

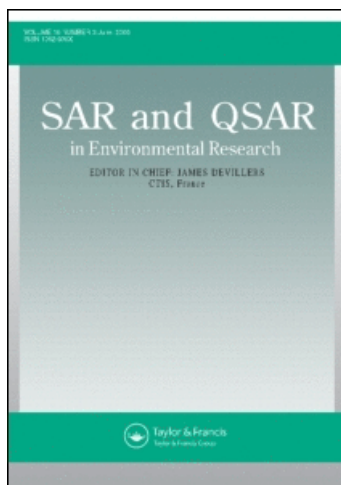
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### Synthesis, antimicrobial activity and QSAR studies of 2,5-disubstituted benzoxazoles

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## Synthesis, antimicrobial activity and QSAR studies of 2,5-disubstituted benzoxazoles

M. Arisoy<sup>a</sup>, O. Temiz-Arpaci<sup>a</sup>, I. Yildiz<sup>a\*</sup>, F. Kaynak-Onurdag<sup>b</sup>, E. Aki<sup>a</sup>, I. Yalcin<sup>a</sup> and U. Abbasoglu<sup>b</sup>

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In this study, a new series of 2,5-disubstituted benzoxazoles was synthesized and their structures were elucidated by elemental analysis, MASS, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and IR spectral data. Newly and previously synthesized 2,5-disubstituted benzoxazole derivatives were evaluated for antibacterial and antifungal activity against standard strains and their drug-resistant isolates. Microbiological results showed that the compounds presented a large spectrum of activity having MIC values of 250–7.8 µg mL<sup>-1</sup> against the tested microorganisms. Among the newly synthesized derivatives **3–22**, compound **11** was the most active against *Candida krusei* out of all; however, it was one dilution less potent than standard drug fluconazole. In addition, all the new and previous compounds were more active than standard drugs ampicillin trihydrate and rifampicin against *Pseudomonas aeruginosa* and its gentamicin-resistant isolate. The 2D-QSAR (Quantitative Structure–Activity Relationship) analysis of a set of newly and previously synthesized benzoxazoles tested for growth inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA) was also performed by using multivariable regression analysis. The activity contributions for substituent effects of these compounds were determined from the correlation equation for predictions of the lead optimization.

**Keywords:** benzoxazoles; antibacterial activity; antifungal activity; QSAR

### 1. Introduction

Infectious diseases caused by bacteria and fungi are still major threats to public health despite tremendous progress in medicinal chemistry. Especially, widespread drug resistance like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) is of major concern. Although there are a number of clinically efficacious antibiotics, they are becoming less effective due to emerging drug resistance [1,2]. Therefore, discovery of new and more effective antimicrobial drugs is very significant and much of the research program efforts is directed towards the design of new agents.

Benzoxazole derivatives have shown various biological activities such as antibacterial, antifungal [3–9], antimycobacterial [10], antitumor [11–15], HIV-1 reverse transcriptase

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[16–22] and topoisomerase I inhibitory activities [23]. A benzoxazole derivative, calcimycin (Figure 1), is an ionophorus carboxylic polyether antibiotic obtained from *Streptomyces chartreusis* (NRRL 3882). It was found very active against Gram-positive bacteria including some *Bacillus* and *Micrococcus* strains [3]. Routiennocin, which is a spiroketal ionophore antibiotic and isolated from a strain of *Streptomyces chartreusis* possessing a benzoxazole ring in its molecular structure, was found very active particularly against some Gram-positive bacteria by acting as a good ionophore, as well [24].

Potent antimicrobial activity of some 2-(*p*-substitutedphenyl)-5-(2-substitutedacetamido)benzoxazoles was reported in our previous study [9]. The encouraging results prompted us to locate a methylene bridge between *p*-substitutedphenyl moiety and benzoxazole ring with the aim of discovering new agents having higher antifungal and antibacterial activity against not only standard strains but also their drug-resistant isolates. Therefore, in this study a new series of 2-(*p*-substitutedbenzyl)-5-(2-substitutedacetamido)benzoxazoles (3–22) carrying morpholine, piperidine, *N*-methylpiperazine and/or *N*-phenylpiperazine groups at the acetamido moiety have been synthesized (Scheme 1) and their structures

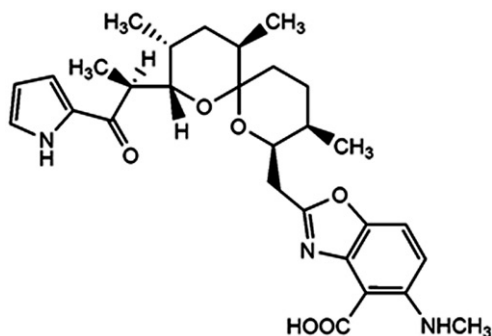
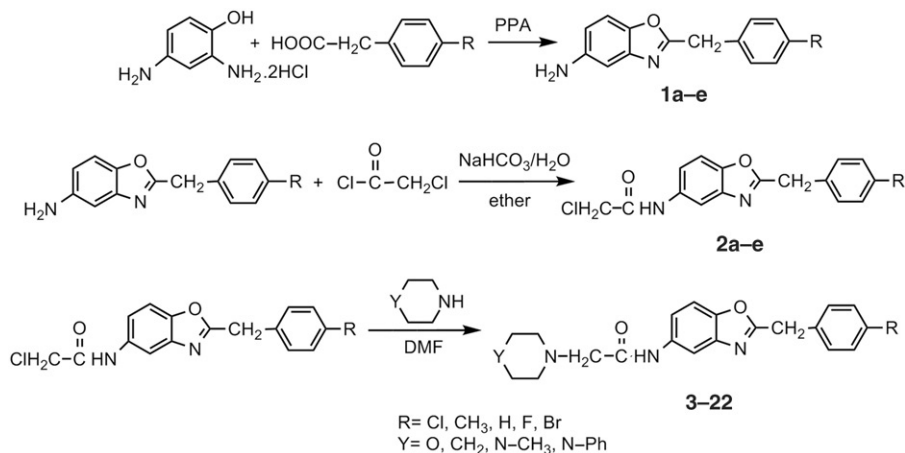


Figure 1. Structure of calcimycin.



Scheme 1. Synthetic pathway of the target compounds.

were elucidated by instrumental analysis methods. The newly (**3–22**) and previously (**23–34**) [9] synthesized compounds were evaluated for their antibacterial and antifungal activity against standard strains and their drug-resistant isolates in comparison with several control drugs. Furthermore, 2D-QSAR (Quantitative Structure–Activity Relationships) analysis of a set of these newly and previously synthesized benzoxazoles tested for antibacterial activity against MRSA was performed by using the multivariable regression analysis. The QSAR study is a method which assumes that differences in the structural or physical properties measured experimentally account for differences in the observed biological or chemical properties [25–27]. For this reason, we performed QSAR analysis of these newly (**3–22**) and previously (**23–34**) [9] synthesized compounds in order to predict the lead optimization for the antibacterial activity against MRSA.

## 2. Materials and methods

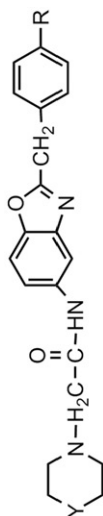
The chemicals were purchased from Aldrich Co. and Fisher Scientific and were used without purification. The reactions were monitored and the purification of the products was checked by thin layer chromatography (TLC). Kieselgel HF 254 chromatoplates (0.3 mm) were used for TLC and the solvent systems were chloroform : methanol (10 : 0.8) for **3–7**, chloroform : methanol (10 : 1.2) for **8–12**, chloroform : methanol (10 : 5) for **13–17**, chloroform : methanol (10 : 0.5) for **18–22**. All the melting points were taken on a Buchi SMP 20 capillary apparatus and are given uncorrected. IR spectra were recorded on a Jasco FT/IR-420 spectrometer as KBr discs.  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR spectra were obtained with a Varian 400 MHz spectrometer in *d*-chloroform ( $\text{CDCl}_3$ ) or dimethylsulfoxide ( $\text{DMSO-d}_6$ ) and tetramethylsilane (TMS) was used as an internal standard. MASS analyses were carried out with a Waters Micromass ZQ by using ESI (+) method. Elemental analyses were performed on LECO 932 CHNS (Leco 932, St. Joseph, MI, USA) instrument and all results were within  $\pm 0.4\%$  of the theoretical values (for exceptions check Table 1). All chemicals and solvents were purchased from Aldrich Chemical Co. or Fischer Scientific.

### 2.1 General procedure for compounds **1a–e** and **2a–e**

Firstly, 5-amino-2-(*p*-substitutedbenzyl)benzoxazoles were synthesized by heating 0.01 mol 2,4-diaminophenol·2HCl with 0.01 mol *p*-substitutedphenyl 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 sulfate and evaporated under diminished pressure. The residue was boiled with 0.2 g charcoal in ethanol and filtered. After the evaporation of solvent *in vacuo*, the crude products (**1a–e**) were obtained and recrystallized from ethanol [7,28,29].

