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Synthesis, Antimicrobial Activities of New Sulfonamidobenzoxazoles and Molecular Docking Studies on *Escherichia coli* TEM-1 β-Lactamase

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Abstract: β-Lactam antibiotics are frequently used for treatment of multi-drug resistant microbial infections and the most common mechanism of resistance against these antibiotics is bacterial β-lactamase production. Herein, we reported the design, synthesis and *in vitro* antimicrobial activities of some new 2-substituted-5-(2,4-dinitrophenylsulfonamido)benzoxazole derivatives. Compounds TN1, TN2, and TN3 were found to be significantly active against *E. coli* isolate which contains extended spectrum β-lactamase enzyme at the MIC value of 8 μg mL^{-1} and that is 4-fold higher than the reference drug ampicillin. We performed molecular docking studies into active site of *Escherichia coli* TEM-1 β-lactamase enzyme in order to predict the protein-ligand interactions. According to the docking results, compounds TN1, TN2, and TN3 showed strong interactions between the important active site residues which are responsible for the catalytic mechanism of TEM-1 β-lactamase enzyme and a good correlation is found with the experimental data.

Keywords: antimicrobial activity, benzoxazole, *Escherichia coli*, β-lactamase, molecular docking, sulfonamide.

INTRODUCTION

THE need for new effective antimicrobial agents to prevent the diseases caused by the fastest-growing prevalence of multi-drug resistant microbial infections, still maintains its importance. For treatment of these infections β-lactam antibiotics are frequently used and the most common mechanism of resistance against these antibiotics is bacterial β-Lactamase production. The Extended Spectrum β-lactamase (ESBL) are enzymes that produced by Enterobacteriaceae family including *Escherichia coli*[1–3] and the enzymes are derived from broad-spectrum beta lactamase TEM-1, TEM-2 or SHV-1 by a limited number of mutations.[4] ESBL enzymes hydrolyze the amide bond of β-lactam ring of penicillins, cephalosporins and related antibiotics, thereby inactivating them and often cause diseases in clinics and hospitals worldwide.[5]

Sulbactam, tazobactam, and clavulanate are known as efficient β-lactamase inhibitors however, their efficiency is restricted to class A β-lactamases.[4,6,7] *Escherichia coli* TEM-1 β-lactamase enzyme belongs to class A β-lactamases and have a broad substrate specificity.[8] In recent years, numerous compounds have been reported as β-lactamase inhibitors. Eidam *et al.* synthesized sulfonamide boronic acid derivatives and reported their higher β-lactamase inhibitory activities.[9]

Additionally, a series of phenylethenesulfonamide derivatives found to be the potent inhibitors of TEM-1 β-lactamase enzyme.[10] On the other hand, the benzoxazoles are known as an important class of heterocyclic compounds exhibiting broad spectrum of antimicrobial activities.[11–15]

Herein, we designed and synthesized some new 2-substituted-5-(2,4-dinitrophenylsulfonamido)benzoxazole derivatives, which consist of both benzoxazole and sulfonamide moieties, and tested their *in vitro* antimicrobial activities. Furthermore we performed molecular docking studies into active site of *Escherichia coli* TEM-1 β-lactamase enzyme in order to predict their protein-ligand interactions.

MATERIALS AND METHODS

Chemistry

All of the solvents and chemicals were purchased from commercial vendors and were used without purification. The melting points were uncorrected and measured on Buchi B540. FTIR spectra were obtained on a Agilent Technologies Cary 630 FTIR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on a VARIAN Mercury 400 MHz FT spectrometer, chemical shifts were expressed as ppm, and coupling constants (*J*) were expressed as hertz. Mass spectra were obtained on a Waters Micromass ZQ using the ESI method. Analytical data were obtained on elemental analyzer system Leco CHNS-932 CHNS-O analyzer and the results (C, H, N, S) were found within ± 0.4 % of the calculated amounts.

GENERAL PROCEDURE FOR THE SYNTHESIS OF 5-(2,4-DINITROPHENYLSULFONAMIDO)BENZOXAZOLE DERIVATIVES (TN1-14)

Firstly, 2-(4-substitutedphenyl)-5-aminobenzoxazole were prepared according to literature data.[14–16] Then 0.95 mmol pyridine and 0.52 mmol 2,4-dinitrobenzene-sulfonyl chloride (**d**) added to a solution of 0.048 mmol 2-(4-substitutedphenyl)-5-aminobenzoxazole in 2 mL dichloromethane. The reaction mixture was stirred for 16 hours at the room temperature. At the end of the reaction, the solid product was filtered and washed with saturated solution of $CuSO_4$ and $NaHCO_3$ in water, then recrystallized from ethyl acetate/*n*-hexan (1:4) mixture.[16, 17] All of the compounds are new.

