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Synthesis and antimicrobial activity of some new 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones

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Abstract

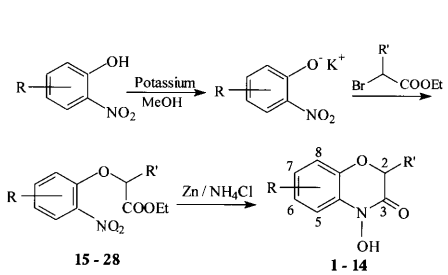
Some 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones were synthesized and evaluated for their antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. Compounds **9**, and **10** exhibited the best activity against *Candida albicans*.

Keywords: 4-Hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one; Antibacterial and antifungal activity

1. Introduction

Cyclic hydroxamic acids derived from 1,4-benzoxazin-3-ones (Hx) have been isolated from extracts of cereals such as wheat, maize and rye [1]. It has been suggested that these molecules play an important role in the protection of plants against insects (aphids), bacteria and fungi [2,3]. It was reported that, these hydroxamic acids inhibit population growth or development of several plant pathogens [4]. These molecules are present naturally in the plants as glucosides from which the aglycones are released rapidly by enzymatic hydrolysis after physical and biological injury of the plants [5]. It is also possible to obtain the aglycones by the aqueous extracts made at room temperature [1]. They exhibit antifungal and insecticide properties.

In view of this, it was considered of interest to synthesize some new 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones with a view to evaluating their antibacterial and antifungal activity in vitro.



No		R'	R
15	1	-H	-H
16	2	-Me	-H
17	3	-Et	-H
18	4	-n-Pr	-H
19	5	-H	(8)-Cl
20	6	-Me	(8)-Cl
21	7	-Et	(8)-Cl
22	8	-n-Pr	(8)-Cl
23	9	-n-Bu	(8)-Cl
24	10	-n-Pen	(8)-Cl
25	11	-H	(6)-OMe
26	12	-Me	(6)-OMe
27	13	-Et	(6)-OMe
28	14	-n-Pr	(6)-OMe

Scheme 1.

2. Chemistry

The targeted compounds have been synthesized by the two steps. (1) Potassium phenolate of the *o*-nitrophenol derivatives reacted with the ethyl ester of α-bromoalkanoic acids (**15**–**28**). (2) Reduction and cyclization of these ether compounds using Zn–NH_4Cl gave the 4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-ones (**1**–**14**) (Scheme 1).

3. Experimental

M.p. were determined with a Büchi SMP-20 melting point apparatus and are uncorrected. The instrumental analysis was performed by TUBITAK (Instrumental analyse Lab., Ankara) by the Bruker AC 400 NMR spectrophotometer, VG Platform II mass spectrometer and Leco CHNS 932 elemental analyser. For the chro-

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Table 1
Some physico-chemical properties and spectral findings of compounds **1**–**14**

