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Synthesis and microbiological activity of some novel 5- or 6-methyl-2-(2,4-disubstituted phenyl)benzoxazole derivatives

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Abstract

The synthesis of a new series of 5- or 6-methyl-2-(2,4-disubstituted phenyl)benzoxazoles (**4**, **5**) is described in order to determine their antimicrobial activities and feasible structure–activity relationships. The synthesized compounds were tested in vitro against three Gram-positive bacteria, three Gram-negative bacteria and the yeast *Candida albicans*, in comparison with several control drugs. Microbiological results exhibited that the synthesized compounds possess a broad spectrum of antibacterial activity against the tested microorganisms. The compounds **4b** and **4c** indicated some antibacterial activity against *Staphylococcus aureus* having a minimum inhibitory concentration (MIC) of 12.5 μg/ml. Moreover, the compound **5a** revealed a significant antibacterial activity against the enterobacter *Pseudomonas aeruginosa* showing a MIC value of 25 μg/ml, i.e. more potent than the control drugs tetracycline and streptomycin. For the antimycotic activity against the yeast *C. albicans*, the derivative **4c** was found to be more active than the other synthesized compounds with a MIC value of 12.5 μg/ml, but one-fold less potent than the control drugs oxiconazole and haloprogin.

Keywords: Antibacterial activity; Antifungal activity; Benzoxazoles

1. Introduction

In the last few years, benzoxazoles and the related heterocycles benzimidazoles and benzothiazoles were studied extensively for their antitumor, antiviral and antibiotic activities as the new non-nucleoside topoisomerase I poisons, HIV-1 reverse transcriptase inhibitors and/or potent DNA gyrase inhibitors [1–6].

A benzoxazole derivative, 3-(4,7-dichlorobenzoxazol-2-ylmethylamino)-5-ethyl-6-methylpyridin-2(1H)-one (L-697,661), was observed as a specific non-nucleoside reverse transcriptase inhibitor for the human immunodeficiency virus type 1 (HIV-1) and combined therapy with zidovudine and L-697,661 achieved a marked decrease of viremia in some primary HIV-infected patients [1–3].

The series of 5-formyl-, 5-(aminocarbonyl)-, and 5- or 6-nitro derivatives of 2-(4-methoxyphenyl)benzimidazoles, benzoxazoles and benzothiazoles were synthesized and studied as topoisomerase I inhibitors [4]. In contrast to bi- and terbenzimidazoles, these substituted benzimidazoles, which were found to be active as topoisomerase I poisons, exhibited minimum or no DNA binding affinity. In evaluating their cytotoxicity, these new topoisomerase I poisons also exhibited no significant cross-resistance against cell lines that express camptothecin-resistant topoisomerase I. Moreover, substituted pyrimido[1,6′-a]benzimidazoles were found to be a new class of potent DNA gyrase inhibitors. However, their antibacterial activity proved to be inferior than the quinolone DNA gyrase inhibitors and antibacterial agents like norfloxacin or fleroxacin [5].

Currently, a new series of 2-(4-aminophenyl)benzothiazoles substituted in the phenyl ring and benzothiazole moiety has been synthesized as antitumor agents and showed potent inhibitory activity against human breast cancer cell lines in vitro and in vivo [6]. Structure–activity relationships derived using these cell types has revealed that activity follows the heterocyclic sequence benzothiazole > benzoxazole > benzimidazole and the 2-(4-amino-3-methylphenyl)-benzothiazole derivative is found as the most potent compound in this series that its activity extends to ovarian, lung and renal cell lines.

Recently, we reported the synthesis and the antimicrobial activity of various 5- or 6-methyl-2-(*p*-substituted benzyl), 2-(*p*-substituted phenoxymethyl), and 2-thiophenoxymethylbenzoxazoles and/or benzimidazoles [7] as given in the general formulae I–IV, against some Gram-positive, Gram-

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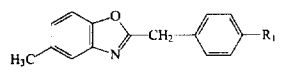
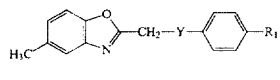
negative bacteria and the yeast *Candida albicans*, indicating a wide variety of in vitro antimicrobial effects providing significant activity, especially against the enterobacter *Pseudomonas aeruginosa* and the yeast *C. albicans* [8–13].