2-PHENYL-5-(2,4-DINITROPHENYLSULFONAMIDO)BENZOXAZOLE (TN1)

32 % yield; m.p 223–224 °C; IR ν_{max} 3337, 3099, 1552, 1477, 1347, 1326, 1157, 1110 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): *d*/ppm = 7.21 (dd, 1H, *J* = 8.8 Hz, *J* = 2.4 Hz, H-6), 7.54 (d, 1H, *J* = 2.4 Hz, H-4), 7.59-7.65 (m, 3H, H-3', H-4' H-5'), 7.76 (d, 1H, *J* = 8.4 Hz, H-7), 8.16-8.18 (m, 2H, H-2', H-6'), 8.24 (d, 1H, *J* = 9.2 Hz, H-6''), 8.60 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz, H-5''), 8.89 (d, 1H, *J* = 2.0 Hz, H-3''), 11.17 (s, 1H, NH); ^{13}C NMR (DMSO-d$_6$): *d*/ppm = 111.62, 113.25, 120.24, 120.46, 126.04, 127.21, 127.32, 129.34, 131.69, 132.21, 132.77, 136.00, 142.01, 147.84, 148.08, 150.05, 163.49; ESIMS *m/z* (%) 441.57 (30) [M+H]$^+$; Anal. Calcd. for $C_{19}H_{12}N_4O_7S$. 0.3HOH: C, 51.19; H, 2.85; N, 12.57; S, 7.19. Found: C, 50.98; H, 2.78; N, 12.74; S, 7.34.

2-(4-CHLOROPHENYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN2)

41 % yield; m.p 206–207 °C; IR (KBr) ν_{max} 3334, 3108, 1539, 1479, 1347, 1306, 1174, 1094 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): *d*/ppm = 7.22 (dd, 1H, *J* = 8.8 Hz, *J* = 2.0 Hz, H-6), 7.54 (d, 1H, *J* = 2.4 Hz, H-4), 7.68 (q, 2H, H-3', H-5'), 7.76 (d, 1H, *J* = 8.8 Hz, H-7), 8.16 (q, 2H, H-2', H-6'), 8.24 (d, 1H, *J* = 8.4 Hz, H-6''), 8.59 (dd, 1H, *J* = 8.4 Hz, *J* = 2.0 Hz, H-5''), 8.89 (d, 1H, *J* = 2.4 Hz, H-3''), 11.09 (s, 1H, NH); ^{13}C NMR (DMSO-d$_6$): *d*/ppm = 111.63, 113.26, 120.24, 120.66, 124.92, 127.21, 129.07, 129.52, 131.69, 132.90, 135.98, 136.99, 141.94, 147.84, 148.10, 150.05, 162.57; ESIMS *m/z* (%) 473.34 (100) [M-H]$^+$, 475.30 (40) [M-H+2]$^+$; Anal. Calcd. for $C_{19}H_{11}ClN_4O_7S$: C, 48.06; H, 2.34; N, 11.80; S, 6.75. Found: C, 48.50; H, 2.69; N, 11.83; S, 6.60.

2-(4-FLUOROPHENYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN3)

37 % yield; m.p 207–209 °C. IR ν_{max} 3341, 3108, 1533, 1480, 1349, 1144, 1098 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): *d*/ppm = 7.21 (dd, 1H, *J* = 9.2 Hz, *J* = 2.4 Hz, H-6), 7.44-7.48 (m, 2H, H-3', H-5'), 7.53 (d, 1H, *J* = 2.0, Hz H-4), 7.75 (d, 1H, *J* = 8.8 Hz, H-7), 8.20-8.25 (m, 3H, H-2', H-6', H-6''), 8.59 (dd, 1H, *J* = 8.4 Hz, *J* = 2.0 Hz, H-5''), 8.89 (d, 1H, *J* = 2.0 Hz, H-3''), 11.15 (s, 1H, NH); ^{13}C NMR (DMSO-d$_6$): *d*/ppm = 111.61, 113.21, 116.60 (J_{C-F} = 22.1 Hz), 120.24, 120.44, 122.72 (J_{C-F} = 3.1 Hz), 127.20, 130.01 (J_{C-F} = 9.1 Hz), 131.68, 132.82, 135.99, 141.99, 147.84, 148.11, 150.04, 162.68, 164.35 (J_{C-F} = 249.1 Hz); ESIMS *m/z* (%) 459.30 (95) [M+H]$^+$; Anal. Calcd. for $C_{19}H_{11}FN_4O_7S$: C, 49.79; H, 2.42; N, 12.22; S, 6.99. Found: C, 49.91; H, 2.52; N, 12.15; S, 6.97.

2-(4-BROMOPHENYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN4)

40 % yield; m.p 219–221 °C; IR ν_{max} 3332, 3106, 1533, 1474, 1345, 1172, 1101cm^{-1}; ^{1}H NMR (DMSO-d$_6$): *d*/ppm = 7.22 (dd, 1H, *J* = 8.8 Hz, *J* = 2.0 Hz, H-6), 7.54 (d, 1H, *J* = 2.4 Hz, H-4), 7.76 (d, 1H, *J* = 8.8 Hz, H-7), 7.82 (q, 2H, H-3', H-5'), 8.08 (q, 2H, H-2', H-6'), 8.23 (d, 1H, *J* = 8.4 Hz, H-6''), 8.59 (dd, 1H, *J* = 9.2 Hz, *J* = 2.4 Hz, H-5''), 8.88 (d, 1H, *J* = 2.0 Hz, H-3''), 11.10 (s, 1H, NH); ^{13}C NMR (DMSO-d$_6$): *d*/ppm = 111.69, 113.25, 120.23, 120.68, 125.26, 125.94, 127.19, 129.17, 131.68, 132.44, 132.94, 135.98, 141.92, 147.82, 148.07, 150.03, 162.67; ESIMS *m/z* 517.95 [M-H]$^+$ (100), 519.14 [M-H+2]$^+$ (100); Anal. Calcd. for $C_{19}H_{11}BrN_4O_7S$: C, 43.95; H, 2.14; N, 10.79; S, 6.17. Found: C, 43.97; H, 2.36; N, 10.79; S, 6.08.