Then, chloroacetylchloride (0.04 mol) was added over a period of 1 h to stirred, ice-cooled mixture of 5-amino-2-(*p*-substitutedbenzyl)benzoxazole (0.04 mol), sodiumbicarbonate (0.04 mol), diethylether (80 mL), and water (40 mL). The mixture was continuously stirred overnight at room temperature and filtered. The precipitate was washed with water, 2 N HCl, water, respectively, and finally with ether. The products (**2a–e**) were recrystallized from ethanol–water mixture and needles were dried *in vacuo* [9].

Table 1. Physical and spectral data of the newly synthesized benzoxazole derivatives 3–22.



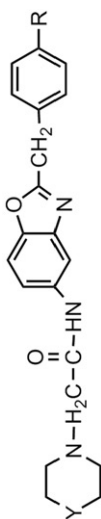
Comp.	R	Y	<i>m.p.</i> (°C)	Yield (%)	<sup>1</sup> H-NMR (δ ppm, <i>J</i> = Hz)	<sup>13</sup> C-NMR (DMSO- <i>d</i> <sub>6</sub> , δ ppm)	MASS <i>m/e</i> (% <i>X</i> ) ( <i>M</i> + <i>H</i> )	IR <i>cm</i> <sup>-1</sup> (C=O)	Formula calculated found
3	Cl	O	107–110	70	(CDCl <sub>3</sub> ): 2.69 (4H, s), 3.22 (2H, s), 3.81 (4H, s), 4.23 (2H, s), 7.31 (4H, s), 7.39–7.48 (2H, m), 7.98 (1H, s), 9.24 (1H, s)	168.74, 166.36, 147.29, 141.62, 136.01, 134.85, 132.50, 131.66, 129.28, 118.07, 110.99, 110.95, 66.75, 62.73, 53.89, 34.08	386(100) 388(33)	1659	C <sub>20</sub> H <sub>20</sub> ClN <sub>3</sub> O <sub>3</sub> C: 62.26 H: 5.22 N: 10.89 C: 62.29 H: 5.34 N: 10.79
					4	CH <sub>3</sub>	O	114–116	76
5	H	O	88–91	60	(CDCl <sub>3</sub> ): 2.73 (4H, s), 3.25 (2H, s), 3.83 (4H, s), 4.27 (2H, s), 7.27–7.37 (5H, m), 7.39–7.49 (2H, m), 7.99 (1H, s), –NH invisible	352(100)	1676	C <sub>20</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> ·0.2H <sub>2</sub> O C: 68.36 H: 6.02 N: 11.96 C: 67.63 H: 5.97 N: 11.70	
					6	F	O	98–100	68

7	Br	O	98–101	55	(CDCl <sub>3</sub> ): 2.68 (4H, s), 3.21 (2H, s), 3.80–3.82 (4H, t), 4.21 (2H, s), 7.24–7.27 (2H, m), 7.39–7.48 (4H, m), 7.97–7.98 (1H, d, $J_m = 1.6$ ), 9.22 (1H, s)	168.77, 166.31, 147.31, 141.64, 136.13, 135.29, 132.23, 132.06, 121.00, 118.09, 111.01, 110.97, 66.77, 62.76, 53.91, 34.17	430(100) 432(100)	1674	C <sub>20</sub> H <sub>20</sub> BrN <sub>3</sub> O <sub>3</sub> · 1H <sub>2</sub> O C: 55.83 H: 4.68 N: 9.77 C: 53.63 H: 4.72 N: 9.43
8	Cl	CH <sub>2</sub>	102–105	30	(CDCl <sub>3</sub> ): 1.50 (2H, s), 1.66–1.68 (4H, t), 2.59 (4H, s), 3.14 (2H, s), 4.23 (2H, s), 7.29–7.33 (4H, m), 7.39–7.41 (1H, d, $J_o = 8.8$ ), 7.47–7.50 (1H, dd, $J_m = 2$ , $J_o = 8.4$ ), 7.97–7.98 (1H, d, $J_m = 1.2$ ), 9.45 (1H, s)	169.27, 166.89, 147.29, 141.73, 136.88, 136.08, 132.79, 129.91, 129.56, 117.87, 110.92, 110.85, 63.39, 54.81, 34.51, 26.13, 24.27, 21.33	384(100) 386(36)	1684	C <sub>21</sub> H <sub>22</sub> ClN <sub>3</sub> O <sub>2</sub> · 0.2H <sub>2</sub> O C: 65.71 H: 5.78 N: 10.95 C: 65.15 H: 5.71 N: 10.85
9	CH <sub>3</sub>	CH <sub>2</sub>	108–109	47	(CDCl <sub>3</sub> ): 1.52 (2H, s), 1.70 (4H, s), 2.33 (3H, s), 2.64 (4H, s), 3.19 (2H, s), 4.22 (2H, s), 7.15–7.17 (2H, d, $J_o = 8$ ), 7.25–7.27 (2H, d, $J_o = 8$ ), 7.37–7.39 (1H, d, $J_o = 8.8$ ), 7.47–7.50 (1H, dd, $J_m = 2$ , $J_o = 8$ ), 7.99 (1H, s), –NH invisible	169.25, 166.70, 147.27, 141.70, 136.09, 135.86, 129.68, 129.35, 127.73, 117.89, 110.94, 110.85, 63.37, 54.78, 34.86, 26.10, 24.24	364(100)	1660	C <sub>22</sub> H <sub>25</sub> N <sub>3</sub> O <sub>2</sub> · 0.3H <sub>2</sub> O C: 72.70 H: 6.93 N: 11.56 C: 71.74 H: 6.92 N: 11.25
10	H	CH <sub>2</sub>	133–136	65	(CDCl <sub>3</sub> ): 1.50 (2H, s), 1.66–1.68 (4H, t), 2.59 (4H, s), 3.13 (2H, s), 4.26 (2H, s), 7.28–7.36 (5H, m), 7.38–7.40 (1H, d, $J_o = 8.4$ ), 7.47–7.49 (1H, dd, $J_m = 1.6$ , $J_o = 8.4$ ), 7.97 (1H, s), 9.44 (1H, s)	169.25, 166.70, 147.27, 141.70, 136.09, 135.86, 129.68, 129.35, 127.73, 117.89, 110.94, 110.85, 63.37, 54.78, 34.86, 26.10, 24.24	350(100)	1681	C <sub>21</sub> H <sub>23</sub> N <sub>3</sub> O <sub>2</sub> · 0.2H <sub>2</sub> O C: 72.18 H: 6.63 N: 12.03 C: 71.40 H: 6.68 N: 11.83
11	F	CH <sub>2</sub>	91–94	74	(CDCl <sub>3</sub> ): 1.50 (2H, s), 1.68 (4H, s), 2.61 (4H, s), 3.15 (2H, s), 4.22 (2H, s), 7.00–7.05 (2H, m), 7.32–7.35 (2H, m), 7.38–7.40 (1H, d, $J_o = 8.4$ ), 7.47–7.49 (1H, dd, $J_m = 2$ , $J_o = 8.4$ ), 7.98 (1H, s), 9.47 (1H, s)	169.25, 166.70, 147.27, 141.70, 136.09, 135.86, 129.68, 129.35, 127.73, 117.89, 110.94, 110.85, 63.37, 54.78, 34.86, 26.10, 24.24	368(100)	1695	C <sub>21</sub> H <sub>22</sub> FN <sub>3</sub> O <sub>2</sub> · 0.1H <sub>2</sub> O C: 68.65 H: 6.04 N: 11.44 C: 68.24 H: 5.66 N: 11.33

(Continued)

Table 1. Continued.

