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Synthesis of some new urea and thiourea derivatives and evaluation of their antimicrobial activities

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In the present study, 18 new compounds were synthesized, 9 of which were urea **(9a-e**, **10a-d)** while the others were thiourea **(11a-e**, **12a-d)** derivatives. These derivatives were prepared by the reaction of 6-amino-5-nonsubstituted/chloro-3-methyl-2(3*H*)-benzoxazolones used as key intermediates with the appropriate isocyanates and isothiocyanates. The chemical structures of new compounds were confirmed by ^{1}H-NMR, mass, and elemental analysis. The synthesized compounds were screened for their antibacterial and antifungal activities against some pathogenic strains. Compounds **9a**, **9b**, **9e**, **10a**, and **10c**, urea derivatives, and compounds **12a and 12d**, thiourea derivatives, exhibited a relatively good inhibitory profile against *E. coli*, with a MIC value of 32 μg/mL when compared with the other target compounds.

Key Words: Urea, thiourea, 2(3*H*)-benzoxazolone, antimicrobial activity

Introduction

Antimicrobial drugs (antibacterial and antifungal) have been successfully used in the treatment of infectious diseases caused by bacteria and fungi since the first half of the 20th century. At the same time, antimicrobial therapy has been effective in prolonging of the average life expectancy by reducing the deaths caused by infectious diseases. However, the increasing use and misuse of antimicrobial drugs have led to the development of more

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resistant pathogens to commonly used antimicrobials. In particular, the emergence of multidrug resistant bacterial strains has made the treatment of bacterial diseases more complex when compared with the first half of last century.[1]

As for fungi, systemic fungal infections have become increasingly common in individuals with suppressed immune systems, such as patients undergoing anticancer chemotherapy or organ transplants and patients with AIDS.[2] In addition, antifungal drugs used in fungal infections have also become less effective owing to the development of fungal resistance.[3]

As a consequence, in recent decades, the incidence of microbial infections has increased at alarming levels in many countries around the world because of antimicrobial resistance. In order to overcome this challenging problem, there is the urgent need for the development of new antimicrobial compounds.

2(3*H*)-Benzoxazolone heterocyclic has received attention due to its capacity to mimic a phenol or a catechol.[4,5] Many 2(3*H*)-benzoxazolone-containing compounds have been reported to possess various biological activities such as analgesic-anti-inflammatory,[6–8] anticonvulsant,[9] antioxidant,[10] and antimicrobial properties.[11,12] Moreover, some authors have reported that the introduction of halogen atoms to the pharmacophore structure can be beneficial for antimicrobial activity.[13,14] Therefore, 2(3*H*)-benzoxazolone and its 5-chloro derivative were chosen to synthesize our target compounds. In addition, some urea and thiourea derivatives are known to be associated with a wide range of biological activities such as analgesic,[15] antitumor,[16,17] anti-HIV,[18,19] and antimicrobial properties.[20–23]

In view of these observations, we have designed new compounds incorporating the above pharmacophores together in order to prepare molecules having enhanced antimicrobial activity.

Therefore, we synthesized new compounds containing urea **(9a-e**, **10a-d)** and thiourea **(11a-e**, **12a-d)** groups at position 6 of 3-methyl-2(3*H*)-benzoxazolone and 5-chloro-3-methyl-2(3*H*)-benzoxazolone rings in order to investigate their antibacterial and antifungal activities.

Experimental

Chemistry

All the chemicals used for the synthesis of the compounds were purchased from Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany). Melting points of the compounds were recorded on an Electrothermal-9200 digital melting points apparatus (Southend, Great Britain) and the values are uncorrected. Microwave irradiation was carried out in a microwave oven (Milestone-MicroSYNTH, Italy). Thin-layer chromatography (TLC) was performed on Merck $60F_{254}$ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm). Flash column chromatography was performed on Kieselgel 60 (0.063-0.2 mm) (Merck). The ^{1}H-NMR spectra (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded employing a Varian Mercury 400 MHz FT spectrometer (Varian Inc., Palo Alto, CA, USA), in DMSO-d_6 at the Faculty of Pharmacy, Ankara University, Ankara, Turkey. The mass spectra were obtained on a Waters ZQ micromass LC-MS spectrometer (Waters Corporation, Milford, MA, USA) using the ESI(+) method at the Faculty of Pharmacy, Ankara University. Elemental analysis was performed on a Leco 932 CHNS instrument (St. Joseph, MI, USA) at the Faculty of Pharmacy, Ankara University, and the results were within $\pm 0.4\%$ of the theoretical

values. The synthesis of 2(3*H*)-benzoxazolone **(1)**,[24] 3-methyl-2(3*H*)-benzoxazolone **(3)**,[24] 5-chloro-3-methyl-2(3*H*)-benzoxazolone **(4)**,[25] 3-methyl-6-nitro-2(3*H*)-benzoxazolone **(5)**,[26] 5-chloro-3-methyl-6-nitro-2(3*H*)-benzoxazolone **(6)**,[27] 6-amino-3-methyl-2(3*H*)-benzoxazolone **(7)**,[26] and 6-amino-5-chloro-3-methyl-2(3*H*)-benzoxazolones **(8)**[27] was previously reported.

