Ilkay Yildiz-Oren[a], Betul Tekiner-Gulbas[a], Ismail Yalcin[a], Ozlem Temiz-Arpaci[a], Esin Akı-Sener[a], Nurten Altanlar[b]

[a] Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Tandogan, Ankara, Turkey
[b] Ankara University, Faculty of Pharmacy, Department of Microbiology, Tandogan, Ankara, Turkey

Synthesis and Antimicrobial Activity of New 2-[p-Substituted-benzyl]-5-[substituted-carbonyl-amino]benzoxazoles

A series of 23 new 2-[p-substituted-benzyl]-5-[p-substituted-phenyl/benzyl-carbonylamino]benzoxazole derivatives has been synthesized by reacting 5-amino-2-[p-substituted-benzyl]benzoxazoles with the appropriate carboxylic acid chlorides. The structures of the synthesized compounds were confirmed by IR and ^{1}H-NMR spectral data. Antimicrobial activities of the compounds were investigated using the twofold serial dilution technique against two gram-positive and two gram-negative bacteria and three *Candida* species in comparison with standard drugs. Microbiological results indicated that the newly synthesized 2-[p-substituted-benzyl]-5-[p-substituted-phenyl/benzyl-carbonylamino]-benzoxazole derivatives (**3**–**25**) possessed a broad spectrum of activity, showing MIC values of 6.25–200 μg/mL against the gram-positive and gram-negative microorganisms tested. Moreover, they showed significant antifungal activity with MIC values of 3.12–100 μg/mL against the *Candida* species tested. Especially, with a MIC value of 3.12 μg/mL, 2-benzyl-5-[p-bromobenzyl-carbonyl-amino]benzoxazole **9** displayed the same activity against *C. glabrata* as the standard drug myconazol.

Keywords: 2-Benzylbenzoxazole; Benzylcarbonylamino-benzoxazole; Phenyl-carbonylamino-benzoxazole; Antibacterial and antifungal activity

Received: August 14, 2003; Accepted: March 12, 2004 [FP851]
DOI 10.1002/ardp.200300851

Introduction

The number of cases of multidrug-resistant bacterial infections is increasing at an alarming rate. Although clinicians now rely on vancomycin as the antibiotic for serious infections resistant to traditional agents [1], there is still need for new classes of antibacterial agents.

Substituted benzoxazole derivatives and their analogues such as benzimidazoles and benzothiazoles have been the aim of many researchers for many years, because they constitute an important class of heterocyclic compounds with antitumor, antiviral and antibiotic activities [2–16].

A benzoxazole derivative, 3-(4,7-dichlorobenzoxazole-2-yl-methylamino)-5-ethyl-6-methylpyridin-2(1H)-one (L-697,661) was identified as a specific non-nucleoside reverse transcriptase inhibitor for the human immunodeficiency virus HIV-1 type, and its use in combined therapy with zidovudine achieved a marked decrease of viremia in some primary HIV-infected patients [6]. Moreover, substituted pyrimido[1,6-a]-benzimidazoles were synthesized as a new class of potent DNA gyrase inhibitors; however, their antibacterial activity was inferior to the quinolone-type antibacterial agents such as norfloxacin or fleroxacin [7]. Recently, a new series of benzothiazoles have been synthesized as antitumor agents that showed potent inhibitory activity against human breast cancer cell lines *in vitro* and *in vivo* [8]. Among them, lysyl-amide of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole has been selected for phase 1 clinical evaluation [17].

A derivative of camptothecin has been prepared wherein the A-ring has been fused to an oxazole ring by Peel et al. [18]. This compound (Figure 1) was found to be significantly more potent as inhibitor of topoisomerase I than camptothecin.

Recently, we reported the synthesis and antimicrobial activity of 2-[p-substituted-phenyl]-5-[substituted-aryl-carbonylamino]benzoxazole derivatives (Figure 2) [19, 20].

In this study, a series of 2-[p-substituted-benzyl]-5-[p-substituted-phenyl/benzyl-carbonylamino]benzoxazole derivatives (**3**–**25**) (Figure 3) has been syn-

Correspondence: Esin Aki-Sener, Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, TR-06100 Tandogan, Ankara, Turkey. Phone: +90 312 223-69-40, Fax: +90 312 223-69-40, e-mail: sener@pharmacy.ankara.edu.tr

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

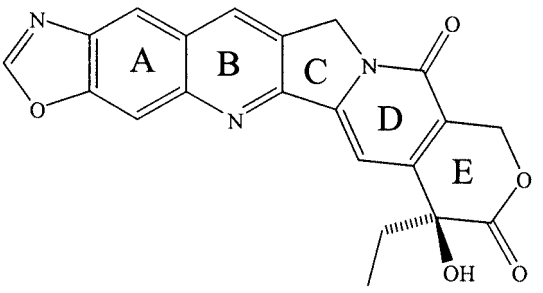
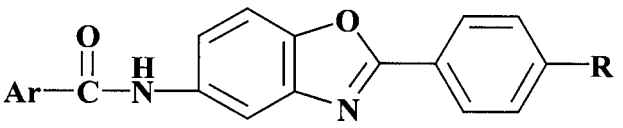


Figure 1.



R = H, C_2H_5, F, $N(CH_3)_2$
Ar = 2-furyl, 2-thienyl, substituted phenyl

Figure 2.

thesized as the target compounds, in order to examine their microbiological activity, and that of the previously synthesized 2-[p-substituted-phenyl]-5-[substituted-aryl-carbonylamino]benzoxazole derivatives [19, 20], against various gram-positive and gram-negative bacteria and diverse fungi in comparison with several control drugs, including structure-activity relationship (SAR) studies.