Comp.	R	Y	m.p. (°C)	Yield (%)	<sup>1</sup> H-NMR (δ ppm, J = Hz)	<sup>13</sup> C-NMR (DMSO-d <sub>6</sub> , δ ppm)	MASS m/e (%X) (M+H)	IR cm <sup>-1</sup> (C=O)	Formula calculated/found
12	Br	CH <sub>2</sub>	102–105	20	(CDCl <sub>3</sub> ): 1.52 (2H, s), 1.69 (4H, s), 2.63 (4H, s), 3.18 (2H, s), 4.21 (2H, s), 7.24–7.26 (2H, d, J <sub>o</sub> = 8.8), 7.38–7.50 (4H, m), 7.99 (1H, s), 9.52 (1H, s)	168.94, 166.37, 147.25, 141.62, 136.11, 134.83, 132.49, 131.66, 129.27, 117.96, 110.97, 110.87, 62.47, 55.17, 55.40, 46.42, 34.07	428(100) 430(100)	1683	C <sub>21</sub> H <sub>22</sub> BrN <sub>3</sub> O <sub>2</sub> · 1.5H <sub>2</sub> O C: 58.89 H: 5.18 N: 9.81 C: 55.44 H: **N: 9.28
13	Cl	N-CH <sub>3</sub>	121–124	37	(DMSO-d <sub>6</sub> ): 2.17 (3H, s), 2.38–2.51 (8H), 3.13 (2H, s), 4.34 (2H, s), 7.42 (4H, s), 7.50–7.53 (1H, dd, J <sub>m</sub> = 1.8 Hz, J <sub>o</sub> = 8.6 Hz), 7.59–7.61 (1H, d, J <sub>o</sub> = 8.4 Hz), 8.06 (1H, s), 9.84 (1H, s)	168.94, 166.37, 147.25, 141.62, 136.11, 134.83, 132.49, 131.66, 129.27, 117.96, 110.97, 110.87, 62.47, 55.17, 55.40, 46.42, 34.07	399(100) 401(37)	1691	C <sub>21</sub> H <sub>23</sub> ClN <sub>4</sub> O <sub>2</sub> · 0.5H <sub>2</sub> O C: 63.23 H: 5.81 N: 14.05 C: 61.73 H: 5.70 N: 13.44
14	CH <sub>3</sub>	N-CH <sub>3</sub>	98–100	56	(DMSO-d <sub>6</sub> ): 2.33 (3H, s), 2.39 (3H, s), 2.60–2.72 (8H), 3.18 (2H, s), 4.22 (2H, s), 7.15–7.17 (2H, d, J <sub>o</sub> = 8.0 Hz), 7.25–7.27 (2H, d, J <sub>o</sub> = 7.6 Hz), 7.38–7.40 (1H, d, J <sub>o</sub> = 8.8 Hz), 7.46–7.48 (1H, dd, J <sub>m</sub> = 1.8 Hz, J <sub>o</sub> = 8.6 Hz), 7.92–7.93 (1H, d, J <sub>m</sub> = 2.4 Hz), 9.18 (1H, s)	168.93, 166.90, 147.29, 141.72, 136.87, 136.08, 132.78, 129.91, 129.56, 117.87, 110.93, 110.87, 62.50, 55.20, 53.41, 46.43, 34.50, 21.33	379(100)	1661	C <sub>22</sub> H <sub>26</sub> N <sub>4</sub> O <sub>2</sub> · 0.2H <sub>2</sub> O C: 69.82 H: 6.92 N: 14.80 C: 69.01 H: 6.80 N: 14.30

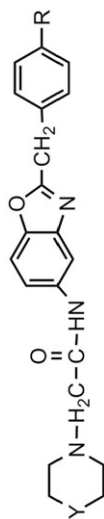


15	H	N-CH <sub>3</sub>	88-91	46	(DMSO-d <sub>6</sub> ): 2.17 (3H, s), 2.38-2.51 (8H), 3.12 (2H, s), 4.32 (2H, s), 7.28-7.37 (5H, m), 7.49-7.60 (2H, m), 8.06 (1H, s), 9.84 (1H, s)	168.95, 166.72, 147.29, 141.71, 136.11, 135.87, 129.71, 129.37, 127.76, 117.92, 110.97, 110.89, 62.51, 55.21, 53.43, 46.45, 34.89	365(100)	1678	C <sub>21</sub> H <sub>24</sub> N <sub>4</sub> O <sub>2</sub> · 0.1H <sub>2</sub> O C: 69.21 H: 6.64 N: 15.37 C: 68.72 H: 6.72 N: 15.03
16	F	N-CH <sub>3</sub>	94-97	37	(DMSO-d <sub>6</sub> ): 2.17 (3H, s), 2.38-2.51 (8H), 3.12 (2H, s), 4.33 (2H, s), 7.17-7.21 (2H, m), 7.41-7.44 (2H, m), 7.50-7.53 (1H, dd, J <sub>m</sub> = 1.8 Hz, J <sub>o</sub> = 8.6 Hz), 7.58-7.60 (1H, d, J <sub>o</sub> = 8.8 Hz), 8.05-8.06 (1H, d, J <sub>m</sub> = 2.0 Hz), 9.82 (1H, s)	168.95, 166.64, 163.23, 160.81, 147.29, 141.68, 136.11, 132.02, 131.99, 131.76, 131.67, 117.95, 116.21, 115.99, 110.98, 110.90, 62.50, 55.20, 53.43, 46.43, 33.97	383(100)	1695	C <sub>21</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>2</sub> · 0.6H <sub>2</sub> O C: 65.95 H: 6.06 N: 14.65 C: 63.93 H: 6.17 N: 14.05
17	Br	N-CH <sub>3</sub>	128-131	14	(DMSO-d <sub>6</sub> ): 2.17 (3H, s), 2.37-2.51 (8H), 3.12 (2H, s), 4.33 (2H, s), 7.34-7.36 (2H, d, J <sub>o</sub> = 8.8 Hz), 7.50-7.61 (4H, m), 8.05-8.06 (1H, d, J <sub>m</sub> = 1.6 Hz), 9.84 (1H, s)	168.93, 166.29, 147.26, 141.62, 136.12, 135.28, 132.21, 132.04, 120.98, 117.96, 110.97, 110.87, 62.48, 55.19, 53.41, 46.43, 34.14	443(95) 445(100)	1689	C <sub>21</sub> H <sub>23</sub> BrN <sub>4</sub> O <sub>2</sub> · 0.7H <sub>2</sub> O C: 56.89 H: 5.23 N: 12.64 C: 55.04 H: 5.00 N: 12.19
18	Cl	N-Ph	146-149	61	(DMSO-d <sub>6</sub> ): 2.67 (4H, s), 3.20 (6H, s), 4.34 (2H, s), 6.75-6.79 (1H, m), 6.93-6.95 (2H, d, J <sub>o</sub> = 7.6 Hz), 7.18-7.22 (2H, m), 7.41 (4H, s), 7.53-7.61 (2H, m), 8.08 (1H, s), 9.92 (1H, s)	168.89, 166.40, 151.71, 147.31, 141.65, 136.15, 134.87, 132.52, 131.69, 129.61, 129.30, 119.50, 118.06, 116.11, 110.99, 62.42, 53.43, 48.78, 34.10	461(100) 463(37)	1671	C <sub>26</sub> H <sub>28</sub> ClN <sub>4</sub> O <sub>2</sub> C: 67.75 H: 5.47 N: 12.15 C: 67.51 H: 5.65 N: 11.94
19	CH <sub>3</sub>	N-Ph	175-176	56	(DMSO-d <sub>6</sub> ): 2.27 (3H, s), 2.67 (4H, s), 3.21 (6H, s), 4.25 (2H, s), 6.75-6.79 (1H, m), 6.93-6.95 (2H, d, J <sub>o</sub> = 8.0 Hz), 7.14-7.25 (6H, m), 7.52-7.59 (2H, m), 8.08 (1H, s), 9.92 (1H, s)	168.90, 166.93, 151.71, 147.32, 141.71, 136.90, 136.08, 132.77, 129.93, 129.62, 129.56, 119.50, 117.97, 116.11, 110.95, 62.41, 53.42, 48.78, 34.50, 21.33	441(100)	1684	C <sub>27</sub> H <sub>28</sub> N <sub>4</sub> O <sub>2</sub> · 0.2H <sub>2</sub> O C: 73.61 H: 6.41 N: 12.72 C: 73.06 H: 6.21 N: 12.44

(Continued)



Table 1. Continued.