General procedure for the synthesis of 5-nonsubstituted/chloro-3-methyl-6-nitro-2(3*H*)-benzoxazolones (5, 6)

3-Methyl-2(3*H*)-benzoxazolone (0.1 mol) or 5-chloro-3-methyl-2(3*H*)-benzoxazolone (0.1) was added in portions to 100 mL of HNO_3 by keeping the internal temperature of flask at 40-50 °C. At the end of addition, the reaction mixture was poured into ice water. The forming precipitate was filtered, dried, and used for the next step without further purification. Yield 91%, mp: 182 °C for compound **5** (Ref. 26; mp: 181-182 °C). Yield 91%, mp: 157 °C for compound **6.**

General procedure for the synthesis of 6-amino-5-nonsubstituted/chloro-3-methyl-2(3*H*)-benzoxazolones (7, 8)

$SnCl_2$ (0.05 mol) was added to the solution of 3-methyl-6-nitro-2(3*H*)-benzoxazolone (0.01 mol) or 5-chloro-3-methyl-6-nitro-2(3*H*)-benzoxazolone (0.01 mol) in 40 mL of ethanol and 10 mL of 6 N HCl and reaction mixture was refluxed for 1.5 h. At the end of this period, the reaction mixture was cooled, and made alkaline with 1 M NaOH. The organic layer was extracted with CH_2Cl_2, dried with $MgSO_4$, and the residue obtained after the solvent evaporation was washed with methanol. The forming precipitate was filtered, dried, and used for next step without further purification. Yield 96%, mp: 154 °C for compound **7** (Ref. 26; mp: 153-154 °C). Yield 86%, mp: 228 °C for compound **8.**

General procedure for the synthesis of urea and thiourea derivatives obtained from 6-amino-3-methyl-2(3*H*)-benzoxazolone (9a-e, 11a-e)

Substituted isocyanate or isothiocyanate derivatives (1 mmol) were added to the solution of 6-amino-3-methyl-2(3*H*)-benzoxazolone (1 mmol) in 10 mL of THF at room temperature. The resulting mixture was stirred at room temperature for 1 h. At the end of this period, the forming precipitate was filtered, dried, and crystallized from appropriate solvent.

General procedure for the synthesis of urea and thiourea derivatives obtained from 6-amino-5-chloro-3-methyl-2(3*H*)-benzoxazolone:

Method A for compounds (10b, 10d)

Substituted isocyanate derivatives (1 mmol) were added to a solution of 6-amino-5-chloro-3-methyl-2(3*H*)-benzoxazolone (1 mmol) in 10 mL of THF at room temperature. The resulting mixture was stirred at room temperature until the starting material had been consumed. At the end of this period, the forming precipitate was filtered, dried, and crystallized from appropriate solvent.

Method B for compounds (10a, 10c, 12b, 12c)

A mixture of substituted isocyanate or isothiocyanate derivatives (3 mmol) and 6-amino-5-chloro-3-methyl-2(3*H*)-benzoxazolone (2.5 mmol) was heated at 65 °C until the amino derivative had been consumed. At the end of this period, the forming precipitate was filtered and either purified by flash column chromatography using ethyl acetate-hexane (50:50) as eluent or crystallized from appropriate solvent.

Method C for compounds (12a, 12d)

A mixture of substituted isothiocyanate derivatives (15 mmol) and 6-amino-5chloro-3-methyl-2(3*H*)-benzoxazolone (2.5 mmol) was heated at 100 °C until the amino derivative had been consumed. At the end of this period, methanol-water was added to reaction medium and the forming precipitate was filtered, dried, and purified by flash column chromatography using ethyl acetate-hexane (50:50) as eluent.

1-Phenyl-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (9a)

Recrystallized from acetic acid, yield 92%, mp: 258 °C. ^{1}H-NMR (DMSO-d$_6$) δ: 8.77 (s, 1H, -NH), 8.70 (s, 1H, -NH), 7.65(s, 1H, H^7), 7.50 (m, 2H, H$^{2'}$, H$^{6'}$), 7.32 (m, 2H, H$^{3'}$, H$^{5'}$), 7.19 (s, 2H, H^5, H^4), 7.02 (m, 1H, H$^{4'}$), 3.35 (s, 3H, -CH$_3$). MS ESI(+) m/e284 (M+H, 100). Anal. ($C_{15}H_{13}N_3O_3$): C, H, N calc. 63.60, 4.63, 14.83 found 63.52, 4.82, 14.65.

