Med Chem Res (2016) 25:553–567
DOI 10.1007/s00044-015-1499-1

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis, molecular docking and antimicrobial evaluation of novel benzoxazole derivatives

**Tugba Ertan-Bolelli[1] · İlkay Yildiz[1] · Selda Ozgen-Ozgacar[2]**

Received: 14 September 2015 / Accepted: 21 December 2015 / Published online: 22 January 2016


**Abstract** In this research, previously and newly synthesized 5-amino-2-(4-substitutedphenyl/benzyl)benzoxazoles (**3a–3l**) and 2-substituted-5-(4-nitro/aminophenylsulfonamido)benzoxazoles (**5a–5l**, **6a–6l**) were evaluated for their antimicrobial activities against *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Mycobacterium tuberculosis* H37RV ATCC 27294 and their drug-resistant isolates *Candida albicans* ATCC 10231 and *Candida krusei* ATCC 6258. The chemical structures of the newly synthesized compounds were characterized by IR, $^{1}$H NMR, $^{13}$C NMR, LC–MS and elemental analysis. Microbiological results indicated that the compounds possessed a broad spectrum of activity against the tested microorganisms at the minimum inhibitory concentration (MIC) values between 256 and 8 μg/mL. Compounds **3a**, **3c** and **3f** exhibited significant antimycobacterial activity showing MIC value of 8 μg/mL against both *M. tuberculosis* and its drug-resistant isolate. InhA, the enoyl-acyl carrier protein reductase from *M. tuberculosis*, is one of the key enzymes in the FASII system involved in mycobacterial fatty acid elongation cycle, which has been validated as an effective antimicrobial target. Molecular docking into active site of InhA was performed on **3FNE.PDB** file to understand ligand–protein interactions. The compounds obtained from this research can be used as scaffolds in the design of new potent drugs.

**Keywords** Antimicrobial activity · Benzoxazole · Enoyl-ACP reductase · Molecular docking · *Mycobacterium tuberculosis* · Sulfonamide

✉ Tugba Ertan-Bolelli
tbolelli@ankara.edu.tr

✉ İlkay Yildiz
iyildiz@pharmacy.ankara.edu.tr

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey

[2] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara, Turkey

## Introduction

In the past few decades, the dramatically rising prevalence of multidrug-resistant microbial infections has caused a serious healthcare problem. In particular, the emergence of multidrug-resistant strains of Gram-positive bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant enterococcal infection is a problem of ever-increasing significance (Dalhoff, 1994; Lee and Hacker, 1999; Livermore, 2000; Poole, 2001; Abbanat *et al.*, 2003). In order to prevent these serious medical problems, there is still need for the new classes of antimicrobial agents.

Tuberculosis (TB), in the past also called phthisis, phthisis pulmonalis or consumption, is a widespread, infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis* (Kumar *et al.*, 2007). The disease has been the leading cause of morbidity and mortality among the infectious diseases. To address these issues, it is necessary to increase research and developmental studies to obtain novel and potent drugs.

The benzoxazole scaffold is a core structure found in a wide class of natural and synthetic compounds. Benzoxazole ring has provided the basis for the design of biologically relevant molecules with broad therapeutic importance

(Prudhomme *et al.*, 1986; Yalcin *et al.*, 1992; Balani *et al.*, 1992; Davey *et al.*, 1993; Kim *et al.*, 1996; Oren *et al.*, 1997; Ueki and Taniguchi, 1997; Oren *et al.*, 1998; Akbay *et al.*, 2003; Plemper *et al.*, 2004; Pinar *et al.*, 2004; Yildiz-Oren *et al.*, 2004a, b; Vinsova *et al.*, 2005; Rida *et al.*, 2005; Varga *et al.*, 2005; Lage *et al.*, 2006; Oksuzoglu *et al.*, 2008; Ertan *et al.*, 2009; Zilifdar *et al.*, 2014). Besides, several benzoxazole derivatives have been reported to possess antitubercular activity (Ileana *et al.*, 2006; Klimesova *et al.*, 2009; Rana *et al.*, 2014).

On the other hand, heterocycles possessing sulfonamido moieties have attracted obvious attention due to their role as pharmacophores (Andrighetti-Fröhner *et al.*, 2009; Akurathi *et al.*, 2010; Lu *et al.*, 2011; Luo *et al.*, 2011; Chandak *et al.*, 2013; Kamal *et al.*, 2013). Studies revealed that sulfonamide compounds had antibacterial effects (Gadad *et al.*, 2000; Ezabadi *et al.*, 2008; Kamal *et al.*, 2013; Azab *et al.*, 2013). In the last few years, we reported the synthesis of several 2,5-(and/or 6)-di(or tri)-substitutedbenzoxazole derivatives as the antimicrobial agents (Yalcin *et al.*, 1992; Oren *et al.*, 1997, 1998; Yildiz-Oren *et al.*, 2004a, b; Temiz-Arpaci *et al.*, 2005; Arisoy *et al.*, 2008; Alper-Hayta *et al.*, 2008; Ertan *et al.*, 2009).

InhA, the enoyl-acyl carrier protein reductase from *M. tuberculosis,* is one of the key enzymes in the FASII system involved in mycobacterial fatty acid elongation cycle, which has been validated as an effective antimicrobial target. Inhibition of mycolic acid biosynthesis is the first event detected in *M. tuberculosis* treated with isoniazid (INH), one of the most effective anti-TB agents (Takayama *et al.*, 1972). At first INH must be activated by KatG, a catalase-peroxidase that oxidizes INH to an acyl-radical which then forms a covalent adduct with co-factor $NAD^+$, the co-substrate for InhA. Then, the INH-NAD adduct functions as a potent inhibitor of InhA (Zhang *et al.*, 1992; Johnsson and Schultz, 1994). The InhA is one of the best validated targets for the development of antitubercular agents. However, the majority of isoniazid (INH)-resistant clinical strains are observed mainly due to the emergence of KatG mutants that do not form an INH-NAD adduct. Thus, compounds that directly inhibit InhA avoiding activation by KatG would be promising candidates for combating MDR-TB (Lu *et al.*, 2010).

Triclosan (5-chloro-2-(2,4-dichloro-phenoxy)phenol ether) is known to inhibit the synthesis of fatty acids targeting InhA directly whose inhibition leads to the lysis of *M. tuberculosis*. In an early study using a structure-based drug design approach, a series of 5-substituted derivatives of triclosan was developed. (5-(2-Pyridylmethyl)-2-(2,4-dichloro-phenoxy)phenol ether) is one of the derivatives of triclosan which are found more potent than triclosan against purified InhA (Freundlich *et al.*, 2009). X-ray crystal structures of InhA in complex with this triclosan derivative become available in Protein Data Bank (3FNE). It is reported that triclosan and its derivatives bind similarly with catalytic residue Tyr158 and co-factor $NAD^+$ (Lu *et al.*, 2010; Shrinivas *et al.*, 2015). Because of that reason binding to Tyr158 and co-factor $NAD^+$ is important for direct inhibition of this enzyme.

In this study, new promising bioactive compounds were designed and synthesized by a simple and efficient method, followed by the evaluation of their biological activities. The synthesis emphasized a strategy that two pharmacologically compatible moieties in one molecule by attaching a sulfonamide group to a benzoxazoles combined. Based on the above considerations, we synthesized some novel 2-substituted-5-(4-nitro/aminophenylsulfonamido)benzoxazole derivatives by using 5-amino-2-(4-substitutedphenyl/benzyl)benzoxazole as an starting compounds. In here, compounds **5b**, **5d**, **5g**, **5j**, **5k**, **5l** and **6a–6d**, **6f–6l** were synthesized for the first time. All of the previously (Ertan-Bolelli *et al.*, 2014) and newly synthesized sulfonamidobenzoxazole and 5-aminobenzoxazole derivatives were evaluated for their in vitro antimicrobial activities against human pathogenic microbes. Molecular docking into active site of InhA was performed to understand ligand–protein interactions.

## Materials and methods

### Chemistry

All chemicals and solvents were purchased from commercial vendors and were used without purification. The progress of the reaction was monitored on ready-made silica gel plates (Merck). The melting points were measured with a capillary melting point apparatus (Buchi B540) and were uncorrected. Yields were calculated after recrystallization. The IR spectra were recorded on a Jasco FT/IR-420 spectrometer as KBr disk. The $^1$H NMR spectra were recorded employing a VARIAN Mercury 400-MHz FT spectrometer, chemical shifts (d) were in parts per million relative to TMS, and coupling constants (*J*) were reported in hertz. Mass spectra were taken on a Waters Micromass ZQ using the ESI method. Elemental analyses were performed by Leco CHNS-932 CHNS-O analyzer. The result of the elemental analyses (C, H, N, S) were within ±0.4 % of the calculated amounts.

### General procedure for the preparation of 2-(4-substitutedphenyl/benzyl)-5-aminobenzoxazoles (3a–3l)

The derivatives were synthesized by heating 2,4-diaminophenol dihydrochloride (**1**) (0.01 mol) with suitable acid (**2a–2l**)

(0.01 mol) in polyphosphoric acid (PPA) (24 g) and stirring at 170–200 °C for 1.5–2.5 h. At the end of the reaction period, the residue was poured into an ice-water mixture and neutralized with an excess of NaOH (10 %) solution, and the residue was filtered and boiled with charcoal (200 mg) in ethanol and filtered. After the evaporation of solvent in vacuo, the crude product was obtained and recrystallized from ethanol–water (1:3) mixture (Yildiz-Oren *et al.*, 2004a; Sener *et al.*, 1987; Wynne *et al.*, 2009).

2-Phenyl-5-aminobenzoxazole (**3a**) (Sener *et al.*, 1987) This compound was prepared by using compounds **1** and **2a** at 180 °C for 2.5 h. It was obtained as creamy solid in 52 % yield; mp 152–154 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 5.13$ (2H, s, $NH_2$), 6.68 (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 6.88 (1H, d, $J = 2.0$ Hz, H-4), 7.43 (1H, d, $J = 8.8$ Hz, H-7), 7.57–7.60 (3H, m, H-3′, H-4′, H-5′), 8.13–8.15 (2H, m, H-2′, H-6′); ESIMS *m/z* 211.7 $[M + H]^+$ (75), 252.7 $[M + H + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{13}H_{10}N_2O$: C, 74.27; H, 4.79; N, 13.33. Found: C, 74.08; H, 4.82; N, 13.03.

2-(4-Chlorophenyl)-5-aminobenzoxazole (**3b**) (Chancellor *et al.*, 2011) This compound was prepared by using compounds **1** and **2b** at 200 °C for 1.5 h. It was obtained as creamy solid in 82 % yield; mp 193–195 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 5.14$ (2H, s, $NH_2$), 6.67 (1H, dd, $J = 8.8$ Hz, *J*: 2.4 Hz, H-6), 6.86 (1H, d, $J = 2.4$ Hz, H-4), 7.40 (1H, d, $J = 8.8$ Hz, H-7), 7.63 (2H, d, $J = 8.4$ Hz, H-3′, H-5′), 8.11 (2H, d, $J = 8.8$ Hz, H-2′, H-6′); ESIMS *m/z* 245.6 $[M + H]^+$ (40), 247.6 $[M + H + 2]^+$ (13), 286.7 $[M + H + 41(CH_3CN)]^+$ (100), 288.7 $[M + H + 2 + 41\ (CH_3CN)]^+$ (36); Anal. Calcd. for $C_{13}H_9ClN_2O$: C, 63.81; H, 3.71; N, 11.45. Found: C, 63.54; H, 3.89; N, 11.39.

2-(4-Fluorophenyl)-5-aminobenzoxazole (**3c**) (Sener *et al.*, 1987) This compound was prepared by using compounds **1** and **2c** at 200 °C for 2 h. It was obtained as creamy solid in 86 % yield; mp 157–159 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 5.14$ (2H, s, $NH_2$), 6.67 (1H, dd, $J = 8.8$ Hz, $J = 2.4$ Hz, H-6), 6.87 (1H, d, $J = 2.4$ Hz, H-4), 7.41–7.45 (3H, m, H-7, H-3′, H-5′), 8.16–8.20 (2H, m, H-2′, H-6′); ESIMS *m/z* 229.7 $[M + H]^+$ (55), 270.8 $[M + H + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{13}H_9FN_2O$: C, 68.42; H, 3.97; N, 12.27. Found: C, 67.95; H, 3.96; N, 11.98.

