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Synthesis and antimicrobial activity of some novel 2,5- and/or 6-substituted benzoxazole and benzimidazole derivatives

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Abstract

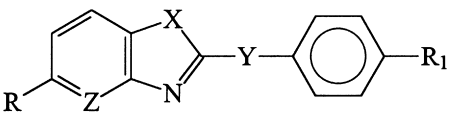
A new series of 2,5- and/or 6-substituted benzoxazoles (**7a–f**), benzimidazoles (**8a–g**) holding cyclohexyl or cyclopentyl moieties at position 2 and 5- or 6-substituted-2-cyclohexylaminomethylbenzoxazoles (**9a**, **b**) was synthesized in order to determine their antimicrobial activities and feasible structure–activity relationships. The synthesized compounds were tested in vitro against three Gram-positive, two Gram-negative bacteria and the yeast *Candida albicans* in comparison with several control drugs. Microbiological results showed that the synthesized compounds were possessing a broad spectrum of antibacterial activity against the tested microorganisms. 5-Chloro-2-(2-cyclohexylethyl)benzimidazole (**8g**) was found as the most active compound against the screened Gram-positive bacteria strains at a minimum inhibitory concentration (MIC) value of 12.5 μg/ml. However, it exhibited lower antibacterial potency than the compared control drugs. On the other side, compounds **7–9** indicated significant antibacterial activity against the Gram-negative enterobacter *Pseudomonas aeruginosa* having MIC values of 50 μg/ml, providing either the same effect as tetracycline or higher activity than streptomycin, but showing less potency than the compared control drug gentamycin. Moreover, the synthesized compounds also possessed antimycotic activity against the yeast *C. albicans* showing MIC values between 25–50 μg/ml.

Keywords: Benzoxazole; Benzimidazole; Synthesis; Antimicrobial activity

1. Introduction

Recent observations suggest that substituted benzimidazoles, benzoxazoles and related heterocycles, which are the structural isosters of nucleotides owing fused heterocyclic nuclei in their structures that allow them to interact easily with the biopolymers, possess potential activity with lower toxicities in the chemotherapeutic approach in man (Haugwitz et al., 1982; Hisano et al., 1982). Moreover, these fused heterocycles were distinctively studied for their antitumor, antiviral and antimicrobial activities as the new nonnucleoside topoisomerase I poisons, human immunodeficiency virus-1 reverse transcriptase inhibitors and/or potent DNA gyrase inhibitors (Hubschwerlen et al., 1992; Perrin et al., 1996; Kim et al., 1996; Shi et al., 1996).

In the last few years, we reported the synthesis and the antimicrobial activity of various 2,5-disubstituted benzoxazoles, benzimidazoles, benzothiazoles and oxazolo(4,5-b)pyridines (Fig. 1), against some Gram-positive, Gram-negative bacteria and the yeast *Candida albicans*, providing a wide variety of in vitro antimicrobial effects especially indicating significant activity against the en-



Z = CH, N

X = O, NH, S

Y = —, CH_2, CH_2CH_2, CH_2O, CH_2S

R = H, CH_3, Cl, NO_2, NH_2

R_1= H, CH_3, C_2H_5, $C(CH_3)_3$, OCH_3, OC_2H_5, Cl, Br, F, NO_2, NH_2, $NHCH_3$, $N(CH_3)_2$, $NHCOCH_3$

Fig. 1. Antimicrobially active 2,5-disubstituted benzoxazoles, benzimidazoles, benzothiazoles and oxazolo(4,5-b)pyridines.

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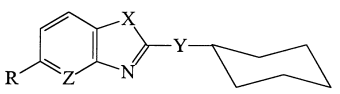
terobacter *Pseudomonas aeruginosa* and the yeast *C. albicans* (Yalçın et al., 1990, 1992; Ören et al., 1997).

The determination of the structure–activity relationships of in vitro antibacterial and antimycotic activities of the previously synthesized compounds revealed that these four related fused heterocyclic nuclei were generally indicating bioisosteric effects for the screened microorganisms. However, oxazolo(4,5-b)pyridine derivatives had the best inhibitory potency for antimicrobial activity against *Klebsiella pneumoniae* and *C. albicans* (Şener et al., 1991; Barrett and Klaubert, 1992; Yalçın and Şener, 1993).

On the other hand, the influence of the substitution of position R on the fused heterocyclic system was found more important than R_1 for affecting the intensity of the activity. It was noted that powerful electron withdrawing substituents at position R improved the potency especially against *C. albicans* (Türker et al., 1990; Şener et al., 1991; Yalçın et al., 1992, 1993, 1995).

