IL FARMACO, 52 (11), 685-689 (1997)

SYNTHESIS AND MICROBIOLOGICAL ACTIVITY OF SOME NOVEL N-(2-HYDROXYL-5-SUBSTITUTEDPHENYL)BENZACETAMIDES, PHENOXYACETAMIDES AND THIOPHENOXYACETAMIDES AS THE POSSIBLE METABOLITES OF ANTIMICROBIAL ACTIVE BENZOXAZOLES

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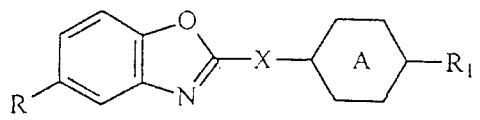
Summary – Synthesis of some novel N-(2-hydroxyl-5-substitutedphenyl)benzacetamides, phenoxyacetamides and thiophenoxyacetamides (**5a-k**) were described in order to determine their *in vitro* antimicrobial activity against 3 Gram-positive, 3 Gram-negative bacteria and the fungus *Candida albicans* comparing with several control drugs. The derivative **5e** was found active at a MIC value of 25μg/ml against the whole tested Gram-positive bacteria strains and the Gram-negative microorganism *Klebsiella pneumoniae*. Moreover, the synthesized compounds **5a-k** exhibited significant antibacterial activity against the enterobacter *Pseudomonas aureginosae* when compared to the control drugs. For the antifungal avtivity against *C. albicans*, the compound **5k** was found more active than the other synthesized derivatives. On the other hand, the antimicrobial activity of some of these acetamide derivatives (**5c, 5d, 5e, 5j** and **5k**) which are the possible metabolites of benzoxazoles, were also compared with their cyclic analogues **6-10.** However, most of the MIC values of the benzoxazole derivatives provided better activity than the compared acetamides, while some others of the acetamide derivatives possessed either one fold improved (**5d, 5e** and **5j**) or the same potency (**5c, 5d, 5e, 5j** and **5k**) against the tested microorganisms.

INTRODUCTION

In our previous studies, we reported the synthesis and the antimicrobial activity of various 2,5-disubstituted benzoxazoles as given in general formula I, providing significant *in vitro* antibacterial activity especially against some enteric Gram-negative rods such as *Klebsiella pneumoniae, Pseudomonas aeruginosae* and the yeast *Candida albicans*[1-6].

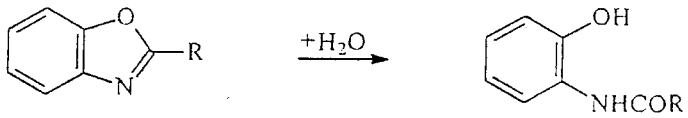


R = H, Cl, NO_2, NH_2, CH_3
R_1 = H, CH_3, C_2H_5, F, Br, Cl, $NHCH_3$, NO_2, NH_2, $C(CH_3)_3$, $NHCOCH_3$, $N(CH_3)_2$, OCH_3
X = CH_2, C_2H_4
A = Phenyl, Cyclohexyl

I

A review of the literature revealed that one of the aspects of the Phase I metabolism pathways of benzoxazoles and 2-methylbenzoxazoles in the rabbit involved rupture of the oxazole ring occurring at the (C–O) linkage of the fused heterocyclic system by mild hydrolysis and produced *o*-formamidophenol and *o*-acetamidophenol as their metabolites respectively[7]. This could be represented as shown in Scheme I, omitting the intermediate stages.

SCHEME I



R= H, CH_3

On the other side, since the discovery of oxyclozanide (**1**) which is an antihelmentic agent effective against *Fasciola hepatica* for the treatment of liver fluke infection[8], there were few amounts of published data on the antibacterial and antifungal activities of the corresponding acetamides. Shioyama *et al.* reported the bactericide and fungicide activity of the N-(2-hydroxylphenyl)phenoxyacetamide (**2**) on rice, with no phytotoxicity[9] and Hirakawa *et al.* noted the protective effect of the N-(n-butyl)-N-(2-hydroxylphenyl)-4'-isopropilbenzamide (**3**) on rice plants against *Pyricularia oryzae*[10]. White[11] synthe-

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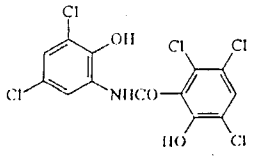
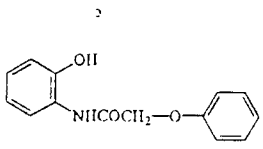
sized the N-(2-hydroxylphenyl)-2'-methylbenzamide (**4**) and found the inhibitory effect on Complex II activity in mitochondria from sporidia of wildtype and carboxin-selected mutant strains of *Ustilago maydis*.