1-(4-Chlorophenyl)-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (9b)

Recrystallized from isopropanol, yield 89%, mp: 338 °C. ^{1}H-NMR (DMSO-d$_6$-RT) δ: 8.78 (s, 1H, -NH), 8.74 (s, 1H, -NH), 7.58 (s, 1H, H^7), 7.45 (d, 2H, H$^{2'}$, H$^{6'}$), 7.29 (d, 2H, H$^{3'}$, H$^{5'}$), 7.13 (s, 2H, H^5, H^4), 3.26 (s, 3H, -CH$_3$). ^{13}C-NMR (DMSO-d$_6$) δ: 154.83, 153.22, 142.69, 139.36, 135.42, 129.29, 127.17, 126.01, 120.44, 144.69, 109.58, 101.85, 28.70. MS ESI(+) m/e318 (M+H, 100). Anal. ($C_{15}H_{12}ClN_3O_3$): C, H, N calc. 56.70, 3.81, 13.23 found 56.75, 3.86, 13.34.

(DMSO-d$_6$ 55 °C) δ: 8.70 (s, 1H, -NH), 8.65 (s, 1H, -NH), 7.57 (d, 1H, H^7), 7.47 (d, 2H, H$^{2'}$, H$^{6'}$), 7.30 (d, 2H, H$^{3'}$, H$^{5'}$), 7.14 (m, 2H, H^5, H^4), 3.35 (s, 3H, -CH$_3$).

(Acetone-d$_6$ RT) δ: 8.24 (s, 1H, -NH), 7.68 (d, 1H, J_m = 2 Hz, H^7), 7.56 (d, 2H, H$^{2'}$, H$^{6'}$), 7.29 (d, 2H, H$^{3'}$, H$^{5'}$), 7.21 (dd, 1H, J_m = 2 Hz, J_o = 8.8 Hz, H^5), 7.08 (d, 1H, J_o = 8.4 Hz, H^4), 3.26 (s, 3H, -CH$_3$).

1-(4-Methoxyphenyl)-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (9c)

Recrystallized from isopropanol, yield 91%, mp: 334 °C. ^{1}H-NMR (DMSO-d$_6$) δ: 8.71 (s, 1H, -NH), 8.53 (s, 1H, -NH), 7.64 (s, 1H, H^7), 7.37 (d, 2H, H$^{2'}$, H$^{6'}$), 7.16 (s, 2H, H^5, H^4), 6.89 (s, 2H, H$^{3'}$, H$^{5'}$), 3.74 (s, 3H, -OCH$_3$), 3.26 (s, 3H, CH$_3$). MS ESI(+) m/e314 (M+H, 100). Anal. ($C_{16}H_{15}N_3O_4$): C, H, N calc. 61.34, 4.83, 13.41 found 61.30, 4.98, 13.33.

1-Benzyl-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (9d)

Recrystallized from isopropanol, yield 92%, mp: 260 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.70 (s, 1H, -NH), 7.64-7.12 (m, 8H, H^7, H^5, H^4, phenyl protons), 6.67 (s, 1H, NH), 4.32 (s, 2H, -CH_2), 3.26 (s, 3H, -CH_3). MS ESI(+) m/e298 (M+H, 100). Anal. ($C_{16}H_{15}N_3O_3$): C, H, N calc. 64.64, 5.09, 14.13 found 64.39, 4.90, 13.69.

1-(2-Phenylethyl)-3-(3-Methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (9e)

Recrystallized from methanol, yield 88%, mp: 238 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.57 (s, 1H, -NH), 7.60 (s, 1H, H^7), 7.33-7.02 (m, 7H, H^5, H^4, phenyl protons), 6.12 (s, 1H, -NH), 3.35 (t, 2H, -CH_2), 3.3 (s, 3H, -CH_3), 2.75 (t, 2H, -CH_2) MS ESI(+) m/e312 (M+H, 100). Anal. ($C_{17}H_{17}N_3O_3$): C, H, N calc. 65.58, 5.50, 13.50 found 65.24, 5.44, 13.25.

1-Phenyl-3-(5-chloro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (10a)

Purification by column chromatography on silica gel using ethyl acetate-hexane (50:50) as eluent, yield 20%, mp: 366 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.36 (s, 1H, -NH), 8.33 (s, 1H, -NH), 8.06 (s, 1H, H^7), 7.52 (s, 1H, H^4), 7.50-6.99 (m, 5H, phenyl protons), 3.32 (s, 3H, -CH_3). MS ESI(+) m/e318 (M+H, 100). Anal. ($C_{15}H_{12}ClN_3O_3$.H_2O): C, H, N calc. 53.66, 4.20, 12.52 found 53.76, 3.88, 2.13.