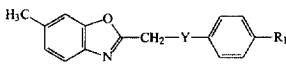
R_1 = H, Cl, Br, NO_2, NH_2

I



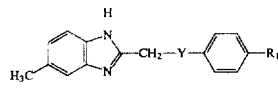
Y = O, S; R_1 = H, Cl

II



Y = —, O, S; R_1 = H, Cl, Br

III



Y = —, O, S, CH_2; R_1 = H, Cl, Br, NH_2

IV

In the present study, a series of novel 5- or 6-methyl-2-(2,4-disubstituted phenyl)benzoxazoles (**4**, **5**) has been synthesized as the target compounds in order to examine their microbiological activities against different Gram-positive, Gram-negative bacteria and the yeast *C. albicans* in comparison with several control drugs.

2. Chemistry

As given in Scheme 1, 5- or 6-methyl-2-(2,4-disubstituted phenyl)benzoxazoles **4**, **5** have been synthesized in excellent yield by the dehydrogenation of the Schiff's bases prepared from the appropriate 2-aminophenol as in Refs. [14–16], respectively, and lead tetraacetate was used for the cyclization of the compounds.

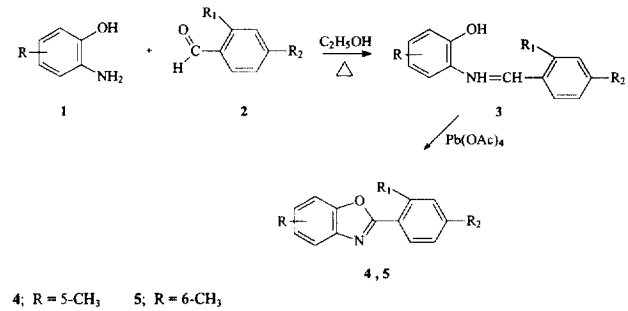
Compounds **4**, **5** were synthesized as new products and the structures of all the derivatives were supported by spectral data. The IR and ^{1}H NMR spectra are in agreement with the proposed structures. Physical and spectral data of the compounds are reported in Table 1.

3. Experimental section

3.1. Chemistry

Kieselgel HF_{254} chromatoplates (0.3 mm) were used for thin-layer chromatography (TLC) and the solvent system was only chloroform. All melting points were taken on a Büchi SMP 20 capillary apparatus and are uncorrected. IR spectra were recorded by a Pye Unicam model SP-1025 apparatus with KBr discs. ^{1}H NMR spectra were obtained with a Bruker 80 MHz spectrometer in d_6-chloroform and tetramethylsilane (TMS) was used as an internal standard. Elemental analyses were carried out with a Perkin-Elmer model 240-C apparatus. The results of the elemental analysis (C, H, N) were within ±0.4% of the calculated amounts.

The compounds **4**, **5** were obtained by treatment of the corresponding Schiff's bases, with lead tetraacetate [14–16].



4; R = 5-CH_3 **5**; R = 6-CH_3

R_1= Cl, F, NO_2, CH_3, OCH_3

R_2= H, Cl, CH_3, OCH_3

Scheme 1. Synthesis of 5- or 6-methyl-2-(2,4-disubstituted phenyl)-benzoxazoles **4**, **5**.

Data for the preparation of the compounds are summarized in Table 1. 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.

3.2. General procedure for the synthesis of 5- or 6-methyl-2-(2,4-disubstituted phenyl)benzoxazoles (4, 5)

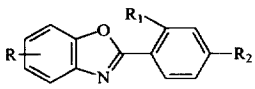
Appropriately substituted benzaldehyde (0.01 mol) was added to the mixture of 4- or 5-methyl-2-aminophenol (0.01 mol) in ethanol (7.5 ml) and boiled for 5 minutes. After the residue was removed by filtration, the Schiff's base was obtained from crystallization in ethanol/water. Then, lead tetraacetate (0.01 mol) was added to the mixture of the corresponding Schiff's base (0.01 mol) in glacial acetic acid (12 ml) and, after waiting a few minutes, the crude product was isolated by filtration and recrystallized from ethanol/water.