Comp.	R	Y	m.p. (°C)	Yield (%)	<sup>1</sup> H-NMR (δ ppm, J = Hz)	<sup>13</sup> C-NMR (DMSO-d <sub>6</sub> , δ ppm)	MASS m/e (%X) (M+H)	IR cm <sup>-1</sup> (C=O)	Formula calculated	Formula found
20	H	N-Ph	115–118	61	(DMSO-d <sub>6</sub> ): 2.65 (4H, s), 3.18 (6H, s), 4.29 (2H, s), 6.73–6.77 (1H, m), 6.91–6.93 (2H, d, J <sub>o</sub> = 8.4 Hz), 7.17–7.35 (7H, m), 7.50–7.52 (1H, d, J <sub>o</sub> = 8.8 Hz), 7.56–7.58 (1H, dd, J <sub>m</sub> = 2.4 Hz, J <sub>o</sub> = 8.8 Hz), 8.05 (1H, s), 9.89 (1H, s)	168.86, 166.71, 151.69, 147.32, 141.70, 136.10, 135.85, 129.68, 129.60, 129.36, 127.74, 119.48, 117.99, 116.09, 110.95, 62.40, 53.41, 48.76, 34.87	427(100)	1671	C <sub>26</sub> H <sub>26</sub> N <sub>4</sub> O <sub>2</sub> · 0.1H <sub>2</sub> O C: 73.22 H: 6.14 N: 13.14 C: 72.79 H: 6.48 N: 12.74	
21	F	N-Ph	144–146	57	(DMSO-d <sub>6</sub> ): 2.67–2.69 (4H, t), 3.21 (6H, s), 4.33 (2H, s), 6.76–6.79 (1H, m), 6.93–6.96 (2H, d, J <sub>o</sub> = 8.8 Hz), 7.17–7.23 (4H, m), 7.41–7.44 (2H, m), 7.53–7.56 (1H, dd, J <sub>m</sub> = 1.8 Hz, J <sub>o</sub> = 9.0 Hz), 7.59–7.61 (1H, d, J <sub>o</sub> = 8.4 Hz), 8.07–8.08 (1H, d, J <sub>m</sub> = 1.6 Hz), 9.92 (1H, s)	168.87, 166.65, 163.23, 160.81, 151.71, 147.31, 141.69, 136.15, 132.03, 131.99, 131.76, 131.68, 129.61, 119.49, 118.01, 116.21, 116.10, 115.99, 110.97, 62.42, 53.43, 48.78, 33.98	445(100)	1673	C <sub>26</sub> H <sub>25</sub> FN <sub>4</sub> O <sub>2</sub> C: 70.25 H: 5.67 N: 12.60 C: 70.15 H: 6.00 N: 12.31	
22	Br	N-Ph	138–141	47	(DMSO-d <sub>6</sub> ): 2.66 (4H, s), 3.20 (6H, s), 4.31 (2H, s), 6.74–6.78 (1H, m), 6.92–6.94 (2H, d, J <sub>o</sub> = 8.0 Hz), 7.18–7.22 (2H, m), 7.32–7.34 (2H, d, J <sub>o</sub> = 8.0 Hz), 7.53–7.60 (4H, m), 8.08 (1H, s), 9.92 (1H, s)	168.89, 166.31, 151.71, 147.31, 141.65, 136.15, 135.29, 132.23, 132.06, 129.61, 121.00, 119.50, 118.06, 116.11, 110.99, 62.42, 53.43, 48.78, 34.17	505(100) 507(85)	1671	C <sub>26</sub> H <sub>25</sub> BrN <sub>4</sub> O <sub>2</sub> · 0.2H <sub>2</sub> O C: 61.79 H: 4.99 N: 11.09 C: 61.29 H: 4.85 N: 10.99	

Note: \*\*\*No satisfactory result.

5-Amino-2-(*p*-substitutedbenzyl)benzoxazoles (**1a–d**) were prepared according to the literature [7,28,29].

**5-Amino-2-(*p*-bromobenzyl)benzoxazole (1e):** Yield: 75%, m.p.: 104–107°C. <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 4.16 (2H, s), 6.63–6.66 (1H, dd, *J*<sub>m</sub> = 2.4 Hz, *J*<sub>o</sub> = 8.4 Hz), 6.95–6.96 (1H, d, *J*<sub>m</sub> = 2.4 Hz), 7.21–7.24 (3H, m), 7.45–7.47 (2H, d, *J*<sub>o</sub> = 8.4 Hz), –NH<sub>2</sub> invisible. MASS [ESI(+), *m/e*]: 303 (M + H, 95%), 305 (100%).

**5-(2-Chloroacetamido)-2-(*p*-chlorobenzyl)benzoxazole (2a):** Yield: 70%, m.p.: 170–173°C. IR (KBr): 1662 cm<sup>-1</sup> (C=O). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 4.22 (2H, s), 4.24 (2H, s), 7.32 (4H, s), 7.44 (2H, s), 7.94 (1H, s), 8.33 (1H, s). MASS [ESI(+), *m/e*]: 335 (M + H, 100%), 337 (63%), 339 (15%). Anal Calcd: C: 57.33, H: 3.61, N: 8.36, Found: C: 57.22, H: 3.73, N: 8.39 for C<sub>16</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>.

**5-(2-Chloroacetamido)-2-(*p*-methylbenzyl)benzoxazole (2b):** Yield: 63%, m.p.: 172–173°C. IR (KBr): 1661 cm<sup>-1</sup> (C=O). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 2.34 (3H, s), 4.22 (2H, s), 4.24 (2H, s), 7.15–7.17 (2H, d, *J*<sub>o</sub> = 8.0 Hz), 7.26–7.28 (2H, d, *J*<sub>o</sub> = 8.0 Hz), 7.44 (2H, s), 7.93 (1H, s), 8.34 (1H, s). MASS [ESI(+), *m/e*]: 315 (M + H, 100%), 317 (30%). Anal Calcd: C: 64.87, H: 4.80, N: 8.90, Found: C: 64.59, H: 4.99, N: 8.80 for C<sub>17</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>2</sub>.

**5-(2-Chloroacetamido)-2-benzylbenzoxazole (2c):** Yield: 73%, m.p.: 126–129°C. IR (KBr): 1660 cm<sup>-1</sup> (C=O). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 4.22 (2H, s), 4.28 (2H, s), 7.29–7.40 (5H, m), 7.44 (2H, s), 7.94 (1H, s), 8.35 (1H, s). MASS [ESI(+), *m/e*]: 301 (M + H, 100%), 303 (34%). Anal Calcd: C: 63.90, H: 4.36, N: 9.31, Found: C: 63.79, H: 4.55, N: 9.26 for C<sub>16</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>2</sub>.

**5-(2-Chloroacetamido)-2-(*p*-fluorobenzyl)benzoxazole (2d):** Yield: 68%, m.p.: 148–151°C. IR (KBr): 1667 cm<sup>-1</sup> (C=O). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 4.22 (2H, s), 4.24 (2H, s), 7.02–7.06 (2H, m), 7.33–7.36 (2H, m), 7.43 (2H, s), 7.94 (1H, s), 8.35 (1H, s). MASS [ESI(+), *m/e*]: 319 (M + H, 100%), 321 (30%). Anal Calcd: C: 60.29, H: 3.79, N: 8.79, Found: C: 60.07, H: 3.77, N: 8.80 for C<sub>16</sub>H<sub>12</sub>ClFN<sub>2</sub>O<sub>2</sub>.

**5-(2-Chloroacetamido)-2-(*p*-bromobenzyl)benzoxazole (2e):** Yield: 80%, m.p.: 184–187°C. IR (KBr): 1663 cm<sup>-1</sup> (C=O). <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>, δ ppm): 4.22 (4H, s), 7.25–7.27 (2H, d, *J*<sub>o</sub> = 7.2 Hz), 7.44–7.49 (4H, m), 7.94 (1H, s), 8.33 (1H, s). MASS [ESI(+), *m/e*]: 379 (M + H, 77%), 381 (100%), 383 (26%). Anal Calcd: C: 50.62, H: 3.19, N: 7.38, Found: C: 50.66, H: 3.30, N: 7.52 for C<sub>16</sub>H<sub>12</sub> BrClN<sub>2</sub>O<sub>2</sub>.

## 2.2 General procedure for the preparation of 2-(*p*-substitutedbenzyl)-5-(2-substitutedacetamido) benzoxazoles (3–22)

A total of 0.002 mol 5-(2-chloroacetamido)-2-(*p*-substitutedbenzyl)benzoxazole derivatives was added to 0.002 mol morpholine/piperidine/*N*-methylpiperazine/*N*-phenylpiperazine and 0.006 mol triethylamine 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, the mixture was poured into ice–water mixture and the precipitate formed was filtered.

Purification by flash chromatography: silica-gel column from appropriate solvents [9]. The chemical, physical, and spectral data of compounds 3–22 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]-propan-sulfonic acid (MOPS) (Sigma), 96-well microplates (Falcon), Transfer pipette (Biohit), Rifampicin (Kocak), Ampicillin trihydrate (Paninkret Chem. Pharm.), Gentamicin sulfate (Deva Ilac Sanayii), Ofloxacin (Zhejiang Huangyan East Asia Chemical CO.), Fluconazole (Nobel), Amphotericin B (Bristol Myers Squibb), Ethanol (Riedel de Haen), DMSO (Riedel de Haen).

2.3.1.1 *Microorganisms*. The following isolates were used: *K. pneumoniae* [has Extended Spectrum Beta Lactamase (ESBL) enzyme], *P. aeruginosa* (resistant to gentamicin), *E. coli* [has ESBL enzyme], *Bacillus subtilis* (resistant to ceftriaxon), *S. aureus* [resistant to meticilline (MRSA)], and *Candida albicans* (biofilm positive).

The following standard strains were considered: *Klebsiella pneumoniae* RSHM 574 (Refik Saydam Hygiene Center Culture Collection), *Pseudomonas aeruginosa* ATCC 25853 (American Type Culture Collection), *Escherichia coli* ATCC 25922, *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *C. albicans* ATCC 10231, and *C. krusei* ATCC 6258.

### 2.3.2 Method

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 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) in the clinical isolates [30].

Standard powders of rifampicin, ampicillin trihydrate, gentamicin sulfate, ofloxacin, fluconazole, and amphotericin B were obtained from the manufacturers. Stock solutions were dissolved in DMSO (ofloxacin), methanol (rifampicin), pH 8 phosphate-buffered saline (PBS) (ampicillin trihydrate), and distilled water (gentamicin sulfate, fluconazole, and amphotericin B).