1-(4-Chlorophenyl)-3-(5-chloro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (10b)

Recrystallized from DMF-H_2O, yield 51%, mp: 262 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.47 (s, 1H, -NH), 8.34 (s, 1H, -NH), 8.02 (s, 1H, H^7), 7.51 (s, 1H, H^4), 7.49 (d, 2H, $H^{2'}$, $H^{6'}$), 7.34 (d, 2H, $H^{3'}$, $H^{5'}$), 3.32 (s, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 180.76, 154.82, 142.11, 139.12, 134.63, 129.66, 128.97, 126.10, 121.09, 109.20, 107.79, 28.78.

MS ESI(+) m/e353 (M+H, 100). Anal. ($C_{15}H_{11}Cl_2N_3O_3$.0.5 H_2O): C, H, N calc. 49.88, 3.34, 11.63 found 49.94, 3.26, 11.75.

1-(4-Methoxyphenyl)-3-(5-chloro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (10c)

Purification by column chromatography on silica gel using ethyl acetate-hexane (50:50) as eluent, yield 15%, mp: 338 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.13 (s, 1H, -NH), 8.18 (s, 1H, -NH), 8.00 (s, 1H, H^7), 7.44 (s, 1H, H^4), 7.31 (d, 2H, $H^{2'}$, $H^{6'}$), 6.83 (d, 2H, $H^{3'}$, $H^{5'}$), 3.66 (s, 3H, -OCH_3), 3.32 (s, 3H,-CH_3). MS ESI(+) m/e348 (M+H, 100). Anal. ($C_{16}H_{14}ClN_3O_4$): C, H, N calc. 55.26, 4.06, 12.08 found 54.80, 4.17, 11.90.

1-Benzy-3-(5-chloro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (10d)

Recrystallized from methanol, yield 17%, mp: 254 °C. ^{1}H-NMR (DMSO-d_6) δ: 8.20 (s, 1H, -NH), 8.10 (s,1H, -NH), 7.50-7.20 (m, 7H, H^4, H^7, phenyl protons), 4.32 (s, 2H, -CH_2), 3.34 (s, 3H, -CH_3). MS ESI(+) m/e332 (M+H, 100). Anal. ($C_{16}H_{14}ClN_3O_3$): C, H, N calc. 57.93, 4.25, 12.67 found 57.95, 4.39, 12.50.

1-Phenyl-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)thiourea (11a)

Recrystallized from acetic acid, yield 92%, mp: 252 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.81 (s, 1H, -NH), 9.79 (s, 1H,-NH), 7.56 (s, 1H, H^7), 7.42 (d, 2H, $H^{2'}$, $H^{6'}$), 7.32 (t, 2H, $H^{3'}$, $H^{5'}$), 7.19 (s, 2H, H^5, H^4), 7.10 (t, 1H, $H^{4'}$), 3.26 (s, 3H, -CH_3) MS ESI(+) m/e300 (M+H, 100). Anal. ($C_{15}H_{13}N_3O_2S$): C, H, N, S calc. 60.18, 4.38, 14.04, 10.71 found 59.98, 4.47, 13.99, 10.56.

1-(4-Chlorophenyl)-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)thiourea (11b)

Recrystallized from methanol, yield 93%, mp: 242 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.89 (s, 1H, -NH), 9.83 (s, 1H, -NH), 7.53-7.22 (m, 7H, H^7, H^5, H^4, phenyl protons), 3.35 (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6) δ: 154.73, 152.90, 141.41, 139.09, 131.08, 129.40, 128.49, 126.31, 120.39, 118.66, 110.22, 104.86, 28.94. MS ESI(+) m/e334 (M+H, 100). Anal. ($C_{15}H_{12}ClN_3O_2S$): C, H, N, S calc. 53.97, 3.62, 12.59, 9.61 found 53.89, 3.86, 12.19, 9.36.

1-(4-Methoxyphenyl)-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)thiourea (11c)

Recrystallized from methanol, yield 91%, mp: 235 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.65 (s, 1H, -NH), 9.60 (s, 1H, -NH), 7.56 (s, 1H, H^7), 7.35 (d, 2H, $H^{2'}$, $H^{6'}$), 7.23 (s, 2H, H^5, H^4), 6.95 (d, 2H, $H^{3'}$, $H^{5'}$), 3.80 (s, 3H, -OCH_3), 3.26 (s, 3H, CH_3). MS ESI(+) m/e330 (M+H, 100). Anal. ($C_{16}H_{15}N_3O_3S$): C, H, N, S calc. 58.34, 4.59, 12.76, 9.74 found 57.94, 4.66, 12.68, 9.40.

1-Benzyl-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)thiourea (11d)

Recrystallized from methanol, yield 91%, mp: 191.5 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.68 (s, 1H, -NH), 8.16 (s, 1H, -NH), 7.51 (s, 1H, H^7), 7.35-7.15 (m, 7H, H^5, H^4, phenyl protons), 4.75 (s, 2H, -CH_2), 3.37 (s, 3H, -CH_3). MS ESI(+) m/e314 (M+H, 100). Anal. ($C_{16}H_{15}N_3O_2S$): C, H, N, S calc. 61.32, 4.82, 13.41, 10.23 found 61.23, 5.05, 13.43, 10.24.

