ELSEVIER

Il Farmaco 57 (2002) 771–775

IL FARMACO

www.elsevier.com/locate/farmac

Short Communication

Synthesis and microbiological activity of some novel *N*-[2-(*p*-substitutedphenyl)-5-benzoxazolyl]-cyclohexyl carboxamide, -cyclohexyl acetamide and -cyclohexyl propionamide derivatives

Özlem Temiz Arpacı [a], Esin Akı Şener [a,*], İsmail Yalçın [a], Nurten Altanlar [b]

[a] *Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, TR-06100 Tandogan, Ankara, Turkey*
[b] *Ankara University, Faculty of Pharmacy Department of Microbiology, TR-06100 Tandogan, Ankara, Turkey*

Received 18 February 2002; accepted 11 May 2002

Abstract

The synthesis and microbiological activity of a new series of *N*-[2-(*p*-substitutedphenyl)-5-benzoxazolyl]-cyclohexyl carboxamide, -cyclohexyl acetamide and -cyclohexyl propionamide derivatives (**4**–**11**) is described. The in vitro microbiological activity of the compounds was determined against Gram-positive, Gram-negative bacteria and the yeast *Candida albicans* in comparison with standard drugs. Microbiological results indicated that the synthesized compounds possessed a broad spectrum of activity against the tested microorganisms.

Keywords: 5-Amide-substituted benzoxazoles; Antibacterial activity; Antifungal activity

1. Introduction

The usage of most antimicrobial agents is limited, not only by the rapidly developing drug resistance, but also by the unsatisfactory status of present treatments of microbial infections and by drug side effects [1–8]. Therefore, the development of new and different antimicrobial drugs is a very important objective and much of the research program efforts are directed toward the design of new agents.

Considering the situation we were encouraged to undertake research on the microbiological activity of some 2,5-disubstituted benzoxazoles and we recently reported the synthesis and microbiological study of a series of 5-benzamido- and 5-phenylacetamido-, 5-phenylpropionamido-, 5-(*p*-substitutedphenyl)oxyacetamido- and 5-phenylthioacetamido-2-(*p*-substitutedphenyl)benzoxazole derivatives (Fig. 1). Among the synthesized compounds, 5-benzamido-, 5-(4-propyloxyphenyl)acetamido- and 5-(2-chlorophenyl)acetamido-2-phenylbenzoxazoles were found active against *Pseudomonas aeruginosa* showing MIC values as low as 25 μg/ml^{-1} [9,10].

In the present study, some novel *N*-[2-(*p*-substitutedphenyl)-5-benzoxazolyl]-cyclohexyl carboxamide, -cyclohexyl acetamide and -cyclohexyl propionamide derivatives have been synthesized in order to examine their in vitro antimicrobial activity against different Gram-positive, Gram-negative bacteria and *C. albicans* as a yeast in comparison with several control drugs and previously synthesized analogs.

The synthesis of compounds **4**–**11** was performed in two steps as shown in Scheme 1. In the first step, 5-amino-2-phenyl- or 5-amino-2-(*p*-ethylphenyl)- or 5-amino-2-(*p*-flourophenyl)-benzoxazoles (**1**–**3**) were obtained by heating benzoic acid or *p*-ethylbenzoic acid or *p*-fluorobenzoic acid with 2,4-diaminophenol in polyphosphoric acid (PPA) as the cyclodehydration reagent [9].

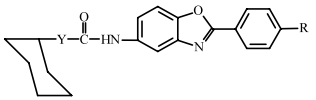
In the second step, the compounds (**4**–**11**) were prepared by reacting 5-amino-2-phenyl- or 5-amino-2-*p*-ethylphenyl)- or 5-amino-2-(*p*-flourophenyl)benzoxazoles with appropriate carboxylic acid chlorides [9,11].

The structures of the synthesized compounds **4**–**11** were supported by spectral data and the IR, ^{1}H NMR spectra are in agreement with the proposed structures.