In order to describe the nature of the mechanism of interactions at the molecular level, the developed quantitative structure-activity relationship QSAR analysis using quantum–chemical calculations revealed that the electrophilic superdelocalizability of the nitrogen atom in the oxazolo moiety of the benzoxazole ring and the lowest unoccupied molecular orbital energy levels of the compounds were related to the activity and the fused heterocyclic system was found as the most important part in the molecule for the interactions (Türker et al., 1990, 1996; Şener et al., 1994; Güllü et al., 1994).

Recently, we described the synthesis and antimicrobial activity of several derivatives of 5-substituted-2-cyclohexyl, 5-substituted-2-cyclohexylmethyl, 5-substituted-2-(2-cyclohexylethyl)benzoxazoles, benzimidazoles, benzothiazoles and oxazolo(4,5-b)pyridines (Yalçın et al., 1992; Şener et al., 1997) as given **1–6** in Fig. 2, in order to determine the conformational influences at position 2 on the heterocyclic nuclei instead of using plenary aromatic rings. The antimicrobial activity results showed that the synthesized compounds which were substituted with cyclohexyl, cyclohexylmethyl and/or 2-cyclohexylethyl moieties at position 2 were also able to inhibit in vitro growth of a number of microorganisms.



1	X = O	Z = CH	Y = —, CH_2	R = H, Cl, NO_2
2	X = NH	Z = CH	Y = —, CH_2	R = H, Cl, NO_2, CH_3
3	X = O	Z = CH	Y = C_2H_4	R = H, Cl, NO_2, NH_2
4	X = O	Z = N	Y = C_2H_4	R = H
5	X = NH	Z = CH	Y = C_2H_4	R = H
6	X = S	Z = CH	Y = C_2H_4	R = H

Fig. 2. Previously synthesized 5-substituted-2-cyclohexyl, 5-substituted-2-cyclohexylmethyl, 5-substiuted-2-(2-cyclohexylethyl)benzoxazoles, benzimidazoles, benzothiazoles and oxazolo(4,5-b)pyridines (Compounds **1–6**).

In the present paper, some novel 2,5- and/or 6-substituted benzoxazoles (**7a–f**), benzimidazoles (**8a–g**) which hold cyclohexyl or cyclopentyl moieties at position 2 and 5- or 6-substituted-2-cyclohexylaminomethylbenzoxazoles (**9a**, **b**) were synthesized as the target compounds in order to examine their in vitro antimicrobial activities against different Gram-positive, Gram-negative bacteria and the yeast *C. albicans* in comparison with several control drugs.

2. Experimental procedures

2.1. Instrumentation and chemicals

Kieselgel HF_{254} (Merck, Darmstadt, Germany) chromatoplates (0.3 mm) were used for TLC and the solvent systems were $CHCl_3$:MeOH (6:0.2) for compounds **7a–f**, AcOEt:*n*-hexane (2:1) for compounds **8a–g**, $CHCl_3$:MeOH:petroleum ether (4:0.3:1) for compounds **9a**, **b**. All melting points (m.p.) were taken on a Buchi SMP 20 capillary apparatus (Buchi, Flawil, Switzerland) and are uncorrected. IR spectra were recorded by Pye Unicam SP-1025 (Pye Unicam, Cambridge, UK) with KBr discs. ^{1}H NMR spectra were obtained with a Bruker 80 MHz spectrometer (Bruker, Billerica, USA) in $CDCl_3$ and tetramethylsilan was used as internal standard. Elemental analyses were carried out with a Perkin Elmer model 240-C apparatus (Perkin Elmer, Norwalk, CT, USA). The results of the elemental analysis (C, H, N) were within ±0.4% of the calculated amounts. The reaction mixtures were protected from moist air by means of a calcium chloride drying tube and stirred magnetically. The starting compounds and the solvents were commercially available products.

2.2. Synthesis

2.2.1. 6-Methyl-2-cyclohexylbenzoxazole (7a)

A mixture of 5-methyl-2-aminophenol (0.01 mol) and cyclohexylcarboxylic acid (0.015 mol) were refluxed at 100–110°C in polyphosphoric acid (PPA) (12.5 g) for 7 h. At the end of the reaction period, the residue was poured into ice–water and neutralized with excess of 10% NaOH solution. The precipitate was collected, washed, dried and extracted with benzene to separate from impurities. The combined benzene extracts were dried over anhydrous sodium sulfate and evaporated in vacuo. The crude product was recrystallized by using ether and petroleum ether. Yield: 15%. m.p.: 56–57°C. ^{1}H NMR ($CDCl_3$): 7.59–7.14 (m, 3H, C-4 H, C-5 H and C-7 H), 2.64–2.45 (m, 14H,

CH_3 and cyclohexyl protons). IR (KBr disc): 3100, 2960, 1610, 1570, 1460, 1260.