1-(2-Phenylethyl)-3-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)thiourea (11e)

Recrystallized from acetic acid, yield 90%, mp: 211 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.50 (s, 1H, -NH), 7.58 (s, 1H, -NH), 7.30-7.10 (m, 8H, H^7, H^5, H^4, phenyl protons), 3.60 (m, 2H, -CH_2), 3.26 (s, 3H, -CH_3), 2.78 (t, 2H, -CH_2). MS ESI(+) m/e328 (M+H, 100). Anal. ($C_{17}H_{17}N_3O_2S$): C, H, N, S calc. 62.36, 5.23, 12.83, 9.79 found 62.16, 5.37, 12.81, 9.79.

1-Phenyl-3-(5-chloro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)thiourea (12a)

Purification by column chromatography on silica gel using ethyl acetate-hexane (50:50) as eluent, yield 17%, mp: 202 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.92 (s, 1H, -NH), 9.46 (s, 1H, -NH), 7.53-7.15 (m, 7H, H^4, H^7, phenyl protons), 3.35 (s, 3H, -CH_3). MS ESI(+) m/e334 (M+H, 100). Anal. ($C_{15}H_{12}ClN_3O_2S$): C, H, N, S calc. 53.97, 3.62, 12.59, 9.61 found 54.04, 3.80, 12.20, 9.20.

1-(4-Chlorophenyl)-3-(5-chloro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (12b)

Recrystallized from acetone, yield, 18%, mp: 189 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.88 (s, 1H, -NH), 9.51 (s, 1H, -NH), 7.48-7.32 (m, 6H, H^4, H^7, phenyl protons), 3.30 (s, 3H, -CH_3). ^{13}C-NMR (DMSO-d_6) δ: 180.75, 153.91, 140.15, 138.13, 131.20, 130.32, 128.44, 128.25, 126.35, 125.39, 111.60, 109.43, 28.25. MS ESI(+) m/e369 (M+H, 100). Anal. ($C_{15}H_{11}Cl_2N_3O_2S$): C, H, N, S calc. 48.93, 3.01, 11.41, 8.71 found 48.80, 3.10, 11.42, 8.72.

1-(4-Methoxyphenyl)-3-(5-chloro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)urea (12c)

Recrystallized from methanol, yield 20%, mp: 188 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.73 (s, 1H, -NH), 9.30 (s, 1H, -NH), 7.52 (s, 2H, H^4, H^7), 7.33 (d, 2H, $H^{2'}$, $H^{6'}$), 6.92 (d, 2H, $H^{3'}$, $H^{5'}$), 3.35 (s, 3H, -CH_3). MS ESI(+) m/e364 (M+H, 100). Anal. ($C_{16}H_{14}ClN_3O_3S$): C, H, N, S calc. 52.82, 3.88, 11.55, 8.81 found 52.39, 3.98, 11.56, 8.53.

1-Benzyl-3-(5-chloro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)thiourea (12d)

Purification by column chromatography on silica gel using ethyl acetate-hexane (50:50) as eluent, yield 10%, mp: 185 °C. ^{1}H-NMR (DMSO-d_6) δ: 9.34 (s, 1H, -NH), 8.20 (s, 1H, -NH), 7.56-7.25 (m, 7H, H^4, H^7, phenyl protons), 4.72 (s, 2H, -CH_2), 3.34 (s, 3H, -CH_3). MS ESI(+) m/e 348 (M+H, 100). Anal. ($C_{16}H_{14}ClN_3O_2S.H_2O$): C, H, N, S calc. 52.53, 4.41, 11.49, 8.76 found 52.29, 4.14, 11.48, 8.89.

Antimicrobial activity

The in vitro minimum inhibitory concentrations (MICs) of the synthesized compounds were determined using the 2-fold serial dilution technique in 96-well microtest plates according to the methods recommended by the Clinical Laboratory Standards Institute (CLSI).[28,29] Standard and the isolated strains of bacteria, *S. aureus* ATCC 29213, MRSA, *E. faecalis* ATCC 29212, *E. faecalis* isolate, *E. coli* ATCC 35218, *E. coli* producing extended spectrum β-lactamase (ESβL), and *P. aeruginosa* ATCC 27853, and its isolate were used to determine antibacterial activity. As for antifungal activity, standard strain of *Candida albicans* ATCC 10231 was used. Ampicillin, amoxicillin/clavulonic acid, gentamicin, and fluconazole were used as references.

Clinical isolates were obtained from Gazi University Hospital, Microbiology Laboratory.