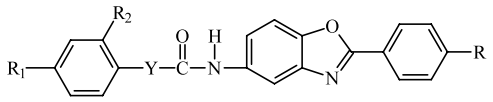
* Corresponding author
E-mail address: sener@pharmacy.ankara.edu.tr (E.A. Şener).

0014-827X/02/$ - see front matter
PII: S0014-827X(02)01278-8

Table 1
Physical properties and spectral data of the compounds **4**–**11**



Comp. no.	Y	R	m.p. (°C)	Yield (%)	Empirical formula	IR (cm^{-1})	^1H NMR δ ppm (J = Hz)
4	–	F	180	17	$C_{20}H_{19}O_2N_2F$	3273, 2920, 1640, 1606, 1563, 1499, 1265, 1050	8.26–8.23 (2H, dd, J = 8.06, J' = 2.73), 7.91–7.90 (1H, d, J = 1.85), 7.56–7.36 (2H, m), 7.24–7.20 (2H, dd, J = 2.06, J' = 8.69), 2.03–1.30 (11H, m)
5	–	H	188	13	$C_{20}H_{20}O_2N_2$	3273, 2920, 1640, 1524, 1499, 1225, 1050	8.26–8.23 (2H, dd, J = 8.79, J' = 2.08), 7.91–7.90 (1H, d, J = 1.70), 7.58–7.38 (3H, m), 7.28–7.20 (2H, dd, J = 8.62, J' = 1.90), 2.29–1.32 (11H, m)
6	–	C_2H_5	185	35	$C_{22}H_{24}O_2N_2$	3294, 6065, 2931, 1655, 1536, 1620, 1480, 1259	8.18–8.16 (2H, d, J = 8.24), 7.88–7.87 (1H, d, J = 1.79), 7.60–7.49 (2H, m), 7.38–7.28 (2H, d, J = 8.21), 2.78–2.72 (2H, q, J = 7.61), 2.29–1.37 (11H, m), 1.33–1.29 (3H, t, J = 7.61)
7	$-CH_2-$	F	192	38	$C_{21}H_{21}O_2N_2F$	3360, 3054, 2923, 1667, 1609, 1567, 1482, 1234, 1054	8.27–8.24 (2H, dd, J = 8.86, J' = 2.05), 7.92–7.91 (1H, d, J = 1.69), 7.55–7.50 (2H, dd, J = 8.68, J' = 2.06), 7.28–7.21 (2H, m), 2.29–2.27 (2H, d, J = 7.08), 1.87–1.32 (11H)
8	$-CH_2-$	C_2H_5	187	26	$C_{23}H_{26}O_2N_2$	3295, 2917, 1651, 1620, 1536, 1482, 1259, 1059	8.17–8.15 (2H, d, J = 8.23), 7.88–7.89 (1H, d, J = 1.81), 7.56–7.49 (2H, m), 7.37–7.35 (2H, d, J = 8.11), 2.76–2.74 (2H, q, J = 7.60), 2.28–2.26 (2H, d, J = 7.10), 2.19–1.29 (14H, m)
9	$-CH_2CH_2-$	F	163	33	$C_{22}H_{23}O_2N_2F$	3491, 3082, 2925, 1667, 1620, 1562, 1481, 1278, 1065	8.26–8.23 (2H, dd, $J = J'$ = 5.42), 7.91–7.92 (1H, d, J = 1.28), 7.56–7.49 (2H, m), 7.24–7.20 (dd, 2H, $J = J'$ = 8.64), 2.44–2.40 (2H, t, J = 7.79), 1.78–1.21 (13H, m)
10	$-CH_2CH_2-$	H	156	22	$C_{22}H_{24}O_2N_2F$	3492, 2926, 1667, 1605, 1562, 1482, 1278, 1066	8.27–8.23 (2H, dd, J = 8.86, J' = 2.05), 7.91–7.92 (1H, d, J = 1.58), 7.56–7.28 (3H, m), 7.25–7.20 (2H, dd, $J = J'$ = 8.65, 2.44–2.40 (2H, t, J = 8.21), 1.78–1.19 (13H, m)
11	$-CH_2CH_2-$	C_2H_5	144	32	$C_{24}H_{28}O_2N_2$	3465, 2920, 1656, 1619, 1561, 1482, 1261, 1058	8.17–8.15 (2H, d, J = 8.17), 7.88–7.89 (1H, d, J = 1.52), 7.55–7.44 (2H, m), 7.37–7.35 (2H, d, J = 8.17), 2.76–2.74 (2H, q, J = 7.59), 2.44–2.40 (2H, t, J = 7.77, 1.77–1.23 (16H, m)