1



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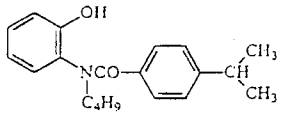

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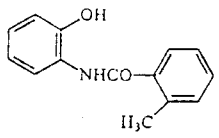


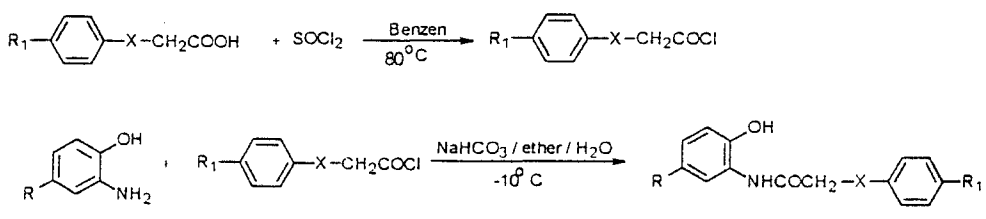
4

In the present paper, some novel N-(2-hydroxyl-5-substitutedphenyl)benzacetamides, phenoxyacetamides and thiophenoxyacetamides (**5a-k**) were synthesized and the *in vitro* antimicrobial activity described with comparing to their cyclic analogues, benzoxazoles (**6-10**), assuming that the synthesized acetamides would be the possible metabolites of these heterocyclic compounds.

CHEMISTRY

The synthesis of the compounds **5a-k** was performed by reacting 4-substituted-2-aminophenols with appropriate carboxylic acid chlorides obtained by treating carboxylic acids with thionyl chloride.

SCHEME II



5a - k

X= - , O, S
R = H, Cl, CH_3
R_1 = H, Cl

Compounds **5a-k** were prepared as new products except **5i**[12]. The structures of all the derivatives **5a-k** were supported by spectral data. The IR and ^1H NMR spectra are in agreement with the proposed structures. Physical and spectral data of the compounds are reported in Table I.

EXPERIMENTAL

A) CHEMISTRY

Kieselgel HF_{254} chromatoplates (0.3 mm) were used for TLC and the solvent systems were chloroform: n-hexane (20:2.5) for **5b-5d, 5f-5i, 5k**, chloroform: n-hexane (20:0.8) for **5a, 5j**, chloroform (20) for **5e**. All the melting points were taken on a Buchi SMP 20 capillary apparatus and are uncorrected. IR spectra were recorded by Perkin Elmer 1330 and Pye Unicam SP-1025 with KBr discs. ^1H NMR spectra were obtained with a Bruker GMBH-DPX 400 MHz spectrometer in d_6-dimethylsulfoxide and TMS was used as an internal standard. Elemental analysis were carried out with a Hewlett Packard 185 CHN Analyzer. The results of the elemental analysis (C, H, N) were within ± 0.4% of the calculated amounts.

General procedure for the synthesis of N-(2-hydroxy-5-substitutedphenyl)benzenacetamides, phenoxyacetamides and thiophenoxyacetamides **(5a-k)**

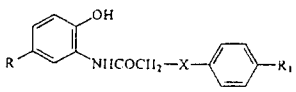
Appropriate acetic acid (5/10 mmol) and thionyl chloride (1.5 ml) were refluxed in benzene (5 ml) at 80 °C for 3 hr. Excess thionyl chloride was then removed *in vacuo*. The residue was dissolved in ether (10 ml) and the solution added during 1hr. to a stirred, ice-cooled mixture of 4-substituted-2-aminophenol (5/10 mmol), sodium bicarbonate (10/20 mmol), ether (10 ml) and water (10 ml). The mixture was kept stirred overnight at room temperature and filtered. The precipitate was washed with water, 2N HCl, again with water and finally with ether to give **5a-k**. The product was recrystallized from aceton-methanol mixture and needles was dried *in vacuo*.