Standard powders of ampicillin (Mustafa Nevzat Pharma), amoxicillin/clavulonic acid (Deva Pharma), gentamicin sulfate (Paninkret Chem. Pharm.), and fluconazole (Sigma) were dissolved in appropriate solvents recommended by CLSI guidelines.[28,29] Stock solutions of the tested compounds were dissolved in DMSO. Stock solutions of the tested compounds and reference drugs were diluted 2-fold in microplate wells. All solvents and diluents, pure microorganisms, and pure media were used in the control wells.

Bacteria were subcultured in Mueller Hinton Agar (MHA) (Merck) plates and incubated overnight at 37 °C and Candida was subcultured in Sabouraud Dextrose Agar (SDA; Merck) plates at 35 °C for 24-48 h.

Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S18. [28] Mueller Hinton Broth (MHB; Merck) was added to each microplate well. Then the tested compounds and reference drugs were added to this medium in wells by 2-fold serial dilution to obtain the required concentrations of 512,

256, 128.....0.5 μg/mL. 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 concentrations were inoculated with a 2-fold diluted solution of the compounds. A 10 μL bacteria inoculum was added to each microplate well. There were 10^4 CFU/mL bacteria in the wells after inoculations.

Fungal susceptibility testing was performed according to the guidelines of CLSI M27-A.[29] Roswell Park Memorial Institute (RPMI)-1640 medium with L-glutamine (Sigma) buffered to pH 7 with 3-(N-morpholino)propanesulfonic acid (MOPS) (Sigma) was added to each microplate well. Then the tested compounds and reference drugs were added to this medium in wells by 2-fold serial dilution to obtain the required concentrations of 512, 256, 128...0.125 μg/mL. The yeast suspensions used for inoculation were prepared at 10^4 CFU/mL by diluting fresh cultures at McFarland 0.5 density (10^6 CFU/mL). Suspensions of the yeast at 10^4 CFU/mL concentrations were inoculated to the 2-fold-diluted solution of the compounds. A 10 μL yeast inoculum was added to each well of the microplates. There were 10^3 CFU/mL yeast in the wells after inoculations.

Microplates were incubated at 37 °C for 24 h for antibacterial activity and at 37 °C for 48 h for antifungal activity. After incubation, the lowest concentration of the compounds that completely inhibits macroscopic growth was determined and reported as MICs. All the experiments were done in 3 parallel series.

Results and discussion

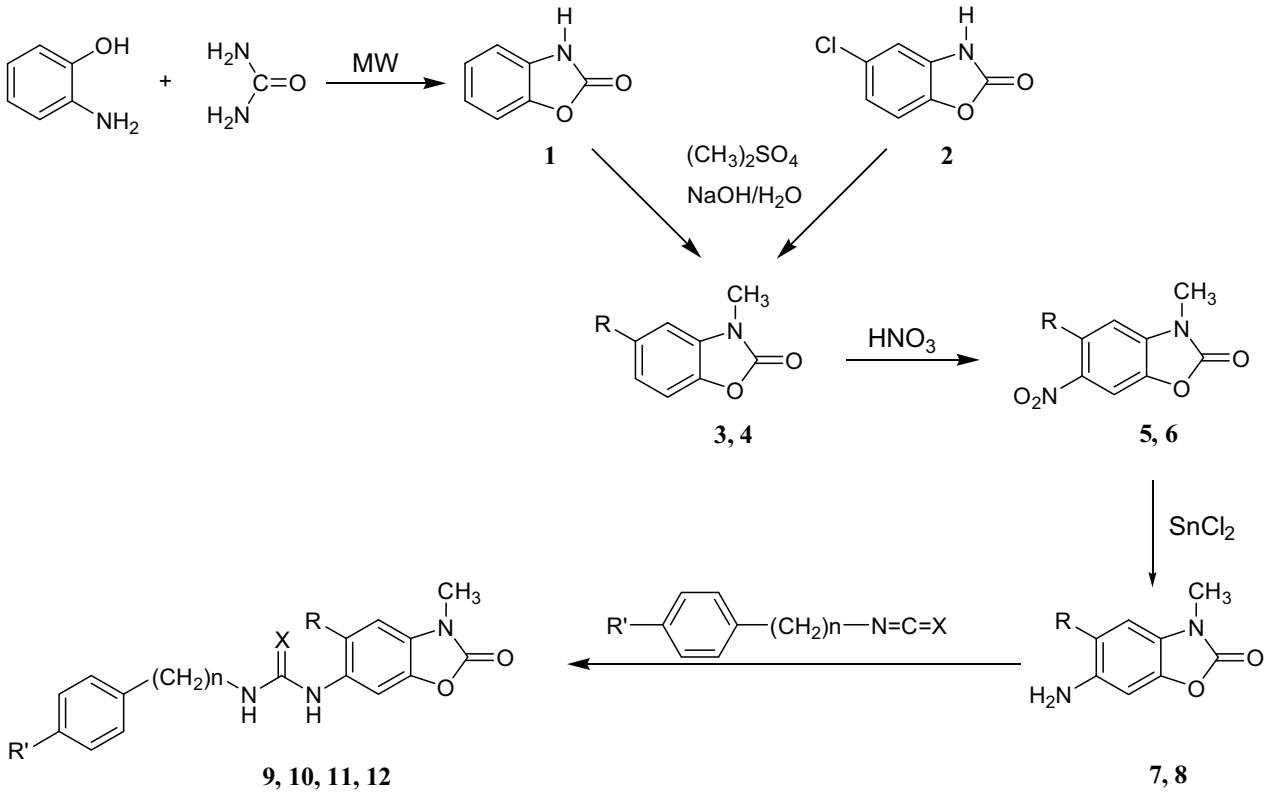
Chemistry

The new compounds (**9a-e**, **10a-d**, **11a-e**, and **12a-d**) were synthesized by a series of reactions as shown in the Scheme. 2(3*H*)-Benzoxazolone **1**,[24] which was prepared by the solvent-free reaction of urea and *o*-aminophenol under microwave irradiation, and commercially available 5-chloro-2(3*H*)-benzoxazolone **2** were used as starting compounds. The treatment of compounds **1** and **2** with dimethyl sulfate yielded 5-nonsubstituted/chloro-3-methyl-2(3*H*)-benzoxazolones, **3**[24] and **4**,[25] respectively. The reaction of compounds **3** and **4** with nitric acid yielded 5-nonsubstituted/chloro-3-methyl-6-nitro-2(3*H*)-benzoxazolones, **5**[26] and **6**,[27] respectively. Then the reduction of the nitro derivatives with $SnCl_2$ gave the corresponding amine derivatives, compounds **7**[26] and **8**,[27] respectively. Finally, 3-methyl-2(3*H*)-benzoxazolone ring-containing urea **9a-e** and thiourea **11a-e** derivatives were readily prepared in very high yields by the reaction of 6-amino-3-methyl-2(3*H*)-benzoxazolone (**7**) with the appropriate isocyanates and isothiocyanates, respectively. However, 5-chloro-3-methyl-2(3*H*)-benzoxazolone ring-containing urea **10a-d** and thiourea **12a-d** derivatives were prepared with 3 different methods in very low yields by the reaction of 6-amino-5-chloro-3-methyl-2(3*H*)-benzoxazolone (**8**) with the appropriate isocyanates and isothiocyanates, respectively.

The chemical structures of the newly synthesized compounds were elucidated by ^{1}H-NMR, mass, and elemental analysis. The ^{1}H-NMR, mass spectra, and elemental analysis data of the compounds are in agreement with the proposed structures.

In the ^{1}H-NMR spectra, the N-H protons of the urea (**9a-e** and **10a-d**) and thiourea (**11a-e** and **12a-d**) derivatives were observed as singlets at 9.47-6.12 ppm and 9.92-7.58 ppm, respectively, which can exchange with D_2O. All other aromatic protons were observed in the expected regions. However, in the ^{1}H-NMR spectra in DMSO-d_6 at room temperature of 3-methyl-2(3*H*)-benzoxazolone ring-containing compounds **9a**, **9b**, **9c**, **11a**,

and **11c**, the splitting of H^7, H^5, and H^4 protons of 2(3*H*)-benzoxazolone was not seen as expected. That is, H^7 proton was seen as a singlet instead of a doublet and H^5 proton was observed as a singlet together with H^4 proton instead of a doublet of doublet. Therefore, compound **9b** was selected for explaining this situation. In the ^{1}H-NMR spectrum in DMSO-d_6 at 55 °C of this compound, H^7 proton was seen as a doublet at 7.57 ppm. H^5 and H^4 protons were seen as a multiplet at 7.14 ppm. In the ^{1}H-NMR spectrum in acetone-d_6 at room temperature of this compound, the splitting of H^7, H^5, and H^4 protons was observed as expected. That is, H^7, H^5, and H^4 protons were seen as a doublet at 7.68 ppm, a doublet of doublet at 7.21 ppm, and a doublet at 7.08 ppm, respectively. This may be attributed to the retention of relaxation time in DMSO-d_6. In addition, 4



Comp.	R	R'	X	n
9a	-H	-H	O	0
9b	-H	-Cl	O	0
9c	-H	-OCH_3	O	0
9d	-H	-H	O	1
9e	-H	-H	O	2
10a	-Cl	-H	O	0
10b	-Cl	-Cl	O	0
10c	-Cl	-OCH_3	O	0
10d	-Cl	-H	O	1

Comp.	R	R'	X	n
11a	-H	-H	S	0
11b	-H	-Cl	S	0
11c	-H	-OCH_3	S	0
11d	-H	-H	S	1
11e	-H	-H	S	2
12a	-Cl	-H	S	0
12b	-Cl	-Cl	S	0
12c	-Cl	-OCH_3	S	0
12d	-Cl	-H	S	1

Scheme. Synthetic route of the title compounds.

of the synthesized compounds (**9b**, **10b**, **11b**, **12b**) were selected for structure elucidation by ^{13}C-NMR. The ^{13}C-NMR spectral data of the compounds are in agreement with the proposed structures.