*2.2.2. 6-Nitro-2-cyclohexylbenzoxazole (**7b**)*

A mixture of 5-nitro-2-aminophenol (0.01 mol) and cyclohexylcarboxylic acid (0.015 mol) were refluxed at 120–130°C in PPA (12.5 g) for 6 h. Compound **7b** was prepared by a similar method to that described for **7a**. Yield: 17%. m.p.: 89–90°C. ^{1}H NMR ($CDCl_3$): 8.39–8.20 (m, 2H, C-5 H, C-7 H), 7.80–7.69 (d, 1H, C-4 H $J_{4,5}$: 8.63), 2.67–1.41 (m, 11H, cyclohexyl protons). IR (KBr disc): 3120, 2980, 1630, 1550, 1460, 1270.

*2.2.3. 5-Chloro-6-nitro-2-cyclohexylbenzoxazole (**7c**)*

A mixture of 4-chloro-5-nitro-2-aminophenol (0.01 mol) and cyclohexylcarboxylic acid (0.015 mol) was refluxed at 120–130°C in PPA (12.5 g) for 6 h. Compound **7c** was prepared by a similar method to that described for **7a**. Yield: 16%. m.p.: 83–84°C. ^{1}H NMR ($CDCl_3$): 8.06 (s, 1H, C-7 H), 7.79 (s, 1H, C-4 H), 3.15–1.38 (m, 11H, cyclohexyl protons). IR (KBr disc): 3120, 2950, 2860, 1610, 1560, 1540, 1450, 1330, 1250.

*2.2.4. 6-Nitro-2-cyclohexylmethylbenzoxazole (**7d**)*

A mixture of 5-nitro-2-aminophenol (0.01 mol) and cyclohexylacetic acid (0.015 mol) was refluxed at 100–110°C in PPA (12.5 g) for 3 h. Compound **7d** was prepared by a similar method to that described for **7a**. Yield: 23%. m.p.: 40–41°C. ^{1}H NMR ($CDCl_3$): 8.41–8.21 (m, 2H, C-5 H, C-7 H), 7.80–7.69 (d, 1H, C-4 H, $J_{4,5}$: 8.67), 2.93–2.84 (d, 2H, CH_2, J: 6.91), 2.75–2.16 (m, 11H, cyclohexyl protons). IR (KBr disc): 3110, 2960, 1610, 1550, 1460, 1250.

*2.2.5. 5-Chloro-6-nitro-2-cyclohexylmethylbenzoxazole (**7e**)*

A mixture of 4-chloro-5-nitro-2-aminophenol (0.01 mol) and cyclohexylacetic acid (0.015 mol) was refluxed at 120–130°C in PPA (12.5 g) for 6 h. Compound **7e** was prepared by a similar method to that described for **7a**. Yield: 16%. m.p.: 64–65°C. ^{1}H NMR ($CDCl_3$): 8.07 (s, 1H, C-7 H), 7.80 (s, 1H, C-4 H), 2.92–2.83 (d, 2H, CH_2, J: 6.91), 1.84–1.26 (m, 11H, cyclohexyl protons). IR (KBr disc): 3110, 2950, 1610, 1560, 1540, 1450, 1330, 1250.

*2.2.6. 5-Chloro-2-cyclopentylethylbenzoxazole (**7f**)*

A mixture of 4-chloro-2-aminophenol (0.01 mol) and 3-cyclopentylpropionic acid (0.015 mol) was refluxed at 120–130°C in PPA (12.5 g) for 6 h. Compound **7f** was prepared by a similar method to that described for **7a**. The crude product was recrystallized from benzene:petroleum ether. Yield: 31%. m.p.: 49°C. ^{1}H NMR ($CDCl_3$): 7.75–7.62 (d, 1H, C-7 H, $J_{7,6}$: 10.5), 7.44–7.15 (m, 2H, C-4 H, C-6 H), 3.02–2.84 (t, 2H, CH_2, J: 7.5), 2.09–1.56 (m, 11H, CH_2 and cyclopentyl protons). IR (KBr disc): 3100, 2950, 1610, 1560, 1450, 1250.