2-(4-ETHYLPHENYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN5)

42 % yield; m.p 190–191 °C; IR ν_{max} 3302, 3095-2968, 1539, 1494, 1345, 1146, 1102 cm^{-1}; ^{1}H NMR (DMSO-d$_6$): *d*/ppm = 1.22 (t, 3H, CH_3), 2.71 (q, 2H, CH_2), 7.19 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz, H-6), 7.45 (d, 2H, *J* = 8.4 Hz, H-3', H-5'), 7.52 (d, 1H, *J* = 2.0 Hz, H-4), 7.38 (d, 1H, *J* = 8.4 Hz, H-7), 8.08 (d, 2H, *J* = 8.4 Hz, H-2', H-6'), 8.24 (d, 1H, *J* = 8.4 Hz, H-6''), 8.59 (dd, 1H, *J* = 8.4 Hz, *J* = 2.0 Hz, H-5''), 8.88 (d, 1H, *J* = 2.4 Hz, H-3''),

11.14 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): *d*/ppm = 15.09 (CH_3), 28.15 (CH_2), 111.49, 113.13, 120.24, 120.25, 123.56, 127.20, 127.41, 128.76, 131.68, 132.74, 136.04, 142.10, 147.84, 148.00, 148.54, 150.03, 163.66; ESIMS *m/z* 469.28 $[M+H]^+$ (100); Anal. Calcd. for $C_{21}H_{16}N_4O_7S$: C, 53.84; H, 3.44; N, 11.96; S, 6.84. Found: C, 53.41; H, 3.53; N, 12.04; S, 6.90.

2-(4-METHYLPHENYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN6)

48 % yield; m.p 232–233 °C; IR ν_{max} 3341, 3106, 1533, 1496, 1349, 1172, 1101 cm^{-1}; ^{1}H NMR (DMSO-d_6): *d*/ppm = 2.40 (s, 3H, CH_3), 7.18 (dd, 1H, *J* = 8.8 Hz, *J* = 2.4 Hz, H-6), 7.41 (d, 2H, *J* = 8.0 Hz, H-3', H-5'), 7.50 (d, 1H, *J* = 2.0 Hz, H-4), 7.72 (d, 1H, *J* = 9.2 Hz, H-7), 8.04 (d, 2H, *J* = 8.0 Hz, H-2', H-6'), 8.22 (d, 1H, *J* = 8.8 Hz, H-6''), 8.58 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz, H-5''), 8.88 (d, 1H, *J* = 2.4 Hz, H-3''), 11.13 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): *d*/ppm = 21.14 (CH_3), 111.48, 113.13, 120.23, 120.27, 123.31, 127.20, 127.30, 129.92, 131.69, 132.72, 136.01, 142.09, 142.49, 147.84, 148.00, 150.03, 163.67; ESIMS *m/z* 455.06 $[M+H]^+$ (100); Anal. Calcd. for $C_{20}H_{14}N_4O_7S$: C, 52.86; H, 3.11; N, 12.33; S, 7.06. Found: C, 53.10; H, 3.44; N, 12.09; S, 6.95.

2-BENZYL-5-(2,4-DINITROPHENYLSULFONAMIDO)BENZOXAZOLE (TN10)

64 % yield; m.p 203–205 °C; IR ν_{max} 3328, 3106, 1530, 1457, 1340, 1148, 1101 cm^{-1}; ^{1}H NMR (DMSO-d_6): *d*/ppm = 4.28 (s, 2H, CH_2), 7.10 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz, H-6), 7.24–7.33 (m, 5H, phenyl'), 7.40 (d, 1H, *J* = 2.4 Hz, H-4), 7.60 (d, 1H, *J* = 8.4 Hz, H-7), 8.16 (d, 1H, *J* = 8.8 Hz, H-6''), 8.54 (dd, 1H, *J* = 8.4 Hz, *J* = 2.0 Hz, H-5''), 8.84 (d, 1H, *J* = 2.4 Hz, H-3''), 11.05 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): *d*/ppm = 34.10 (CH_2), 111.24, 113.16, 119.94, 120.21, 127.09, 127.17, 128.64, 129.07, 131.64, 132.29, 134.87, 135.99, 141.40, 147.81, 148.23, 150.00, 166.79; ESIMS *m/z* 455.00 $[M+H]^+$ (100); Anal. Calcd. for $C_{20}H_{14}N_4O_7S$: C, 52.86; H, 3.11; N, 12.33; S, 7.06. Found: C, 52.83; H, 3.40; N, 12.22; S, 6.99.

2-(4-CHLOROBENZYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN11)

46 % yield; m.p 191–192 °C; IR ν_{max} 3101, 1537, 1492, 1349, 1172, 1098 cm^{-1}; ^{1}H NMR (DMSO-d_6): *d*/ppm = 4.30 (s, 2H, CH_2), 7.11 (dd, 1H, *J* = 8.4 Hz, *J* = 2.0 Hz, H-6), 7.35–7.40 (m, 5H, phenyl', H-4), 7.60 (d, 1H, *J* = 8.4 Hz, H-7), 8.16 (d, 1H, *J* = 8.8 Hz, H-6''), 8.54 (dd, 1H, *J* = 8.4 Hz, *J* = 2.0 Hz, H-5''), 8.84 (d, 1H, *J* = 2.0 Hz, H-3''), 11.05 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): *d*/ppm = 33.23 (CH_2), 111.19, 113.10, 119.92, 120.14, 127.10, 128.49, 130.99, 131.56, 131.78, 132.24, 133.80, 135.92, 141.27, 147.74, 148.14, 149.94, 166.39; ESIMS *m/z* 489.30 $[M+H]^+$ (100), 491.07 $[M+H+2]^+$ (36); Anal. Calcd. for $C_{20}H_{13}ClN_4O_7S$: C, 49.14; H, 2.68; N, 11.46; S, 6.56. Found: C, 49.17; H, 2.94; N, 11.71; S, 6.60.

2-(4-FLUOROBENZYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN12)

59 % yield; m.p 178–179 °C; IR ν_{max} 3185, 3108, 1535, 1466, 1336, 1170, 1099 cm^{-1}; ^{1}H NMR (DMSO-d_6): *d*/ppm = 4.28 (s, 2H, CH_2), 7.07 (d, 1H, *J* = 9.2 Hz, H-6), 7.12–7.17 (m, 2H, H-2', H-6'), 7.36-7.39 (m, 3H, H-4, H-3', H-5'), 7.56 (d, 1H, *J* = 8.4 Hz, H-7), 8.15 (d, 1H, *J* = 8.8 Hz, H-6''), 8.52 (dd, 1H, *J* = 8.8 Hz, *J* = 2.4 Hz, H-5''), 8.81 (s, 1H, H-3''), 11.04 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): *d*/ppm = 33.12 (CH_2), 111.00, 112.86, 115.30 ($J_{C\text{-}F}$ = 21.8 Hz), 119.96 ($J_{C\text{-}F}$ = 5.8 Hz), 126.94, 130.98, 131.01, 131.05, 131.51, 141.28, 147.76, 147.79, 149.71, 161.24 ($J_{C\text{-}F}$ = 241.7 Hz), 166.44; ESIMS *m/z* 473.31 $[M+H]^+$ (100); Anal. Calcd. for $C_{20}H_{13}FN_4O_7S$: C, 50.85; H, 2.77; N, 11.86; S, 6.79. Found: C, 50.96; H, 3.04; N, 11.95; S, 6.86.

2-(4-BROMOBENZYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN13)

61 % yield; m.p 124–126 °C; IR ν_{max} *3095*-3073, 1537, 1464, 1364, 1172, 1098 cm^{-1}; ^{1}H NMR (DMSO-d_6): *d*/ppm = 4.29 (s, 2H, CH_2), 7.11 (dd, 1H, *J* = 8.4 Hz, *J* = 2.4 Hz, H-6), 7.29–7.32 (m, 2H, H-2', H-6'), 7.39 (d, 1H, *J* = 1.6 Hz, H-4), 7.50–7.53 (m, 2H, H-3', H-5'), 7.60 (d, 1H, *J* = 8.4 Hz, H-7), 8.16 (d, 1H, *J* = 8.8 Hz, H-6''), 8.54 (dd, 1H, *J* = 8.8 Hz, *J* = 2.0 Hz, H-5''), 8.84 (d, 1H, *J* = 1.6 Hz, H-3''), 11.04 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): *d*/ppm = 33.30 (CH_2), 111.19, 113.09, 119.92, 120.14, 120.26, 127.10, 131.36, 131.43, 131.56, 132.25, 134.21, 135.92, 141.26, 147.74, 148.14, 149.94, 166.32; ESIMS *m/z* 533.24 $[M+H]^+$ (100), 535.06 $[M+H+2]^+$ (100); Anal. Calcd. for $C_{20}H_{13}BrN_4O_7S$: C, 45.04; H, 2.46; N, 10.51; S, 6.01. Found: C, 45.22; H, 2.77; N, 10.65; S, 6.04.

2-(4-METHYLBENZYL)-5-(2,4-DINITROPHENYLSULFONAMIDO)-BENZOXAZOLE (TN14)

31 % yield; m.p 192–193 °C; IR ν_{max} 31110-3037, 1537, 1472, 1349, 1172, 1105 cm^{-1}; ^{1}H NMR (DMSO-d_6): *d*/ppm = 2.24 (s, 3H, CH_3), 4.22 (s, 2H, CH_2), 7.08-7.12 (m, 3H, H-6, H-3', H-5'), 7.20 (d, 2H, *J* = 8.0 Hz, H-2', H-6'), 7.39 (d, 1H, *J* = 2.0 Hz, H-4), 7.59 (d, 1H, *J* = 8.4 Hz, H-7), 8.16 (d, 1H, *J* = 8.8 Hz, H-6''), 8.54 (dd, 1H, *J* = 8.8 Hz, *J* = 2.0 Hz, H-5''), 8.83 (d, 1H, *J* = 2.4 Hz, H-3''), 11.04 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): *d*/ppm = 20.52 (CH_3), 33.64 (CH_2), 111.13, 113.07, 119.83, 120.13, 127.08, 128.85, 129.11, 131.57, 131.71, 132.19, 135.93, 136.16, 141.34, 147.74, 148.15, 149.93, 166.88; ESIMS *m/z* 469.32 $[M+H]^+$ (100); Anal. Calcd. for $C_{21}H_{16}N_4O_7S$: C, 53.84; H, 3.44; N, 11.96; S, 6.84. Found: C, 53.72; H, 3.53; N, 11.97; S, 6.81.

Antimicrobial Evaluation

Standard strains of *E. coli* ATCC 25922, *E. coli* isolate (ESBL: Extended spectrum *β*-lactamase, resistant to all *β*-lactam antibiotics), *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* isolate (resistant to ciprofloxacin), *Acinetobacter*

baumannii ATCC 17978, *Acinetobacter baumannii* isolate (resistant to ciprofloxacin), *Staphylococcus aureus* ATCC 29213, *S. aureus* isolate [MRSA (meticillin resistant *S.aureus*)], *Enterococcus faecalis* ATCC 29212, *E. faecalis* isolate (resistant to vancomycin) and *Candida albicans* ATCC 10231 were used in the study.

Microdilution Method

Mueller Hinton Agar (MHA), Cation Adjusted Mueller Hinton Broth (CAMHB), Sabouraud Dextrose Agar (SDA), Sabouraud Liquid Medium (SLM) and RPMI-1640 medium with L-glutamine (Sigma) buffered with MOPS (pH7) media were used during the study. Microdilution method was used to determine the susceptibilities of the microorganisms according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) M100-S25[18] and M27-A3[19] standards. For bacteria 100 µL of CAMHB for Candida RPMI-1640 medium with L-glutamine buffered with MOPS (pH7) were added to each well of the microplates.

McFarland 0.5 turbidity standard was used to standardize the inoculum density of the microorganisms. Saline suspension of 16–20 hours grown pure colonies of bacteria or fungi were adjusted to achieve McFarland turbidity. The inoculum concentrations resulted in suspensions containing 1–2 × 10^8 CFU mL^{-1} bacteria. Bacterial inoculum suspensions were diluted 1:20 to yield 1×10^6 CFU mL^{-1} and the final suspension in the wells of the microplate was 10^5 CFU mL^{-1}. Inoculum suspension of *Candida albicans* was diluted 1:100 and 1:20 respectively and 2.5 × 10^3 CFU mL^{-1} were inoculated to the twofold-diluted solution of the compounds.

Standard powders of ampicillin (Sigma), gentamycin (Sigma), ciprofloxacin (Sigma), meropenem (Sigma) and fluconazole (Sigma) were used as control. Stock solutions of the tested compounds were dissolved in DMSO (Merck). Standard antibiotic solutions were dissolved in appropriate solvents recommended by CLSI guidelines.[18]

Antibiotics	Diluent	Solvent
ampicillin	0.1 mol dm^3 pH 6 PBS	0.1 mol dm^3 pH 8 PBS
gentamycin	distilled water	distilled water
ciprofloxacin	distilled water	distilled water
fluconazole	1 : 9 water : ethanol	distilled water

A hundred microliters of the stock solutions of standard drugs and the tested compounds were added to the first wells of the microplates and diluted two-fold in the wells of the microplates. The solution of the synthesized compounds and standard drugs were prepared at 1024, 512, 256, 128, 64, 32, 16, 8 µg mL^{-1} and 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 µg mL^{-1} concentrations, respectively. All solvents and diluents, pure media and pure microorganisms were used in control wells.

Finally, a 10 µL microorganism inoculums were added to each well of the microplates. Bacteria were incubated for 16–20 hours at 37 °C and fungi were incubated for 24–48 hours at 35 °C. After incubation, the lowest concentration of the compounds that completely inhibits macroscopic growth was determined and reported as minimum inhibitory concentrations (MICs).

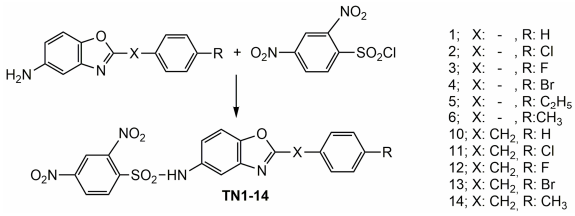
Molecular Docking

The crystal structure of the TEM-1 *β*-lactamase enzyme of *E. coli* was selected for molecular docking studies (PDB ID: 1ERQ).[20] Protein and ligands were prepared by using Accelrys Discovery Studio 3.5 software.[21] Target protein, TEM-1 *β*-lactamase enzyme was taken, hydrogens were added the ligand was extracted, and optimized using the all atom CHARMm forcefield and the Adopted Basis set Newton Raphson method until the root mean deviation (RMS) gradient was < 0.05 kcal / mol / $Å^2$. By using the binding site module, minimized protein was defined as the receptor. The binding site was defined from the cavity finding method and modified that contain all of the important active site residues of the *β*-lactamase enzyme. Binding sphere (40.78, 36.48, 31.78, 8.27) was selected from the active site. The most active compounds against *E. coli* isolate (**TN1**, **TN2**, **TN3)** and the boronate inhibitor that is the ligand of 1ERQ.pdb crystal structure were sketched, all atom CHARMm forcefield parameterization was assigned and then minimized using the ABNR method as described above. Conformational searches of the ligands were performed using a simulated annealing molecular dynamics approach. The ligands were heated to a temperature of 700 K and then annealed to 200 K. CDOCKER method was performed by using Discovery Studio 3.5.[22] TEM-1 *β*-lactamase enzyme was held rigid but the ligands were allowed to be flexible during refinement. At first the methodology was validated by docking of boronate inhibitor. The docked position of boronate inhibitor overlaps well with an RMSD of 1.3 Å with the crystal structure position. Afterwards molecular docking studies were performed on the compounds **TN1**, **TN2**, **TN3**. All docked poses were scored by applying Analyze Ligand Poses subprotocol and binding energies were calculated by using in situ ligand minimization step (ABNR method) and using implicit solvent model (GBMV) in Discovery Studio 3.5 software. The lowest binding energy was taken as the best-docked conformation of the compounds for the macromolecule. The pictures were taken by using Discovery Studio 4.1 visualizer.

RESULTS AND DISCUSSION

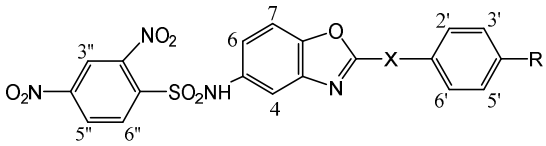
Chemistry

In this study, 2-(4-substitutedphenyl/benzyl)-5-(2,4-dinitrophenylsulfonamido)benzoxazole derivatives (**TN1**–**TN14**) were synthesized for the first time. At first step, 5-amino-2-substitutedbenzoxazoles were obtained.[14–16] Then 2,4-dinitrobenzenesulfonyl chloride and 5-amino-2-substitutedbenzoxazole derivatives were treated in pyridine and dichloromethane to obtain 5-(2,4-dinitrophenyl-sulfonamido)benzoxazole derivatives (**TN1**–**TN14**) (Scheme 1).[16,17] Synthesized structures were characterized by ^{1}H NMR, ^{13}C NMR, Mass Spectra and Elemental Analysis and the results are in agreement with the proposed structures. According to the ^{1}H NMR spectra of the compounds the signals of NH (SO_2NH) proton of the compounds was observed at 11.05–11.17 ppm. Aromatic CH_3 and benzylic CH_2 protons were appeared at 2.24–2.40 ppm and 2.71–4.30 ppm, respectively. All the aromatic protons were observed at 7.07–8.39 ppm. ^{13}C NMR spectra were appropriate to formulas of the synthesized compounds and Mass spectra showed $M^+ + H$ peaks in accordance with their formulas. Additionally, elemental analyses results of C, H, N, S were found within ± 0.4 % of the calculated amounts.



Scheme 1. Synthetic pathway of 5-(2,4-dinitrophenylsulfon-amido) benzoxazole (**TN1**–**14**) derivatives. *Reagents and conditions:* Pyridine and

Table 1. The structures of the tested benzoxazoles and their *in vitro* antimicrobial activities as MIC values (μg mL^{-1}).



Compounds			Microorganisms										
Code	X	R	*E.c.*	*E.c.**	*P.a.*	*P.a.**	*A.b*	*A.b**	*S.a.*	*S.a.**	*E.f.*	*E.f.**	*C.a.*
TN1	–	H	128	8	256	128	128	128	256	256	128	128	128
TN2	–	Cl	128	8	256	128	128	128	256	256	32	16	128
TN3	–	F	128	8	256	128	128	128	256	256	32	128	128
TN4	–	Br	128	128	256	128	128	128	256	512	128	128	128
TN5	–	C_2H_5	128	128	256	128	128	128	16	16	32	64	128
TN6	–	CH_3	128	256	256	128	128	128	256	256	256	256	128
TN10	CH_2	H	128	16	256	128	128	128	16	16	64	32	128
TN11	CH_2	Cl	128	128	256	128	128	128	16	16	64	128	128
TN12	CH_2	F	128	32	256	128	128	64	16	256	64	64	128
TN13	CH_2	Br	128	128	256	128	128	128	16	16	128	64	128
TN14	CH_2	CH_3	128	128	128	128	128	64	32	32	64	128	128
Ciprofloxacin			< 0.125	1	0.5	0.25	> 32	> 32	0.5	> 32	1	> 32	–
Ampicillin			4	> 32	–	–	–	–	2	> 32	2	8	–
Meropenem			< 0.125	0.125	1	1	8	> 32	< 0.125	> 32	8	32	–
Gentamycin			1	4	1	2	> 32	32	1	> 32	16	> 32	–
Fluconazole			–	–	–	–	–	–	–	–	–	–	1

[a] ***Abbreviations:*** *E.c.*:*Escherichia coli* ATCC 25922; *E.c.**: *E. coli* isolate; *P.a.*: *Pseudomonas aeruginosa* ATCC 25758; *P.a.**: *P. aeruginosa* isolate; *A.b.*: *Acinetobacter baumannii* ATCC 17978; *A.b.**: *Acinetobacter* isolate; *S.a.*: *Staphylococcus aureus* ATCC 29213; *S.a.**: *S. aureus* isolate; *E.f.*: *Enterococcus faecalis* ATCC 29212; *E.f.**: *E. faecalis* isolate; *C.a.*: *Candida albicans* ATCC 10231.

In vitro Antimicrobial Evaluation

All of the synthesized 2-(4-substitutedphenyl/benzyl)-5-(2,4-dinitrophenylsulfonamido)benzoxazoles (**TN1**–**TN14**), were tested for their *in vitro* antimicrobial activities against *Escherichia coli* ATCC 25922, *E. coli* isolate (ESBL), *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* isolate (resistant to ciprofloxacin), *Acinetobacter baumannii* ATCC 17978, *A. baumannii* isolate (resistant to ciprofloxacin) as Gram-negative bacteria, *Staphylococcus aureus* ATCC 29213, *S. aureus* isolate [MRSA (meticillin resistant *S. aureus*)], *Enterococcus faecalis* ATCC 29212, *E. faecalis* isolate (resistant to vancomycin) as Gram-positive bacteria and *Candida albicans* ATCC 10231 as fungus. The standard drugs, ciprofloxacin, ampicillin, meropenem, gentamycin for antibacterial activity, and fluconazole for antifungal activity were screened under identical conditions for quality control and comparison. Microdilution method was used for determination of Minimum Inhibitory Concentration (MIC) values as seen in Table 1.

In vitro biological results demonstrated that all of the synthesized compounds showed a wide spectrum of activity against the tested microorganisms at MIC values between 8 and 512 μg mL^{-1}. Most of the tested compounds showed significant antibacterial activities against *S. aureus*, *E. faecalis* and their drug resistant isolates. Compounds **TN5**, **TN10, TN11** and **TN13** showed high antibacterial activity against drug resistant isolate of *S. aureus* with the MIC value of 16 μg mL^{-1} and they were found to be 2-fold more effective than all of the reference drugs. Compounds **TN2** and **TN10** showed better activity than the reference drugs ciprofloxacin, meropenem and gentamycin against drug resistant isolate of *E. faecalis* with the MIC values of 16-32 μg mL^{-1}.

Moreover, compounds **TN1**, **TN2** and **TN3** showed significant antibacterial activity against *E. coli* isolate with the MIC value of 8 μg mL^{-1} and that is 4-fold higher than the reference drug ampicillin. According to obtained data, CH_2 bridge which placed on the 2nd position of benzoxazole ring seems to decrease the activity against *E. coli* isolate.

Molecular Docking Studies

In this study, compounds **TN1**, **TN2**, and **TN3** were found to be significantly active at the MIC value of 8 μg mL^{-1} against *E. coli* isolate which contains extended spectrum *β*-lactamase enzyme. Molecular docking studies were performed on these compounds in order to understand the interactions between the compounds and TEM-1 *β*-lactamase enzyme by using CDOCKER method.[20,21]

TEM-1 is the most common *β*-lactamase in Gram-negative bacteria, belonging to Class A beta-lactamases 2b group and ampicillin resistance is highly associated with the production of TEM-1 in *E. Coli.*[4] The active site of TEM-1 *β*-lactamase enzyme including the residues: Ser70, Lys73, Ser130, Asn132, Glu166, Lys234, Ala237. It is reported that all of these residues play important roles in catalytic mechanism of the *β*-lactamase enzyme. Ser70 covalently bound to *β*-lactam ring as an acyl-enzyme intermediate and Lys73 acts as a general base in abstracting a proton from Ser70 and transferring it to the thiazolidine ring of *β*-lactam antibiotic *via* Ser130.[23,24]

According to the docking results, boronate inhibitor revealed H bonds with Ser70, Ser130, Asn170, Ala237 and Arg243; revealed pi-alkyl interaction with Tyr105 that are in accordance with the X-ray structure of 1ERQ.pdb. One of the most active compounds **TN1** revealed H bonds with Ser130 and Arg243; revealed pi-pi stacking with Tyr105, pi-donor interaction with Asn132, pi-alkyl interaction with Val 216 and Ala237, pi-sulfur interaction with Met270 (Figure 1b). **TN2** revealed halogen bond with Asn132; revealed pi-alkyl interactions with Pro219, Ala237 and Met270 (Figure 1c). **TN3** revealed H bond with Ser70; revealed pi-pi stacking with Tyr105, pi-alkyl interaction with Val216 and Ala237, pi-sulfur interaction with Met270 (Figure 1d). Binding energies of the compounds **TN1**, **TN2**, **TN3** and boronate inhibitor are –3.60, –2.85, –5.43 and –11.38, respectively (Table 2). *In vitro* biological results demonstrated that **TN1**, **TN2** and **TN3** exhibited promising antimicrobial activity against *E. coli* isolate and the docking results were also correlated with the microbiological data.

Table 2. Docking results.

Code	Binding Energy / kcal mol^{-1}	Interacted Residues in < 4 Å
TN1	–3.60	Ser70, Lys73, Tyr105,[a] **Ser130** (2.98 Å), Asn132,[c] Glu166, Asn170, Val216,[b] Ser235, Gly236, Ala237,[b] Gly238, **Arg243** (2.04 Å), Met270[d]
TN2	–2,85	Ser70, Lys73, Tyr105, Ser130, Asn132[x] (3.06 Å), Asn170, Lys215, Val216, Gly218, Pro219,[b] Ala237,[b] Gly238, Met270[b]
TN3	–5.43	**Ser70** (2.31 Å), Lys73, Tyr105,[a] Met129, Ser130, Asn132, Glu166, Asn170, Lys215, Val216,[b] Pro219, Ala237,[b] Gly238, Met270[b]
boronate inhibitor	–11.38	Met69, **Ser70** (2.10, 2.29 Å), Lys73, Tyr105,[b] **Ser130** (2.00, 2.11 Å), Asn132, **Asn170** (1.80 Å), Val216, Lys234, Ser235, Gly236, **Ala237** (2.11, 2.64 Å), Gly238, Glu239, **Arg243** (1.82 Å), **H$_2$O510** (water mediated H-bonds with Val216 (1.96 Å) and Arg243 (1.86 Å)

Bold: H-bonds;
[a] pi-pi interactions.
[b] pi-alkyl interactions.
[c] pi-donor interactions.
[d] pi-sulfur interactions.
[x] halogen bonds.

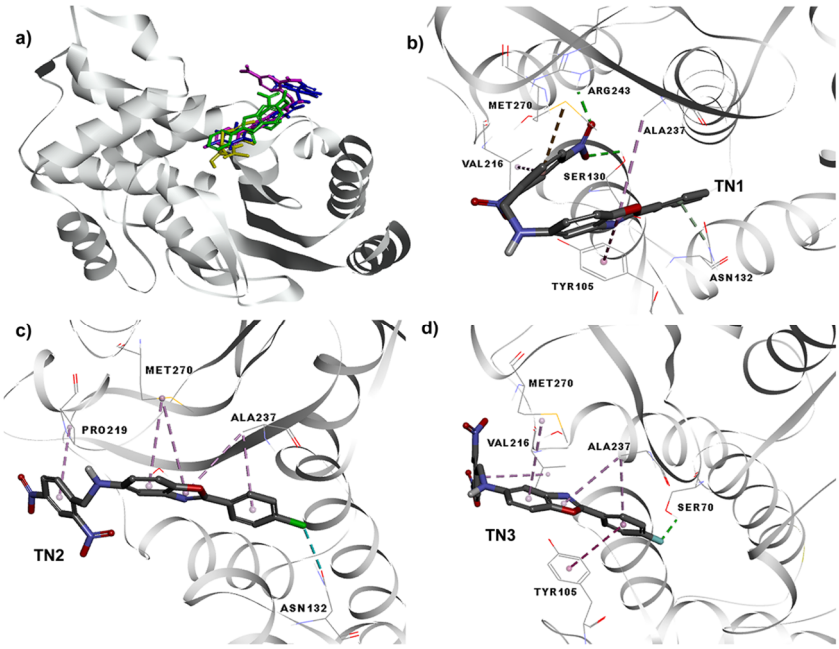


Figure 1. (a) Docked position of boronate inhibitor (yellow) and **TN1** (green), **TN2** (blue), **TN3** (pink); (b) Docked position of **TN1**: compound revealed H bonds with Ser130 and Arg243, showed interactions with Tyr105 (pi-pi interaction), Asn132 (pi-donor interaction), Val216 (pi-alkyl interaction), Ala237 (pi-alkyl interaction), Met270 (pi-sulfur interaction); (c) docked position of **TN2**: compound revealed halogen bond with Asn132, showed pi-alkyl interactions with Pro219, Ala237 and Met270; (d) docked position of **TN3**: compound revealed H bond with Ser70, showed interactions with Tyr105 (pi-pi interaction), Val216 (pi-alkyl interaction), Ala237 (pi-alkyl interaction), Met270 (pi-alkyl interaction).

CONCLUSION

Herein, we designed and synthesized some new 2-substituted-5-(2,4-dinitrophenylsulfonamido)benzoxazoles and tested their *in vitro* antimicrobial activities. All of the tested compounds showed significant activity against the tested microorganisms especially extended spectrum *β*-lactamase containing *E. coli* isolate. Compounds **TN1**, **TN2**, and **TN3** were found to be significantly active with the MIC value of 8 μg mL^{-1} against the extended spectrum *β*-lactamase containing *E. coli* isolate, which was resistant to all *β*-lactam antibiotics. In order to predict the protein-ligand interactions we performed molecular docking studies into active site of *E. coli* TEM-1 *β*-lactamase enzyme. According to the docking results, compounds **TN1**, **TN2**, and **TN3** showed strong interactions between the important active site residues which are responsible for the catalytic mechanism of TEM-1 *β*-lactamase enzyme, such as Ser70, Ser130, and Asn132. A good correlation was noticed between the docking scores and the microbiological data. It can be concluded that these compounds could show their activity by inhibiting the *β*-lactamase enzyme. The compounds obtained from this study can be useful in designing of new potent *β*-lactamase inhibitors by using them as lead compounds.

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