Chemistry

5-Amino-2-[p-substituted-benzyl]benzoxazoles (**1**, **2**) were obtained by heating p-substituted phenylacetic acids with 2,4-diaminophenol in PPA (polyphosphoric acid) [19, 20].

Compounds (**3**–**25**) were obtained from 5-amino-2-[p-substituted-benzyl]benzoxazoles with p-substituted benzoic acid or p-substituted phenylacetic acid chlorides obtained by treating appropriate carboxylic acids with thionyl chloride [21, 22] as given in Scheme 1.

Compounds **3**–**25** are new, and their structures were supported by spectral data (Table 1).

Results and discussion

A series of 23 new 2-[p-substituted-benzyl]-5-[substituted-phenyl/benzyl-carbonylamino]-benzoxazole derivatives (**3**–**25**) has been synthesized by using a two-step procedure as shown in Scheme 1 [19–22]. All of the derivatives (**3**–**25**) 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.

In order to determine the antimicrobial activity of the synthesized compounds (**3**–**25**), two gram-positive bacteria, two gram-negative bacteria and three *Candida* species were screened using the twofold serial dilution technique [23, 24]. All the biological results of the compounds are given in Table 2. The combined data reported that the newly synthesized compounds showing MIC values between 200–3.12 μg/mL were able to inhibit the *in vitro* growth of the microorganisms screened.

In this study, our goal was to investigate the role of the second position of the benzoxazole ring for antimicrobial activity. Therefore, we put p-substituted-benzyl instead of p-substituted-phenyl [19, 20] at position 2 of benzoxazole. Additionally, we examined the effect of the 5-carbonylamino substitutents of the benzoxazole ring system on antimicrobial activity. Consequently, the newly presented compounds **3**–**25** were compared with previously prepared compounds **26**–**64** [19, 20] with regard to their antibacterial and antifungal activity (Table 2).

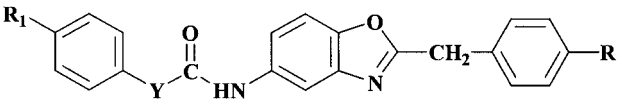
According to Table 2, all of the new compounds **3**–**25** showed lower antibacterial activity against the screened gram-positive bacteria *S. aureus* or *B. subtilis* (MIC values between 200–12.5 μg/mL) than the control drugs. With a MIC value of 12.5 μg/mL against *S. aureus*, only compound **21** was found to be more active than the others.

Furthermore, the antibacterial activity of compounds **3**–**25** against the gram-negative bacteria *E. coli* and *P. aeruginosa* results in MIC values between 25 and 100 μg/mL. While none of the compounds showed lower activity than the standard drugs against *E. coli,* some of the compounds (**5**–**10**, **13**, **16**, **21**–**25**) were found to be more active against *P. aeruginosa* (with MIC values of 25 μg/mL) than the standard drugs tetracycline and streptomycin.

Compounds **3**–**25** were also tested against *C. albicans, C. krusei* and *C. glabrata* for their antimycotic activity, and most of the compounds showed significant antimycotic activity displaying MIC values between 3.12 and 100 μg/mL. Compound **9**, having a MIC value of 3.12 μg/mL against *C. glabrata*, was more active than the other compounds tested. Moreover, compound **9** showed the same activity as the control drug myconazole. Compounds **13** and **23** were

Full Paper

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim



Y = —, CH_2

R = H, Cl

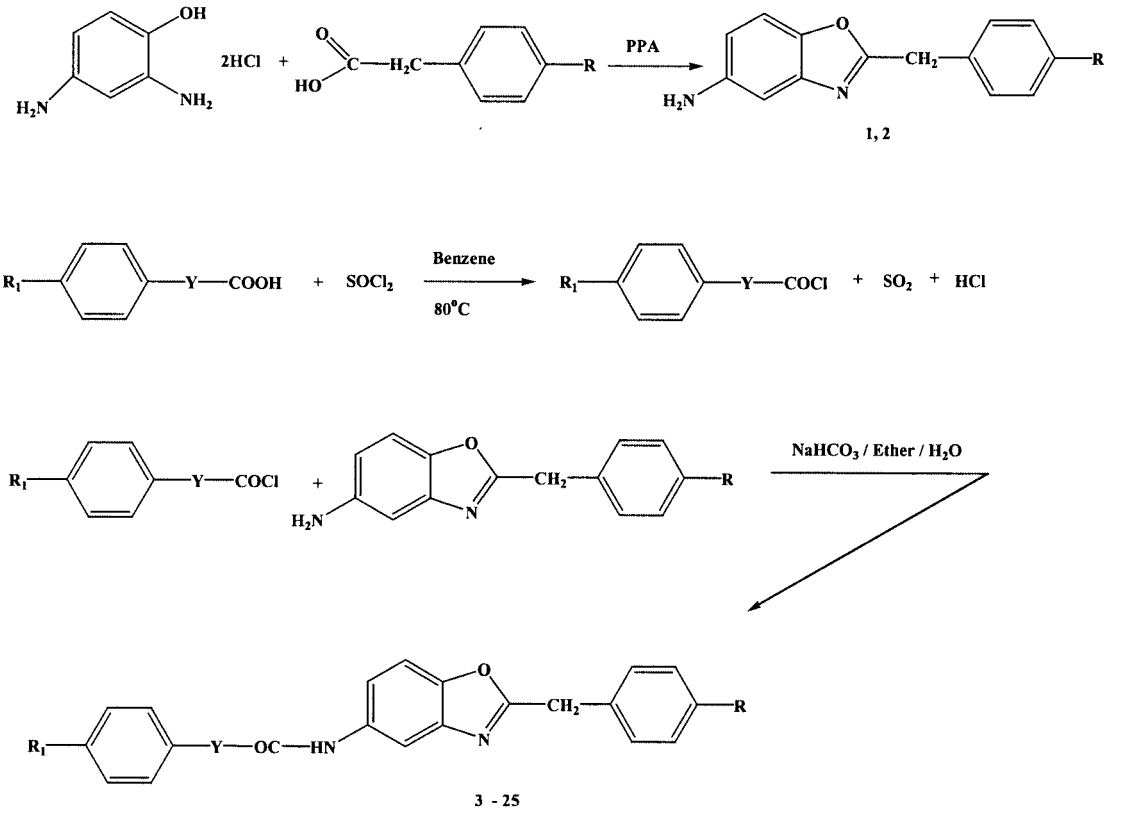
R_1 = H, Cl, Br, F, CH_3, C_2H_5, NO_2, $C(CH_3)_3$