Antimicrobial activity

As shown in the Table, the synthesized compounds were tested for their antibacterial activity against 4 gram-positive bacteria (*Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA, isolate), *Enterococcus faecalis*, *E. faecalis* isolate), 4 gram-negative bacteria (*Escherichia coli*, *E. coli* producing extended spectrum β-lactamase, *Pseudomonas aeruginosa*, *P. aeruginosa* isolate), and for their antifungal activity against *Candida albicans* using microdilution. Ampicillin, amoxicillin/clavulonic acid, gentamicin, and fluconazole were used as references.

Regarding the antibacterial activity of the target compounds against gram-positive bacteria, the compounds had no significant activity against *S. aureus,* its isolate, or *E. faecalis* isolate. However, compounds **10a** and **10b**, urea derivatives, showed the best inhibitory activity against *E. faecalis* with a MIC value of 16 μg/mL. The higher activity of these compounds might be attributed to chloro substituent at the 5-position of 3-methyl-2(3*H*)-benzoxazolone ring. The rest of the compounds showed activity against *E. faecalis*, with MIC values ranging from 32 to 256 μg/mL. In terms of the antibacterial activity of the target compounds against gram-negative bacteria, the compounds had no significant activity against *P. aeruginosa*, its isolate, or *E. coli* isolate. However, compounds **9a**, **9b**, **9e**, **10a**, and **10c**, urea derivatives, and compounds **12a** and **12d**, thiourea derivatives, exhibited a relatively good inhibitory profile against *E. coli*, with a MIC value of 32 μg/mL when compared with the other compounds. In other words, their antibacterial activity was 25% of that of amoxicillin/clavulonic acid against *E. coli*. These compounds may be modified for further studies with the hope of obtaining better antibacterial agents against *E. coli*. The rest of the compounds showed activity against *E. coli*, with MIC values ranging from 64 to 128 μg/mL.

As for antifungal activity of the synthesized derivatives, compounds **9b**, **9e**, **10b**, and **10d**, urea derivatives, and compounds **11a**, **11e**, **12a** and **12d**, thiourea derivatives, displayed activity against *C. albicans* with a MIC value of 64 μg/mL.

As regards the relationships between the structure and the detected antibacterial activity, one might say that gram-negative *E. coli* and gram-positive *E. faecalis* were generally more sensitive to the title compounds in comparison with the other used Gram bacteria. When the chemical structures of the active compounds were taken into consideration, one can say that the urea derivatives were generally more active than the thiourea derivatives against *E. faecalis* and *E. coli* and that chloro substitution at the 5-position of the 3-methyl-2(3*H*)-benzoxazolone ring did not cause any remarkable change in antibacterial activity of the target compounds, except for **10a** and **10b**. In addition, one can say that structural differences do not play a critical role in antifungal activity.

Table. Antimicrobial activity of the synthesized compounds (MICs, μg/mL).

Compounds	*S. aureus* ATCC 29213	*S. aureus* isolate	*E. faecalis* ATCC 29212	*E. faecalis* isolate	*E. coli* ATCC 35218	*E.coli* isolate	*P.aeruginosa* ATCC 27853	*P.aeruginosa* isolate	*C. albicans* ATCC 10231
9a	512	128	32	256	32	256	128	128	128
9b	256	128	64	256	32	256	128	128	64
9c	512	128	32	256	64	256	128	128	128
9d	512	128	64	256	64	256	128	128	128
9e	256	128	64	256	32	256	128	128	64
10a	256	128	16	256	32	256	128	128	128
10b	256	128	16	256	128	256	128	128	64
10c	512	128	64	256	32	256	256	256	256
10d	256	128	64	256	64	256	256	128	64
11a	256	128	128	256	128	256	128	128	64
11b	256	128	32	256	64	256	128	128	128
11c	256	128	128	256	64	256	128	128	128
11d	256	128	128	256	128	256	128	128	128
11e	256	128	64	256	64	256	128	128	64
12a	256	128	256	256	32	256	128	128	64
12b	512	128	128	256	64	256	128	256	256
12c	512	128	256	256	128	256	128	256	128
12d	256	128	128	256	32	256	128	128	64
Ampicillin	0.5	-	2	0.5	-	1024	-	-	-
Gentamicin	0.5	16	16	32	-	1024	1	256	-
Amoxicillin: Clavulanic Acid (2:1)	0.125	8	0.25	0.5	8	4	-	-	-
Fluconazole	-	-	-	-	-	-	-	-	1

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