Y ; —, -CH_2-, -SCH_2-, OCH_2-, - CH_2CH_2-,

R ; -H, -C_2H_5, F

R_1 ; -H, -F, -Cl, -Br, -OCH_3, -CH_3, -C_2H_5, -NO_2, -$C(CH_3)_3$, -OC_3H_7

R_2 ; -H, -Cl, -OCH_3, -CH_3

Fig. 1.

Physical and spectral data of the compounds are reported in Table 1.

2. Experimental

2.1. Chemistry

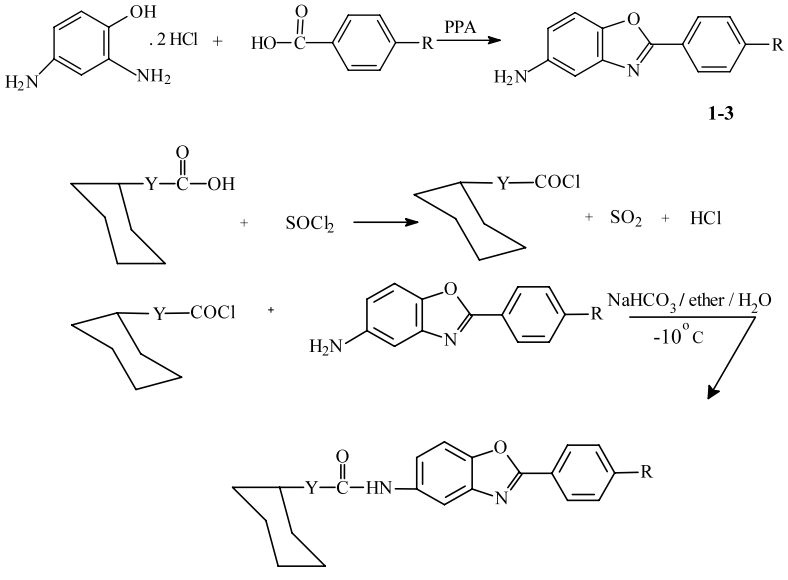
Silica gel HF_{254} chromatoplates (0.3 mm) were used for TLC and the solvent systems were chloroform:-methanol (15:0.5) for compounds **4**–**11**. All the melting points were taken on a Buchi SMP 20 capillary apparatus and are uncorrected. IR spectra were recorded by FT/IR-420 with KBr discs. ^{1}H NMR spectra were obtained with a Bruker 400 MHz spectrometer in chloroform-d_6 and tetramethylsilan (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 analyses (C, H, N) were within $\pm$0.4% of the calculated amounts.

2.2. General procedure for the synthesis of 5-amino-2-(p-substitutedpheny)benzoxazole derivatives (1–4)

5-Amino-2-(*p*-substitutedphenyl)benzoxazole derivatives were synthesized by heating 0.01 mol 2,4-diaminophenol·2HCl with 0.01 mol *p*-substituted benzoic acid in 24 g PPA and stirring for 2.5 h. At the end of the reaction period, the residue was poured into ice-water mixture and neutralized with excess of % 10 NaOH solution extracted with benzene, the benzene 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 the solvent in vacuo, the crude product was obtained and recrystallized.