*2.2.7. 2-Cyclopentylbenzimidazole (**8a**)*

A mixture of 2-phenylenediamine (0.01 mol), cyclopentylcarboxylic acid (0.01 mol) and 6 M HCl (10 ml) was refluxed at 80–100°C for 14 h. At the end of the reaction period, the reaction mixture was poured into ice–water and neutralized with excess of $NaHCO_3$. The precipitate was collected, washed, dried and extracted with benzene to separate from impurities. After the evaporation of solvent in vacuo, the crude product was obtained and recrystallized by using ethanol and distilled water. Yield: 24%. m.p.: 246–248°C (lit. 251–253°C). ^{1}H NMR ($CDCl_3$): 8.85 (s, 1H, NH), 7.58–7.01 (m, 4H, C-4 H, C-5 H, C-6 H, C-7 H), 3.24–1.71 (m, 9H, cyclopentyl protons). IR (KBr disc): 3080, 2980, 1620, 1550, 1450, 1260.

*2.2.8. 5-Chloro-2-cyclopentylbenzimidazole (**8b**)*

A mixture of 4-chloro-2-phenylenediamine (0.01 mol), cyclopentylcarboxylic acid (0.01 mol) and 6 M HCl (10 ml) was refluxed at 80–100°C for 14 h. Compound **8b** was prepared by a similar method to that described for **8a**. Yield: 20%. m.p.: 188°C. ^{1}H NMR ($CDCl_3$): 9.91 (s, 1H, NH), 7.48–7.19 (m, 2H, C-4 H, C-7 H), 7.14–7.01 (dd, 1H, C-6 H $J_{6,7}$: 8.56, $J_{6,4}$: 1.92), 3.36–1.79 (m, 9H, cyclopentyl protons). IR (KBr disc): 3100, 3000, 1610, 1570, 1530, 1430, 1260.

*2.2.9. 2-Cyclopentylmethylbenzimidazole (**8c**)*

A mixture of 2-phenylenediamine (0.01 mol), cyclopentylacetic acid (0.01 mol) and 6 M HCl (10 ml) was refluxed at 80–100°C for 12 h. Compound **8c** was prepared by a similar method to that described for **8a**. Yield: 26%. m.p.: 205–206°C. ^{1}H NMR ($CDCl_3$): 9.57 (s, 1H, NH), 7.63–7.16 (m, 4H, C-4 H, C-5 H, C-6 H, C-7 H), 3.04–2.95 (d, 2H, CH_2, J: 8.21), 1.72–1.53 (m, 9H, cyclopentyl protons). IR (KBr disc): 3120, 2920, 1610, 1580, 1530, 1420, 1250.

*2.2.10. 5-Chloro-2-cyclopentylmethylbenzimidazole (**8d**)*

A mixture of 4-chloro-2-phenylenediamine (0.01 mol), cyclopentylacetic acid (0.01 mol) and 6 M HCl (10 ml) was refluxed at 80–100°C for 12 h. Compound **8d** was prepared by a similar method to that described for **8a**. Yield: 24%. m.p.: 152–153°C. ^{1}H NMR ($CDCl_3$): 9.78 (s, 1H, NH), 7.55–7.26 (m, 2H, C-4 H, C-7 H), 7.25–7.12 (dd, 1H, C-6 H $J_{6,7}$: 8.64, $J_{6,4}$: 1.92), 3.01–2.92 (d, 2H, CH_2, J: 7.20), 1.75–1.53 (m, 9H, cyclopentyl protons). IR (KBr disc): 3085, 2980, 1630, 1600, 1550, 1420, 1290.

*2.2.11. 2-(2-Cyclopentylethyl)benzimidazole (**8e**)*

A mixture of 2-phenylenediamine (0.01 mol), 3-cyclopentylpropionic acid (0.01 mol) and 6 M HCl (10 ml) was refluxed at 110°C for 12 h. Compound **8e** was prepared by a similar method to that described for **8a**. Yield: 20%. m.p.: 158°C. ^{1}H NMR ($CDCl_3$): 10.32 (s, 1H, NH), 7.60–7.14 (m, 4H, C-4 H, C-5 H, C-6 H, C-7 H), 3.06–2.88 (t, 2H, CH_2, J: 6.77), 1.95–1.09 (m, 11H, CH_2

and cyclopentyl protons). IR (KBr disc): 3100, 2995, 1610, 1500, 1420, 1280.

*2.2.12. 5-Chloro-2-(2-cyclopentylethyl)benzimidazole (**8f**)*

A mixture of 4-chloro-2-phenylenediamine (0.01 mol), 3-cyclopentylpropionic acid (0.01 mol) and 6 M HCl (10 ml) was refluxed at 120°C for 12 h. Compound **8f** was prepared by a similar method to that described for **8a**. Yield: 19%. m.p.: 116–114°C. ^{1}H NMR ($CDCl_3$): 10.13 (s, 1H, NH), 7.50–7.11 (m, 3H, C-4 H, C-6 H, C-7 H), 3.05–2.88 (t, 2H, CH_2, *J*: 7.20), 1.93–1.45 (m, 11H, CH_2 and cyclopentyl protons). IR (KBr disc): 3100, 2990, 1620, 1580, 1540, 1450, 1280.