2-(4-Bromophenyl)-5-aminobenzoxazole (**3d**) (Sener *et al.*, 1987) This compound was prepared by using compounds **1** and **2d** at 200 °C for 2.5 h. It was obtained as creamy solid in 60 % yield; mp 197–200 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 5.14$ (2H, s, $NH_2$), 6.67 (1H, dd, $J = 8.8$ Hz, $J = 2.4$ Hz, H-6), 6.85 (1H, d, $J = 2.4$ Hz, H-4), 7.40 (1H, d, $J = 9.2$ Hz, H-7), 7.77 (2H, d, $J = 8.4$ Hz, H-3′, H-5′), 8.04 (2H, d, $J = 8.8$ Hz, H-2′, H-6′); ESIMS *m/z* 289.6 $[M + H]^+$ (58), 291.7 $[M + H + 2]^+$ (48), 330.7 $[M + H + 41(CH_3CN)]^+$ (92), 332.7 $[M + H + 2 + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{13}H_9BrN_2O$: C, 54.00; H, 3.14; N, 9.69. Found: C, 53.70; H, 3.08; N, 9.37.

2-(4-Ethylphenyl)-5-aminobenzoxazole (**3e**) (Sener *et al.*, 1987) This compound was prepared by using compounds **1** and **2e** at 180 °C for 2 h. It was obtained as creamy solid in 81 % yield; mp 127–128 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 1.23$ (3H, t, $CH_3$), 2.70 (2H, q, $CH_2$), 5.11 (2H, s, $NH_2$), 6.66 (1H, dd, $J = 8.4$ Hz, $J = 2.0$ Hz, H-6), 6.86 (1H, d, $J = 1.6$ Hz, H-4), 7.41 (3H, t, H-7, H-3′, H-5′), 8.05 (2H, d, $J = 8.4$ Hz, H-2′, H-6′); ESIMS *m/z* 239.7 $[M + H]^+$ (75), 280.8 $[M + H + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.39; H, 5.97; N, 11.56.

2-(4-Methylphenyl)-5-aminobenzoxazole (**3f**) (Wynne *et al.*, 2009) This compound was prepared by using compounds **1** and **2f** at 170 °C for 1.5 h. It was obtained as creamy solid in 92 % yield; mp 172–174 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 2.40$ (3H, s, $CH_3$), 5.12 (2H, s, $NH_2$); 6.66 (1H, dd, $J = 8.8$ Hz, $J = 2.4$ Hz, H-6); 6.87 (1H, d, $J = 2.4$ Hz, H-4), 7.40–7.41 (3H, m, H-7, H-3′, H-5′), 8.02 (2H, d, *J*: 8.0, H-2′, H-6′); ESIMS *m/z* 225.7 $[M + H]^+$ (98), 266.7 $[M + H + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{14}H_{12}N_2O$: C, 74.98; H, 5.39; N, 12.49. Found: C, 75.15; H, 5.28; N, 12.02.

2-(4-Methoxyphenyl)-5-aminobenzoxazole (**3g**) (Chen *et al.*, 2011) This compound was prepared by using compounds **1** and **2g** at 120 °C for 2 h. It was obtained as creamy solid in 41 % yield; mp 156–158 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 3.86$ (3H, s, $CH_3$), 5.09 (2H, s, $NH_2$), 6.64 (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 6.85 (1H, d, $J = 2.0$ Hz, H-4), 7.13 (2H, d, $J = 9.2$ Hz, H-3′, H-5′), 7.38 (1H, d, $J = 8.8$ Hz, H-7), 8.07 (2H, d, $J = 8.4$ Hz, H-2′, H-6′); ESIMS *m/z* 241.6 $[M + H]^+$ (100), 282.6 $[M + H + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.73; H, 4.83; N, 11.45.

2-Benzyl-5-aminobenzoxazole (**3h**) (Yildiz-Oren *et al.*, 2004a) This compound was prepared by using compounds **1** and **2h** at 180 °C for 1.5 h. It was obtained as creamy solid in 61 % yield; mp 79–81 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 4.22$ (2H, s, $CH_2$), 5.08 (2H, s, $NH_2$), 6.59 (1H, dd, $J = 9.2$ Hz, $J = 2.4$ Hz, H-6), 6.79 (1H, d, $J = 2.4$ Hz, H-4), 7.26–7.35 (6H, m, Ar); ESIMS *m/z* 225.6 $[M + H]^+$ (68), 266.6 $[M + H + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{14}H_{12}N_2O$. 0.75 $H_2O$: C, 70.72; H, 5.72; N, 11.78. Found: C, 70.50; H, 5.90; N, 11.20.

2-(4-Chlorobenzyl)-5-aminobenzoxazole (**3i**) (Yildiz-Oren *et al.*, 2004a) This compound was prepared by using compounds **1** and **2i** at 200 °C for 1.5 h. It was obtained as creamy solid in 75 % yield; mp 83–85 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 4.24$ (2H, s, $CH_2$), 5.03 (2H, s, $NH_2$), 6.58 (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 6.77 (1H, d, $J = 2.0$ Hz, H-4), 7.55 (1H, d, $J = 8.8$ Hz, H-7), 7.37–7.43 (4H, m, phenyl); ESIMS *m/z* 259.7 $[M + H]^+$ (32), 261.7 $[M + H + 2]^+$ (10), 300.8 $[M + H + 41(CH_3CN)]^+$ (100), 302.7 $[M + H + 2 + 41(CH_3CN)]^+$ (35); Anal. Calcd. for $C_{14}H_{11}ClN_2O$. 0.25 $H_2O$: C, 63.88; H, 4.40; N, 10.64. Found: C, 63.76; H, 4.42; N, 10.90.

2-(4-Fluorobenzyl)-5-aminobenzoxazole (**3j**) (Oksuzoglu *et al.*, 2007) This compound was prepared by using compounds **1** and **2j** at 200 °C for 1.5 h. It was obtained as creamy solid in 72 % yield; mp 76–77 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 4.23$ (2H, s, $CH_2$), 5.02 (2H, s, $NH_2$), 6.57 (1H, dd, $J = 8.4$ Hz, $J = 2.0$ Hz, H-6), 6.77 (1H, d, $J = 2.4$ Hz, H-4), 7.15–7.20 (2H, m, H-3′, H-5′); 7.27 (1H, d, $J = 8.4$ Hz, H-7), 7.37–7.41 (2H, m, H-2′, H-6′); ESIMS *m/z* 243.7 $[M + H]^+$ (58), 284.7 $[M + H + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{14}H_{11}FN_2O$: C, 69.41; H, 4.58; N, 11.56. Found: C, 69.23; H, 4.35; N, 11.48.

2-(4-Bromobenzyl)-5-aminobenzoxazole (**3k**) (Arisoy *et al.*, 2008) This compound was prepared by using compounds **1** and **2k** at 200 °C for 1.5 h. It was obtained as creamy solid in 89 % yield; mp 101–104 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 4.22$ (2H, s, $CH_2$), 5.03 (2H, s, $NH_2$), 6.58 (1H, dd, $J = 8.4$ Hz, $J = 2.0$ Hz, H-6), 6.76 (1H, d, $J = 2.0$ Hz, H-4), 7.27 (1H, d, $J = 8.4$ Hz, H-7), 7.32 (2H, d, $J = 8.0$ Hz, H-3′, H-5′), 7.55 (2H, d, $J = 8.8$ Hz, H-2′, H-6′); ESIMS *m/z* 303.5 $[M + H]^+$ (30), 305.5 $[M + H + 2]^+$ (40), 346.7 $[M + H + 2 + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{14}H_{11}BrN_2O$: C, 55.47; H, 3.66; N, 9.24. Found: C, 55.80; H, 3.83; N, 9.69.

2-(4-Methylbenzyl)-5-aminobenzoxazole (**3l**) (Ertan-Bolelli *et al.*, 2014) This compound was prepared by using compounds **1** and **2l** at 180 °C for 1.5 h. It was obtained as creamy solid in 61 % yield; mp 82–84 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 2.27$ (3H, s, $CH_3$), 4.16 (2H, s, $CH_2$), 5.01 (2H, s, $NH_2$), 6.57 (1H, dd, $J = 8.4$ Hz, $J = 2.0$ Hz, H-6), 6.76 (1H, d, $J = 2.4$ Hz, H-4), 7.14 (2H, d, $J = 8.0$ Hz, H-3′, H-5′), 7.22 (2H, d, $J = 8.0$ Hz, H-2′, H-6′), 7.25 (1H, d, $J = 8.8$ Hz, H-7); ESIMS *m/z* 239.7 $[M + H]^+$ (50), 280.7 $[M + H + 41(CH_3CN)]^+$ (100); Anal. Calcd. for $C_{15}H_{14}N_2O$. 1 $H_2O$: C, 70.29; H, 6.29; N, 10.93. Found: C, 70.56; H, 5.99; N, 10.55.

### General procedure for the preparation of 5-(4-nitrophenylsulfonamido)benzoxazole derivatives (5a–5l)

Pyridine (0.95 mmol) and 4-nitrobenzenesulfonyl chloride (**4**) (0.52 mmol) were added to a solution of 2-(4-substitutedphenyl)-5-aminobenzoxazole (**3a–3l**) (0.048 mmol) in dichloromethane (2 mL). The reaction mixture was stirred at the room temperature for 16 h. At the end of the reaction, the residue was filtered and washed with saturated solution of $CuSO_4$ and $NaHCO_3$ in water (Wynne *et al.*, 2009) and then recrystallized with a mixture of ethyl acetate/*n*-hexan (1:4). The crystals were dried *in vacuo*. Only compounds **5b**, **5d**, **5g**, **5j**, **5k** and **5l** are new. The remaining compounds **5a**, **5c**, **5e**, **5f**, **5h** and **5i** were published by us in 2014 (Ertan-Bolelli *et al.*, 2014).

2-Phenyl-5-(4-nitrophenylsulfonamido)benzoxazole (**5a**) (Ertan-Bolelli *et al.*, 2014) This compound was prepared by using **3a**. It was obtained as creamy solid in 57 % yield; mp 247–250 °C.

2-(4-Chlorophenyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5b**) This compound was prepared by using **3b**. It was obtained as creamy solid in 65 % yield; mp 263–265 °C; IR (KBr) $\nu_{max}$ 3275, 3131–3081, 1528, 1480, 1349, 1314, 1161, 1085 $cm^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 7.17$ (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 7.53 (1H, d, $J = 2.0$ Hz, H-4), 7.66 (2H, d, $J = 8.8$ Hz, H-3′, H-5′), 7.71 (1H, d, $J = 9.2$ Hz, H-7), 8.02 (2H, d, $J = 8.4$ Hz, H-2″, H-6″), 8.14 (2H, d, $J = 8.8$ Hz, H-2′, H-6′), 8.38 (2H, d, $J = 9.2$ Hz, H-3″, H-5″), 10.75 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, 400 MHz,): $\delta = 111.51$, 112.51, 120.01, 124.58, 124.91, 128.29, 128.96, 129.43, 133.92, 136.86, 141.83, 144.43, 147.70, 149.78, 162.39 (15C, Ar–C); ESIMS *m/z* (%) 428.20 (100) $[M-H]^+$, 430.22 (40) $[M-H + 2]^+$; Anal. Calcd. for $C_{19}H_{12}ClN_3O_5S$: C 53.09, H 2.81, N 9.78, S 7.46. Found: C 52.92, H 3.08, N 9.84, S 7.52.

2-(4-Fluorophenyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5c**) (Ertan-Bolelli *et al.*, 2014) This compound was prepared by using **3c**. It was obtained as creamy solid in 46 % yield; mp 230–232 °C.