All bacterial isolates were subcultured in MHA plates and incubated overnight 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 and standard drugs were prepared at 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.8, 3.9, 1.95, 0.98, 0.48, 0.24, 0.12, 0.06  $\mu\text{g mL}^{-1}$  concentrations in the wells of microplates by diluting in the liquid media. Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S16 [31]. The bacterial suspensions used for inoculation were prepared at  $10^5 \text{ cfu mL}^{-1}$  by diluting fresh cultures at MacFarland 0.5 density ( $10^7 \text{ cfu mL}^{-1}$ ). Suspensions of the bacteria at  $10^5 \text{ cfu mL}^{-1}$  concentration were inoculated to the two-fold diluted solutions of the compounds. There were  $10^4 \text{ cfu mL}^{-1}$  bacteria in the wells after inoculations. MHB was used for diluting the

bacterial suspension and for two-fold dilution of the compound. Eighty percent DMSO and 20% EtOH, methanol, DMSO, PBS, pure microorganisms, and pure media were used as control wells. A 10  $\mu\text{L}$  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.

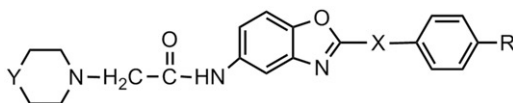
All *Candida* isolates were subcultured in SDA plates, incubated at 35°C for 24–48 h prior to antifungal susceptibility testing, and passaged at least twice to ensure purity and viability. Susceptibility testing was performed in RPMI-1640 medium with L-glutamine buffered, pH 7, with MOPS and culture suspensions were prepared through the guideline of CLSI M27-A [32]. The yeast suspensions used for inoculation were prepared at 10<sup>4</sup> cfu mL<sup>-1</sup> by diluting fresh cultures at MacFarland 0.5 density (10<sup>6</sup> cfu mL<sup>-1</sup>). Suspensions of the yeast at 10<sup>4</sup> cfu mL<sup>-1</sup> concentration were inoculated to the 2-fold diluted solution of the compounds. There were 10<sup>3</sup> cfu mL<sup>-1</sup> yeasts in the wells after inoculations. A 10  $\mu\text{L}$  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 MICs were reported.

#### 2.4 QSAR analysis

Chemical structures and antibacterial activity against *S. aureus* isolate (MRSA) of the previously [9] and newly synthesized compounds shown as MIC values, are given in Tables 2 and 3. The potency was defined as  $\log 1/C$  where C was the MIC value expressed in molar concentration units. A training set including compounds 3, 4, 7, 9–12, 15–19, 23–25, 29–31, 33, 34 and a test set consisting of compounds 5, 13, 14, 27, 28, 32 were considered. The variables used as independent descriptors in the QSAR analysis were hydrophobic, electronic, steric, and structural parameters. The structural variable  $I_X$  presented a value of 1 for the presence of a methylene bridge between *p*-substituted phenyl group and benzoxazole ring and 0 otherwise. Similarly,  $I_{YO}$ ,  $I_{YNCH_3}$ ,  $I_{YNH}$  variables showed a value of 1 for the presence of morpholine, *N*-methylpiperazine, and piperazine group, respectively, and 0 for the absence of the corresponding group. The screened physicochemical parameters in this QSAR study were  $\log P$ ,  $\pi$  for the hydrophobic effects,  $\sigma$ ,  $F$  (field effect),  $R$  (resonance effect), as the electronic influences and Verloop's STERIMOL descriptors ( $L$ ,  $B_1$ ,  $B_4$ ) for the steric interactions of the substituents at the position  $R$ . Values of the physicochemical parameters used in this QSAR study were taken from the table of Hansch and Leo [33] except  $\log P$  which was calculated by using the Accelrys's Cerius<sup>2</sup> [34] program. The values of the parameters used in the correlation equations related to the activity among the candidate set of variables in the training set are shown in Table 2. Multivariable regression analysis of the QSAR study was run on a PC using the program BILIN (<http://www.kubinyi.de/bilin-program.html>) and Minitab (MINITAB Release 15, <http://www.minitab.com>) program package.

Multivariable regression analysis that involves finding the best fit of dependent variable (antibacterial activity) to a combination of independent variables (descriptors)

Table 2. Training set of compounds and the parameters used in Equations (1) and (2).

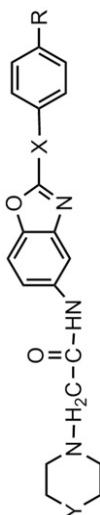


Comp. no	X	R	Y	$I_X$	$I_{YO}$	$I_{YNCH_3}$	$I_{YNH}$	$BI_R$	$\pi_R$
3	CH <sub>2</sub>	Cl	O	1	1	0	0	1.8	0.71
4	CH <sub>2</sub>	CH <sub>3</sub>	O	1	1	0	0	1.52	0.58
7	CH <sub>2</sub>	Br	O	1	1	0	0	1.95	0.86
9	CH <sub>2</sub>	CH <sub>3</sub>	CH <sub>2</sub>	1	0	0	0	1.52	0.58
10	CH <sub>2</sub>	H	CH <sub>2</sub>	1	0	0	0	1	0
11	CH <sub>2</sub>	F	CH <sub>2</sub>	1	0	0	0	1.35	0.14
12	CH <sub>2</sub>	Br	CH <sub>2</sub>	1	0	0	0	1.95	0.86
15	CH <sub>2</sub>	H	N-CH <sub>3</sub>	1	0	1	0	1	0
16	CH <sub>2</sub>	F	N-CH <sub>3</sub>	1	0	1	0	1.35	0.14
17	CH <sub>2</sub>	Br	N-CH <sub>3</sub>	1	0	1	0	1.95	0.86
18	CH <sub>2</sub>	Cl	N-Ph	1	0	0	0	1.8	0.71
19	CH <sub>2</sub>	CH <sub>3</sub>	N-Ph	1	0	0	0	1.52	0.58
23	-	H	O	0	1	0	0	1	0
24	-	F	O	0	1	0	0	1.35	0.14
25	-	C <sub>2</sub> H <sub>5</sub>	O	0	1	0	0	1.52	1.02
29	-	C <sub>2</sub> H <sub>5</sub>	NH	0	0	0	1	1.52	1.02
30	-	C(CH <sub>3</sub> ) <sub>3</sub>	NH	0	0	0	1	2.59	1.98
31	-	H	N-CH <sub>3</sub>	0	0	1	0	1	0
33	-	C <sub>2</sub> H <sub>5</sub>	N-CH <sub>3</sub>	0	0	1	0	1.52	1.02
34	-	C(CH <sub>3</sub> ) <sub>3</sub>	N-CH <sub>3</sub>	0	0	1	0	2.59	1.98

were used by the least squares method. The statistically significant correlation Equations (1) and (2) obtained from multivariable regression analysis to describe the QSAR analysis are given in Table 4.

In the equations, the figures in parentheses are the 95% confidence intervals of the regression coefficients and the constant term,  $n$  is the number of compounds,  $r^2$  is the square of the correlation coefficient,  $s$  is the standard error of estimate and  $F$  is the significance test.

In order to judge the validity of the predictive power of the QSAR analysis, the cross-validation method was also applied to the original dataset by removing a compound from the data in such a way that each observation (compound) is deleted only once. For each reduced dataset a model was developed and the response values of the deleted observations were predicted from this model. Finally, the resulting predictive residual sum of squares (PRESS) and  $Q^2$  (the square of the cross validation regression coefficient) were calculated for the equations [27,35–37].  $s$ -PRESS value represents the SD of the cross-validation predictions. The search for the simple correlation coefficients which are given in Table 5 also reveals that there is no intercorrelation between the independent variables in any case entered in the correlation equations. The predicted  $\log 1/C$  values with residuals of the training set determined from equations are given in Table 6.

Table 3. Compounds, parameters, observed, and calculated  $\log I/C$  values, residuals of the test set by using Equations (1) and (2).

Comp. no	X	R	Y	R	$I_X$	$I_{YO}$	$I_{YNCH_3}$	$I_{YNH}$	$BI_R$	$\pi_R$	$\pi_R$	Equation (1)			Equation (2)		
												Obs. $\log I/C$	Calc. $\log I/C$	Residuals	Calc. $\log I/C$	Residuals	Residuals
5	CH <sub>2</sub>	H	O	H	1	1	0	0	1	0	0	3.449	3.137	0.312	3.400	0.049	
13	CH <sub>2</sub>	Cl	N-CH <sub>3</sub>	Cl	1	0	0	0	1.8	0.71	0.71	3.504	3.779	-0.275	3.855	-0.351	
14	CH <sub>2</sub>	CH <sub>3</sub>	N-CH <sub>3</sub>	CH <sub>3</sub>	1	0	1	0	1.52	0.58	0.58	3.481	3.666	-0.185	3.819	-0.338	
27	-	H	NH	H	0	0	0	1	1	0	0	3.731	4.011	-0.280	4.125	-0.394	
28	-	F	NH	F	0	0	0	1	1.35	0.14	0.14	3.754	4.152	-0.398	4.163	-0.409	
32	-	F	N-CH <sub>3</sub>	F	0	0	1	0	1.35	0.14	0.14	3.770	4.152	-0.382	3.997	-0.227	

Table 4. Correlation equations obtained by QSAR analysis using multivariable regression analysis.