*2.2.13. 5-Chloro-2-(2-cyclohexylethyl)benzimidazole. HCL (**8g**)*

A mixture of 4-chloro-2-phenylenediamine (0.01 mol), 3-cyclohexylpropionic acid (0.01 mol) and 6 M HCl (10 ml) was refluxed at 110°C for 12 h. Compound **8g** was prepared by a similar method to that described for **8a**. HCl salts: dry HCl gas, crystallized from EtOH–diethyl ether. Yield: 45%. m.p.: 269–274°C (dec.). ^{1}H NMR ($CDCl_3$): 7.82 (d, 1H, C-4 H, $J_{4,6}$: 1.34), 7.75 (d, 1H, C-7 H, $J_{7,6}$: 8.74), 7.45–7.30 (dd, 1H, C-6 H, $J_{6,7}$: 8.70, $J_{6,4}$: 1.63), 3.25 (t, 2H, CH_2, *J*: 8.16), 2.60 (m, 2H, CH_2), 1.97–0.98 (m, 11H, cyclohexyl protons). IR (KBr disc): 3050, 2924, 2850, 1619, 1569, 1474, 1333, 1215, 1058, 925–596.

*2.2.14. 5-Chloro-2-cyclohexylaminomethylbenzoxazole (**9a**)*

A mixture of 4-chloro-2-aminophenol (0.01 mol) and chloroacetic acid (0.015 mol) was refluxed at 110–120°C in PPA (12.5 g) for 5 h. 5-Chloro-2-chloromethylbenzoxazole was prepared by a similar method to that described for **7a**. Then, the mixture of 5-chloro-2-chloromethylbenzoxazole (0.01 mol) and cyclohexylamine (0.01 mol) in dimethylformamide DMF (1 ml) was refluxed at 60°C for 3.5 h. At the end of the reaction period, 10% NaOH was added to the reaction mixture and extracted with $CHCl_3$, then it was evaporated. After purification by flash chromatography in a silica gel column with $CHCl_3$, the crude product was recrystallized from ethanol:distilled water. Yield: 45%. m.p.: 47.5°C. ^{1}H NMR ($CDCl_3$): 7.68 (d, 1H, C-4 H, $J_{4,6}$: 1.97), 7.42–7.40 (d, 1H, C-7 H, $J_{7,6}$: 8.63), 7.30–7.27 (dd, 1H, C-6 H, $J_{6,7}$: 8.76, $J_{6,4}$: 2.00), 4.01 (s, 2H, CH_2), 2.60–1.00 (m, 11H, cyclohexyl protons). IR (KBr disc): 3340, 3020, 2924, 1624, 1562, 1470, 1320, 1210, 920–600.

*2.2.15. 6-Nitro-2-cyclohexylaminomethylbenzoxazole (**9b**)*

A mixture of 5-nitro-2-aminophenol (0.01 mol) and chloroacetic acid (0.015 mol) was refluxed at 110–120°C in PPA (12.5 g) for 3.5 h. 6-Nitro-2-chloromethylbenzoxazole (m.p.: 61–62°C) was prepared by a similar method to that described for **7a**. Then, the mixture of 6-nitro-2-chloromethylbenzoxazole (0.01 mol) and cyclohexylamine (0.01 mol) in DMF (1 ml) was refluxed at 60°C for 3.5 h. Compound **9b** was prepared by a similar method to that described for **9a**. The crude product was recrystallized from ethanol:distilled water. Yield: 37%. m.p.: 182°C. ^{1}H NMR ($CDCl_3$): 7.90–7.80 (dd, 1H, C-5 H, $J_{5,4}$: 8.67, $J_{5,7}$: 2.50), 7.73–7.70 (d, 1H, C-7 H, $J_{7,5}$: 2.48), 7.13–7.09 (d, 1H, C-4 H, $J_{4,5}$: 8.66), 4.43 (s, 2H, CH_2), 2.20–1.10 (m, 11H, cyclohexyl protons). IR (KBr disc): 3344, 3030, 2924, 1625, 1541, 1490, 1299, 1255, 1214, 924–751.