Figure 3.

found to be more active than the others with a MIC value of 6.25 μg/mL against *C. krusei*.

Table 2 indicated that substitution at position 5 of the benzoxazole ring with 4-chloro / fluoro / methoxy-benzamido having fluorine or ethyl groups at the para position of 2-phenylbenzoxazole (compounds **52**–**56**) generally causes an increase in the activity against *S. aureus* resulting in MIC values of 25 μg/mL. Heterocyclic rings such as 2-furyl and 2-thienyl attached at 5-carbonylamino of 2-(p-dimethylamino-phenyl)benzoxazole also increase the activity against *S. aureus* (compounds **63**, **64**). With MIC values of 25 μg/mL, most of the compounds were found to be significantly active against *B. subtilis*.

Furthermore, the antibacterial activity of compounds **3**–**64** showed lower activity (MIC values of 25–200 μg/mL) than the standard drugs against *E. coli*. Compounds **5**–**10**, **13**, **16**, **21**–**26**, **46**, **50**, and **54** were found to be more active against *P. aeruginosa* (MIC values of 25 μg/mL) than the standard drugs tetracycline and streptomycin. Most of the 2-benzylbenzoxazole derivatives showed significant activity against *P. aeruginosa*.



Y = —, CH_2

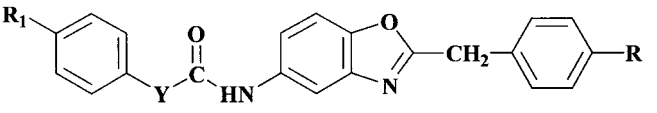
R = H, Cl

R_1 = H, Cl, Br, F, CH_3, C_2H_5, NO_2, $C(CH_3)_3$

Scheme 1.

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 1. Physical, preparation and spectral data of the synthesized compounds **3**–**25.**



Comp. No:	Y	R	R_1	m.p. (°C)	Yield (%)	Empirical formula	IR (cm^{-1})	^{1}H-NMR δppm (*J* = Hz)
3	–	H	H	136	54	$C_{21}H_{16}O_2N_2$	3295, 1573–1529, 3032, 1491–1470, 2940, 1295–1025, 1647, 1619, 961–691	4.00–4.10 (2H, s), 7.25–8.05 (14H, m)
4	–	H	C_2H_5	143	29.74	$C_{23}H_{20}O_2N_2$	3376, 3030, 2960–2870, 1653, 1622, 1570–1507, 1481, 1289–1017, 966–668	1.20–1.30 (3H, t, J = 7.57), 2.70–2.80 (2H, q, J = 7.61), 4.25–4.30 (2H, s), 7.20–7.42 (7H, m), 7.42–7.50 (1H, dd, $J_{7,6}$ = 8.74), 7.55–7.65 (1H, dd, $J_{6,7}$ = 8.74, $J_{6,4}$ = 1.82), 7.78–7.85 (2H, d, $J_{2'',3''} = J_{6'',5''}$ = 8.60), 7.85–7.90 (1H, s), 7.92–8.00 (1H, d, $J_{4,6}$ = 1.70)
5	–	H	NO_2	205–206	57.57	$C_{21}H_{15}O_4N_3$	3308 , 3067, 2900–2840, 1648, 1620, 1601–1536, 1484–1430, 1295–1012, 968–693	4.20–4.40 (2H, s), 7.25–7.45 (5H, m), 7.45–7.55 (1H, d, $J_{7,6}$ = 8.78), 7.65–7.75 (1H, d, $J_{6,7}$ = 8.37), 8.00–8.05 (1H, s), 8.05–8.15 (2H, d, $J_{2'',3''} = J_{6'',5''}$ = 8.45), 8.25–8.40 (3H, m)
6	–	H	$C(CH_3)_3$	144–145	37.25	$C_{25}H_{24}O_2N_2$	3407, 3026, 2958–2868, 1662, 1620, 1566–1535, 1481, 1268–1027, 964–693	1.30–1.40 (9H, s), 4.20–4.30 (2H, s), 7.20–7.90 (12H, m), 7.90–8.05 (1H, s)
7	–	H	Br	197–198	11.23	$C_{21}H_{15}O_2N_2Br$	3271, 3085, 2930–2850, 1672, 1625, 1588–1558, 1485, 1297–1010, 970–695	4.30–4.50 (2H, s), 7.40–7.55 (5H, m), 7.55–7.65 (1H, d, $J_{7,6}$ = 8.75), 7.65–7.75 (1H, dd, $J_{6,7}$ = 8.77, $J_{6,4}$ = 1.9), 7.75–7.85 (2H, d, $J_{3'',2''} = J_{5'',6''}$ = 8.46), 7.85–7.95 (2H, d, $J_{6'',5''} = J_{2'',3''}$ = 8.47), 8.00–8.05 (1H, s), 8.05–8.15 (1H, d, $J_{4,6}$ = 1.84)
8	–	H	F	153–154	27.81	$C_{21}H_{15}O_2N_2F$	3360, 3068, 2950–2900, 1648, 1624, 1602–1540, 1455–1423, 1269–1027, 960–695	4.00–4.10 (2H, s), 6.90–7.75 (12H, m), 7.75–7.85 (1H, s)
9	CH_2	H	Br	181–182	53.25	$C_{22}H_{17}O_2N_2Br$	3263, 3062, 2960–2890, 1652, 1620, 1574–1542, 1482–1454, 1271–1013, 967–675	3.70″380 (2H, s), 420–4.30 (2H, s), 7.20–725 (2H, d, $J_{3'',2''} = J_{5'',6''}$ = 8.22), 7.25–7.40 (8H, m), 7.45–7.60 (2H, d, $J_{2'',3''} = J_{6'',5''}$ = 8.14), 7.75–7.85 (1H, s)
10	CH_2	H	F	140–141	34.56	$C_{22}H_{17}O_2N_2F$	3256, 3073, 2924, 1649, 1622, 1573–1511, 1482–1455, 1279–1017, 977–695	3.70–3.80 (2H, s), 4.30–4.40 (2H, s), 7.00–7.50 (12H ,m), 7.80–7.90 (1H, s)
11	CH_2	H	H	123	12.19	$C_{22}H_{18}O_2N_2$	3255, 3061–3027, 2930, 1649, 1620, 1573–1540, 1480–1454, 1259–1029, 975–671	3.70–3.80 (2H, s), 4.20–4.30 (2H, s), 7.20–7.50 (13H, m), 7.75–7.85 (1H, s)
12	CH_2	H	Cl	171–172	38.60	$C_{22}H_{17}O_2N_2Cl$	3264, 3063, 2960, 1652, 1620, 1575–1542, 1482–1455, 1271–1017, 980–695	3.70–3.80 (2H, s), 4.20–4.30 (2H, s), 7.30–7.45 (12H, m), 7.70–7.80 (1H, s)
13	CH_2	H	CH_3	138–139	29.37	$C_{23}H_{20}O_2N_2$	3270, 3050, 2920, 1615, 1567–1538, 1483–1455, 1267–1029, 1662, 972–694	2.30–2.40 (3H, s), 3.70–3.80 (2H, s), 4.20–4.30 (2H, s), 7.10–7.40 (12H, m), 7.80 (1H, s)
14	–	Cl	H	160	44.99	$C_{21}H_{15}O_2N_2Cl$	3296, 3058, 2930, 1648, 1619, 1574–1533, 1482, 1299–1015, 967–692	4.50–4.70 (1H, s), 7.25–8.05 (13H, m)