B) MICROBIOLOGY

For both the antibacterial and the antimycotic assays, the compounds **5a-k** and **6-10** were dissolved in absolute ethanol (0.8 mg/ml)[13]. 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 (MIC) were determined using the method of two-fold serial dilution technique[1,13,14]. 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 a fungus *Candida 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, *Klebsiella pneumoniae NTCC* 52211, and *Pseudomonas 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

TABLE I - Physical properties, preparation and spectral data of the compounds **5a-k**



Comp.	X	R	R_1	m.p. (°C)	Yield (%)	IR (cm^{-1})	^1H NMR δ ppm (J = Hz)
5a	-	Cl	H	168	22.9	3180, 3060, 2830-2900, 1680, 1570-1315,1325-1055, 1180, 935-710	10.20(s,1H), 9.30 (s,1H), 8.00 (s,1H), 7.20-7.40 (m,5H), 6.90 (d, 1H,J=8.54), 6.80 (d,1H,J=8.76), 3.80 (s, 2H)
5b	-	H	H	145-148	40.5	3380, 3040, 2840-2900, 1640, 1585-1400,1350-1035, 960-695	9.75(s,1H), 9.30 (s,1H), 7.80 (d,1H), 7.20-7.40 (m,5H), 6.95 (t, 1H, J=7.50), 6.85 (d, 1H,J=7.94), 6.75 (t,1H,J=7.56), 3.75 (s, 2H)
5c	-	CH_3	H	146	33.0	3300, 3080, 2720-2900, 1630, 1570-1420,1360-1020, 965-695	9.50(s,1H), 9.25 (s,1H), 7.58 (s,1H), 7.20-7.40 (m,5H), 6.70 (dd, 2H), 3.75 (s, 2H), 2.25 (s,3H)
5d	O	Cl	Cl	183	41.6	3410, 3120, 1660, 1600-1495,1370-1020, 1100-1080, 880-660	10.30(s,1H), 9.20 (s,1H), 8.20 (s,1H), 7.30-7.42 (dd,2H,J=8.77), 7.03-7.10 (dd,2H, J=8.59), 7.00 (dd, 1H), 6.89 (d, 1H), 4.90 (s, 2H)
5e	O	H	Cl	164-165	43.2	3360, 3220, 1660, 1590-1435,1350-1030, 1100, 930-685	9.90(s,1H), 9.20 (s,1H), 7.90 (d,1H,J=7.87), 7.32-7.40 (dd,2H,J=8.81), 7.02-7.10(d,2H J=8.75), 6.94 (t, 1H, J=7.40), 6.90 (d,1H,J=7.37), 6.80(t,1H,J=7.51), 4.75 (s, 2H)
5f	O	Cl	H	193-194	54.0	3385, 3090, 1660, 1590-1430,1360-1030, 1080, 920-685	10.35(s,1H), 9.20 (s,1H), 8.10 (s,1H), 7.00-7.40 (m,5H), 6.90 (d, 1H, J=10.92), 6.89 (d 1H,J=8.36), 4.80 (s, 2H)
5g	O	CH_3	Cl	158-160	34.3	3380, 3120, 2720-2920, 1660, 1600-1445 1365-1010, 1120, 980-665	9.66(s,1H), 9.10 (s,1H), 7.80 (s,1H), 7.34-7.41 (d,2H,J=7.98),7.01-7.10 (d,2H,J=9.05), 6.76 (dd, 2H,J=8.29), 4.75 (s, 2H), 2.20(s,3H)
5h	O	CH_3	H	158-159	46.6	3380, 3080, 2720-2920,1660, 1600-1495, 1380-1055, 875-680	9.72(s,1H), 9.10 (s,1H), 7.85 (s,1H), 7.34 (t,2H,J=7.41),7.03(m,3H), 6.78 (dd, 2H, J=8.09), 4.70 (s, 2H), 2.20 (s, 3H)
5i	O	H	H	142-144	60.0	3395, 3290, 1655, 1580-1430,1345-1035, 970-670	10.00(s,1H), 9.10 (s,1H), 8.00 (d,1H,J=7.83), 7.34 (t,2H),7.04(m,3H), 6.95 (dd, 1H, J=7.00), 6.89 (d, 1H,J=7.54), 6.79(t,1H,J=7.59), 4.75 (s, 2H)
5j	S	Cl	H	166-168	23.8	3300, 3080, 1640, 1580-1420,1370-1020, 1120, 915-645	10.25(s,1H), 9.40 (s,1H), 8.00 (s,1H), 7.42 (d,2H,J=7.84),7.33(t,2H, J=7.58), 7.21(t, 1H,J=7.24), 6.96 (dd, 1H,Jo=6.58,Jm=1.76), 6.88 (d, 1H,J=8.94), 4. (s, 2H)
5k	S	CH_3	H	133	21.9	3300, 3100, 2860-2900, 1640, 1595-1500 1305-1115, 970-685	9.60(s,1H), 9.35 (s,1H), 7.80 (s,1H), 7.42 (d,2H,J=7.82),7.33(t,2H, J=7.62), 7.21(t,1H,J=7.25), 6.75 (dd, 2H,Jo=8.22,Jm=2.05), 3.86 (s, 2H), 2.25 (s, 3H)