2.3. Microbiology

For both the antibacterial and the antimycotic assays, the compounds were dissolved in absolute ethanol (0.8 mg/ml) (Charles et al., 1979). Further dilutions of the compounds and standard drugs in the test medium were prepared at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 μg/ml concentrations. The minimum inhibitory concentration (MIC) values were determined using the method of twofold serial dilution (Charles et al., 1979; Shadomy and Espinel, 1980). In order to ensure that the solvent per se had no effect on bacterial growth, a control test was also performed containing inoculated broth supplemented with only ethanol at the same dilutions used in our experiments and found inactive in culture medium.

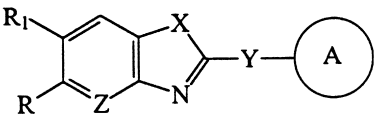
All the compounds were tested for their in vitro growth inhibitory activity against different bacteria and the yeast *C. albicans* RSKK 628. There origin of bacterial strains were *Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 10541 and *Bacillus subtilis* ATCC 6033 as Gram-positive and *Escherichia coli* ATCC 10536, and *P. aeruginosa* RSKK 355 as Gram-negative bacteria. RSKK strains of the microorganisms used in this study were obtained from the culture collection of Refik Saydam Health Institution of Health Ministry, Ankara and maintained at the Microbiology Department of Faculty of Pharmacy of Ankara University.

Ampicillin, amoxycillin, tetracycline, streptomycin, gentamycin, oxiconazole, and haloprogin were used as control drugs. The observed data on the antimicrobial activity of the compounds and the control drugs are given in Table 1.

2.3.1. Antibacterial assay

The cultures were obtained in Mueller–Hinton broth (Difco, MI, USA) for all the bacteria after 24 h of incubation at 37±1°C. Testing was carried out in Mueller–Hinton broth at pH 7.4 and the twofold serial dilution technique was applied. The final inoculum size was 10^5 colony forming units (CFU)/ml. A set of tubes containing only inoculated broth was kept as controls. After incubation for 24 h at 37±1°C, the last tube with no growth of microorganism was recorded to represent MIC expressed

Table 1
The in vitro antimicrobial activity of the compounds **7–9** and the previously synthesized derivatives **1–6** with the comparison of standard drugs (MIC in μg/ml)



							Microorganisms[a]					
Comp.	R	R_1	X	Y	Z	A	Sa	Sf	Bs	Ec	Pa	Ca
1a	H	H	O	–	CH	Cyclohexyl	50	50	>200	50	50	12.5
1b	Cl	H	O	–	CH	Cyclohexyl	25	50	50	50	25	50
1c	NO_2	H	O	–	CH	Cyclohexyl	25	25	3.12	25	25	12.5
1d	Cl	H	O	CH_2	CH	Cyclohexyl	25	50	>200	50	50	12.5
1e	NO_2	H	O	CH_2	CH	Cyclohexyl	50	50	3.12	25	12.5	50
2a	H	H	NH	–	CH	Cyclohexyl	50	50	6.25	25	12.5	25
2b	Cl	H	NH	–	CH	Cyclohexyl	25	50	25	50	25	50
2c	NO_2	H	NH	–	CH	Cyclohexyl	25	50	25	50	25	50
2d	CH_3	H	NH	–	CH	Cyclohexyl	50	50	>200	50	25	12.5
2e	H	H	NH	CH_2	CH	Cyclohexyl	25	25	6.25	25	12.5	25
2f	Cl	H	NH	CH_2	CH	Cyclohexyl	25	50	12.5	50	25	25
2g	NO_2	H	NH	CH_2	CH	Cyclohexyl	12.5	50	25	50	50	50
2h	CH_3	H	NH	CH_2	CH	Cyclohexyl	25	50	25	50	25	50
3a	H	H	O	C_2H_4	CH	Cyclohexyl	50	50	25	50	25	12.5
3b	Cl	H	O	C_2H_4	CH	Cyclohexyl	50	50	25	50	25	12.5
3c	NO_2	H	O	C_2H_4	CH	Cyclohexyl	50	50	25	50	12.5	12.5
3d	NH_2	H	O	C_2H_4	CH	Cyclohexyl	50	50	25	50	12.5	12.5
4	H	H	O	C_2H_4	N	Cyclohexyl	50	50	25	50	25	12.5
5	H	H	S	C_2H_4	CH	Cyclohexyl	50	50	25	50	25	12.5
6	H	H	NH	C_2H_4	CH	Cyclohexyl	50	50	25	50	25	12.5
7a	H	CH_3	O	–	CH	Cyclohexyl	50	50	100	50	100	25
7b	H	NO_2	O	–	CH	Cyclohexyl	50	50	50	50	50	50
7c	Cl	NO_2	O	–	CH	Cyclohexyl	50	50	100	50	50	50
7d	H	NO_2	O	CH_2	CH	Cyclohexyl	50	50	50	50	50	25
7e	Cl	NO_2	O	CH_2	CH	Cyclohexyl	50	50	50	50	50	25
7f	Cl	H	O	C_2H_4	CH	Cyclopentyl	50	50	25	50	50	25
8a	H	H	NH	–	CH	Cyclopentyl	50	50	100	50	50	50
8b	Cl	H	NH	–	CH	Cyclopentyl	50	100	100	50	50	50
8c	H	H	NH	CH_2	CH	Cyclopentyl	50	50	50	50	50	50
8d	Cl	H	NH	CH_2	CH	Cyclopentyl	50	50	50	50	50	25
8e	H	H	NH	C_2H_4	CH	Cyclopentyl	50	50	25	50	50	25
8f	Cl	H	NH	C_2H_4	CH	Cyclopentyl	25	25	25	50	50	25
8g	Cl	H	NH	C_2H_4	CH	Cyclohexyl	12.5	12.5	12.5	50	50	25
9a	Cl	H	O	CH_2NH	CH	Cyclohexyl	50	50	25	50	50	25
9b	H	NO_2	O	CH_2NH	CH	Cyclohexyl	50	50	50	50	50	25
Ampicillin							0.78	0.78	0.78	3.12	>200	–
Amoxycillin							0.78	0.78	0.78	3.12	>200	–
Tetracycline							0.78	0.78	0.78	3.12	50	–
Gentamycin							0.78	12.5	0.78	3.12	12.5	–
Streptomycin							3.12	100	50	1.56	100	–
Oxiconazole							–	–		–	–	6.25
Haloprogin												6.25