No.	M.p. (°C)	Formula (C, H, N)	Yield (%)	NMR (δ ppm)	MS (70 eV, EI)
1	167–169 [6] (169)	$C_8H_7NO_3$			
2	148–149 [8] (152)	$C_9H_9NO_3$			
3	128–130	$C_{10}H_{11}NO_3$	41	1.06 (t, 3H, $J = 7.2$), 1.92 (m, 2H), 4.63 (s, 1H), 6.96 (m, 1H), 7.05 (m, 2H), 7.33 (1H), 9.0 (br.s, 1H)	193 (M^+, 28), 148 (100), 136 (25), 120 (56), 106 (18), 93 (23), 80 (59)
4	87–89	$C_{11}H_{13}NO_3$ ·0.25 HOH	35	0.94 (t, 3H, $J = 7.4$), 1.54 (m, 2H), 1.86 (m, 2H), 4.72 (dd, 1H), 6.94 (m, 1H), 7.04 (m, 2H), 7.35 (m, 1H)	207 (M^+, 73), 191 (11), 162 (100), 148 (15), 136 (39), 120 (98), 106 (19), 93 (13), 80 (55), 78 (34)
5	213–215	$C_8H_6ClNO_3$	40	4.63 (s, 2H), 6.96 (t, 1H, $J = 8.1$), 7.04 (dd, 1H, $J_o = 8.1$ $J_m = 1.4$), 7.27 (dd, 1H, $J_o = 8.1$ $J_m = 1.4$)	199 (M^+, 36), 201 (12), 154 (100), 156 (33), 127 (24), 99 (45), 101 (15)
6	136–138	$C_9H_8ClNO_3$ ·HOH	30	1.62 (d, 3H, $J = 6.8$), 4.91 (q, 1H, $J = 6.8$), 6.99 (t, 1H, $J_o = 8.1$), 7.1 (dd, 1H), 7.27 (dd, 1H)	213 (M^+, 30), 215 (10), 168 (100), 170 (33), 127 (24), 99 (43)
7	110–112	$C_{10}H_{10}ClNO_3$	24	1.1 (t, 3H, $J = 7.4$), 1.91 (m, 2H), 4.74 (dd, 1H, $J = 6.5$), 6.98 (t, 1H, $J = 8.1$), 7.10 (dd, 1H, $J_o = 8.2$ $J_m = 1.4$), 7.28 (dd, 1H, $J_o = 8.2$ $J_m = 1.4$)	227 (M^+, 51), 229 (17), 182 (100), 184 (33), 170 (47), 172 (16), 154 (78), 156 (26), 127 (30), 99 (47)
8	134–136	$C_{11}H_{12}ClNO_3$	43	0.96 (t, 3H, $J = 7.4$), 1.58 (m, 2H), 1.85 (m, 2H), 4.81 (dd, 1H, $J = 6.5$), 6.98 (t, 1H, $J = 8.1$), 7.10 (dd, 1H, $J_o = 8.2$ $J_m = 1.4$), 7.27 (dd, 1H, $J_o = 8.2$ $J_m = 1.4$)	241 (M^+, 12), 225 (16), 196 (75), 198 (25), 183 (73), 185 (24), 154 (14), 99 (21), 41 (100)
9	132–3	$C_{12}H_{14}ClNO_3$· 0.4 HOH	35	0.9 (t, 3H, $J = 7.3$), 1.4 and 1.6 (m, 4H), 1.9 (m, 2H), 4.84 (dd, 1H, $J = 4.7$), 7.03 (t, 1H, $J = 8.1$), 7.13 (dd, 1H, $J_o = 8$ $J_m = 1.2$), 7.31 (dd, 1H, $J_o = 8$ $J_m = 1.2$)	255 (M^+, 33), 257 (9.5), 239 (7), 210 (28), 212 (9), 198 (9), 183 (14), 170 (18), 154 (26), 55 (24), 32 (100).
10	120–1	$C_{13}H_{16}ClNO_3$· 0.8 HOH	16	0.9 (t, 3H, $J = 7.3$), 1.3, 1.55 and 1.63 (m, 6H), 1.9 (m, 2H), 4.85 (dd, 1H, $J = 4.7$), 7.02 (t, 1H, $J = 8.06$) 7.13 (dd, 1H, $J_o = 8.2$ $J_m = 1.4$), 7.28 (dd, 1H, $J_o = 8.2$ $J_m = 1.4$)	269 (M^+, 42), 271 (13), 253 (44), 255 (12), 224 (33), 226 (9), 183 (61), 170 (24), 154 (52), 55 (87), 32 (100).
11	147–149 [9] (147)	$C_9H_9NO_4$			
12	116–118	$C_{10}H_{11}NO_4$	17	1.54 (d, 3H, $J = 6.8$), 3.8 (s, 3H), 4.73 (q, 1H, $J = 6.8$), 6.56 (dd, 1H, $J_o = 8.8$ $J_m = 2.5$), 6.86 (d, 1H, $J_o = 8.8$), 6.96 (d, 1H, $J_m = 2.5$)	209 (M^+, 96), 193 (11), 164 (100), 136 (24), 123 (33), 108 (25), 95 (32)
13	92–94	$C_{11}H_{13}NO_4$	9.5	1.05 (t, 3H,$J = 7.4$), 1.90 (m, 2H), 3.79 (s, 3H), 4.57 (dd, 1H), 6.55 (dd, 1H, $J_o = 8.8$ $J_m = 2.5$), 6.86 (d, 1H, $J_o = 8.8$), 6.93 (d, 1H, $J_m = 2.5$)	223 (M^+, 67), 178 (100), 150 (44), 136 (23), 123 (26), 108 (28), 95 (24)
14	85–87	$C_{12}H_{15}NO_4$	19	0.92 (t, 3H, $J = 7.4$), 1.50 (m, 2H), 1.80 (m, 2H), 3.73 (s, 3H), 4.59 (dd, 1H), 6.51 (dd, 1H, $J_o = 8.8$ $J_m = 2.5$), 6.79 (d, 1H, $J_o = 8.8$), 6.97 (d, 1H, $J_m = 2.5$)	237 (M^+, 10), 221 (42), 192 (18), 178 (12), 150 (59), 136 (11), 123 (17), 108 (18), 107 (13), 95 (23), 55 (100)