Institution of Health Ministry, Ankara and maintained at the Microbiology Department of Faculty of Pharmacy of Ankara University.

Ampicillin, amoxycillin, tetracycline, gentamycin, 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 II.

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 microorganism was recorded to represent MIC expressed in μg/ml.

Antimycotic assay

The yeast *Candida 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.

TABLE II - The *in vitro* antimicrobial activity of the compounds **5a-k** and the standard drugs (MIC in μg/ml)

Comp.	Microorganisms[a]						
	Gram-positive			Gram-negative			Fungus
	Sa	*Sf*	*Bs*	*Ec*	*Kp*	*Pa*	*Ca*
5a	50	50	50	50	50	50	50
5b	50	50	50	50	50	50	50
5c	100	50	50	50	50	50	50
5d	25	50	25	100	100	50	100
5e	25	25	25	50	25	50	50
5f	50	100	25	50	50	50	50
5g	50	100	50	50	50	50	100
5h	100	50	50	50	25	50	50
5i	50	50	50	50	50	50	50
5j	25	100	50	50	50	50	50
5k	50	50	50	50	50	50	25
Ampicillin	0.78	0.78	0.78	3.12	12.5	>200	-
Amoxycillin	0.78	0.78	0.78	3.12	12.5	>200	-
Tetracycline	0.78	0.78	0.78	3.12	3.12	50	-
Gentamycin	0.78	12.5	0.78	3.12	1.56	12.5	-
Streptomycin	3.12	100	50	1.56	1.56	100	-
Oxiconazole	-	-		-	-	-	6.25
Haloprogin	-	-		-	-	-	6.25

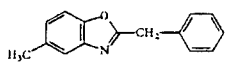
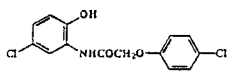
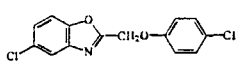
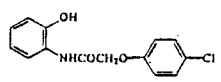
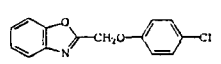
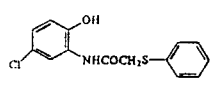
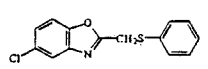
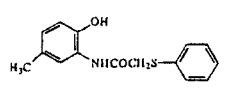
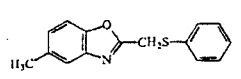
([a]) Abbreviations; *Sa, Staphylococcus aureus; Sf, Streptococcus faecalis; Bs, Bacillus subtilis; Ec, Escherichia coli; Kp, Klebsiella pneumoniae; Pa, Pseudomonas aeruginosa; Ca, Candida albicans.*