[a] Abbreviations; Sa, *Staphylococcus aureus*; Sf, *Streptococcus faecalis*; Bs, *Bacillus subtilis*; Ec, *Escherichia coli*; Pa, *Pseudomonas aeruginosa*; Ca, *Candida albicans*.

in μg/ml. Every experiment in the antibacterial assay was replicated twice in order to define the MIC values.

2.3.2. Antimycotic assay

The yeast *C. albicans* was maintained in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25±1°C. Testing was performed in Sabouraud dextrose broth at pH 7.4 and the twofold serial dilution technique was applied. The final inoculum size was 10^4 CFU/ml. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25±1°C, the last tube with no growth of yeast was recorded to represent MIC expressed in μg/ml. Every experiment in the antimycotic assay was replicated twice in order to define the MIC values.

3. Results and discussion

3.1. Chemistry

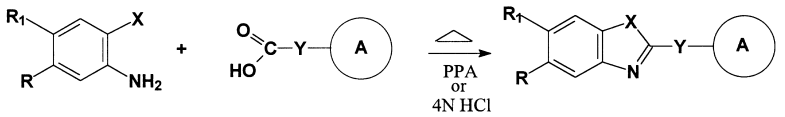
The synthesis of 2,5- and/or 6-substituted benzoxazoles (**7a–f**) and benzimidazoles (**8a–g**) was performed through heating corresponding carboxylic acids with appropriate 4- and/or 5-disubstituted-2-aminophenols or 4- and/or 5-disubstituted-2-phenylenediamines by means of dehydrating agents in an one-step procedure (Route A in Fig. 3).

PPA was used as the cyclodehydration reagent in the synthesis of the compounds **7a–f** and in the first step of the compounds **9a** and **b**. During the synthesis of **8a–g**, aqueous hydrochloric acid was used as the condensation reagent according to the well known Phillips' method (Phillips, 1928).

The derivatives 5- or 6-substituted-2-cyclohexylaminomethylbenzoxazoles (**9a** and **b**) were synthesized in two steps (Route B in Fig. 3). At the first step 5- or 6-substituted-2-chloromethylbenzoxazole was prepared by heating 2-chloroacetic acid with 4- or 5-substituted-2-aminophenol in PPA. Then, 5- or 6-substituted-2-chloromethylbenzoxazole was treated with cyclohexylamine in order to obtain the compounds **9a** and **b**.