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 1. (continued).

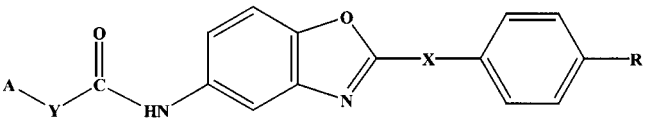
Comp. No:	Y	R	R_1	m.p. (°C)	Yield (%)	Empirical formula	IR (cm^{-1})	^{1}H-NMR δppm (*J* = Hz)
15	–	Cl	C_2H_5	172–173	28.20	$C_{23}H_{19}O_2N_2Cl$	3271, 3080, 2968–2931, 1623, 1572, 1481, 1298–1015, 1671, 969–697	1.00–1.10 (3H, t, *J*= 7.60), 2.50–2.60 (2H, q, *J* = 7.60), 4.00–4.10 (2H, s), 7.05–7.20 (6H, m), 7.20–7.30 (1H, d, $J_{7,6}$ = 8.75), 7.35–7.45 (1H, dd, $J_{6,7}$ = 8.77, $J_{6,4}$ = 1.99), 7.55–7.65 (2H, d, $J_{2'',3''} = J_{6'',5''}$ = 8.18), 7.70–7.80 (2H, m, $J_{4,6}$ = 2.7)
16	–	Cl	NO_2	205–207	50.86	$C_{21}H_{14}O_4N_3Cl$	3316, 3079, 2850, 1651, 1618, 1603–1529, 1481, 1290–1015, 973–683	4.00–4.20 (2H, s), 7.00–7.50 (7H, m), 7.75–7.85 (1H, s), 7.85–8.20 (4H, m)
17	–	Cl	$C(CH_3)_3$	145	21.54	$C_{25}H_{23}O_2N_2Cl$	3296, 2950–2850, 2964, 1638, 1615, 1572–1528, 1480, 1267–1015, 968–689	1.00–1.40 (9H, s), 4.00–4.20 (2H, s), 7.00–7.90 (12H, m)
18	–	Cl	Br	197	41.26	$C_{21}H_{14}O_2N_2ClBr$	3270, 3082, 2980–2900, 1673, 1624, 1588–1558, 1482, 1275–1012, 970–683	4.00–4.10 (2H, s), 7.10–7.20 (4H, m), 7.20–7.30 (1H, d, $J_{7,6}$ = 8.75), 7.35–7.40 (1H, dd, $J_{6,7}$ = 8.74), 7.40–7.50 (2H, d, $J_{3'',2''} = J_{5'',6''}$ = 8.53), 7.50–7.65 (2H, d, $J_{2'',3''} = J_{6'',5''}$ = 8.43), 7.70 (1H, s), 7.75–7.80 (1H, s)
19	–	Cl	F	173–174	20.78	$C_{21}H_{14}O_2N_2ClF$	3397, 3073, 2930, 1660, 1619, 1602–1550, 1485, 1286–1015, 972–692	4.00–4.10 (2H, s), 6.90–7.00 (2H, q), 7.10–7.20 (4H, q), 7.20–7.30 (1H, d, $J_{7,6}$ = 8.77), 7.35–7.45 (1H, dd, $J_{6,7}$ = 8.76, $J_{6,4}$ = 1.87), 7.65–7.90 (4H, q)
20	CH_2	Cl	Br	178	55.73	$C_{22}H_{16}O_2N_2ClBr$	3264, 3054, 2980–2920, 1662, 1618, 1590–1536, 1487, 1266–1013, 972–678	3.80–3.95 (2H, s), 4.30–4.45 (2H, s), 7.25–7.75 (11H, m), 7.90–8.00 (1H, s)
21	CH_2	Cl	F	139–140	51.72	$C_{22}H_{16}O_2N_2ClF$	3261, 3096, 2920, 1666, 1623, 1569–1509, 1482, 1278–1014, 982–694	3.70–3.85 (2H, s), 4.20–4.30 (2H, s), 7.00–7.50 (11H, m), 7.80–7.90 (1H, s)
22	CH_2	Cl	H	164	8.15	$C_{22}H_{17}O_2N_2Cl$	3265, 3071, 2950–2900, 1682, 1621, 1572, 1478, 1297–1015, 973–694	3.60–3.80 (2H, s), 4.10–4.20 (2H, s), 7.00–7.50 (12H, m), 7.70–7.80 (1H, d, $J_{4,6}$ = 1.67)
23	CH_2	Cl	Cl	167–168	13.12	$C_{22}H_{16}O_2N_2Cl_2$	3262, 3055, 2360, 1663, 1618, 1596–1539, 1482, 1267–1015, 973–674	3.80–3.95 (2H, s), 4.35–4.45 (2H, s), 7.30–7.60 (11H, m), 7.95–8.00 (1H, d, $J_{4,6}$ = 1.63)
24	CH_2	Cl	NO_2	232–233	15.05	$C_{22}H_{16}O_4N_3Cl$	3265–3219, 3073, 2980–2860, 1687, 1624, 1567–1509, 1482, 1255–1015, 982–728	3.40–3.60 (2H, s), 3.90–4.00 (2H, s), 7.00–7.06 (2H, m), 7.06–7.12 (1H, d, $J_{7,6}$ = 8.79), 7.20–7.25 (1H, dd, $J_{6,7}$ = 8.8, $J_{6,4}$ = 2.07), 7.25–7.35 (2H, d, $J_{6'',5''} = J_{2'',3''}$ = 8.72), 7.35–7.45 (2H, s), 7.70–7.75 (1H, d, $J_{4,6}$ = 1.96), 7.85–7.95 (2H, dd, $J_{3'',2''} = J_{5'',6''}$ = 8.76, $J_{3'',5''} = J_{5'',3''}$ = 1.89), 9.80–10.00 (1H, s)
25	CH_2	Cl	CH_3	169–171	32.49	$C_{23}H_{19}O_2N_2Cl$	3249, 3026, 2920–2890, 1644, 1617, 1571–1535, 1480, 1268–1015, 971–686	2.50–2.65 (3H, s), 3.80–3.90 (2H, s), 4.35–4.50 (2H, s), 7.30–7.55 (11H, m), 7.90–8.00 (1H, d, $J_{4,6}$ = 1.71)

On the other side, with MIC values of 12.5–50 μg/mL, all the compounds showed notable activity against *C. albicans*, except for **32** and **34**.

The SAR of the synthesized compounds revealed that compounds possessing p-bromo / chloro / methyl-phenyl-acetamido groups at position 5 of the fused hetero-

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 2. Antimicrobial activity results (MIC, μg/mL) of the newly and previously [19, 20] synthesized compounds as well as the standard drugs.