<i>Nb.</i>	<i>Equations and statistical results</i>		
(1)	$\log 1/C = +3.609 (\pm 0.25) - 0.554 (\pm 0.12)I_X - 0.320 (\pm 0.13)I_{YO} + 0.402 (\pm 0.13)B1_R$		
	$n = 20$	$r^2 = 0.910$	$s = 0.126$
	$F = 54.539$	$Q^2 = 0.876$	$PRESS = 0.355$
	$s\text{-PRESS} = 0.149$	$SSY = 2.870$	$PRESS/SSY = 0.123$
(2)	$\log 1/C = +3.697 (\pm 0.19) - 0.298 (\pm 0.17)I_X + 0.262 (\pm 0.17)I_{YCH_3} + 0.428 (\pm 0.31)I_{YNH} + 0.273 (\pm 0.15)\pi_R$		
	$n = 20$	$r^2 = 0.872$	$s = 0.156$
	$F = 25.615$	$Q^2 = 0.743$	$PRESS = 0.737$
	$s\text{-PRESS} = 0.222$	$SSY = 2.870$	$PRESS/SSY = 0.256$

Table 5. Correlation matrix of the variables used in Equations (1) and (2).

	$I_X$	$I_{YO}$	$I_{YNCH_3}$	$I_{YNH}$	$B1_R$
$I_{YO}$	-0.134				
$I_{YNCH_3}$	-0.134	-0.429			
$I_{YNH}$	-0.408	-0.218	-0.218		
$B1_R$	-0.084	-0.097	-0.031	0.344	
$\pi_R$	-0.336	-0.123	0.009	0.489	0.918

Table 6. Observed and calculated  $\log 1/C$  values with residuals obtained from the Equations (1) and (2).

<i>Comp. no</i>	<i>Obs. log 1/C</i>	<i>Equation (1)</i>		<i>Equation (2)</i>	
		<i>Calc. log 1/C</i>	<i>Residuals</i>	<i>Calc. log 1/C</i>	<i>Residuals</i>
3	3.489	3.459	0.031	3.593	-0.104
4	3.466	3.346	0.120	3.558	-0.092
7	3.537	3.519	0.018	3.634	-0.098
9	3.464	3.666	-0.203	3.558	-0.094
10	3.446	3.457	-0.011	3.400	0.047
11	3.769	3.598	0.171	3.438	0.331
12	3.836	3.839	-0.003	3.634	0.201
15	3.465	3.457	0.007	3.661	-0.196
16	3.787	3.598	0.189	3.699	0.087
17	3.851	3.839	0.012	3.896	-0.045
18	3.567	3.779	-0.212	3.593	-0.027
19	3.547	3.666	-0.119	3.558	-0.011
23	3.732	3.691	0.041	3.697	0.035
24	3.755	3.832	-0.077	3.735	0.019
25	3.767	3.900	-0.133	3.976	-0.209
29	4.368	4.220	0.148	4.404	-0.036
30	4.702	4.650	0.052	4.666	0.036
31	4.050	4.011	0.039	3.959	0.091
33	4.083	4.220	-0.137	4.237	-0.154
34	4.717	4.650	0.067	4.499	0.218

### 3. Results and discussion

#### 3.1 Chemistry

In the present study, some new 2-(*p*-substitutedbenzyl)-5-(2-substitutedacetamido) benzoxazoles (**3–22**) were synthesized by using a three-step procedure as shown in Scheme 1. Firstly, 5-amino-2-substitutedbenzoxazoles (**1a–e**) were obtained by heating appropriate acids with 2,4-diaminophenol dihydrochloride in PPA [7,9]. Then, 5-amino-2-substitutedbenzoxazole derivatives were treated with chloroacetylchloride in order to obtain amide compounds (**2a–e**). Finally, compounds **3–22** were prepared by reacting amide compounds with morpholine/piperidine/4-methylpiperazine or 4-phenylpiperazine group. The structures of **3–22** were supported by spectral data. The IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, MASS spectra and elemental analysis results are in agreement with the proposed structures. Physical and spectral data of these compounds are given in Table 1.

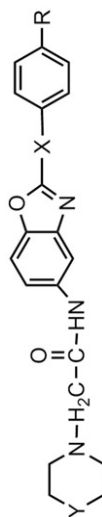
According to the spectroscopic data of the final compounds the IR spectra showed characteristic C=O (amide) stretching bands in the 1659–1695 region. In the <sup>1</sup>H-NMR spectrum of the compound **1e**, signals of NH<sub>2</sub> protons were invisible while in the spectra of the compound **2a–e** the signal of NH proton was observed at 8.33–8.35 ppm as a singlet band; benzylic and aliphatic CH<sub>2</sub> protons were observed at 4.22–4.28 ppm as two singlet bands except compound **2e** that protons of methylene groups of this derivative actually were observed overlapped at 4.22 ppm as a singlet band.

In the <sup>1</sup>H-NMR spectra of the compounds **5**, **6**, and **9** signal of NH protons, too, were invisible while the same protons of the other newly synthesized benzoxazole compounds were observed at 9.18–9.92 ppm as singlet bands. Aromatic methyl protons appeared at 2.27–2.39 ppm and methyl protons attached to piperazine ring were observed at 2.17–2.33 ppm as singlet bands, as well. Aromatic protons of benzoxazole ring were observed at the expected regions but some of them appeared as singlet bands (overlapped). All the other aromatic and aliphatic protons were observed at the typical regions, however, in most cases it was impossible to observe the multiplet bands of piperazine, morpholine, and piperidine protons since they were in the cyclic ring. Besides, <sup>13</sup>C-NMR spectra of the compounds **3**, **7**, **9**, **10**, and **13–22** were appropriate to their structures and MASS spectra of the compounds showed M + H peaks in accordance with their formulas since the electrospray ionization method was employed.

#### 3.2 In vitro antibacterial and antifungal activity

Antibacterial and antifungal activity of all the compounds (**3–34**) were assayed *in vitro* for antibacterial activity against *K. pneumoniae* RSHM 574, *P. aeruginosa* ATCC 25853, *E. coli* ATCC 25922, *K. pneumoniae* isolate [has ESBL enzyme], *P. aeruginosa* isolate (resistant to gentamicin), *E. coli* isolate [has ESBL enzyme] as Gram-negative bacteria; *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *B. subtilis* isolate (resistant to ceftriaxon), *S. aureus* isolate [resistant to meticilline (MRSA)] as Gram-positive bacteria and the antifungal activity was evaluated against *C. krusei* ATCC 6258, *C. albicans* ATCC 10231, *C. albicans* isolate (biofilm positive). The MIC values were determined by two-fold serial dilution technique in MHB and SDA for the antibacterial and antifungal assay, respectively. For comparison of antimicrobial activity, rifampicin, ampicillin, trihydrate, gentamycin sulfate, ofloxacin were used as reference antibacterial agents and fluconazole, amphotericin B were employed as reference antifungal agents. All the biological results of the tested compounds are given in Table 7.



Table 7. The *in vitro* antimicrobial activity of newly and previously synthesized benzoxazole derivatives comparing with the control drugs (MIC in  $\mu\text{g mL}^{-1}$ ).