The compounds **7a–f**, **8a–g**, **9a** and **b** were synthesized as new products except **8a** (Pellicciari et al., 1985). The

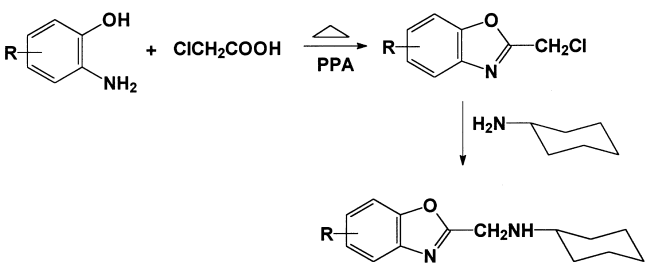
Route A;



7 and **8**

7a;	X = O,	Y= –,	A= cyclohexyl,	R= H,	$R_1 = CH_3$
7b;	X = O,	Y= –,	A= cyclohexyl,	R= H,	$R_1 = NO_2$
7c;	X = O,	Y= –,	A= cyclohexyl,	R= Cl,	$R_1 = NO_2$
7d;	X = O,	$Y=CH_2$,	A= cyclohexyl,	R= H,	$R_1 = NO_2$
7e;	X = O,	$Y=CH_2$,	A= cyclohexyl,	R= Cl,	$R_1 = NO_2$
7f;	X = O,	$Y=C_2H_4$,	A= cyclopentyl,	R= Cl,	$R_1 = H$
8a;	X = NH,	Y= –,	A= cyclopentyl,	R= H,	$R_1 = H$
8b;	X = NH,	Y= –,	A= cyclopentyl,	R= Cl,	$R_1 = H$
8c;	X = NH,	$Y=CH_2$,	A= cyclopentyl,	R= H,	$R_1 = H$
8d;	X = NH,	$Y=CH_2$,	A= cyclopentyl,	R= Cl,	$R_1 = H$
8e;	X = NH,	$Y=C_2H_4$,	A= cyclopentyl,	R= H,	$R_1 = H$
8f;	X = NH,	$Y= C_2H_4$,	A= cyclopentyl,	R= Cl,	$R_1 = H$
8g;	X = NH,	$Y=C_2H_4$,	A= cyclohexyl,	R= H,	$R_1 = H$

Route B ;



9

9a; R= 5-Cl
9b; R= 6-NO_2

Fig. 3. Synthesis of compounds **7a–f**, **8a–g**, **9a** and **b**.

structures of all the synthesized compounds were supported by spectral data and the IR, ^{1}H NMR spectra are in agreement with the proposed structures.

3.2. Antimicrobial activity

The synthesized compounds **7–9** were in vitro tested against three Gram-positive, two Gram-negative bacteria and the yeast *C. albicans* by using the twofold serial dilution technique and compared to their antimicrobial activity with previously synthesized compounds **1–6** (Yalçın et al., 1992; Şener et al., 1997) and several control drugs as given in Table 1. The results reported in Table 1 indicate that the synthesized compounds **7–9** are able to inhibit in vitro growth of screened microorganisms showing MIC values between 12.5–100 μg/ml.

Table 1 reveals that the synthesized compounds **7–9** provided antibacterial activity against *S. aureus*, *Str. faecalis* and *B. subtilis* possessing MIC values between 25–100 μg/ml, except the derivative 5-chloro-2-(2-cyclohexylethyl)benzimidazole (**8g**), which was found active at a MIC value of 12.5 μg/ml against each of the screened Gram-positive bacteria strains. However, all the synthesized compounds exhibited lower antibacterial potencies than the compared control drugs.

On the other hand, for the determination of the antibacterial activity in Gram-negative bacteria, the synthesized compounds also revealed lower potencies than the compared control drugs against *E. coli*. But, for the Gram-negative microorganism *P. aeruginosa*, which is present in nosocomial infections and often resistant to antibiotic therapy, the compounds **7–9** were indicated significant antibacterial activity having MIC values of 50 μg/ml, exhibiting one dilution step lower potency than the compared control drugs gentamycin, while providing either the same effect as tetracycline or higher activity than streptomycin.

Moreover, the synthesized compounds were also tested against *C. albicans* for their antimycotic activities and possessed MIC values between 25–50 μg/ml, whereas antimycotic potencies of the compared control drugs oxiconazole and haloprogin were observed to be better than the corresponding compounds, showing MIC values of 6.25 μg/ml.

In conclusion, structure–activity relationships of the compounds **1–9** given in Table 1 reveal that substitution of position 2 on the fused heterocyclic system with a cyclohexyl ring causes increases in the antimicrobial activity compared to substitution with cyclopentyl. If the benzimidazole ring holds a 2-cyclohexylethyl moiety on position 2 together with a chlorine atom at the fifth position (see compound **8g**), the activity against *Str. faecalis* is increased. Furthermore, compound **8g** and 5-nitro-2-cyclohexylmethylbenzimidazole (compound **2g**) are found to be the most active derivatives for antibacterial activity against *S. aureus*. On the other side, having a nitro group at position 5 on the benzoxazole nucleus substituted with 2-cyclohexyl or 2-cyclohexylmethyl moieties (see compounds **1c** and **e**) increases the potency against *B. subtilis*.

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