** were as potent as ofloxacin which indicates that a methylene bridge between the benzoxazole ring and the phenyl group at position 2 of the benzoxazole structure enhance the activity against this pathogen. The tested compounds possessed low antifungal activity against *C. albicans* in comparison with the antifungal reference drugs fluconazole and amphotericin B.

In conclusion, the novel 2-(*p*-substituted phenyl)-5-(2-{4-[(*p*-chloro-fluorophenyl)/phenyl]piperazin-1-yl}-acetamido)-benzoxazoles **3**–**17** were synthesized in a three-step procedure. The structures of all derivatives were supported by the analytical data. The antibacterial activities of the new compounds against

standard bacterial strains were inferior to those of standard antibiotics, but equal to those of the standard drugs against drug-resistant strains. Further modifications in 2-(*p*-substituted benzyl/phenyl)-5-[2-(4-substituted piperazin-1-yl)acetamido]-benzoxazole derivatives may eventually lead to compounds being more potent against drug-resistant clinical bacterial strains than the standard antibiotics.

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