						Microorganisms*						
			gram-positive			gram-negative				fungus		
Comp. No.	**Ref.**	**A**	**X**	**Y**		***Sa***	***Bs***	***Ec***	***Pa***	***Ca***	***Ck***	***Cg***
3		Phenyl	CH_2	–	H	200	50	100	100	50	12.5	25
4		4-Ethylphenyl	CH_2	–	H	50	50	50	50	25	25	12.5
5		4-Nitrophenyl	CH_2	–	H	50	25	100	25	12.5	12.5	12.5
6		4-*tert*-Butylphenyl	CH_2	–	H	200	50	25	25	50	25	25
7		4-Bromophenyl	CH_2	–	H	50	50	50	25	25	25	25
8		4-Fluorophenyl	CH_2	–	H	50	25	50	25	12.5	50	25
9		4-Bromophenyl	CH_2	CH_2	H	25	25	25	25	25	12.5	3.12
10		4-Fluorophenyl	CH_2	CH_2	H	50	25	25	25	50	12.5	12.5
11		Phenyl	CH_2	CH_2	H	25	25	25	50	12.5	12.5	25
12		4-Chlorophenyl	CH_2	CH_2	H	100	100	50	50	25	25	25
13		4-Methylphenyl	CH_2	CH_2	H	25	25	25	25	25	6.25	12.5
14		Phenyl	CH_2	–	Cl	200	50	100	100	50	25	25
15		4-Ethylphenyl	CH_2	–	Cl	50	50	50	50	25	25	25
16		4-Nitrophenyl	CH_2	–	Cl	100	50	25	25	50	25	25
17		4-*tert*-Butylphenyl	CH_2	–	Cl	25	50	50	50	50	50	50
18		4-Bromophenyl	CH_2	–	Cl	50	50	50	50	50	100	100
19		4-Fluorophenyl	CH_2	–	Cl	50	25	25	50	12.5	12.5	12.5
20		4-Bromophenyl	CH_2	CH_2	Cl	50	25	50	50	12.5	25	50
21		4-Fluorophenyl	CH_2	CH_2	Cl	12.5	100	50	25	50	25	50
22		Phenyl	CH_2	CH_2	Cl	50	25	50	25	25	25	25
23		4-Chlorophenyl	CH_2	CH_2	Cl	50	25	50	25	50	6.25	12.5
24		4-Nitrophenyl	CH_2	CH_2	Cl	50	50	50	25	12.5	25	25
25		4-Methylphenyl	CH_2	CH_2	Cl	100	25	50	25	12.5	12.5	12.5
26*	[19]	Phenyl	–	–	H	50	50	50	25	25	–	–
27*	[19]	4-Fluorophenyl	–	–	H	100	100	200	200	50	–	–
28	[19]	4-Bromophenyl	–	–	H	50	50	50	50	50	–	–
29	[19]	4-Chlorophenyl	–	–	H	50	50	50	50	50	–	–
30	[19]	4-Methoxyphenyl	–	–	H	50	50	50	50	50	–	–
31	[19]	4-Methylphenyl	–	–	H	50	50	50	50	25	–	–
32	[19]	4-Ethylphenyl	–	–	H	100	50	100	50	100	–	–
33	[19]	4-Nitrophenyl	–	–	H	100	200	100	50	25	–	–
34	[19]	4-*tert*-Butylphenyl	–	–	H	100	100	100	100	100	–	–
35	[19]	Phenyl	–	–	C_2H_5	50	50	50	50	50	–	–
36	[19]	4-Methylphenyl	–	–	C_2H_5	100	100	50	50	50	–	–
37	[19]	4-Ethylphenyl	–	–	C_2H_5	50	50	50	50	50	–	–
38	[19]	2-Methoxyphenyl	–	–	H	25	25	50	50	25	–	–
39	[19]	2-Chlorophenyl	–	–	H	50	50	50	50	25	–	–
40	[19]	2,4-Dimethoxyphenyl	–	–	H	100	200	50	100	25	–	–