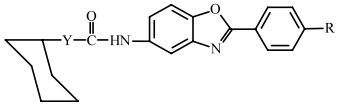
2.3. General procedure for amide derivatives (4–11)

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 then removed in vacuo. The residue was dissolved in ether (10 ml) and solution added during 1 h to a stirred, ice-cold mixture of 5-amino-2-(*p*-substituted-phenyl)benzoxazoles (0.5 mmol), sodium bicarbonate (0.5 mmol), diethyl ether (10 ml) and water (10 ml). The mixture was kept stirred overnight at room temperature and filtered. The precipitate was washed with water, 2 N HCl and water, respectively, and finally with ether to give **4**–**11**. The products were recrystallized from ethanol–water mixture and needles were dried in vacuo. The chemical, physical and spectral data of compounds **4**–**11** are reported in Table 1.



Scheme 1.

Table 2
The in vitro antimicrobial activity of the compounds **4**–**11** and the control drugs (MIC in μg ml^{-1})



Comp. No.	Sa	Sf	Bs	Ec	Ca
4	100	12.5	50	50	12.5
5	50	12.5	50	50	25
6	50	12.5	50	100	50
7	50	12.5	25	50	25
8	50	12.5	50	25	25
9	50	12.5	50	25	50
10	50	12.5	50	25	25
11	50	25	50	50	50
Ampicillin	1.56	1.56	1.56	12.5	–
Amoxycillin	1.56	1.56	1.56	3.12	–
Tetracycline	1.56	1.56	1.56	3.12	–
Streptomycin	3.12	100	50	1.56	–
Clotrimazole	–	–	–	–	6.2
Haloprogin	–	–	–	–	3.1

Sa, *Staphylococcus aureus*; Ec, *Escherichia coli*; Sf, *Streptococcus faecalis*; Bs, *Bacillus subtilis*, Ca, *Candida albicans*.

2.4. Microbiology

The compounds were dissolved in absolute ethanol (0.8 mg/ml^{-1}) for both the antibacterial and antimycotic assays. 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^{-1} concentrations with Mueller–Hinton broth and Sabouraud dextrose broth. The minimum inhibitory concentrations (MIC) were determined using the method of two-fold serial dilution technique [12,13]. 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. Origin of bacterial strains are *Staphylococcus aureus* ATCC 6538, *Streptococcus faecalis* ATCC 10541 and *Bacillus subtilis* ATCC 6033 as Gram-positive and *Escherichia coli* ATCC 10536 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.

2.5. 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. The yeast *C. albicans* was 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 two-fold serial dilution technique was applied. The final inoculum size was 10^5 CFU ml^{-1} for the antibacterial assay and 10^4 CFU ml^{-1} for the antifungal assay. A set of tubes containing only inoculated broth was kept 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^{-1}. Every experiment in the antibacterial and antifungal assays was replicated twice in order to define the MIC values.

Ampicillin, amoxycillin, tetracycline, streptomycin, ketoconazole and fluconazole 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. Results and discussion

The chemical, physical and spectral data of the synthesized compounds **4**–**11** are reported in Table 1. The antimicrobial activity of the compounds was investigated against *S. aureus*, *S. faecalis*, *B. subtilis* as Gram-positive, *E. coli* as Gram-negative bacteria strains and the yeast *C. albicans* using two-fold serial dilution technique in comparison to control drugs and the results reported at Table 2.

The synthesized compounds showed some antibacterial activity against the Gram-positive bacteria such as *S. aureus* and *B. subtilis* possessing MIC values 25–100 μg/ml^{-1}. All the compounds showed weak to moderate antibacterial activity against *S. faecalis* possessing MIC values in the range 12.5–25 μg/ml^{-1}.

The compounds **4**–**11** were also tested against *C. albicans* for their antimycotic activity and most of the compounds indicated significant antimycotic activity (MIC values around 12.5–50 μg/ml^{-1}), but generally inferior to that of antimycotic potencies of clotrimazole and haloprogin (MIC values of 6.2 and 3.1 μg ml^{-1}, respectively).