3.3. Microbiology

For both the antibacterial and the antimycotic assays, the compounds were dissolved in absolute ethanol (0.8 mg/ml) [17]. 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 concentrations (MICs) were determined using the two-fold serial dilution technique [10,17,18]. 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 as used in our experiments and found to be 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. The bacterial strains used are *Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 10541 and *Bacillus subtilis* ATCC 6033 as Gram-positive and *Escherichia coli* ATCC 10536, *Klepsiella pneumoniae* NTCC 52211, and *Ps. aeruginosa* RSKK 355 as Gram-neg-

Table 1
Physical properties, preparation and spectral data of the compounds **4**, **5**



Comp.	R	R_1	R_2	M.p. (°C)	Yield (%)	IR (cm^{-1})	^1H NMR: δ (ppm)
4a	5-CH_3	Cl	H	75–76	66.6	3100, 2940, 1600, 1560, 1470, 1260, 730–910	8.19–8.08 (1H, m), 8.06–7.16 (6H, m), 2.50 (3H, s)
4b	5-CH_3	OCH_3	H	87–88	64.0	3100, 2985, 1610, 1560, 1460, 1260, 700–910	8.18–8.05 (1H, dd, J = 8.23 and 2.21 Hz), 7.59–7.00 (6H, m), 4.00 (3H, s), 2.47 (3H, s)
4c	5-CH_3	F	H	78–79	52.86	3095, 2945, 1630, 1550, 1480, 1260, 690–920	8.14–8.11 (1H, m), 7.60–7.13 (6H, m), 2.49 (3H, s)
4d	5-CH_3	NO_2	H	119–120	47.24	3095, 2960, 1630, 1550, 1540, 1480, 1370, 1200, 700–950	8.19–8.03 (1H, m), 7.94–7.26 (6H, m), 2.49 (3H, s)
4e	5-CH_3	Cl	Cl	112–113	71.9	3100, 2960, 1600, 1560, 1460, 1270, 690–940	8.15–8.04 (1H, d, J = 8.53 Hz), 7.63–7.15 (5H, m), 2.49 (3H, s)
4f	5-CH_3	CH_3	CH_3	94–95	50.63	3060, 2940, 1620, 1550, 1480, 1260, 690–920	8.10–7.99 (1H, d, J = 8.38 Hz), 7.58–7.08 (5H, m), 2.76 (3H, s), 2.47 (3H, s), 2.38 (3H, s)
4g	5-CH_3	OCH_3	OCH_3	91–92	10.41	3110, 2980, 1610, 1550, 1480, 1220, 700–960	8.13–8.01 (1H, d, J = 9.26 Hz), 7.58–6.52 (5H, m), 3.98 (3H, s), 3.85 (3H, s), 2.45 (3H, s)
5a	6-CH_3	Cl	H	78–79	65.84	3100, 2985, 1630, 1550, 1450, 1270, 700–940	8.19–8.07 (1H, m), 7.76–7.14 (6H, m), 2.51 (3H, s)
5b	6-CH_3	OCH_3	H	74–75	64	3100, 2995, 1620, 1550, 1480, 1260, 700–940	8.17–8.05 (1H, dd, J = 8.05 and 2.05 Hz), 7.73–7.02 (6H, m), 4.01 (3H, s), 2.49 (3H, s)
5c	6-CH_3	F	H	95–96	44.05	3080, 2940, 1620, 1540, 1450, 1250, 700–950	8.28–8.09 (1H, m), 7.74–7.10 (6H, m), 2.50 (3H, s)
5d	6-CH_3	NO_2	H	103–104	62.99	3095, 2940, 1630, 1550, 1540, 1460, 1350, 1240, 1270, 710–940	8.19–8.09 (1H, m), 7.98–7.15 (6H, m), 2.50 (3H, s)
5e	6-CH_3	Cl	Cl	97–98	86.33	3100, 2960, 1610, 1560, 1460, 1250, 700–940	8.15–8.04 (1H, d, J = 8.53 Hz), 7.73–7.14 (5H, m), 2.51 (3H, s)
5f	6-CH_3	CH_3	CH_3	78–79	25.32	3065, 2940, 1620, 1550, 1490, 1240, 690–940	8.09–7.99 (1H, d, J = 8.53 Hz), 7.69–7.18 (5H, m), 2.76 (3H, s), 2.50 (3H, s), 2.38 (3H, s)
5g	6-CH_3	OCH_3	OCH_3	81–82	14.87	3100, 2980, 1620, 1570, 1450, 1260, 730–950	8.13–8.01 (1H, d, J = 9.41 Hz), 7.87–6.53 (5H, m), 3.99 (3H, s), 3.86 (3H, s), 2.47 (3H, s)

ative bacteria. RSKK strains of the microorganisms used in this study were obtained from the culture collection of the Refik Saydam Health Institution of the Health Ministry, Ankara, and maintained at the Microbiology Department of the Faculty of Pharmacy of Ankara University.

Ampicillin, amoxycillin, tetracycline, streptomycin, 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 2.

3.3.1. Antibacterial assay

The cultures were obtained in Mueller-Hinton broth (Difco) 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 two-fold serial dilution technique was applied. 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 microorganisms was recorded to represent MIC expressed in μg/ml.

3.3.2. 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 two-fold serial dilution technique was applied. 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.

4. Results and discussion

The chemical, physical and spectral data of the synthesized compounds **4** and **5** are reported in Table 1. The antimicrobial activity of the compounds in comparison with that of some control drugs is shown in Table 2 and indicates that the compounds **4**, **5** are able to inhibit in vitro growth of a number of microorganisms, exhibiting MIC values between 50 and 12.5 μg/ml.

Table 2 reveals that the synthesized compounds showed some antibacterial activity against the Gram-positive bacteria *S. aureus*, possessing MIC values between 25 and 50 μg/ml, except the derivatives **4b** and **4c** which were active at 12.5 μg/ml MIC values. All the synthesized compounds indicated

Table 2
The in vitro antimicrobial activity of the compounds **4**, **5** and the standard drugs (MIC in μg/ml)

Comp.	Microorganisms [a]						
	Gram-positive			Gram-negative			Fungus
	Sa	Sf	Bs	Ec	Kp	Pa	Ca
4a	25	50	25	50	50	50	25
4b	12.5	50	25	50	50	50	25
4c	12.5	50	25	50	50	50	12.5
4d	50	50	25	50	50	50	25
4e	50	50	25	50	50	50	25
4f	50	50	25	50	50	50	25
4g	50	50	25	50	25	50	25
5a	50	50	25	25	25	25	25
5b	50	50	25	50	50	50	25
5c	50	50	25	50	50	50	25
5d	50	50	50	50	50	50	25
5e	50	50	25	50	50	50	25
5f	50	50	50	50	50	50	25
5g	50	50	25	50	25	50	25
Ampicillin	1.56	1.56	1.56	12.5	25	>200	–
Amoxycillin	1.56	1.56	1.56	3.12	12.5	>200	–
Tetracycline	1.56	1.56	1.56	3.12	3.12	50	–
Streptomycin	3.12	100	50	1.56	1.56	100	–
Oxiconazole	–	–		–	–	–	6.25
Haloprogin	–	–		–	–	–	6.25

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

the same potency, showing 50 μg/ml MIC values against *Strep. faecalis* and most of the compounds were found active at a MIC value of 25 μg/ml against *B. subtilis*. However, they exhibited lower antibacterial potencies than the compared control drugs ampicillin, amoxycillin and tetracycline against the screened Gram-positive bacteria strains.

Furthermore, the determination of the antibacterial activity of the compounds **4**, **5** against *E. coli* and *K. pneumoniae* as Gram-negative bacteria also revealed lower potencies than the compared control drugs. But, against the Gram-negative enterobacter *Ps. aeruginosa*, which is effective in nosocomial infections and often resistant to antibiotic therapy, the derivative 6-methyl-2-(2-chlorophenyl)benzoxazole **5a** indicated significant activity, showing a MIC value of 25 μg/ml, being thus more potent than the compared control drugs tetracycline and streptomycin.

The compounds **4** and **5** were also tested against *C. albicans* for their antimycotic activities and the compound 5-methyl-2-(2-fluorophenyl)benzoxazole **4c** was found to be more active than the other tested compounds, having a MIC value of 12.5 μg/ml. In spite of that, antimycotic potencies of the compared control drugs oxiconazole and haloprogin were one dilution better than the corresponding compound **4c**, showing MIC values of 6.25 μg/ml.

In conclusion, structure–activity relationships of the synthesized compounds reveal that holding a methyl group at position 5 instead of 6 on the fused heterocyclic system while substituting the *ortho* position of the 2-phenyl moiety at the benzoxazole ring by a fluorine atom increases the antimycotic activity against *C. albicans* and the antibacterial activity against *S. aureus*. However, a methyl group at position 6 on the benzoxazole ring was found to be more effective to improve the intensity of the antibacterial activity against the screened Gram-negative bacteria, especially for the enterobacter *Ps. aeruginosa*.

Acknowledgements

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