41	[19]	2,4-Dimethylphenyl	–	–	H	50	25	50	100	25	–	–

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 2. (continued).

Comp. No.	Ref.	A	X	Y		*Sa*	*Bs*	*Ec*	*Pa*	*Ca*	*Ck*	*Cg*
42	[19]	Phenyl	–	CH_2	H	100	50	100	100	50	–	–
43	[19]	4-Bromophenyl	–	CH_2	H	50	50	50	50	25	–	–
44	[19]	4-Chlorophenyl	–	CH_2	H	100	50	100	50	50	–	–
45	[19]	4-Nitrophenyl	–	CH_2	H	50	200	50	50	25	–	–
46	[19]	4-Propyloxyphenyl	–	CH_2	H	50	200	25	25	25	–	–
47	[19]	Phenyl	–	CH_2	C_2H_5	50	50	50	50	50	–	–
48	[19]	4-Bromophenyl	–	CH_2	C_2H_5	50	50	50	50	50	–	–
49	[19]	4-Chlorophenyl	–	CH_2	C_2H_5	50	50	50	50	50	–	–
50	[19]	2-Chlorophenyl	–	CH_2	H	50	100	25	25	25	–	–
51	[19]	3,5-Dimethoxyphenyl	–	–	H	100	200	50	50	25	–	–
52	[20]	4-Chlorophenyl	–	–	F	25	25	25	50	25	–	–
53	[20]	4-Chlorophenyl	–	–	C_2H_5	25	25	25	50	25	–	–
54	[20]	4-Methoxyphenyl	–	–	C_2H_5	25	25	25	25	12.5	–	–
55	[20]	4-Fluorophenyl	–	–	F	25	25	25	50	25	–	–
56	[20]	4-Fluorophenyl	–	–	C_2H_5	25	25	25	50	25	–	–
57	[20]	2-Thienyl	–	–	F	50	100	50	100	50	–	–
58	[20]	2-Thienyl	–	–	H	50	50	50	50	50	–	–
59	[20]	2-Furyl	–	–	F	50	25	25	50	25	–	–
60	[20]	2-Furyl	–	–	H	50	25	50	50	25	–	–
61	[20]	2-Thienyl	–	–	C_2H_5	50	100	200	200	50	–	–
62	[20]	2-Furyl	–	–	C_2H_5	25	25	100	100	50	–	–
63	[20]	2-Thienyl	–	–	$N(CH_3)_2$	25	25	25	50	12.5	–	–
64	[20]	2-Furyl	–	–	$N(CH_3)_2$	25	50	50	50	25	–	–
Ampicillin						1.56	1.56	12.5	>200	–	–	–
Amoxycillin						1.56	1.56	3.12	>200	–	–	–
Tetracycline						1.56	1.56	3.12	50	–	–	–
Streptomycin						3.12	50	1.56	100	–	–	–
Ciprofloxacine						3.12	1.56	3.13	0.78	–	–	–
Gentamisin						3.12	1.56	12.5	12.5	–	–	–
Myconazole						–	–	–	–	3.12	1.56	3.12
Clotrimazole						–	–	–	–	6.25	–	–
Haloprogin						–	–	–	–	3.12	–	–

***Sa:** *Staphylococcus aureus* (ATCC 25923); ***Bs**: *Bacillus subtilis* (ATCC 6633); ***Ec**: *Escherichia coli* (ATCC 23556); ***Pa**: *Pseudomonas auruginosa* (ATCC 10145); ***Ca**: *Candida albicans*; ***Ck**: *Candida krusei* (ATCC 6258); ***Cg**: *Candida glabrata* (isolate)

cyclic system and a chlorine substituent at the para position of the 2-benzyl moiety of the benzoxazole had increased antimycotic activity against *C. krusei* and *C. glabrata*.