No.	X	R	Y	K.p.*	P.a.*	E.c.*	B.s.*	S.a.*	K.p.	P.a.	E.c.	B.s.	S.a.	C.a.	C.a.*	C.k.
3	CH <sub>2</sub>	Cl	O	125	62.5	125	125	125	62.5	62.5	62.5	125	62.5	62.5	125	125
4	CH <sub>2</sub>	CH <sub>3</sub>	O	125	62.5	125	125	125	62.5	62.5	62.5	125	125	62.5	62.5	62.5
5	CH <sub>2</sub>	H	O	125	62.5	125	125	125	62.5	62.5	62.5	125	125	62.5	62.5	125
6	CH <sub>2</sub>	F	O	125	62.5	125	125	62.5	62.5	62.5	15.625	125	125	62.5	125	125
7	CH <sub>2</sub>	Br	O	125	125	125	125	125	62.5	62.5	62.5	125	125	62.5	62.5	125
8	CH <sub>2</sub>	Cl	CH <sub>2</sub>	125	62.5	125	125	62.5	62.5	62.5	62.5	125	31.25	62.5	125	125
9	CH <sub>2</sub>	CH <sub>3</sub>	CH <sub>2</sub>	125	62.5	125	125	125	62.5	62.5	62.5	125	31.25	62.5	62.5	125
10	CH <sub>2</sub>	H	CH <sub>2</sub>	125	125	125	125	125	62.5	62.5	62.5	125	125	62.5	62.5	125
11	CH <sub>2</sub>	F	CH <sub>2</sub>	62.5	62.5	125	125	62.5	62.5	62.5	62.5	125	125	62.5	62.5	31.25
12	CH <sub>2</sub>	Br	CH <sub>2</sub>	125	125	125	125	125	62.5	62.5	62.5	125	125	62.5	62.5	125
13	CH <sub>2</sub>	Cl	N-CH <sub>3</sub>	125	62.5	125	125	125	62.5	62.5	62.5	125	62.5	62.5	125	125
14	CH <sub>2</sub>	CH <sub>3</sub>	N-CH <sub>3</sub>	125	62.5	125	250	125	62.5	62.5	62.5	125	62.5	62.5	125	125
15	CH <sub>2</sub>	H	N-CH <sub>3</sub>	125	125	125	125	125	62.5	62.5	62.5	125	250	62.5	62.5	125
16	CH <sub>2</sub>	F	N-CH <sub>3</sub>	125	62.5	125	125	62.5	62.5	62.5	15.625	125	125	62.5	125	125
17	CH <sub>2</sub>	Br	N-CH <sub>3</sub>	125	125	125	125	125	62.5	62.5	62.5	125	125	62.5	62.5	125
18	CH <sub>2</sub>	Cl	N-Ph	125	62.5	125	250	125	62.5	62.5	62.5	125	125	62.5	125	125
19	CH <sub>2</sub>	CH <sub>3</sub>	N-Ph	125	62.5	125	250	125	62.5	62.5	62.5	125	125	62.5	125	125
20	CH <sub>2</sub>	H	N-Ph	125	125	125	125	125	62.5	62.5	62.5	125	250	62.5	62.5	125
21	CH <sub>2</sub>	F	N-Ph	125	125	125	125	125	62.5	62.5	62.5	125	125	62.5	62.5	125
22	CH <sub>2</sub>	Br	N-Ph	125	125	125	125	125	62.5	62.5	62.5	125	250	62.5	62.5	125
23 <sup>(9)</sup>	-	H	O	125	62.5	125	125	125	62.5	62.5	15.625	125	125	62.5	125	125

24 <sup>(9)</sup>	-	F	O	125	62.5	125	62.5	62.5	62.5	125	62.5	62.5	62.5	62.5
25 <sup>(9)</sup>	-	C <sub>2</sub> H <sub>5</sub>	O	125	62.5	250	62.5	62.5	62.5	250	62.5	62.5	125	125
26 <sup>(9)</sup>	-	C(CH <sub>3</sub> ) <sub>3</sub>	O	125	62.5	125	62.5	62.5	15.625	125	62.5	62.5	62.5	62.5
27 <sup>(9)</sup>	-	H	NH	125	62.5	125	62.5	62.5	62.5	125	62.5	62.5	62.5	31.25
28 <sup>(9)</sup>	-	F	NH	125	62.5	125	62.5	62.5	15.625	125	62.5	62.5	125	125
29 <sup>(9)</sup>	-	C <sub>2</sub> H <sub>5</sub>	NH	62.5	62.5	62.5	15.625	62.5	62.5	31.25	15.625	31.25	31.25	31.25
30 <sup>(9)</sup>	-	C(CH <sub>3</sub> ) <sub>3</sub>	NH	62.5	125	31.25	7.8	62.5	62.5	15.625	15.625	7.8	7.8	7.8
31 <sup>(9)</sup>	-	H	N-CH <sub>3</sub>	125	62.5	125	31.25	62.5	31.25	125	62.5	31.25	62.5	31.25
32 <sup>(9)</sup>	-	F	N-CH <sub>3</sub>	62.5	62.5	125	62.5	62.5	62.5	125	62.5	125	125	125
33 <sup>(9)</sup>	-	C <sub>2</sub> H <sub>5</sub>	N-CH <sub>3</sub>	125	62.5	125	31.25	62.5	62.5	125	31.25	62.5	125	125
34 <sup>(9)</sup>	-	C(CH <sub>3</sub> ) <sub>3</sub>	N-CH <sub>3</sub>	125	62.5	15.625	7.8	62.5	62.5	15.625	15.625	62.5	62.5	7.8
Ampicilin trihydrate				15.625	>500	>15.625	0.48	0.48	>500	0.48	0.48	-	-	-
Gentamicin				7.8	62.5	15.625	0.12	7.8	31.25	0.48	0.48	-	-	-
Rifampicin				7.8	>500	3.9	3.9	0.9	>500	1.9	0.06	-	-	-
Ofloxacin				3.9	62.5	7.8	3.9	1.9	62.5	0.12	0.12	-	-	-
Fluconazole				-	-	-	-	-	-	-	-	1.9	0.48	15.625
Amphotericin B				-	-	-	-	-	-	-	-	0.48	0.24	1.9

Notes: K.p.\*: *K. pneumoniae* isolate.

P.a.\*: *P. aeruginosa* isolate.

E.c.\*: *E. coli* isolate.

B.s.\*: *B. subtilis* isolate.

S.a.\*: *S. aureus* isolate.

K.p.: *K. pneumoniae* isolate.

P.a.: *P. aeruginosa* ATCC 25853.

E.c.: *E. coli* ATCC 25922

B.s.: *B. subtilis* ATCC 6633.

S.a.: *S. aureus* ATCC 25923.

C.a.: *C. albicans* ATCC 210231.

C.a.\*: *C. albicans* isolate.

C.k.: *C. krusei* ATCC 6258.

According to Table 7, the newly synthesized compounds (**3–22**) show a broad spectrum of activity with MIC values of 250–31.25  $\mu\text{g mL}^{-1}$  against the tested microorganisms. All the new compounds show weak activity with MIC values of 250–125  $\mu\text{g mL}^{-1}$  against *B. subtilis* and its isolate. Among all the new and previous compounds, **34** is the most active against *B. subtilis* isolate and compounds **30** and **34** are the most active against *B. subtilis* and *S. aureus* but they are less active than the standard drugs. According to the observed activity against *S. aureus* isolate (MRSA), **30** and **34**, too, are the most potent among all the compounds and they have the same activity as gentamicin. It can be concluded that compounds without  $\text{CH}_2$  bridge at the second position of benzoxazole ring are generally more active than those with  $\text{CH}_2$  bridge against Gram-positive bacteria. Additionally, holding *t*-butyl group on position R with together piperazine and *N*-methylpiperazine groups attached to the acetamido moiety significantly help to inhibit the growth of MRSA.

All the new compounds **3–22** exhibit same antibacterial activity against *E. coli*, *K. pneumoniae*, and *P. aeruginosa* possessing a MIC value of 62.5  $\mu\text{g mL}^{-1}$  except compounds **6** and **16** which have a MIC value of 15.625  $\mu\text{g mL}^{-1}$  against *E. coli*. All the compounds present a moderate inhibitory effect with MIC values 125–62.5  $\mu\text{g mL}^{-1}$  against *E. coli* isolate, *K. pneumoniae* isolate and it is weaker than the activity of standard drugs. All the newly and previously synthesized compounds are more active than rifampicin and ampicillin trihydrate against *P. aeruginosa* and its gentamicin resistant isolate with MIC values of 125–31.25  $\mu\text{g mL}^{-1}$ . They have the potency as well as ofloxacin with a MIC value of 62.5  $\mu\text{g mL}^{-1}$  against *P. aeruginosa* except derivative **31**, which presents a MIC value of 31.25  $\mu\text{g mL}^{-1}$  and is even more potent than ofloxacin and as potent as gentamicin. In addition, most of the new and previous compounds are as active as ofloxacin and gentamicin against *P. aeruginosa* isolate. The activity observed against *K. pneumoniae* is the same for all the compounds which reveals that differences in the chemical structures of the compounds do not affect the activity against the mentioned microorganism.

In the past 10 years there has been a major development of antifungal drugs; however, there are still weaknesses in the range and scope of current antifungal chemotherapy [38]. All the newly synthesized derivatives (**3–22**) are less active than standard drugs amphotericin B and fluconazole against fungi. The most active one against *C. krusei* among them is compound **11**, however, it is one dilution less potent than standard drug fluconazole. Compounds **30** and **34** are the most active against *C. krusei* among all the compounds. Moreover, the results against *C. krusei* for derivatives **30** and **34** are quite encouraging due to the fact that they are one dilution more potent than fluconazole.

### 3.3 QSAR study against *S. aureus* (MRSA)

In this study, QSAR analysis was performed using the extra-thermodynamic method, correlating the antibacterial activity against MRSA with various physicochemical and structural parameters in order to reveal predictions for the lead optimization in the training set of compounds of newly and previously [9] synthesized 2,5-disubstituted benzoxazole derivatives.