2-(4-Bromophenyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5d**) This compound was prepared by using **3d**. It was obtained as creamy solid in 67 % yield; mp 266–267 °C; IR (KBr) $\nu_{max}$ 3275, 3128, 1526, 1477, 1348, 1312, 1161, 1071; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 7.19$ (1H, d, $J = 8.0$ Hz, H-6), 7.54 (1H, s, H-4), 7.70

(1H, d, $J = 8.8$ Hz, H-7), 7.79 (2H, d, $J = 8.4$ Hz, H-3′, H-5′), 8.02–8.06 (4H, m, Ar), 8.39 (2H, d, $J = 8.0$ Hz, H-3″, H-5″), 10.76 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, 400 MHz,): $\delta = 111.57$, 112.55, 120.08, 124.64, 125.29, 125.87, 128.33, 129.13, 132.40, 133.99, 141.88, 144.48, 147.74, 149.82, 162.56 (15C, Ar–C); ESIMS *m/z* 472.13 $[M\text{-}H]^+$ (100), 474.13 $[M\text{-}H + 2]^+$ (95); Anal. Calcd. for $C_{19}H_{12}BrN_3O_5S$: C, 48.12; H, 2.55; N, 8.86; S, 6.76. Found: C, 47.85; H, 2.86; N, 8.91; S, 6.84.

2-(4-Ethylphenyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5e**) (Ertan-Bolelli *et al.*, 2014) This compound was prepared by using **3e**. It was obtained as creamy solid in 53 % yield; mp 217–219 °C.

2-(4-Methylphenyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5f**) (Ertan-Bolelli *et al.*, 2014) This compound was prepared by using **3f**. It was obtained as creamy solid in 43 % yield; mp 244–245 °C.

2-(4-Methoxyphenyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5g**) This compound was prepared by using **3g**. It was obtained as yellowish solid in 71 % yield; mp 227–230 °C; IR (KBr) $\nu_{max}$ 3264, 1526, 1498, 1349, 1308, 1160; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 3.86$ (3H, s, $CH_3$), 7.08 (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 7.15 (2H, d, $J = 8.8$ Hz, H-3′, H-5′), 7.45 (1H, d, $J = 2.0$ Hz, H-4), 7.66 (1H, d, $J = 8.4$ Hz, H-7), 7.98 (2H, d, $J = 8.8$ Hz, H-2″, H-6″), 8.09 (2H, d, $J = 9.2$ Hz, H-2′, H-6′), 8.37 (2H, d, $J = 9.2$ Hz, H-3″, H-5″), 10.68 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, 400 MHz,): $\delta = 55.50$ (1C, $CH_3$), 111.19, 112.29, 114.79, 118.39, 119.32, 124.62, 128.32, 129.18, 133.65, 142.18, 144.51, 147.65, 149.81, 162.31, 163.51 (15C, Ar–C); ESIMS *m/z* 426.80 $[M + H]^+$ (100); Anal. Calcd. for $C_{20}H_{15}N_3O_6S$: C, 56.47; H, 3.55; N, 9.88; S, 7.54. Found: C, 56.56; H, 3.71; N, 10.09; S, 7.53.

2-Benzyl-5-(4-nitrophenylsulfonamido)benzoxazole (**5h**) (Ertan-Bolelli *et al.*, 2014) This compound was prepared by using **3h**. It was obtained as creamy solid in 55 % yield; mp 145–146 °C.

2-(4-Chlorobenzyl)-5-(4-nitrophenylsulfonamido)benzoxazole (*5i*) (Ertan-Bolelli *et al.*, 2014) This compound was prepared by using **3i**. It was obtained as creamy solid in 40 % yield; mp 179–181 °C.

2-(4-Fluorobenzyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5j**) This compound was prepared by using **3j**. It was obtained as creamy solid in 53 % yield; mp 151–153 °C; IR (KBr) $\nu_{max}$ 3252, 3128–3039, 2924, 1527, 1480, 1348, 1308, 1223, 1161; $^1$H NMR (DMSO-$d_6$, 400 MHz,) $\delta = 4.30$ (2H, s, $CH_2$), 7.06 (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 7.15–7.19 (2H, m, H-3′, H-5′), 7.39–7.42 (3H, m, Ar), 7.58 (1H, d, $J = 8.4$ Hz, H-7), 7.96 (2H, d, $J = 9.2$ Hz, H-2″, H-6″), 8.35 (2H, d, $J = 8.8$ Hz, H-3″, H-5″), 10.64 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, 400 MHz,): $\delta = 33.12$ (1C, $CH_2$), 111.05, 112.46, 115.20, 115.41, 119.29, 124.54, 128.24, 130.94–131.07, 133.29, 141.25, 144.43, 147.82, 149.74, 160.03, 162.45 (15C, Ar–C); ESIMS *m/z* 428.48 $[M + H]^+$ (100); Anal. Calcd. for $C_{20}H_{14}FN_3O_5S$: C, 56.20; H, 3.30; N, 9.83; S, 7.50. Found: C, 55.95; H, 3.52; N, 9.88; S, 7.57.

2-(4-Bromobenzyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5k**) This compound was prepared by using **3k**. It was obtained as creamy solid in 41 % yield; mp 187–189 °C; IR (KBr) $\nu_{max}$ 3115, 3057, 2856, 1525, 1488, 1348, 1312, 1165, 1068; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 4.30$ (2H, s, $CH_2$), 7.06 (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 7.33 (2H, d, $J = 8.0$ Hz, H-3′, H-5′), 7.39 (1H, d, $J = 2.0$ Hz, H-4), 7.54 (2H, d, $J = 8.8$ Hz, H-2′, H-6′), 7.58 (1H, d, $J = 8.8$ Hz, H-7), 7.96 (2H, d, $J = 9.2$ Hz, H-2″, H-6″), 8.35 (2H, d, $J = 8.8$ Hz, H-3″, H-5″), 10.64 (1H, s, NH); $^{13}$C NMR (DMSO-$d_6$, 400 MHz,): $\delta = 33.31$ (1C, $CH_2$), 111.07, 112.45, 119.32, 120.26, 124.55, 128.24, 131.35, 131.42, 133.31, 134.24, 141.22, 144.43, 147.81, 149.74, 166.18 (15C, Ar–C) ESIMS *m/z* 488.37 $[M + H]^+$ (85), 490.37 $[M + H + 2]^+$ (100); Anal. Calcd. for $C_{20}H_{14}BrN_3O_5S$: C, 49.19; H, 2.89; N, 8.61; S, 6.57. Found: C, 49.24; H, 3.17; N, 8.65; S, 6.68.

2-(4-Methylbenzyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5l**) This compound was prepared by using **3l**. It was obtained as creamy solid in 57 % yield; mp 158–160 °C; IR (KBr) $\nu_{max}$ 3258, 3076, 2924–2790, 1527, 1472, 1349, 1310, 1166; $^1$H NMR (DMSO-$d_6$, 400 MHz,) $\delta = 2.27$ (3H, s, $CH_3$), 4.23 (2H, s, $CH_2$), 7.03 (1H, dd, $J = 8.4$ Hz, $J = 2.4$ Hz, H-6), 7.14 (2H, d, $J = 8.0$ Hz, H-3′, H-5′), 7.22 (2H, d, $J = 8.4$ Hz, H-2′, H-6′), 7.37 (1H, d, $J = 2.0$ Hz, H-4), 7.56 (1H, d, $J = 8.8$ Hz, H-7), 7.94 (2H, d, $J = 8.8$ Hz, H-2″, H-6″), 8.34 (2H, d, $J = 8.8$ Hz, H-3″, H-5″), 10.63 (1H, s, NH); $^{13}$C NMR DMSO-$d_6$: $\delta = 20.59$ (1C, $CH_3$), 33.72 (1C, $CH_2$), 111.08, 112.51, 119.32, 124.61, 128.30, 128.92, 129.18, 131.80, 133.35, 136.22, 141.36, 144.52, 147.88, 149.80, 166.82 (15C, Ar–C); ESIMS *m/z* 424.70 $[M + H]^+$ (100); Anal. Calcd. for $C_{21}H_{17}N_3O_5S$: C, 59.57; H, 4.05; N, 9.92; S, 7.57. Found: C, 59.44; H, 4.12; N, 9.98; S, 7.44.

### General procedure for the preparation of 5-(4-aminophenylsulfonamido)benzoxazole derivatives (6a, 6e–h, 6l)

0,5 mmol 2-(4-Substitutedphenyl/benzyl)-5-(4-nitrophenyl sulfonamido)benzoxazole (**5a**, **5e–h**, **5l**) in ethanol (50 mL) was reduced by hydrogenation using 40 psi of $H_2$ and 10 % Pd–C (40 mg) until uptake of $H_2$ ceased (Zheng *et al.*, 2007). The catalyst was filtered on a bed of celite and washed with ethanol, and the filtrate was concentrated in

vacuo. The crude product was purified by recrystallization with a mixture of ethyl acetate/*n*-hexan (1:4) to obtain **6a**, **6e–h**, **6l**. The crystals were dried in vacuo. All the compounds are new except **6e** (Ertan-Bolelli *et al.*, 2014).

2-Phenyl-5-(4-aminophenylsulfonamido)benzoxazole (**6a**) This compound was prepared by using **5a**. It was obtained as white solid in 41 % yield; mp 266–269 °C; IR (KBr) $\nu_{max}$ 3343, 3247, 1486, 1307, 1142; $^1$H NMR (DMSO-d$_6$, 400 MHz,) $\delta$ = 5.98 (2H, s, NH$_2$), 6.51 (2H, d, $J$ = 8.8 Hz, H-3″, H-5″), 7.11 (1H, dd, $J$ = 8.4 Hz, $J$ = 2.0 Hz, H-6), 7.38 (2H, d, $J$ = 8.8 Hz, H-2″, H-6″), 7.43 (1H, d, $J$ = 2.0 Hz, H-4), 7.60–7.66 (4H, m, Ar), 8.16 (2H, dd, $J$ = 7.6 Hz, $J$ = 1.6 Hz, H-2′, H-6′), 9.97 (1H, s, NH); $^{13}$C NMR DMSO-d$_6$: $\delta$ = 110.87, 110.97, 112.49, 118.70, 123.90, 126.20, 127.16, 128.68, 129.20, 131.91, 135.59, 141.72, 146.83, 152.78, 162.99 (15C, Ar–C); ESIMS $m/z$ 366.40 [M + H]$^+$ (100); Anal. Calcd. for $C_{19}H_{15}N_3O_3S$. 0.1 $CH_3COOC_2H_5$: C, 62.27; H, 4.26; N, 11.23; S, 8.57. Found: C, 61.96; H, 4.20; N, 11.43; S, 8.64.

2-(4-Ethylphenyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6e**) (Ertan-Bolelli *et al.*, 2014) This compound was prepared by using **5e**. It was obtained as white solid in 60 % yield; mp 251–254 °C.

2-(4-Methylphenyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6f**) This compound was prepared by using **5f**. It was obtained as white solid in 33 % yield; mp 289–291 °C; IR (KBr) $\nu_{max}$ 3406–3335, 3108, 1466, 1309, 1187; $^1$H NMR (DMSO-d$_6$, 400 MHz,): $\delta$ = 2.40 (3H, s, CH$_3$), 5.97 (2H, s, NH$_2$), 6.52 (2H, d, $J$ = 9.2 Hz, H-3″, H-5″), 7.09 (1H, dd, $J$ = 8.8 Hz, $J$ = 2.0 Hz, H-6), 7.39 (5H, t, Ar), 7.62 (1H, d, $J$ = 8.8 Hz, H-7), 8.04 (2H, d, $J$ = 8.4 Hz, H-2′, H-6′), 9.93 (1H, s, NH); ESIMS $m/z$ 380.80 [M + H]$^+$ (100); Anal. Calcd. for $C_{20}H_{17}N_3O_3S$. 0.1 $CH_3COOC_2H_5$: C, 63.11; H, 4.62; N, 10.82; S, 8.26. Found: C, 62.75; H, 4.61; N, 11.06; S, 8.23.