Finally, we compared the antimicrobial activity of synthesized benzamide and phenylacetamide derivatives [11] with their cyclohexyl analogues **4**–**11**. It appears that benzamide and phenylacetamide derivatives showed same or better antifungal and antibacterial activities against *C. albicans*, *S. aureus*, *B. subtilis* and

E. coli than the corresponding cyclohexyl analogues **4**–**11**. Although, compounds **4**–**10** exhibited a somewhat higher potency against *S. faecalis* than the corresponding synthesized benzamide and phenylacetamide derivatives.

Acknowledgements

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

References

[1] C. Hubschwerlen, P. Pflieger, J.L. Specklin, K. Gubernator, H. Gmunder, P. Angehrn, I. Kompis, Pyrimido[1,6-*a*]benzimidazoles: A new class of DNA gyrase inhibitors, J. Med. Chem. 35 (1992) 1385–1392.

[2] Ö. Temiz, İ. Ören, E. Şener, İ. Yalçın, N. Uçartürk, Synthesis and microbiological activity of some novel 5- or 6-methyl-2-(2,4-disubstitutedphenyl)benzoxazole derivatives, Farmaco 53 (1998) 337–341.

[3] İ. Ören, Ö. Temiz, İ. Yalçın, E. Şener, N. Altanlar, Synthesis and antimicrobial activity of some novel 2,5- and/or 6-substituted benzoxazole and benzimidazole derivatives, Eur. J. Pharm. Sci. 7 (1998) 153–160.

[4] C.Y. Hong, Discovery of gemifloxacin: a quinolone of new a generation, Farmaco 56 (2001) 41–44.

[5] M. Takemura, I. Hayakawa, Recent advances in the field of quinolones, Farmaco 56 (2001) 37–40.

[6] A. Carta, P. Sanna, L. Gherardini, D. Usai, S. Zanetti, Novel functionalized pyrido[2,3-*g*]quinoxalinones as antibacterial, antifungal and anticancer agents, Farmaco 56 (2001) 933–938.

[7] D.P. Fidler, Legal issues associated with antimicrobial drug resistance, Emerging Infectious Diseases 4 (1998) 1–11.

[8] Y. Arakawa, Y. Ike, M. Nagasawa, N. Shibata, Y. Doi, K. Shibayama, T. Yagi, T. Kurata, Emerging Infectious Diseases 6 (2000) 1–7.

[9] E.A. Sener, O.T. Arpaci, I. Yalcin, N. Altanlar, Synthesis and microbiological activity of some novel 5-benzamido- and 5-phenylacetamido- substituted 2-phenylbenzoxazole derivatives, Farmaco 55 (2000) 397–405.

[10] Ö. Temiz-Arpacı, İ. Ören, N. Altanlar, Synthesis and antimicrobial activity of some novel 2-(*p*-substituted-phenyl)-5-substituted-carbonylaminobenzoxazoles, Farmaco (2002) 57 (2002) 175–181.

[11] İ. Yalçın, B.K. Kaymakçıoğlu, İ. Ören, E. Şener, Ö. Temiz, A. Akın, N. Altanlar, Synthesis and microbiological activity of some novel *N*-(2-hydroxyl-5-substitutedphenyl)benzacetamides, phenoxyacetamides and thiophenoxyacetamides as the possible metabolites of antimicrobial active benzoxazoles, Farmaco 52 (1997) 685–689.

[12] E.S. Charles, V.K. Agrawal, S. Sharma, R.N. Iyer, Synthesis of 2,5-disubstituted benzimidazoles as potential antihookworm and antimicrobial agents, Eur. J. Med. Chem., Chim. Ther. 14 (1979) 435–438.

[13] S. Shadomy, A. Espinel, Manual of Clinical Microbiology, American Society Microbiology, Washington, DC, 1980, p. 647.