RESULTS AND DISCUSSION

The synthesized compounds **5a-k** were screened against three Gram-positive, three Gram-negative bacteria strains and the yeast *C. albicans* using two fold serial dilution technique. The results reported in Table II indicate that compounds **5a-k** exhibited some antibacterial activity against the screened Gram-positive bacteria *S. aureus, S. faecalis* and *B. subtilis*, lower than the compared control drugs ampicillin, amoxycillin and tetracycline, showing MIC values between 100-25μg/ml. Among the synthesized compounds, N-(2-hydroxylphenyl)-4'-chlorophenoxyacetamide (**5e**) was the only derivative that possessed an activity of 25μg/ml MIC value against the tested Gram-positive bacteria strains.

The activity of the compounds **5a-k** were also tested against *E. coli, K. pneumoniae* and *P. aeruginosae* as Gram-negative bacteria. The antibacte-

TABLE III - Comparison of the antimicrobial activity of the synthesized acetamides **5c, 5d, 5e, 5j** and **5k** with their cyclic analogues **6-10** (MIC in μg/ml)

Comp. No:	Comp.	Microorganisms[a]						
		Gram-positive			Gram-negative			Fungus
		Su	*Sf*	*Bs*	*Ec*	*Kp*	*Pa*	*Ca*
5c		100	50	50	50	50	50	50
6		12.5	50	12.5	50	12.5	25	12.5
5d		25	50	25	100	100	50	100
7		50	50	25	50	25	25	50
5e		25	25	25	50	25	50	50
8		50	50	50	50	50	50	25
5j		25	100	50	50	50	50	50
9		50	50	6.25	50	25	50	12.5
5k		50	50	50	50	50	50	25
10		25	25	25	25	25	25	25

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

rial activity of these compounds against *E. coli* and *K. pneumoniae* exhibited lower potency than the compared control drugs tetracycline, gentamycine and streptomycin. The compounds, N-(2-hydroxylphenyl)-4'-chlorophenoxyacetamide (**5e**) and N-(2-hydroxyl-5-methylphenyl)phenoxyacetamide (**5h**) were found more active than the other synthesized derivatives, having MIC values of 25μg/ml against *K. pneumoniae*.

For the antibacterial activity against the enterobacter *P. aeruginosae*, the synthesized compounds **5a-k** indicated significant activity with 50μg/ml MIC values and possessed better potency than the compared control drug streptomycin, but showing one fold less activity than gentamycine.

Moreover, the antifungal activity of the synthesized compounds were tested against the yeast *C. albicans* and exhibited MIC values between 100-25μg/ml. However, N-(2-hydroxyl-5-methylphenyl) thiophenoxyacetamide (**5k**) was found more active than the other synthesized compounds, showing 25μg/ml MIC value; however, the control drugs oxiconazole and haloprogin possessed one dilution better potency.

On the other side, we compared the antimicrobial activities of the synthesized acetamide derivatives **5c, 5d, 5e, 5j** and **5k** to their heterocyclic analogues **6-10**, given in Table III, assuming that they are the possible metabolites of benzoxazoles. The compared benzoxazoles **6-10** have been recently synthesized in our laboratories[15].

Table III reveals that although most of the MIC values of the benzoxazole derivatives (**6-10**) provided better antimicrobial activity than the com-

pared acetamides, some of the acetamide derivatives possessed either same or one fold improved potency (**5d, 5e** and **5j**). For instance, the compound **5e** indicated one dilution better antibacterial activity than the compared benzoxazole derivative **8** against the tested Gram-positive bacteria strains and *K. pneumoniae*. For the antifungal activity, all the benzoxazoles were found more active than their possible metabolite amide forms except **5k,** which is the only compound showing the same potency of its cyclic analogue **10** against *C. albicans*.

In conclusion, if the synthesized acetamide derivatives are the possible opened hydrolyzed metabolites of these cyclized compounds in the organism, then, prolonged antimicrobial efficacy could be expected for benzoxazoles against the screened microorganisms.

We would like to thank The Turkish Scientific and Technical Research Association (TUBITAK) for the financial support (Grant No: SBAG-AYD-120) in this research.

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Received April 7, 1997; accepted June 30, 1997