2-(4-Methoxyphenyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6g**) This compound was prepared by using **5g**. It was obtained as white solid in 43 % yield; mp 273–274 °C; IR (KBr) $\nu_{max}$ 3396–3329, 3056, 2838, 1465, 1310, 1187; $^1$H NMR (DMSO-d$_6$, 400 MHz,) $\delta$ = 3.86 (3H, s, CH$_3$), 5.97 (2H, s, NH$_2$), 6.51 (2H, d, $J$ = 8.8 Hz, H-3″, H-5″), 7.06 (1H, dd, $J$ = 8.4 Hz, $J$ = 1.6 Hz, H-6), 7.14 (2H, d, $J$ = 8.8 Hz, H-3′, H-5′), 7.37 (3H, d, $J$ = 8.8 Hz, H-2″, H-6″), 7.60 (1H, d, $J$ = 9.2 Hz, H-7), 8.09 (2H, d, $J$ = 8.8 Hz, H-2′, H-6′), 9.91 (1H, s, NH); $^{13}$C NMR DMSO-d$_6$: $\delta$ = 55.42 (1C, CH$_3$), 110.66, 110.68, 112.47, 114.67, 118.16, 118.54, 123.95, 128.67, 129.05, 135.41, 141.92, 146.75, 152.74, 162.11, 163.13 (15C, Ar–C); ESIMS $m/z$ 396.70 [M + H]$^+$ (100); Anal. Calcd. for $C_{20}H_{17}N_3O_4S$: C, 60.75; H, 4.33; N, 10.63; S, 8.11. Found: C, 60.79; H, 4.31; N, 10.75; S, 8.01.

2-Benzyl-5-(4-aminophenylsulfonamido)benzoxazole (**6h**) This compound was prepared by using **5h**. It was obtained as creamy solid in 68 % yield; mp 154–156 °C; IR (KBr) $\nu_{max}$ 3410–3344, 3154, 3031, 1455, 1311, 1186; $^1$H NMR (DMSO-d$_6$, 400 MHz,): $\delta$ = 4.25 (2H, s, CH$_2$), 5.92 (2H, s, NH$_2$), 6.47 (2H, d, $J$ = 8.4 Hz, H-3″, H-5″), 6.99 (1H, dd, $J$ = 8.8 Hz, $J$ = 2.4 Hz, H-6), 7.25–7.32 (8H, m, Ar), 7.47 (1H, d, $J$ = 8.4 Hz, H-7), 9.83 (1H, s, NH); $^{13}$C NMR DMSO-d$_6$: $\delta$ = 34.05 (1C, CH$_2$), 110.55, 110.91, 112.45, 118.13, 123.92, 126.95, 128.55, 128.62, 128.96, 134.92, 135.06, 141.08, 146.99, 152.71, 166.16 (15C, Ar–C); ESIMS $m/z$ 380.80 [M + H]$^+$ (100); Anal. Calcd. for $C_{20}H_{17}N_3O_3S$. 0.1 $CH_3COOC_2H_5$: C, 63.11; H, 4.62; N, 10.82; S, 8.26. Found: C, 62.67; H, 4.43; N, 11.15; S, 8.40.

2-(4-Methylbenzyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6l**) This compound was prepared by using **5l**. It was obtained as creamy solid in 34 % yield; mp 192–193 °C; IR (KBr) $\nu_{max}$ 3463–3412, 3216, 1477, 1311, 1183; $^1$H NMR (DMSO-d$_6$, 400 MHz,): $\delta$ = 2.27 (3H, s, CH$_3$), 4.21 (2H, s, CH$_2$), 5.95 (2H, s, NH$_2$), 6.49 (2H, d, $J$ = 8.8 Hz, H-3″, H-5″), 7.01 (1H, dd, $J$ = 9.2 Hz, $J$ = 2.4 Hz, H-6), 7.14 (2H, d, $J$ = 8.0 Hz, H-3′, H-5′), 7.22 (2H, d, $J$ = 8.0 Hz, H-2′, H-6′), 7.30 (1H, d, $J$ = 2.0 Hz, H-4), 7.33 (2H, d, $J$ = 9.2 Hz, H-2″, H-6″), 7.49 (1H, d, $J$ = 8.4 Hz, H-7), 9.85 (1H, s, NH); $^{13}$C NMR DMSO-d$_6$: $\delta$ = 20.61 (1C, CH$_3$), 33.74 (1C, CH$_2$), 110.59, 110.99, 112.52, 118.19, 123.99, 128.69, 128.90, 129.17, 131.91, 135.11, 136.15, 141.16, 147.06, 152.77, 166.42 (15C, Ar–C); ESIMS $m/z$ 394.80 [M + H]$^+$ (100); Anal. Calcd. for $C_{21}H_{19}N_3O_3S$: C, 64.11; H, 4.87; N, 10.68; S, 8.15. Found: C, 63.98; H, 4.95; N, 10.67; S, 7.98.

## General procedure for the preparation of 5-(4-aminophenylsulfonamido)benzoxazole derivatives (6b–d, 6i–k)

The reduction of the nitro group for compounds bearing halogen atoms was carried out with iron powder and ammonium chloride. 2-(4-Substitutedphenyl/benzyl)-5-(4-nitrophenylsulfonamido)benzoxazole (**5b–d**, **5i–k**) (4.65 mmol) was solved in methanol (20 mL), and then iron powder (0.78 g, 13.65 mmol) and an aqueous solution of ammonium chloride (1.3 g, 23.3 mmol, 20 mL) were added in it. The reaction mixture was stirred at 70 °C for 2.5 h. The reaction mixture was cooled and filtered to remove the inorganic residues and then evaporated (Habens *et al.*, 2005). The precipitate was dissolved in ethyl acetate and extracted with water and then recrystallized with a mixture of ethyl acetate/*n*-hexan (1:4) to obtain **6b–d**, **6i–k**. The crystals were dried in vacuo. All the compounds are new.

2-(4-Chlorophenyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6b**) This compound was prepared by using **5b**. It was obtained as creamy solid in 31 % yield; mp 314–315 °C; IR (KBr) $\nu_{max}$ 3419–3349, 3124, 1482, 1311, 1160, 1093; $^1$H NMR (DMSO-$d_6$, 400 MHz,) $\delta = 5.92$ (2H, s, $NH_2$), 6.47 (2H, d, $J = 8.4$ Hz, H-3″, H-5″), 7.08 (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 7.34 (2H, d, $J = 8.8$ Hz, H-2″, H-6″), 7.39 (1H, d, $J = 1.6$ Hz, H-4), 7.60–7.65 (3H, m, Ar), 8.11 (2H, d, $J = 8.0$ Hz, H-2′, H-6′), 9.92 (1H, s, NH); ESIMS *m/z* 400.70 $[M + H]^+$ (100), 402.70 $[M + H + 2]^+$ (40); Anal. Calcd. for $C_{19}H_{14}ClN_3O_3S$: C, 57.07; H, 3.53; N, 10.51; S, 8.02. Found: C, 57.10; H, 3.63; N, 10.78; S, 7.90.

2-(4-Fluorophenyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6c**) This compound was prepared by using **5c**. It was obtained as creamy solid in 33 % yield; mp 280–282 °C; IR (KBr) $\nu_{max}$ 3408–3341, 3106, 1465, 1313, 1237, 1162; $^1$H NMR (DMSO-$d_6$, 400 MHz,) $\delta = 5.96$ (2H, s, $NH_2$), 6.51 (2H, d, $J = 8.8$ Hz, H-3″, H-5″), 7.11 (1H, dd, J = 8.8 Hz, $J = 2.4$ Hz, H-6), 7.38 (2H, d, $J = 8.8$ Hz, H-2″, H-6″), 7.42–7.47 (3H, m, Ar), 7.64 (1H, d, $J = 8.8$ Hz, H-7), 8.19–8.22 (2H, m, H-2′, H-6′), 9.95 (1H, s, NH); $^{13}$C NMR DMSO-$d_6$: $\delta = 110.82$, 110.96, 112.48, 116.33, 116.56, 118.67, 122.86, 122.89, 123.88, 128.67, 129.77, 129.87, 135.63, 141.68, 146.86, 152.77, 162.18, 162.91, 165.40 (15C, Ar–C); ESIMS *m/z* 384.80 $[M + H]^+$ (100); Anal. Calcd. for $C_{19}H_{14}FN_3O_3S$: C, 59.52; H, 3.68; N, 10.96; S, 8.36. Found: C, 59.42; H, 3.76; N, 11.22; S, 8.32.

2-(4-Bromophenyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6d**) This compound was prepared by using **5d**. It was obtained as white solid in 46 % yield; mp 304–305 °C; IR (KBr) $\nu_{max}$ 3349, 1477, 1310, 1160, 1071; $^1$H NMR (DMSO-$d_6$, 400 MHz,) $\delta = 5.97$ (2H, s, $NH_2$), 6.51 (2H, d, $J = 8.8$ Hz, H-3″, H-5″), 7.12 (1H, dd, $J = 8.8$ Hz, $J = 2.0$ Hz, H-6), 7.38 (2H, d, $J = 9.2$ Hz, H-2″, H-6″), 7.43 (1H, d, $J = 2.4$ Hz, H-4), 7.65 (1H, d, $J = 8.4$ Hz, H-7), 7.81 (2H, d, $J = 8.4$ Hz, H-3′, H-5′), 8.07 (2H, d, $J = 8.4$ Hz, H-2′, H-6′), 9.97 (1H, s, NH); $^{13}$C NMR DMSO-$d_6$: $\delta = 111.55$, 111.62, 114.98, 119.40, 126.70, 127.70, 129.18, 129.75, 132.48, 135.90, 142.26, 147.47, 150.50, 163.56 (15C, Ar–C); ESIMS *m/z* 444.80 (100) $[M + H]^+$; 446.80 (80) $[M + H + 2]^+$; Anal. Calcd. for $C_{19}H_{14}BrN_3O_3S$: C 51.36, H 3.18, N 9.46, S 7.22. Found: C 51.19, H 3.28, N 9.71, S 7.06.

2-(4-Chlorobenzyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6i**) This compound was prepared by using **5i**. It was obtained as creamy solid in 32 % yield; mp 176–178 °C; IR (KBr) $\nu_{max}$ 3464–3327, 3218, 1478, 1311, 1183, 1091; $^1$H NMR (DMSO-$d_6$, 400 MHz,) $\delta = 4.30$ (2H, s, $CH_2$), 5.95 (2H, s, $NH_2$), 6.50 (2H, d, $J = 8.8$ Hz, H-3″, H-5″), 7.03 (1H, dd, $J = 8.4$ Hz, $J = 2.0$ Hz, H-6), 7.31–7.40 (7H, m, Ar), 7.51 (1H, d, $J = 8.8$ Hz, H-7), 9.87 (1H, s, NH); $^{13}$C NMR DMSO-$d_6$: $\delta = 33.25$ (1C, $CH_2$), 110.57, 110.86, 112.44, 118.15, 123.88, 128.46, 128.62, 130.95, 131.69, 133.92, 135.11, 141.01, 146.95, 152.70, 165.82 (15C, Ar–C); ESIMS *m/z* 414.70 $[M + H]^+$ (100), 416.70 $[M + H + 2]^+$ (40); Anal. Calcd. for $C_{20}H_{16}ClN_3O_3S$: C, 58.04; H, 3.90; N, 10.15; S, 7.75. Found: C, 58.13; H, 3.89; N, 10.34; S, 7.51.

2-(4-Fluorobenzyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6j**) This compound was prepared by using **5j**. It was obtained as creamy solid in 77 % yield; mp 187–190 °C; IR (KBr) $\nu_{max}$ 3464–3327, 3216, 1478, 1311, 1182, 1223; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 4.28$ (2H, s, $CH_2$), 5.95 (2H, s, $NH_2$), 6.50 (2H, d, $J = 8.8$ Hz, H-3″, H-5″), 7.03 (1H, dd, $J = 8.8$ Hz, $J = 2.4$ Hz, H-6), 7.15–7.19 (2H, m, H-3′, H-5′), 7.31–7.42 (5H, m, Ar), 7.50 (1H, d, $J = 8.8$ Hz, H-7), 9.86 (1H, s, NH); $^{13}$C NMR DMSO-$d_6$: $\delta = 33.14$ (1C, $CH_2$), 110.56, 110.89, 112.45, 115.18, 115.39, 118.15, 123.90, 128.63, 130.95–131.09, 135.09, 141.06, 146.98, 152.71, 160.01, 162.42, 166.09 (15C, Ar–C); ESIMS *m/z* 398.70 $[M + H]^+$ (100); Anal. Calcd. for $C_{20}H_{16}FN_3O_3S$: C, 60.44; H, 4.06; N, 10.57; S, 8.07. Found: C, 60.36; H, 3.98; N, 10.80; S, 7.95.