In conclusion, 2-benzyl-benzoxazole derivatives showed significant activities against gram-positive and gram-negative bacteria. However, the alternates of the substituents attached at 5-carbonylamino of benzoxazoles made no important difference for the antimicrobial activity. It could be pointed out that 2-benzyl-benzoxazole derivatives generally indicated better activities than 2-phenyl-benzoxazole derivatives against *C. albicans* and that they showed notable activities against *Candida* species such as *C. glabrata* and *C. krusei*, and these observations could guide us to design further new lead antifungal compounds.

Acknowledgments

We thank the Research Fund of Ankara University (Grant No. 2001-08-03-27) for financial support of this research.

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Experimental

Chemistry

Silicagel HF_{254} chromatoplates (0.3 mm) were used for TLC. The solvent systems were chloroform : methanol (15 : 0.5) for compounds **3**–**25**. Melting points were taken on a Buchi SMP 20 capillary apparatus and are uncorrected. IR spectra were recorded by FT/IR–420 in KBr discs. ^{1}H-NMR spectra were obtained with a Bruker 80 MHz spectrometer in d_6-chloroform; tetramethylsilan (TMS) was used as an internal standard. Elemental analyses were carried out with a Perkin Elmer model 240-C apparatus.

*General procedure for the synthesis of 5-amino-2-[p-substituted-benzyl]benzoxazole (**1**, **2**) [19, 20]*

5-amino-2-[p-substituted-benzyl]benzoxazole was synthesized by heating 0.01 mol 2,4-diaminophenol 2HCl with 0.01 mol suitable phenyl acetic acid in 12.5 g polyphosphoric acid (PPA) and stirring 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 10 M NaOH solution extracted with benzene. Then, this solution was dried over anhydrous sodium sulfate and evaporated under diminished pressure. The residue was boiled with 200 mg charcoal in ethanol and filtered. After the evaporation of solvent *in vacuo*, the crude product was obtained and recrystallized from ethanol.

2-Benzyl-5-aminobenzoxazole

Reaction time: 2.5 h. Reaction temperature: 150–160 °C. Yield: 66 %. MW: 224. M.p.: 82–83 °C. ^{1}H-NMR ($CDCl_3$): 4.00–4.10 (s, 2H, CH_2), 6.40–6.60 (dd, 1H, C-6 H $J_{6,7}$ = 8.56, $J_{6,4}$ = 2.25), 6.70–6.80 (d, 1H, C–4 H, $J_{4,6}$ = 2.22), 7.00–7.05 (d, 1H, C-7 H, $J_{7,6}$ = 8.58), 7.05–7.20 (m, 7H, C-2′, C-3′, C-4′, C-5′, C-6′, NH). IR (KBr disc): 3385, 1617, 1562, 1486, 1452, 1270, 1190.

2-(p-Chlorobenzyl)-5-aminobenzoxazole

Reaction time: 1.5 h. Reaction temperature: 195–198 °C. Yield: 100 %. MW: 224. M.p.: 85–87 °C. ^{1}H-NMR ($CDCl_3$): 4.10–4.30 (s, 2H, CH_2), 6.70–6.80 (dd, 1H, C-6 H $J_{6,7}$ = 8.56, $J_{6,4}$ = 2.66), 6.95–7.00 (d, 1H, C-4 H,$J_{4,6}$ = 2.21), 7.20–7.25 (d, 1H, C-7 H, $J_{7,6}$ = 8.6), 7.25–7.40 (m, 6H, C-2′, C-3′, C-5′, C-6′, NH). IR (KBr disc): 3381, 2210, 1915, 1615, 1563, 1486, 1452, 1298, 1271, 1191.

*General procedure for 2-[p-substituted-benzyl]-5-[p-substituted-phenyl/benzyl-carbonylamino]benzoxazole derivatives **3**–**25** [21, 22]*

Appropriate carboxylic acid (0.5 mmol) and thionyl chloride (1.5 mL) were refluxed in benzene (5 mL) at 80 °C for 3 h. Excess thionyl chloride was removed *in vacuo*. The residue was dissolved in ether (10 mL), and this solution was added during 1 h to a stirred, ice-cold mixture of 5-amino-2-[p-substituted-benzyl]benzoxazoles **1**, **2** (0.5 mmol), sodium bicarbonate (0.5 mmol), diethyl ether (10 mL) and water (10 mL). The mixture was continuously stirred overnight at room temperature and filtered. The precipitate was washed with water, 2 N HCl, again water and finally with ether to give **3**–**25**. The products were recrystallized from ethanol-water as needles which were dried *in vacuo*. The chemical, physical and spectral data of compounds **3**–**25** are reported in Table 1.

Microbiology

For the antibacterial and antimycotic assays, the compounds were dissolved in absolute ethanol (0.8 mg/mL). Further dilutions of the compounds and standard drugs in the test medium were prepared at the required quantities at concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg/mL with Mueller-Hinton broth and Sabouraud dextrose broth. The minimum inhibitory concentrations (MIC) were determined using the twofold serial dilution technique [23, 24]. A control test with inoculated broth supplemented with only ethanol at the same dilutions as used in our experiments was also performed, and this ethanol-supplemented broth was found to be inactive in the culture medium. All the compounds were tested for their *in vitro* growth inhibitory activity against different bacteria and the yeasts *Candida albicans* (ATCC 10145), *Candida krusei* (ATCC 6258), and *Candida glabrate* (isolated). Origins of bacterial strains are *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633) as gram-positive and *Escherichia coli* (ATCC 23556) and *Pseudomonas aeruginosa* (ATCC 10145) as gram-negative bacteria. ATCC 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 the Faculty of Pharmacy of Ankara University.