Table 2
The in vitro antibacterial and antifungal activity of **1–14** [a]

No*	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*
1			
2		10	
3			
4			9
5		9	9
6		13	
7		10	9
8	9	12	14
9	10	10	15
10	13	11	16
11		9	9
12		10	10
13		10	10
14		12	11
Fluconazole			16
Ketoconazole			28
Ampicilline	29	22	

[a] Growth-inhibition zone diameter (mm).

matographic analysis Merck Silica Gel 60 (230–400 mesh ASTM) was used.

3.1. General procedure for the synthesis of **15–28**

A suspension of related powdered potassium 2-nitrophenolates (0.03 mol, prepared by the treatment of metallic potassium and 2-nitrophenoles, precipitated with ether, red coloured) in related ethyl α-alkylbromoacetate (0.045 mol) is heated under reflux until the colour has turned from orange to yellow (30 min). After cooling, the reaction mixture was diluted with CH_2Cl_2. The potassium bromide that precipitated was filtered off. The evaporation of CH_2Cl_2 gave **15–28**. The compounds **18–28** were obtained as yellow coloured oily residues and used for the further step without purification. Compounds **15** [6], **16** [7] and **17** [7] were obtained as solids.

3.2. General procedure for the synthesis of **1–14**

After adding a solution of NH_4Cl (0.05mol) in water (18 ml) to a solution of the esters **15–28** (0.012 mol) in EtOH (90 ml) under vigorous stirring, zinc dust (2.72 g) is added at a rate which does not allow the temperature to rise above 40°C. After stirring for 2 h, the precipitate formed is separated by filtration and shaken thoroughly with 1 M NaOH (35 ml). The alkaline extract is filtered, and the filtrate is acidified under cooling with conc. HCl. The precipitate formed is isolated, washed with water, and crystallised from EtOH to give **1–14** as colourless needles. Some physico-chemical properties, yields and spectral findings of **1–14** are given in Table 1.

4. Antimicrobial activity technique

Antimicrobial activity of the synthesised compounds was determined with the previously reported standard method [10]. A paper disc (8 mm in diameter) was soaked in a 2000 μg/ml solution of the test compound in propylene glycol **1–14** and placed on an agar plate containing fungi or bacteria cells, which was incubated at a temperature of 37°C for 24 h. Propylene glycol as a blank has no inhibition zone. The diameter of the growth inhibition zone around the paper disc was measured.

5. Results and discussion

Compounds **1–14** were evaluated for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in vitro. Table 2 shows the results of in vitro activity determination by a paper disc assay (measuring the diameter of the inhibition zone around a paper disc soaked in a solution of test compounds). All of the compounds have no important inhibitory activity towards *S. aureus* and *E. coli*. However, on the whole chlorosubstituted derivatives **5–10**, exhibited better activity than others. Among them, compounds having long alkyl chain on the second position of the benzoxazine ring **9** and **10** showed good antifungal activity which was comparable to that of fluconazole. The antifungal activity increases with the length of alkyl chain on the C-2. While **1–4** have no inhibitory activity against *C. albicans*, **11–14** were slightly active.

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