2-(4-Bromobenzyl)-5-(4-aminophenylsulfonamido)benzoxazole (**6k**) This compound was prepared by using **5k**. It was obtained as creamy solid in 66 % yield; mp 170–172 °C; IR (KBr) $\nu_{max}$ 3478–3380, 3308, 1490, 1302, 1187, 1074; $^1$H NMR (DMSO-$d_6$, 400 MHz,): $\delta = 4.28$ (2H, s, $CH_2$), 5.95 (2H, s, $NH_2$), 6.50 (2H, d, $J = 8.4$ Hz, H-3″, H-5″), 7.03 (1H, dd, $J = 8.4$ Hz, $J = 2.0$ Hz, H-6), 7.31-7.35 (5H, m, Ar), 7.51 (1H, d, $J = 8.8$ Hz, H-7), 7.54 (2H, d, $J = 8.4$ Hz, H-2′, H-6′), 9.86 (1H, s, NH); $^{13}$C NMR DMSO-$d_6$: $\delta = 33.32$ (1C, $CH_2$), 110.57, 110.87, 112.45, 118.17, 120.20, 123.89, 128.63, 131.32, 131.40, 134.34, 135.11, 141.02, 146.97, 152.71, 165.75 (15C, Ar–C); ESIMS *m/z* 458.70 $[M + H]^+$ (100), 460.70 $[M + H + 2]^+$ (100); Anal. Calcd. for $C_{20}H_{16}BrN_3O_3S$: C, 52.41; H, 3.52; N, 9.17; S, 6.99. Found: C, 52.21; H, 3.62; N, 9.53; S, 6.86.

## Antimicrobial evaluation

Standard strains of *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *M. tuberculosis* H37RV ATCC 27294 and clinical isolates of these microorganisms, *Candida albicans* ATCC 10231 ve *Candida krusei* ATCC 6258, were included in the study.

### Microdilution method

Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S181. MHB was added to each well of the microplates. The bacterial suspensions used for inoculation were prepared at $10^5$ CFU/mL by diluting fresh cultures at McFarland 0.5 density ($10^7$ CFU/mL). Suspensions of the bacteria at $10^5$ CFU/mL concentration were inoculated to the twofold diluted solution of the compounds. A 10-μL bacteria inoculum was added to each well of the microplates. There were $10^4$ CFU/mL bacteria in the wells after inoculations. Microplates were incubated at 37 °C overnight (CLSI, 2006a).

Fungal susceptibility testing was performed according to the guidelines of CLSI M27-A32. RPMI-1640 medium with L-glutamine (Sigma) buffered to pH 7 with MOPS was added to each well of the microplates. The colonies were suspended in sterile saline, and the resulting suspension was adjusted to McFarland 0.5 density ($10^6$ CFU/mL). A working suspension was prepared by a 1:100 dilution followed by a 1:20 dilution of the stock suspension. A 10 μL of this suspension at $10^3$ CFU/mL was inoculated to the twofold diluted solution of the compounds. Microplates were incubated at 35 °C for 24–48 h (CLSI, 2006b).

After incubation, the lowest concentration of the compounds that completely inhibits macroscopic growth was determined and reported as minimum inhibitory concentrations (MIC). All solvents and diluents, pure microorganisms and pure media were used in control wells. All the experiments were done in 3 parallel series.

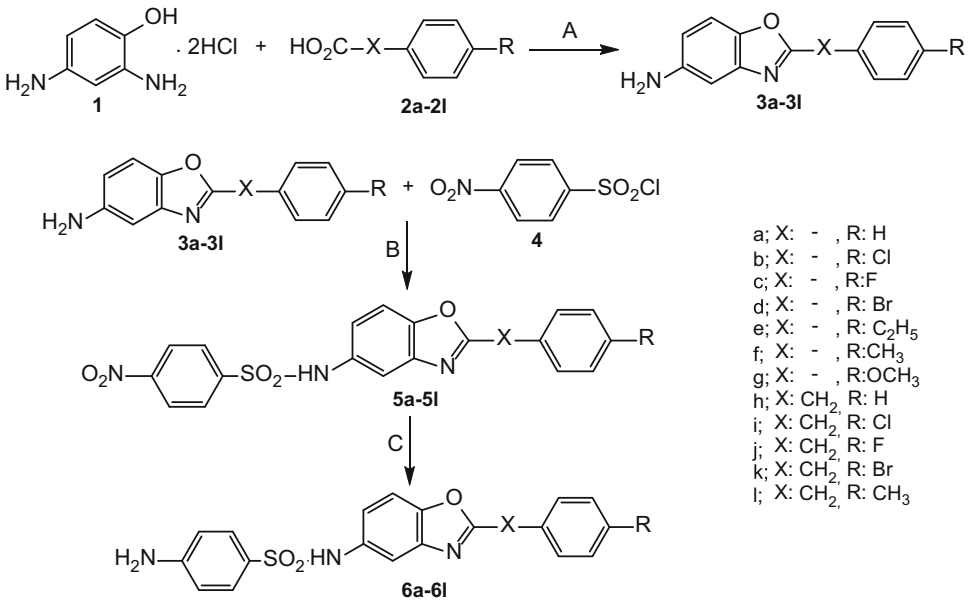
### Microplate Alamar Blue Assay (MABA)

*Mycobacterium tuberculosis* H37Rv ATCC 27294 (American Type Culture Collection) was subcultured on Middlebrook 7H11 agar (Becton–Dickinson). Suspensions were prepared in 0.04 % (vol/vol) Tween 80–0.2 % bovine serum albumin so that their turbidities matched that of a McFarland no. 1 turbidity standard. Isoniazid (INH) and ethambutol (EMB) were obtained from Sigma. Stock solutions of INH and EMB were prepared in deionized water.

Sterile deionized water was added to all outer-perimeter wells of sterile 96-well plates to minimize evaporation of the medium in the test wells during incubation. One hundred microliters of six drug solutions were added to the wells in rows B to G in columns 2 and 3 by using a multichannel pipette, 100 μL was transferred from column 3 to column 4, and the contents of the wells were mixed well. Identical serial 1:2 dilutions were continued through column 10, and 100 μL of excess medium was discarded from the wells in column 10. Final drug concentration ranges were as follows: for INH, 0.031 to 8.0 μg/mL and for EMB, 0.5 to 128 μg/mL. One hundred microliters of *M. tuberculosis* inoculum was added to the wells in rows B to G in columns 2–11. The plates were sealed with Parafilm and were incubated at 37 °C for 5 days. Fifty microliters of a freshly prepared 1:1 mixture of 10X Alamar Blue reagent and 10 % Tween 80 was added to well B11. The plates were reincubated at 37 °C for 24 h. If well B11 turned pink, the reagent mixture was added to all wells in the microplate. The microplates were resealed with Parafilm and were incubated for an additional 24 h at 37 °C, and the colors of all wells were recorded. A blue color in the well was interpreted as no growth, and a pink color was scored as growth. A few wells appeared violet after 24 h of incubation, but they invariably changed to pink after another day of incubation and thus were scored as growth. The MIC was defined as the lowest drug concentration which prevented a color change from blue to pink (Franzblau *et al.*, 1998).

### Molecular docking

The crystal structure of the InhA was retrieved from the Protein Data Bank (**PDB ID: 3FNE**) (Freundlich *et al.*, 2009). Accelrys Discovery Studio 3.5 (Accelrys Inc., 2012) software was used for preparation of protein and ligands. The target protein was taken, the ligand was extracted, hydrogens were added, and their positions were optimized using the all-atom CHARMm forcefield and the Adopted Basis set Newton–Raphson (ABNR) method available in Discovery Studio 3.5 protocol until the root mean deviation (RMS) gradient was $<0.05$ kcal/mol/Å$^2$. The minimized protein was defined as the receptor using the binding site module. The binding site was defined from the cavity finding method which was modified to accommodate all the important interacting residues in the active site of the enzyme. Binding sphere for **3FNE** (27.49, 3.71, 10.20, 13.16) was selected from the active site using the binding site tools. The most antimycobacterial active compounds **3a**, **3c**, **3f** and the triclosan derivative, which is the ligand of **3FNE.PDB** crystal structure, were sketched, and all-atom CHARMm forcefield parameterization was assigned and then minimized using the ABNR method as described above. Conformational searches of the ligands were carried out using a simulated annealing molecular dynamics (MD) approach. The ligands were heated to a temperature of 700 K and then annealed to 200 K. CDOCKER (Wu *et al.*, 2003) method was performed by using Discovery Studio 3.5 (Accelrys Inc., 2012). The protein is held rigid, while the ligands are allowed to be flexible during refinement. The docking and scoring methodology was first validated by docking of triclosan derivative. The docked position of triclosan derivative overlaps well with the crystal structure position, with an RMSD of 0.53 Å. Afterward, molecular




**Scheme 1** Synthetic pathway of 2-(substitutedbenzyl/phenyl)-5-aminobenzoxazole (**3a–3l**) and 5-(4-nitro/aminophenyl-sulfonamido)benzoxazole (**5a–5l**, **6a–6l**) derivatives. *Reagents and conditions:* A) PPA, 170-200 °C, 1.5-2.5 h; B) Pyridine and dichloromethane, RT, 16 h; C) Pd–C/$H_2$, RT (for **6a**, **6e–h**, **6l**); methanol, iron powder and ammonium chloride, 70 °C, 2.5 h (for **6b–d**, **6i–k**)

docking studies were performed on the antimycobacterial active compounds (**3a**, **3c**, **3f**). Finally, all docked poses were scored by applying Analyze Ligand Poses subprotocol, and binding energies were calculated by applying Calculate Binding Energy subprotocol in Discovery Studio 3.5 by using in situ ligand minimization step (ABNR method) and implicit solvent model (GBMV). The lowest binding energy was taken as the best-docked conformation of the compounds for the macromolecule.

## Results and discussion

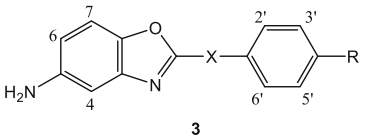
### Chemistry

In this study, 17 new 2-(4-substitutedphenyl)-5-(4-nitro/aminophenylsulfonamido)benzoxazole derivatives (**5b**, **5d**, **5g**, **5j**, **5k**, **5l** and **6a–6d**, **6f–6l**) were synthesized for the first time. For preparing them at first, 5-amino-2-(4-substitutedphenyl/benzyl)benzoxazoles (**3a–3l**) were obtained by heating appropriate acid with 2,4-diaminophenol in PPA (Yildiz-Oren *et al.*, 2004b; Sener *et al.*, 1987; Wynne *et al.*, 2009). After, 5-amino-2-(4-substitutedphenyl/benzyl)benzoxazole derivatives (**3a–3l**) and 4-nitrobenzenesulfonyl chloride (**4**) were treated in pyridine and dichloromethane to prepare 5-(4-nitrophenyl sulfonamido)benzoxazole derivatives (**5a–5l**) (Wynne *et al.*, 2009). Afterward, 5-(aminophenyl/benzylsulfonamido)benzoxazoles (**6a–6l**) were obtained by reduction using Pd–C/$H_2$ (Zheng *et al.*, 2007) or iron powder/ammonium chloride (Habens *et al.*, 2005) of compounds **5a–5l** as given in Scheme 1 (Ertan-Bolelli *et al.*, 2014). The newly synthesized structures were supported by spectral data. The IR, $^1$H NMR, $^{13}$C NMR, mass spectra and elemental analysis results are in agreement with the proposed structures. According to the spectroscopic data of the compounds **5b**, **5d**, **5g**, **5j**, **5k**, **5l** and **6a–6d**, **6f–6l,** the IR spectra showed that characteristic NH ($SO_2NH$) stretching bands were in the 3106–3308 $cm^{-1}$ region. In the $^1$H NMR spectra of the compounds **6a–6d**, **6f–6l**, the signals of $NH_2$ protons were observed at 5.92–5.98 ppm as a singlet band; the signal of NH ($SO_2$ NH) proton of the compounds **5b**, **5d**, **5g**, **5j**, **5k**, **5l** and **6a–6d**, **6f–6l** was observed at 9.83–10.76 ppm. Besides, benzylic $CH_2$ and aromatic $CH_3$ protons appeared at 4.21–4.30 ppm and 2.27–2.40 ppm as a singlet, respectively. Moreover, all the aromatic protons were observed at 6.47–8.39 ppm. $^{13}$C NMR spectra of the compounds were appropriate to their formulas. Mass spectra of the compounds showed $M^+ + H$ peaks in accordance with their formulas, since the electrospray ionization method was employed. On the other hand, the results of the elemental analyses (C, H, N, S) were within ±0.4 % of the calculated amounts.