Ampicillin, amoxycillin, tetracycline, streptomycin, ciprofloksazin, gentamycin, myconazole, clotrimazaole, and haloprogin were used as control drugs. The data on the antimicrobial activity of the compounds and the control drugs as MIC values (μg/mL) are given in Table 2.

Antibacterial and antifungal assay

The cultures were obtained from Mueller-Hinton broth (Difco) for all the bacterial strains after 24 h of incubation at 37 ± 1 °C. *Candida albicans*, *Candida krusei and Candida glabrate* were maintained in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25 ± 1 °C. Testing was carried out in Mueller-Hinton broth and Sabouraud dextrose broth (Difco) at pH 7.4, and the twofold serial dilution technique was applied. The final inoculum size was 10^5 CFU/mL for the antibacterial assay and 10^4 CFU/mL for the antifungal assay. A set of tubes containing only inoculated broth was used as controls. For the antibacterial assay after incubation for 24 h at 37 ± 1 °C and after incubation for 48 h at 25 ± 1 °C for the antifungal assay, the last tube with no growth of microorganism and/or yeast was recorded to represent the MIC expressed in μg/mL. All experiments in the antibacterial and antifungal assays were replicated twice.

References

[1] S. K. Fridkin, R. P. Gaynes, *Clinics in Chest Medicine* **1999**, *20*, 303–316.

[2] T. Hisano, M. Ichikawa, K. Tsumoto, M. Tasaki, *Chem. Pharm. Bull.* **1982**, *30*, 2996–3004.

[3] M. Prudhomme, J. Guyot, G. Jeminet, *J. Antibiotics* **1986**, *39*, 934–937.

[4] S. Ersan, S. Nacak, R. Berkem, T. Özden, *Arzneim. Forsch.* **1997**, *47*, 963–965.

[5] H. M. El-Shaaer, S. A. Abdel-Aziz, H. A. Allimony, R. M. Abdel-Rahman, *Pharmazie* **1997**, *52*, 585–589.

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[6] L. Perrin, A. Rakik, S. Yearly, C. Baumberger, S. Kinlochde Loies, M. Pechiere, B. Hirschel, *AIDS* **1996**, *10*, 1233−1237.

[7] C. Hubschwerlen, P. Pflieger, J. L. Specklin, K. Gubernator, H. Gmunder, P. Angehrn, I. Kompis, *J. Med. Chem.* **1992**, *35*, 1385−1388.

[8] D. F. Shi, T. D. Bradshaw, S. Wrigley, C. J. McCall, P. Lelieveld, I. Fichtner, M. F. G. Stevens, *J. Med. Chem.* **1996**, *39*, 3375−3384.

[9] M. B. Reynolds, M. DeLuca, S. Kerwin, *Bioorg. Chem.* **1999**, 27, 326−337.

[10] Z. M. Nofal, M. El-Zahar, S. S.Abd El-Karim, *Molecules* **2000**, *5*, 99−153.

[11] S. Sato, T. Kajiura, M. Noguchi, K. Takehana, T. Kobayashi, T. Tsuji, *J. Antibiotics* **2001**, *54*, 102−104.

[12] E. Şener, İ. Yalçın, E. Sungur, *Quant. Struc. Act. Relat.* **1991**, *10*, 223−228.

[13] E. Şener, İ. Yalçın, Ö. Temiz, İ. Ören, A. Akın, N. Uçartürk, *Farmaco* **1996**, *52*, 99−103.

[14] İ. Ören, Ö. Temiz, İ. Yalçın, E. Şener, A. Akın, N. Uçartürk, *Arzneim. Forsch.* **1997**, *47*, 1393−1397.

[15] Ö. Temiz, İ. Ören, E. Şener, İ. Yalçın, N. Uçartürk, *Farmaco* **1998**, *53*, 337−341.

[16] İ. Yalçın, İ. Ören, E. Şener, A. Akın, N. Uçartürk, *Eur. J. Med. Chem.* **1992**, *27*, 401−406.

[17] I. Hutchinson, S. A. Jennings, B. R. Vishnuvajjala, A. D. Westwell, M. F. G. Stevens, *J. Med. Chem.* **2002**, *45*, 744−747.

[18] M. R. Peel, M. W. Milstead, D. D. Sternbach, M. Besterman, P. Leitner, B. Morton, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2129−2132.

[19] E. A. Sener, Ö. T. Arpaci, I. Yalcin, N. Altanlar, *Farmaco* **2000**, *55*, 397−405.

[20] Ö. T. Arpaci, E. A. Sener, I. Yalcin, N. Altanlar, *Arch. Pharm. Pharm. Med. Chem.* **2002**, *6*, 283−288.

[21] İ. Yalçın, B. K. Kaymakcıoğlu, İ. Ören, E. Şener, Ö. Temiz, A. Akın, N. Altanlar, *Farmaco* **1997**, *52*, 685−689.

[22] L. E. Totton, C. L. Raiford, *J. Am. Chem. Soc.* **1954,** *76*, 5127−5130.

[23] E. S. Charles, V. K. Agrawal, S. Sharma, R. N. Iyer, *Eur. J. Med. Chem. Chim. Ther.* **1979**, *14*, 435−438.

[24] S. Shadomy, A. Espinel, In: *Manual of Clinical Microbiology.* Am. Soc. Microbiol., Washington DC, **1980,** p. 647.

© 2004 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim