Indian Journal of Chemistry
Vol. 42B, April 2003, pp. 905-909

Synthesis and antimicrobial activity of some novel 2,6,7-trisubstituted-2*H*-3,4-dihydro-1,4-benzoxazin-3-one derivatives

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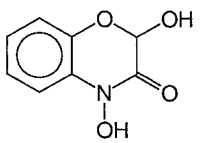
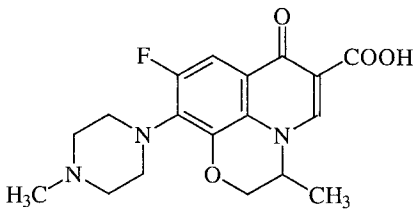
Received 20 December 2001; accepted (revised) 20 August 2002

The synthesis of a new series of 2*H*-3,4-dihydro-1,4-benzoxazin-3-one derivatives **1-8** is accomplished in order to determine their antimicrobial activities and study their structure-activity relationships (SAR). The synthesized compounds have been tested *in vitro* against two Gram-positive, three Gram-negative bacteria and the fungus *Candida albicans*. The *in vitro* activity results show that the synthesized compounds exibit MIC values between 50-12.5 μg/mL for the antimicrobial activity against the tested microorganisms. The antibacterial and antifungal activities of the compounds **1-8** are also compared to several standard drugs.

Virtanen *et al*[1-4] have isolated 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) as a glycoside from rye plant. Since then a number of analogous of 1,4-benzoxazine have been found in nature. Only, limited number of compounds containing 1,4-benzoxazine ring system have been studied for their chemotherapeutic activity. The most interesting observation that ofloxacin molecule posseses the 1,4-benzoxazine ring system in its structure[5,6], prompted us to investigate this heterocyclic nuclei to ascertain if it would offer any advantage over the other known clinically used antimicrobial drugs.

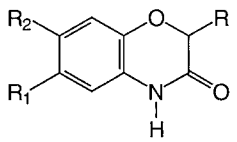


DIBOA



Ofloxacin

In this study, 3,4-dihydro-2,6,7-trisubstituted-2*H*-1,4-benzoxazin-3-one derivatives **I** were prepared and subjected to antimicrobial activity against different Gram-positive and Gram-negative bacteria and the fungus *C. albicans*.



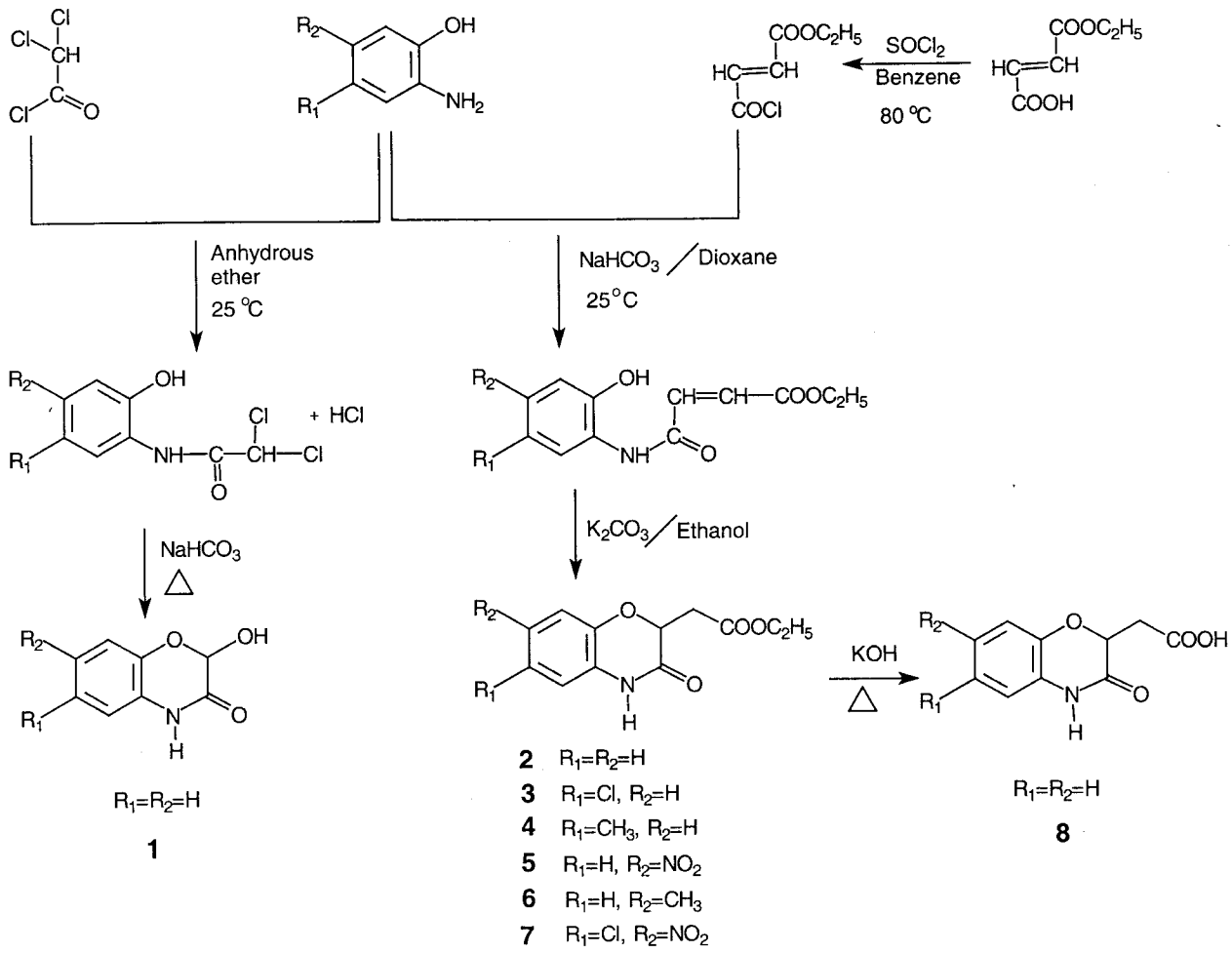
R: -OH,-CH_2COOH,-$CH_2COOC_2H_5$
R_1: -H,-Cl,-CH_3, R_2: -H,-NO_2,-CH_3

I

Results and Discussion

Dichloroacetyl chloride was added to a suspension *o*-aminophenol in anhydrous diethyl ether and stirred for 2 hr to obtain *N*-dichloroacetyl-2-aminophenol which was boiled in aqueus $NaHCO_3$ to give compound[7-9] **1**. For the synthesis of compounds **2-7**, first of all, monoethylfumaryl chloride was prepared using monoethylfumaric acid and thionyl chloride in benzene[10] and was added dropwise to a suspension of appropriate *o*-aminophenol and $NaHCO_3$ in dry dioxan to give ethyl 3-[(4- and/or 5-substituted-2-hydroxyphenyl)carbamoyl]acrylate derivatives which subsequently on stirring in EtOH with K_2CO_3 gave compounds[11] **2-7** (**Scheme I**). Synthesis of the compound **8**, was accomplished by heating compound **2** with 2*N* KOH [12,13].

The physical and spectral data of the synthesized compounds **1-8** are reported in **Table I**. The antimicrobial activity of these compounds and the



Scheme I

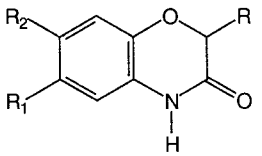
standard drugs are shown in **Table II**. The results indicate that the compounds **1-8** are able to inhibit *in vitro* growth of a number of microorganisms exibiting MIC values between 50 and 12.5 μg/mL .

The data in the **Table II** reveal that the synthesized compounds show antibacterial activity with a MIC value of 50 μg/mL against the Gram-positive bacteria *Staphylococcus aureus*. Interestingly, the compounds **2**, **5** and **8** were active even at 25 μg/mL. Compounds **5** and **7** were found to be more active than the other compounds at a MIC value of 25 μg/mL against *Bacillus subtilis*. However, they exibited lower antibacterial potencies when compared to the standard drugs ampicillin, amoxycillin and tetracycline against the Gram-positive bacteria strains.

Furthermore, the antibacterial activity of the compounds **1-8** against the Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* at a MIC value of 25 μg/mL was found to possess lower potency when compared with drugs. But, the compounds **1**, **3**, **4** and **8** indicated significant activity, having a MIC value of 25 μg/mL against the Gram-negative enterobacter *Pseudomonas aeruginosa*, which is effective in nosocomial infections and often resistant to antibiotic therapy. Thus they showed more potency than the compared drugs, tetracycline and streptomycin.

Moreover, the antifungal activity of the synthesized compounds against *C. albicans* showed MIC values as 25-12.5 μg/mL. The compounds ethyl 3,4-dihydro-6-chloro-3-oxo-2*H*-1,4-benzoxazin-2-acetate **3** and ethyl 6-chloro-7-nitro-3-oxo-2*H*-3,4-dihydro-1,4-benzoxazin-2-acetate **7** showing a MIC value of 12.5 μg/mL were found to be more potent than the other compounds.

Table I—Physical properties and spectral data of the compounds **1-8**.



Compd.	R	R_1	R_2	Mol. formula	m.p. (°C)	Yield (%)	^{1}H NMR (δ, ppm; *J* in Hz)
1	-OH	-H	-H	$C_8H_6O_3N$	201-03[7]	76.40	5.60 (s, 1H,*CH*), 6.90-7.00 (m, 4H, *Ar-H*), 10.30 (s, 1H, *NH*), 7.40 (s, 1H, *OH*).
2	$-CH_2COOC_2H_5$	-H	-H	$C_{12}H_{12}O_4N$	106-07[8]	53.33	1.25 (t, *J* = 6.63, 3H, $CH_2COOCH_2CH_3$), 3.00 (m, 2H, $CH_2COOCH_2CH_3$), 4.20 (q, 2H, $CH_2COOCH_2CH_3$), 5.00 (m, 1H, *CH*), 6.80-7.10 (m, 4H, *Ar-H*), 9.40 (s, 1H, *NH*)
3	$-CH_2COOC_2H_5$	-Cl	-H	$C_{12}H_{11}O_4NCl$	143-45[8]	21.10	1.30 (t, *J* = 7.14, 3H, $CH_2COOCH_2CH_3$), 3.10 (m, 2H, $CH_2COOCH_2CH_3$), 4.20 (q, 2H, $CH_2COOCH_2CH_3$), 4.95 (q, 1H, *CH*), 6.90 (m, 3H, *Ar-H*), 9.50 (s, 1H, *NH*)
4	$-CH_2COOC_2H_5$	$-CH_3$	-H	$C_{13}H_{14}O_4N$	115-16[8]	35.64	1.25 (t, *J* = 7.09, 3H, $CH_2COOCH_2CH_3$), 2.25 (s, 3H, CH_3), 3.00 (d, 2H, $CH_2COOCH_2CH_3$), 4.20 (q, *J* = 7.13, 2H, $CH_2COOCH_2CH_3$), 5.00 (q, *J* = 7.66, 1H, *CH*), 6.60 (s, 1H, 5-*H*), 6.75-6.85 (m, 2H, *7-H, 8-H*), 9.00 (s, 1H, *NH*).
5	$-CH_2COOC_2H_5$	-H	$-NO_2$	$C_{12}H_{11}O_6N_2$	172	40.14	1.30 (t, *J* = 6.89, 3H, $CH_2COOCH_2CH_3$), 3.00 (m, 2H, $CH_2COOCH_2CH_3$), 4.20 (q, 2H, $CH_2COOCH_2CH_3$), 5.00 (q, 1H, *CH*), 7.10 (d, $J_{5,6}$ = 8.97, 1H, *5-H*), 7.80-7.90 (m, 2H, *6-H, 8-H*), 7.80 ($J_{6,5}$ = 9.31, $J_{6,8}$ = 2.15), 7.90 δ ppm ($J_{8,6}$ = 1.95), 11.10 (s, 1H, *NH*).
6	$-CH_2COOC_2H_5$	-H	$-CH_3$	$C_{13}H_{14}O_4N$	115-16	60.24	1.30 (t, *J* = 7.13, 3H, $CH_2COOCH_2CH_3$), 2.30 (s, 3H, *7-H* CH_3),3.00 (d, 2H, $CH_2COOCH_2CH_3$), 4.20 (q, 2H, $CH_2COOCH_2CH_3$), 5.00 (t, 1H, *CH*), 6.60-6.90 (m, 3H, *Ar-H*), 9.10 (s, 1H, *NH*).
7	$-CH_2COOC_2H_5$	-CI	$-NO_2$	$C_{12}H_{10}O_6N_2Cl$	162-65	38.79	1.25 (t, *J* = 7.9, 3H, $CH_2COOCH_2CH_3$), 3.00 (m, 2H, $CH_2COOCH_2CH_3$), 4.20 (q, 2H, $CH_2COOCH_2CH_3$), 5.00 (q, 1H, *CH*), 7.10 (s, 1H, *5-H*), 7.60 (s, 1H, *8-H*), 11.20 (s, 1H, *NH*).
8	$-CH_2COOH$	-H	-H	$C_{12}H_8O_4N$	170-73[11]	12.74	2.80-3.11 (m, 2H, CH_2), 4.90 (q, 1H, *CH*), 6.85 (s, 4H, *Ar-H*), 10.10 (s, 1H, *NH*).

Table II—Antimicrobial activity of 2,6,7-trisubstituted-2*H*-3,4-benzoxazine derivatives **1-8** against some Gram-positive, Gram-negative bacteria and *C. albicans* (MIC in μg/mL)

	Microorganisms					
	Gram-positive		Gram- negative		Fungus	
Compd	*S.aureus*	*B.subtilis*	*E.coli*	*K.pneumoniae*	*P.aeruginosa*	*C.albicans*
1	50	50	25	25	25	25
2	25	50	50	50	50	25
3	50	50	25	25	25	12.5
4	50	50	25	25	25	25
5	25	25	25	25	50	25
6	50	50	50	50	50	25
7	50	25	25	25	50	12.5
8	25	50	50	25	25	25

(—*contd*)

Table II—Antimicrobial activity of 2,6,7-trisubstituted-2*H*-3,4-benzoxazine derivatives **1-8** against some Gram-positive, Gram-negative bacteria and *C. albicans* (MIC in μg/mL).(—*contd*)

	Microorganisms					
	Gram-positive		Gram- negative		Fungus	
Compd	*S.aureus*	*B.subtilis*	*E.coli*	*K.pneumoniae*	*P.aeruginosa*	*C.albicans*
Ampicillin	1.56	1.56	12.5	25	>200	-
Amoxycillin	1.56	1.56	3.12	12.5	>200	-
Streptomycin	3.12	50	1.56	1.56	100	-
Tetracycline	1.56	1.56	3.12	3.12	50	-
Oxiconazole	-	-	-	-	-	6.25
Haloprogin	-	-	-	-	-	3.1

In conclusion, structure-activity relationships of the synthesized compounds reveal that possessing a chlorine atom at position-6 on the fused heterocyclic system with a substitution of ethyl acetate group at position-2 increases antifungal activity against *C. albicans*.

Experimental Section

Kieselgel-60 GF_{254} chromatoplates (0.3 mm) were used for TLC and the solvent systems were chloroform:methanol (20:2.0) for compounds **1-7**, chloroform:methanol:glacial acetic acid (20:5:0.02) for compound **8**. All the melting points were taken on a Buchi SMP 20 capillary apparatus and are uncorrected. Microanalyses were performed on a Perkin Elmer model 240-c apparatus and satisfactory results (± 0.4 % of calculated values) for C,H,N were obtained. IR spectra were recorded on a Perkin Elmer Model 782 with KBr discs; and ^{1}H NMR spectra on a Bruker GmbH D PX-400 (400 MHz) High Performance Digital FT-NMR Spectrometer in $CDCl_3$ / DMSO-d_6 using TMS as an internal standard.

General procedure for synthesis of 2-hydroxy-2*H*-3,4-dihydro-1,4-benzoxazin-3-one 1. Dichloroacetyl chloride (4.8 mL, 5 mmoles) was added dropwise to a suspension of *o*-aminophenol (1.090 g, 10 mmoles) in anhyd. ether (15 ml). The reaction mixture was stirred for 2 hr at room temperature, then the ether was evaporated and residue was crystallised from ethanol. *N*-dichloroacetyl-2-aminophenol (0.440 g, 2 mmoles) thus obtained was heated in aqueous $NaHCO_3$ solution for 1.5 hr, cooled, and acidified with dil. HCl. The mixture was extracted with ether and dried over anhyd. sodium sulphate and evaporated *in vacuo*. The crude product thus obtained was recrystallised from ethanol (m.p 201-03 °C)[7].

General procedure for synthesis of ethyl 3,4-dihydro-2,6,7-trisubstituted-3-oxo-2*H*-1,4-benzoxazin-2-acetate 2-7. Monoethyl fumaric acid (1.44 g, 10 mmoles) was added dropwise to thionyl chloride (1.5 mL, 20 mmoles) in benzene (15 mL). The reaction mixture was stirred for 3 hr at 80 °C. At the end of the reaction the excess thionyl chloride was evaporated. The residue was added dropwise to a suspension of appropriate *o*-aminophenol (1.09 g, 10 mmoles) and $NaHCO_3$ (1 g, 10 mmoles) in dry dioxane. The reaction mixture was stirred for 24 hr at room temperature, then poured into water and extracted with EtOAc. The extract was washed with water, dried and evaporated. The residue was recrystallised from EtOH to give ethyl 3-[(4- and/or 5-substituted-2-hydroxyphenyl)carbamoyl]acrylate derivatives. A suspension of these compounds (10 mmoles) and K_2CO_3 (5 mmoles) in EtOH (70 mL) was stirred for 3 hr at room temperature and then poured into water and extracted with EtOAc. The extracts were washed with water, dried and evaporated. The residue was recrystallised from EtOH .

General procedure for the synthesis of 2*H*-3,4-dihydro-1,4-benzoxazin-3-oxo-2-acetic acid 8. Ethyl 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazin-2-acetate **2** was heated with 2*N* alc. KOH for 20 min[13]. The reaction mixture was cooled, acidified with 2*N* HCl, and then extracted with EtOAc. The extract was dried and evaporated *in vacuo*. The residue was recrystallised from EtOH.

Biological activity

For determining both the antibacterial and antifungal activities, the compounds were dissolved in absolute ethanol[14] (0.8 μg/mL) . Further dilutions of the compounds and standard drugs in the test medium were furnished 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 tecnique[14-17]. 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 fungus *Candida albicans* RSKK 628. The origin of bacterial strains were *Staphylococcus aureus* RSKK 250, and *Bacillus subtilis* ATCC 6633 as Gram-positive bacteria and *Escherichia coli* RSKK 313, *Klebsiella pneumoniae* RSKK 256, and *Pseudomonas aeruginosa* RSKK 356 as a Gram-negative bacteria. RSKK strains of the microorganisms used in this study were obtained from the culture collection of Refik Saydam Institution of Health Ministry, Ankara and maintained at the Microbiology Department of 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 standard drugs are given in **Table II**.

Antibacterial assay

The cultures were obtained in Mueller-Hinton broth (Difco) for all the bacteria after 24 hr of incubation at 37 ± 1 °C. Testing was carried out in Mueller-Hinton broth at *p*H 7.4 and the two- fold serial dilution technique was applied. The final inoculum size was 10^5 CFU/mL . A set of tubes containing only inoculated broth was kept as controls. After incubation for 24 hr at 37 ± 1 °C, the last tube with no growth of microorganism was recorded to represent MIC expressed in μg/mL.

Antifungal assay

The yeast *Candida albicans* was maintained in sabouraud dextrose broth (Difco) after incubation for 24 hr at 25 ± 1 °C. Testing was performed in sabouraud dextrose broth at *p*H 7.4 and the two-fold serial dilution technique was applied. The final inoculum size was 10^4 CFU/mL. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 hr at 25 ± 1 °C, the last tube with no growth of yeast was recorded to represent MIC expressed in μg/mL.

Acknowledgement

Authors thank the Research Fund of Ankara University (Grant No: 98-03-00-06) for the financial support.

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