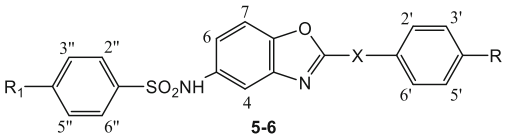
### In vitro antimicrobial evaluation

All of the previously and newly synthesized 5-amino-2-(4-substitutedphenyl/benzyl)benzoxazoles (**3a–3l**), 2-(4-substitutedphenyl)-5-(4-nitrophenylsulfonamido)benzoxazoles (**5a–5l**), 2-(4-substitutedphenyl)-5-(4-aminophenylsulfonamido)-benzoxazoles (**6a–6l**) were tested for their in vitro antimicrobial activity against *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 as Gram-positive bacteria, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as Gram-

**Table 1** Structures of the tested benzoxazoles and their in vitro antimicrobial activities as MIC values (μg/mL)




3




5-6

| Compounds | | | | Microorganisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | X | R | $R_1$ | Gram-negative | | | | Gram-positive | | | | Fungi | | Mycobacteria | |
| | | | | *E.c.* | *E.c.** | *P.a.* | *P.a.** | *S.a.* | *S.a.** | *E.f.* | *E.f.** | *C.a.* | *C.k.* | *M.t.* | *M.t.** |
| **3a** | – | H | – | 64 | 64 | 32 | 32 | 64 | 64 | 64 | 32 | 64 | 64 | 8 | 8 |
| **3b** | – | Cl | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 32 | 64 | 16 | 8 |
| **3c** | – | F | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 32 | 64 | 8 | 8 |
| **3d** | – | Br | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 32 | 64 | 8 | 32 |
| **3e** | – | $C_2H_5$ | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 32 | 64 | 16 | 8 |
| **3f** | – | $CH_3$ | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 64 | 64 | 8 | 8 |
| **3g** | – | $OCH_3$ | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 64 | 64 | 64 | 8 |
| **3h** | $CH_2$ | H | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 64 | 64 | 32 | 32 |
| **3i** | $CH_2$ | Cl | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 64 | 64 | 16 | 8 |
| **3j** | $CH_2$ | F | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 64 | 64 | 16 | 32 |
| **3k** | $CH_2$ | Br | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 64 | 64 | 16 | 16 |
| **3l** | $CH_2$ | $CH_3$ | – | 64 | 64 | 32 | 64 | 64 | 64 | 64 | 32 | 64 | 64 | 32 | 64 |
| **5a** | – | H | $NO_2$ | 128 | 128 | 128 | 32 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 32 |
| **5b** | – | Cl | $NO_2$ | 128 | 128 | 128 | 32 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 32 |
| **5c** | – | F | $NO_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 64 |
| **5d** | – | Br | $NO_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 32 |
| **5e** | – | $C_2H_5$ | $NO_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 16 |
| **5f** | – | $CH_3$ | $NO_2$ | 64 | 128 | 128 | 32 | 128 | 128 | 128 | 128 | 128 | 64 | 64 | 64 |
| **5g** | – | $OCH_3$ | $NO_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 64 | 64 | 64 | 16 |
| **5h** | $CH_2$ | H | $NO_2$ | 128 | 128 | 128 | 64 | 128 | 128 | 128 | 128 | 64 | 64 | 32 | 16 |
| **5i** | $CH_2$ | Cl | $NO_2$ | 128 | 128 | 128 | 64 | 128 | 128 | 128 | 128 | 32 | 64 | 32 | 16 |
| **5j** | $CH_2$ | F | $NO_2$ | 128 | 128 | 128 | 64 | 128 | 128 | 128 | 128 | 256 | 64 | 32 | 16 |
| **5k** | $CH_2$ | Br | $NO_2$ | 128 | 128 | 128 | 64 | 128 | 128 | 128 | 128 | 128 | 128 | 32 | 16 |
| **5l** | $CH_2$ | $CH_3$ | $NO_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 64 | 64 | 32 | 16 |
| **6a** | – | H | $NH_2$ | 128 | 128 | 128 | 32 | 128 | 128 | 128 | 128 | 128 | 64 | 64 | 32 |
| **6b** | – | Cl | $NH_2$ | 128 | 128 | 128 | 32 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 64 |
| **6c** | – | F | $NH_2$ | 128 | 128 | 128 | 32 | 256 | 128 | 128 | 64 | 128 | 64 | 64 | 64 |
| **6d** | – | Br | $NH_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 64 |
| **6e** | – | $C_2H_5$ | $NH_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 16 |
| **6f** | – | $CH_3$ | $NH_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 128 | 64 | 64 | 64 |
| **6g** | – | $OCH_3$ | $NH_2$ | 128 | 128 | 128 | 64 | 64 | 128 | 128 | 128 | 64 | 64 | 64 | 64 |
| **6h** | $CH_2$ | H | $NH_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 64 | 64 | 32 | 16 |
| **6i** | $CH_2$ | Cl | $NH_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 64 | 64 | 16 | 16 |
| **6j** | $CH_2$ | F | $NH_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 128 | 128 | 32 | 16 |
| **6k** | $CH_2$ | Br | $NH_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 64 | 128 | 16 | 16 |
| **6l** | $CH_2$ | $CH_3$ | $NH_2$ | 128 | 128 | 128 | 64 | 256 | 128 | 128 | 128 | 128 | 128 | 16 | 16 |
| Meropenem | | | | <2 | <2 | <2 | 8 | <2 | <2 | 8 | 8 | – | – | – | – |
| Ampicillin | | | | 8 | 64 | – | – | <2 | 32 | <2 | 4 | – | – | – | – |
| Ceftriaxone | | | | <2 | 128 | 16 | 32 | 8 | 64 | – | – | – | – | – | – |

**Table 1** continued

| Compounds | | | | Microorganisms | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | X | R | $R_1$ | Gram-negative | | | | Gram-positive | | | | Fungi | | Mycobacteria | |
| | | | | *E.c.* | *E.c.** | *P.a.* | *P.a.** | *S.a.* | *S.a.** | *E.f.* | *E.f.** | *C.a.* | *C.k.* | *M.t.* | *M.t.** |
| Gentamicin | | | | <2 | 128 | <2 | 32 | <2 | 64 | 4 | 4 | – | – | – | – |
| Tetracycline | | | | <2 | 128 | 16 | 32 | 1 | 32 | 2 | 64 | – | – | – | – |
| Trimethoprim-Sulfamethoxazole | | | | <2 | 128 | 32 | 32 | <2 | 32 | <2 | <2 | – | – | – | – |
| Sulfamethoxazole | | | | 16 | 128 | – | – | <2 | 8 | 64 | 64 | – | – | 8 | 8 |
| Ofloxacin | | | | <2 | 16 | <2 | 32 | <2 | <2 | <2 | <2 | – | – | – | – |
| Ciprofloxacin | | | | <2 | 16 | <2 | 16 | <2 | <2 | <2 | <2 | – | – | – | – |
| Fluconazole | | | | – | – | – | – | – | – | – | – | 1 | 64 | – | – |
| Amphotericin B | | | | – | – | – | – | – | – | – | – | <0.25 | 0.5 | – | – |
| Isoniazide | | | | – | – | – | – | – | – | – | – | – | – | <0.25 | <0.25 |
| Ethambutol | | | | – | – | – | – | – | – | – | – | – | – | 2 | 2 |

*E.c.*, *E. coli* ATCC 25922; *E.c.**, *E. coli* isolate (include ESBL); *P.a.*, *Pseudomonas aeruginosa* ATCC 27853; *P.a.**, *P. aeruginosa* isolate (rezistant of gentamicin); *S.a.*, *Staphylococcus aureus* ATCC 29213; *S.a.**, *S. aureus* isolate (MRSA); *E.f.*, *Enterococcus faecalis* ATCC 29212, *E.f.**, *E. faecalis* isolate (rezistant of vancomycin); *C.a.*, *Candida albicans* ATCC 10231; *C.k.*, *C. krusei* ATCC 6258; *M.t.*, *M. tuberculosis* H37RV ATCC 27294; *M.t.**, *M. tuberculosis* isolate

negative bacteria strains, *M. tuberculosis* H37RV ATCC 27294 as Mycobacteria and their drug-resistant isolates *C. albicans* ATCC 10231 and *C. krusei* ATCC 6258 as fungus. The standard drugs meropenem, ampicillin, ceftriaxone, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, sulfamethoxazole, ofloxacin and ciprofloxacin for antibacterial activity, isoniazid and ethambutol for antimycobacterial activity, and fluconazole and amphotericin B for antifungal activity were also screened under identical conditions for quality control and comparison. The minimum inhibitory concentration (MIC) values were determined by twofold serial dilution technique in Mueller–Hinton Broth (MHB) and Sabouraud Dextrose Agar (SDA) for the antibacterial and antifungal assays, respectively. Microplate Alamar Blue Assay (MABA) in Middlebrook 7H11 Agar was used in order to obtain the MIC values for antimycobacterial effect. All of the biological results are given in Table 1.

Microbiological results indicated that previously and newly synthesized compounds possessed a broad spectrum of activity against the tested microorganisms at MIC values between 8 and 256 μg/mL.

According to the obtained data, all of the 2-(4-substitutedbenzyl/phenyl)-5-aminobenzoxazole derivatives (**3a**–**3l**) showed better antibacterial activity against *E. coli*, *S. aureus* and *E. faecalis* with their drug-resistant isolates and *P. aeruginosa* than their sulfonamide derivatives except compounds **5f**, **6g** and **6c**. The tested compounds indicated significant inhibitory effect against the Gram-negative enterobacter drug-resistant isolate of *P. aeruginosa* with MIC values of 32–64 μg/mL. Among the compounds, derivatives **3a**, **5a**, **5b**, **5f** and **6a**–**6c** showed good activities against *P. aeruginosa* clinical isolate, which is effective in nosocomial infections, comparable to that of ceftriaxone, gentamicin, tetracycline, trimethoprim-sulfamethoxazole and ofloxacin.

Moreover, **3a**–**3l** compounds were found to be more potent than the other compounds against drug-resistant isolate of *E. faecalis* with MIC value of 32 μg/mL. Even they were more effective than the standard drugs tetracycline and sulfamethoxazole. It could be noticed that an amine group at the 5th position of benzoxazole ring was very important for improving the activity.

On the other hand, although compounds **3b**–**3e** and **5i** indicated significant activity against *C. albicans* with MIC value of 32 μg/mL, they exhibited less effect than the standard drugs. Surprisingly, antifungal activities against *C. krusei* of all derivatives except **5k** and **6j**–**6l** are comparable to fluconazole.

Furthermore, most of the compounds exhibited very important activity against *M. tuberculosis* and its clinical isolate with MIC values between 8 and 64 μg/mL comparing with other human pathogenic microbes. Surprisingly, compounds **3a**, **3c**, **3d** and **3f** against *M. tuberculosis* and compounds **3a**–**3c**, **3e**–**3g** and **3i** against drug-resistant *M. tuberculosis* showed the same activity with standard drug sulfamethoxazole with a MIC value of 8 μg/mL.