From the data as seen in Table 2, the QSAR Equations (1) and (2) (Table 4) of the training set of compounds were developed by using multivariable regression analysis and

were found to be the best fit for the predictions according to the statistical data analysis results.

As can be deduced from Table 4, the goodness of fit of Equations (1) and (2) are significant, possessing a high  $r^2$  value (0.910 and 0.872, respectively) and a small  $s$  value (0.126 and 0.156, respectively) with an overall  $F$ -test value of 54.539 for Equation (1) and 25.615 for Equation (2) at a significance level  $p < 0.05$ . The correlation coefficients which are given in Table 5 reveal that there is no colinearity between the independent variables used in Equations (1) and (2). In order to avoid the risk of chance correlation, some circumstances which were pointed out by Kubinyi [39], have been taken into consideration in the study. Cross-validation was applied to the original data set and the resulting PRESS values were calculated. The calculated overall PRESS values for Equations (1) and (2) are 0.355 and 0.737, respectively. PRESS values are smaller to SSY (sum of the squares of the response values of the total observations) values (2.870) of the equations that means the models predict better than chance and are statistically significant [36]. Additionally, PRESS/SSY values for Equations (1) and (2), which is the approximate confidence interval for a prediction, are smaller than 0.4. This is also the proof that the observed models are valid [36,37].

Experimentally observed  $\log 1/C$  values along with the calculated  $\log 1/C$  values and residuals of the training set compounds through the developed QSAR equations are given in Table 6. The plot of the predicted *versus* observed  $\log 1/C$  values of the training set compounds for the Equations (1) and (2) are also given in Figures 2 and 3, respectively.

The obtained QSAR equations were screened by using a test set (Table 7) which was not included in the developed models. The observed and calculated  $\log 1/C$  values along with residuals of the test set compounds obtained by Equations (1) and (2) are given in Table 3. Figures 4 and 5 represent the graph of the obtained *versus* calculated  $\log 1/C$  values of the test set molecules for the Equations (1) and (2), which have  $r$  values of 0.882 and 0.838, respectively.

Quantitative Structure–Activity Relationships analysis revealed that attaching 4-substitutedphenyl instead of 4-substitutedbenzyl moiety to the second position of

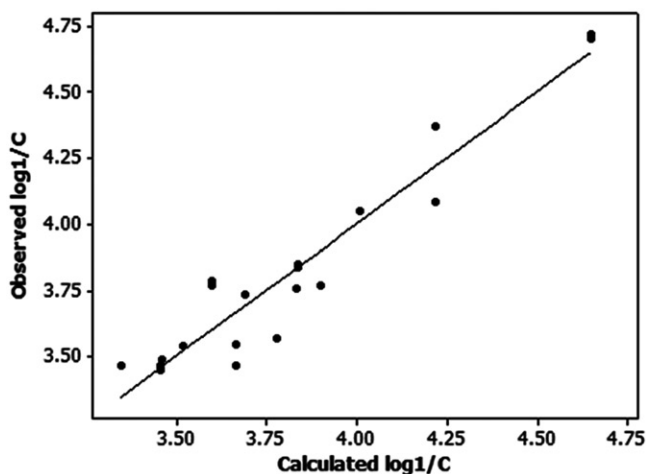


Figure 2. Plot of observed *vs.* calculated  $\log 1/C$  values of the training set compounds obtained from Equation (1).

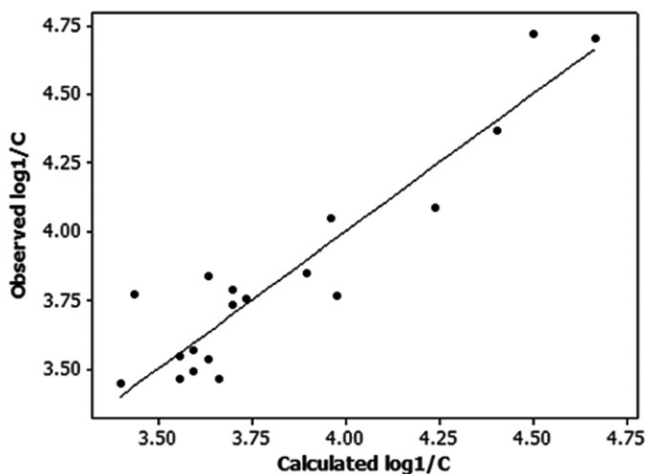


Figure 3. Plot of observed vs. calculated  $\log 1/C$  values of the training set compounds obtained from Equation (2).

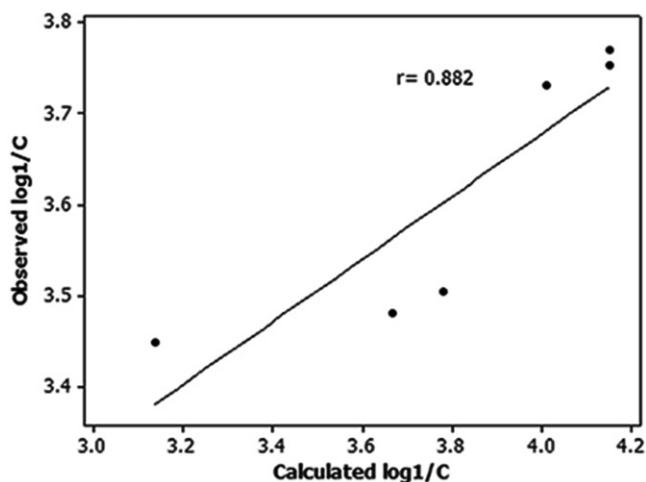


Figure 4. Plot of observed vs. calculated  $\log 1/C$  values of the test set compounds obtained from Equation (1).

benzoxazole ring increases the activity against *S. aureus* (MRSA) according to the obtained Equations (1) and (2). In addition, QSAR study demonstrated that substituents at the position *R* are important for the activity. While Equation (1) indicates that the width of the substituent at this position has a positive effect over the potency, Equation (2) determines that the hydrophobicity of this position is also significant and enhances the activity against *S. aureus* (MRSA). Furthermore, in Equation (1), it is stated that if morpholine moiety is attached to the acetamido group, which is at the fifth position of the benzoxazole ring, the potency decreases. But if this acetamido group is substituted with a piperazine or *N*-methylpiperazine moiety then the activity increases according to the Equation (2).

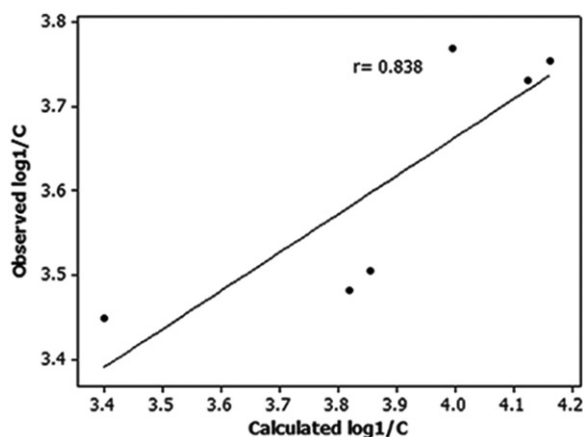


Figure 5. Plot of observed vs. calculated log<sub>1</sub>/C values of the test set compounds obtained from Equation (2).

#### 4. Conclusion

We have discovered moderate active compounds of a series of 2-(*p*-substitutedbenzyl)-5-(2-substitutedacetamido)benzoxazoles as antimicrobial agents. Although antimicrobial activity of the previously synthesized benzoxazole derivatives (**23–34**) were assayed in our former study [9], we wanted to observe their activity against not only standard strains but also their drug-resistant isolates along with the new and similar benzoxazole compounds (**3–22**) and under the same conditions. This let us to evaluate the antimicrobial activity of this set of benzoxazole compounds more accurately.

This present study provides encouraging results for the inhibitory activity against particularly *P. aeruginosa* and its gentamicin-resistant isolate since all the newly and previously synthesized benzoxazole derivatives show comparable antibacterial potency with the standard drugs. In addition, it was observed that antimicrobial activity against some of the microorganisms does not vary although there are structural differences between benzoxazole compounds. The antimicrobial results against *C. krusei* for **30** and **34** are quite promising due to the fact that these compounds are one dilution more potent than fluconazole.

We have also performed a QSAR analysis of this set of benzoxazole derivatives against MRSA. The QSAR study reveals that the width and the hydrophobicity of the substituent at the position *R* and inhibitory activity against this isolate are directly proportional. A methylene bridge should not take place between benzoxazole nuclei and *p*-substituted-phenyl group. While attaching morpholine moiety to the acetamido group of the fifth position of benzoxazole ring decreases the activity, binding up piperazine and/or *N*-methylpiperazine moiety to the same acetamido group enhances the potency. These observations can guide to design new lead antibacterial compounds against *S. aureus* (MRSA).

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