## Molecular docking studies

The NADH-dependent InhA has been validated as the primary molecular target of the frontline antitubercular drugs. As shown in Table 1, compounds **3a**, **3c** and **3f** were found to be significantly active against both *M. tuberculosis* H37RV ATCC 27294 and its clinical isolate at the MIC value of 8 μg/mL. Here, these compounds (**3a**, **3c** and **3f**)

**Table 2** Docking results

| Comp. code | Binding energy (kcal/mol) | Interacted residues* |
|---|---|---|
| **3a** | −16.5561 | **Gly96** (2.25 Å), Phe97, Met98, Met103, Phe149, Tyr158, Met161, Pro193, Ala198, Met199, Ile215, Leu218, **NAD$^+$** (2.75 Å) |
| **3c** | −18.0974 | **Gly96** (2.45 Å), Phe97, Met98, Met103, Phe149, Tyr158, Met161, Pro193, Thr196, Ala198, Met199, Ile215, Leu218, **NAD$^+$**(2.63 Å) |
| **3f** | −15.7779 | **Gly96** (2.44 Å), Phe97, Met103, Phe149, Tyr158[b], Met161, Pro193, Ala198, Met199, Ile215, Leu218, Glu219, **NAD$^+$**(2.58 Å) |
| Triclosan derivative | −11.9466 | Gly96, Phe97, Met98, Met103, Phe149[b], Met155, Pro156, Ala157, **Tyr158**[a] (1.86 Å), Met161, Lys165[b], Pro193, Try196, Ala198, Met199, Ile215, Leu218, Glu219, **NAD$^+$** (2.15 Å) |

* Van der Waals contact distance <4 Å

Bold: H bonds; [a] $\pi$–$\pi$ interactions; [b] $\pi$–$\sigma$ interactions

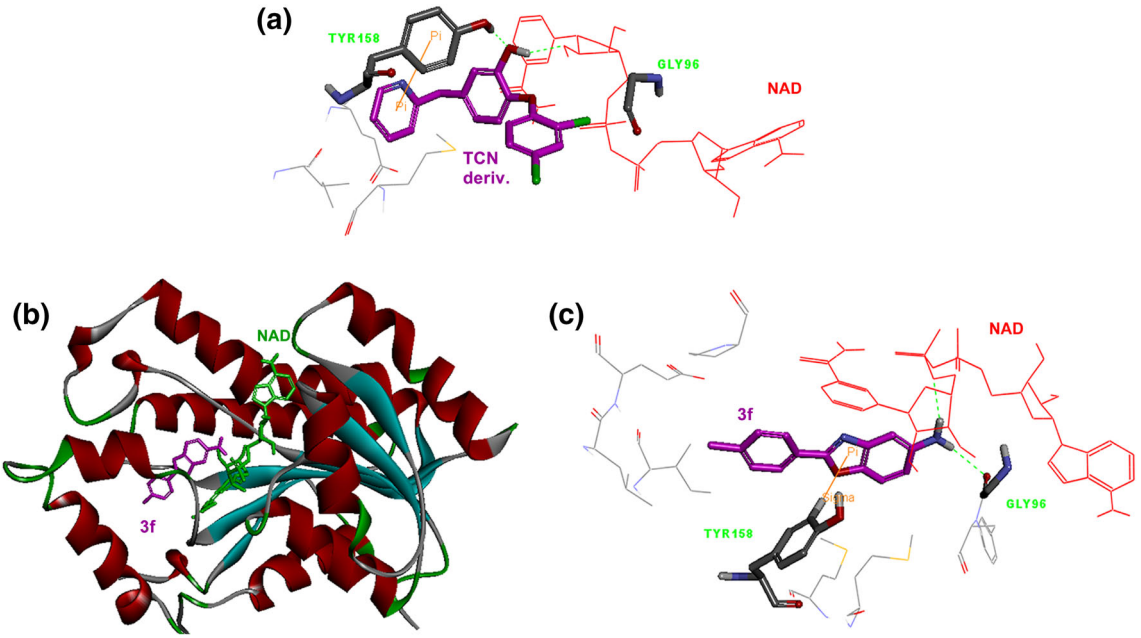



**Fig. 1** **a** Docked position of triclosan (TCN) derivative (C atoms are purple): Hydroxyl group has H bonds with Tyr158 and NAD$^+$ (red), and pyridine ring has pi–pi interaction with Tyr158. **b):** Docked position of **3f** (purple) with NAD$^+$ (green) in 3FNE **c):** Docked position of **3f** (C atoms are purple): Amino group has H bonds with Gly96 and NAD$^+$ (red), and oxazole ring has pi–sigma interaction with Tyr158

were docked into active site of InhA (**PDB ID: 3FNE**) by using CDOCKER method (Wu *et al.*, 2003; Accelrys Inc., 2012) in order to understand ligand–protein interactions. Firstly, one of the triclosan derivatives (**3FNE.PDB** ligand), which are the known specific inhibitor of InhA, was docked. After then, molecular docking studies were performed for the compounds **3a**, **3c** and **3f**. The molecular docking studies of the examined compounds showed that their binding to InhA active site with the position and orientation was found to be very close to the result of the crystal structure of triclosan derivative complex in InhA. All the docking scores are given in Table 2. According to the docking studies, triclosan derivative revealed H bonds with Tyr158 and co-factor NAD$^+$, which are in accordance with the X-ray structure binding features (**3FNE**). As for the most active benzoxazole derivatives **3a**, **3c** and **3f,** the amino group at the 5th position of their benzoxazole ring interacted with an active site residue Gln96 and co-factor NAD$^+$. Unlike other compounds, derivative **3f** showed also pi–sigma interaction between Tyr158 and the oxazole ring of it as shown in Fig. 1. Binding energies of these compounds **3a**, **3c**, **3f** and triclosan derivative are −16.5561, −18.0974, −15.7779 and −11.9466, respectively. *In vitro* studies demonstrated that **3a**, **3c** and **3f** exhibited promising antitubercular activity, and the docking results were also correlated with the experimental data.

## Conclusion

In this study, the synthesis of 17 novel 2-substituted-5-(4-nitro/aminophenylsulfonamido)benzoxazole derivatives (**5b**, **5d**, **5g**, **5j**, **5k**, **5l** and **6a–6d**, **6f–6l)** were described by using 2-(4-substitutedphenyl)-5-aminobenzoxazole (**3a–3l**). In addition, in vitro antimicrobial activities against human pathogenic microbes of all previously and newly synthesized benzoxazole derivatives (**3a–3l**, **5a–5l**, **6a–6l**) were investigated. Microbiological results indicated that all the tested compounds possessed a broad spectrum of activity. Generally, 2-(4-substitutedphenyl)-5-aminobenzoxazoles (**3a–3l**) were found to be more potent than their sulfonamido derivatives for all tested microorganisms. In particular, compounds **3a**, **3b**, **3c**, **3e**, **3f**, **3g** and **3i** revealed very important effect against drug-resistant isolate of *M. tuberculosis* with MIC value of 8 μg/mL. The structure–activity relationship showed that amino group at the 5th position together with phenyl ring instead of benzyl ring on the 2nd position of benzoxazole played very important role for improving antituberculosis activity.

According to in vitro microbiological studies, 2-phenyl-5-aminobenzoxazole (**3a**), 2-(4-fluorophenyl)-5-aminobenzoxazole (**3c**) and 2-(4-methylphenyl)-5-aminobenzoxazole (**3f**) exhibited significantly antitubercular activity against both *M. tuberculosis* and its drug-resistant isolate. For that reason, molecular docking of compounds **3a**, **3c** and **3f** to the active site of InhA, which has been validated as the primary molecular target of the frontline antitubercular drugs, was carried out using CDOCKER method (Accelrys Inc., 2012) in order to understand ligand–protein interactions. Previous reports revealed that the catalytic residue Tyr158 and co-factor $NAD^+$ played important role for the activity of InhA (Lu *et al.*, 2010; Shrinivas *et al.*, 2015). Molecular docking studies showed that promising antitubercular compounds **3a**, **3c** and **3f** directly interacted with InhA enzyme by binding to Tyr158 and/or co-factor $NAD^+$, and these results were correlated with the experimental data. It can be concluded that compounds **3a**, **3c** and **3f** could be considered as scaffolds for designing new potent antitubercular drugs.

**Acknowledgments** We thank the Research Fund of Ankara University (Grant No. 12B3336002) for the financial support of this research.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

Abbanat D, Macielag M, Bush K (2003) Novel antibacterial agents for the treatment of serious Gram-positive infections. Expert Opin Investig Drugs 12:379–399

Accelrys Inc (2012) Discovery Studio 3.5. http://accelrys.com/products/collaborative-science/biovia-discovery-studio/. Accessed 18 Dec 2015

Akbay A, Oren I, Temiz-Arpaci O, Aki-Sener E, Yalcin I (2003) Synthesis and HIV-1 reverse transcriptase inhibitor activity of some 2,5,6-substituted benzoxazole, benzimidazole, benzothiazole and oxazolo(4,5-*b*)pyridine derivatives. Arzneim Forsch 53(4):266–271

Akurathi V, Dubois L, Lieuwes NG, Chitneni SK, Cleynhens BJ, Vullo D, Supuran CT, Verbruggen AM, Lambin P, Bormans GM (2010) Synthesis and biological evaluation of a 99mTc-labelled sulfonamide conjugate for in vivo visualization of carbonic anhydrase IX expression in tumor hypoxia. Nucl Med Biol 37:557–564

Alper-Hayta S, Arisoy M, Temiz-Arpaci O, Yildiz I, Aki E, Ozkan S, Kaynak F (2008) Synthesis, antimicrobial activity, pharmacophore analysis of some new 2-(substitutedphenyl/benzyl)-5-[(2-benzofuryl)carboxamido] benzoxazoles. Eur J Med Chem 43(11):2568–2578

Andrighetti-Fröhner CR, de Oliveira KN, Gaspar-Silva D, Pacheco LK, Joussef AC, Steindel M, Simões CMO, de Souza AMT, Magalhaes UO, Afonso IF, Rodrigues CR, Nunes RJ, Castro HC (2009) Synthesis, biological evaluation and SAR of sulfonamide 4-methoxychalcone derivatives with potential antileishmanial activity. Eur J Med Chem 44:755–763

Arisoy M, Temiz-Arpaci O, Yildiz I, Kaynak-Onurdag F, Aki E, Yalcin I, Abbasoglu U (2008) Synthesis, antimicrobial activity and QSAR studies of 2,5-disubstitutedbenzoxazoles. SAR QSAR Environ Res 19(5–6):589–612

Azab M, Youssef M, El-Bordany E (2013) Synthesis and antibacterial evaluation of novel heterocyclic compounds containing a sulfonamido moiety. Molecules 18:832–844

Balani SK, Pitzenberger SM, Kauffman LR, Arison BH, Ramjit HG, Goldman ME, O'Brien JA, King JD, Hoffman JM, Rooney Theoharides AD (1992) Metabolism of a new HIV-1 reverse transcriptase inhibitor, 3-[2-(benzoxazol-2-yl)ethyl]-5-ethyl-6-methylpyridin-2(1H)-one (L-696,229), in rat and liver slices. Drug Metab Dispos 20(6):869–876

Chancellor DR, Davies KE, De Moor O, Dorgan CR, Johnson PD, Lambert AG, Lawrence D, Lecci C, Maillol C, Middleton PJ, Nugent G, Poignant SD, Potter AC, Price RPD, Pye J, Storer R, Tinsley JM, Van Well R, Vickers R, Vile J, Wilkes FJ, Wilson FX, Wren SP, Wynne GM (2011) Discovery of 2-arylbenzoxazoles as upregulators of utrophin production fort he treatment of Duchenne muscular dystrophy. J Med Chem 54:3241–3250

Chandak N, Bhardwaj JK, Sharma R, Sharma PK (2013) Inhibitors of apoptosis in testicular germ cells: synthesis and biological evaluation of some novel IBTs bearing sulfonamide moiety. Eur J Med Chem 59:203–208

Chen CJ, Wang I-W, Sheu H-S, Lee GH, Lai CK (2011) Metallomesogens derived from benzoxazoles-salicylaldimine conjugates. Tetrahedron 67(42):8120–8130

Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) (2006a) Performance Standards for Antimicrobial Susceptibility Testing; 16th Informational Supplement. CLSI M100-S16. Clinical and Laboratory Standarts Institute, 940 West Valley Road, Wayne, Pennsylvania, USA

Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) (2006b) Reference method for broth dilution antifungal susceptibility testing yeast; approved standard, M27-A. Clinical and Laboratory Standarts Institute, 940 West Valley Road, Wayne, Pennsylvania, USA

Dalhoff A (1994) Quinolone resistance in *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Development during therapy and clinical significance. Infection 22:111–121

Davey RT, Dewar RL, Reed GF, Vasudevachari MB, Polis MA, Kovacs JA, Falloon J, Walker RE, Masur H, Haneiwich SE,

O'Neill DG, Decker MR, Metcalf JA, Deloria MA, Laskin OL, Salzman N, Lane HC (1993) Plasma viremia as a sensitive indicator of the antiretroviral activity of L-697,661. Proc Natl Acad Sci 90(12):5608–5612

Ertan T, Yildiz I, Tekiner-Gulbas B, Bolelli K, Temiz-Arpaci O, Ozkan S, Kaynak F, Yalcin I, Aki E (2009) Synthesis, biological evaluation and 2d-QSAR analysis of benzoxazoles as antimicrobial agents. Eur J Med Chem 44:501–510

Ertan-Bolelli T, Musdal Y, Bolelli K, Yilmaz S, Aksoy Y, Yildiz I, Aki-Yalcin E, Yalcin I (2014) Synthesis and biological evaluation of 2-substituted-5-(4-nitrophenylsulfonamido)benzoxazoles as human GST P1-1 inhibitors, and description oft he binding site features. ChemMedChem 9:984–992

Ezabadi IR, Camoutsis C, Zoumpoulakis P, Geronikaki A, Soković M, Glamočilija J, Ćirić A (2008) Sulfonamide-1,2,4-triazole derivatives as antifungal and antibacterial agents: Synthesis, biological evaluation, lipophilicity, and conformational studies. Bioorg Med Chem 16:1150–1161

Franzblau SG, Witzig RS, Mclaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH (1998) Rapid, low-technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate Alamar blue assay. J Clin Microbiol 36(2):362–366

Freundlich JS, Wang F, Vilcheze C, Gulten G, Langley R, Schiehser GA, Jacobus DP, Jacobs WR, Sacchettini JC (2009) Triclosan derivatives: towards potent inhibitors of drug-sensitive and drug-resistant *Mycobacterium tuberculosis*. ChemMedChem 4(2):241–248

Gadad AK, Mahajanshetti CS, Nimbalkar S, Raichurkar A (2000) Synthesis and antibacterial activity of some 5-guanylhydrazone/thiocyanato-6-arylimidazo[2,1-*b*]-1,3,4-thiadiazole-2-sulfonamide derivatives. Eur J Med Chem 35:853–857

Habens F, Srinivasan N, Oakley F, Mann DA, Ganesan A, Packham G (2005) Novel sulfasalazine analogues with enhanced NF-kB inhibitory and apoptosis promoting activity. Apoptosis 10(3):481–491

Ileana IR, Rodriguez AD, Wang Y, Franzblau SG (2006) Ileabethoxazole: a novel benzoxazole alkaloid with antimycobacterial activity. Tetrahedron Lett 47:3229–3232

Johnsson K, Schultz PG (1994) Mechanistic studies of the oxidation of isoniazid by the catalase peroxidase from *Mycobacterium tuberculosis*. J Am Chem Soc 116:7425–7426

Kamal A, Swapna P, Shetti RV, Shaik AB, Narasimha Rao MP, Gupta S (2013) Synthesis, biological evaluation of new oxazolidino-sulfonamides as potential antimicrobial agents. Eur J Med Chem 62:661–669

Kim JS, Sun Q, Gatto B, Yu C, Liu A, Liu LF, LaVoie EJ (1996) Structure-activity relationships of benzimidazoles and related heterocycles as topoisomerase I poisons. Bioorg Med Chem 4:621–630

Klimesova V, Koci J, Waisser K, Kaustova J, Mollmann U (2009) Preparation and in vitro evaluation of benzylsulfanyl benzoxazole derivatives as potential antituberculosis agents. Eur J Med Chem 44:2286–2293

Kumar V, Abbas AK, Fausto N, Mitchell RN (2007) Robbins basic pathology, 8th edn. Saunders Elsevier, Philadelphia, pp 516–522

Lage H, Aki-Sener E, Yalcin I (2006) High antineoplastic activity of new heterocyclic compounds in cancer cells with resistance against classical DNA topoisomerase II-targeting drugs. Int J Cancer 119(1):213–220

Lee V, Hacker S (1999) Antibiotic resistance versus small molecules, the chemical evolution. J Med Chem 19:521–542

Livermore D (2000) Antibiotic resistance in *staphylococci*. Int J Antimicrob Agents 16:3–10

Lu XY, You QD, Chen YD (2010) Recent progress in the identification and development of InhA direct inhibitors of *Mycobacterium tuberculosis*. Mini-Rev Med Chem 10:182–193

Lu X, Zhang H, Li X, Chen G, Li QS, Luo Y, Ruan BF, Chen XW, Zhu HL (2011) Design, synthesis and biological evaluation of pyridine acyl sulfonamide derivatives as novel COX-2 inhibitors. Bioorg Med Chem 19:6827–6832

Luo Y, Qiu KM, Lu X, Liu K, Fu J, Zhu HL (2011) Synthesis, biological evaluation, and molecular modeling of cinnamic acyl sulfonamide derivatives as novel antitubulin agents. Bioorg Med Chem 19:4730–4738

Oksuzoglu E, Temiz-Arpaci O, Tekiner-Gulbas B, Eroglu H, Sen G, Alper S, Yildiz I, Diril N, Aki-Sener E, Yalcin I (2007) A study on the genotoxic activities of some new benzoxazoles. Med Chem Res 16(1):1–14

Oksuzoglu E, Tekiner-Gulbas B, Alper S, Temiz-Arpaci O, Ertan T, Yildiz I, Diril N, Sener-Aki E, Yalcin I (2008) Some benzoxazoles and benzimidazoles as DNA topoisomerase I and II inhibitors. J Enzyme Inhib Med Chem 23(1):37–42

Oren I, Temiz O, Yalcin I, Sener E, Akin A, Ucarturk N (1997) Synthesis and microbiological activity of 5 (or 6)-methyl-2-substituted benzoxazole and benzimidazole derivatives. Arzneim Forsch/Drug Res 47(2):1393–1397

Oren I, Temiz O, Yalcin I, Sener E, Altanlar N (1998) Synthesis and antimicrobial activity of some novel 2,5- and/or 6-substituted benzoxazole and benzimidazole derivatives. Eur J Pharm Sci 7:153–160

Pinar A, Yurdakul P, Yildiz-Oren I, Temiz-Arpaci O, Acan NL, Aki-Sener E, Yalcin I (2004) Some fused heterocyclic compounds as eukaryotic topoisomerase II inhibitors. Biochem Biophys Res Commun 317(2):670–674

Plemper RK, Erlandson KJ, Lakdawala AS, Sun A, Prussia A, Boonsombat J, Aki-Sener E, Yalcin I, Yildiz I, Temiz-Arpaci O, Tekiner BP, Liotta D, Snyder JP (2004) A novel target site for template-based design of measles virus entry inhibitors. Proc Natl Acad Sci 101:5628–5633

Poole K (2001) Multidrug resistance in Gram-negative bacteria. Curr Opin Microbiol 4:500–508

Prudhomme M, Guyot J, Jeminet G (1986) Semi-synthesis of A 23187 (Calcimycin) Analogs. IV. Cation carier properties in mitochondria of analogs with modified benzoxazole rings. Antimicrobial activity. J Antibiot 39:934–937

Rana DN, Chhabria MT, Shah NK, Brahmkshatriya PS (2014) Pharmacophore combination as a useful strategy to discover new antitubercular agents. Med Chem Res 23:370–381

Rida SM, Ashour FA, El-Hawash S, El-Semary M, Badr MH, Shalaby MA (2005) Synthesis of some novel benzoxazole derivatives as anticancer, anti HIV-1 and antimicrobial agents. Eur J Med Chem 40:949–959

Sener E, Yalcin I, Ozden S, Ozden T, Akin A, Yildiz S (1987) Synthesis and antimicrobial activities of 5-amino-2-(*p*-substitutedphenyl)benzoxazole derivatives. Turk J Med Pharm 11(3):391–396

Shrinivas DJ, Uttam AM, Deepak P, Tejraj MA, Andanappa KG, Mallikarjuna NN, Rahul J (2015) Design, synthesis of quinolinyl Schiff bases and azetidinones as enoyl ACP-reductase inhibitors. Med Chem Res 24:3892–3911

Takayama K, Wang L, David HL (1972) Effect of isoniazid on the in vivo mycolic acid synthesis, cell growth, and viability of *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2:29–35

Temiz-Arpaci O, Ozdemir A, Yalcin I, Yildiz I, Aki-Sener E, Altanlar N (2005) Synthesis and antimicrobial activity of some5-[2-(morpholin-4-yl)acetamido] and/or 5-[2-(4-substitutedpiperazin-1-yl)acetamido]-2-(*p*-substitutedphenyl) benzoxazoles. Arch Pharm Chem Life Sci 338:105–111

Ueki M, Taniguchi M (1997) UK-1 a novel cytotoxic metabolite from *Streptomyces* sp. 517-02. III. Antibacterial action of demethyl UK-1. J Antibiot 50(9):788–790

Varga A, Aki-Sener E, Yalcin I, Temiz-Arpaci O, Tekiner-Gulbas B, Cherepnev G, Molnar J (2005) Induction of apoptosis and necrosis by resistance benzazoles and benzoxazines on tumour cell line mouse lymphoma L5718 $Mdr^{+}$ cells. In Vivo 19(6):1087–1092

Vinsova J, Horak V, Buchta V, Kaustova J (2005) Highly lipophilic benzoxazoles with potential antibacterial activity. Molecules 10:783–793

Wu G, Robertson DH, Brooks CL, Vieth M (2003) Detailed analysis of grid-based molecular docking: a case study of CDOCKER—a CHARMm-based MD docking algorithm. J Comput Chem 24(13):1549–1562

Wynne GM, Wren SP, Johnson PD, Price PD, De Moor O, Nugent G, Storer R, Pye RJ, Dorgan CR (2009) Treatment of Duchenne muscular dystrophy. US Patent 2009-075938, filled Feb 9, 2007, issued Mar 19, 2009

Yalcin I, Oren I, Sener E, Akin A, Ucarturk N (1992) The synthesis and the structure-activity relationships of some substituted benzoxazoles, oxazolo(4,5-*b*)pyridines, benzothiazoles and benzimidazoles as antimicrobial agents. Eur J Med Chem 27:401–406

Yildiz-Oren I, Tekiner-Gulbas B, Yalcin I, Temiz-Arpaci O, Aki-Sener E, Altanlar N (2004a) Synthesis and antimicrobial activity of new 2-[*p*-substituted-benzyl]-5-[substituted-carbonylamino]benzoxazoles. Arch Pharm 337(7):402–410

Yildiz-Oren I, Yalcin I, Aki-Sener E, Ucarturk N (2004b) Synthesis and structure-activity relationships of new antimicrobial active multisubstituted benzazole derivatives. Eur J Med Chem 39:291–298

Zhang Y, Heym B, Allen B, Young D, Cole S (1992) The catalase peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. Nature 358:591–593

Zheng X, Oda H, Takamatsu K, Sugimoto Y, Tai A, Akaho E, Ali HI, Oshiki T, Kakuta H, Sasaki K (2007) Analgesic agents without gastric damage: design and synthesis of structurally simple benzenesulfonanilide-type cyclooxygenase-1-selective inhibitors. Bioorg Med Chem 15(2):1014–1021

Zilifdar F, Alper-Hayta S, Yilmaz S, Kaplan-Ozen C, Foto E, Aydogan Z, Yildiz I, Aki E, Yalcin I, Diril N (2014) Genotoxic potentials and eukaryotic DNA topoisomerase I inhibitory effects of some benzoxazine derivatives. Med Chem Res 23(1):480–486