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Short communication

Synthesis and antibacterial and antifungal properties of thiazolinoethyl-2(3*H*)-benzoxazolone derivatives. II

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Summary — Cyano derivatives of 6-acyl-2(3*H*)-benzoxazolones were reacted with cysteamine HCl in ethanol to give the corresponding 6-acyl-3-thiazolinoethyl-2(3*H*)-benzoxazolones and their antibacterial and antifungal activities were investigated. The chemical structures were proved by means of their IR and ^{1}H-NMR spectra and elemental analysis. Investigation of antimicrobial activity of the compounds was carried out by tube dilution and disc techniques using bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Streptococcus faecalis* ATCC 29212) and yeast-like fungi (*Candida parapsilosis*, *C albicans*, *C pseudotropicalis* and *C stellatoidea*). Inhibitory effects were observed for many compounds against *S aureus* and *Bacillus subtilis*. Compounds **13** and **15** had minimum inhibitory concentrations (MIC) of 8.4 and 4.2 μg/mL respectively. The antifungal studies against *C albicans* (**10** and **16**, MIC = 67.5 μg/mL), *C parapsilosis* (**15**, MIC = 67.5 μg/mL) and *C stellaatoidea* (**9**, MIC = 67.5 μg/mL) were more successful in comparison.

thiazolinoethyl-2(3*H*)-benzoxazolone derivative / synthesis / in vitro study / antibacterial agent / antifungal agent

Introduction

The preparation and evaluation of antimicrobial properties of a number of thiazolinoalkyl-4(1*H*)-pyridones and thiazolinomethyl-2(3*H*)-benzoxazolones have been reported from our research laboratories [1, 2]. 6-Acyl-2(3*H*)-benzoxazolone derivatives have been found to show good biological actvity [3–7]. In recent years, thiazoline derivatives have also been reported to exhibit a broad spectrum of biological activity [8–15]. Caujolle et al [16] and Erol et al [1, 2] explained the microbiological importance of thiazolines and synthesized several new compounds with substituted aryl alkyl thiazoline derivatives. In view of this we aimed to investigate the effect of several derivatives of thiazolinoalkyl-2(3*H*)-benzoxazolones with either an acyl functional group or a chloride at position 5 of the 2-benzoxazolinone ring, and we now report the antimicrobial activities associated with members of this series.

Chemistry

6-Acyl derivatives of 2(3*H*)-benzoxazolone and chloroxazone were synthesized by heating 2(3*H*)-benzoxazolone with substituted benzoic acids in the presence of polyphosphoric acid for 7 h. Reaction of the 6-acyl-2(3*H*)-benzoxazolone derivatives with cysteamine HCl yielded the anticipated thiazolinoethyl-2(3*H*)-benzoxazolone derivatives (**9–16**) (table I). The basic structures of these compounds were confirmed by IR and ^{1}H-NMR spectral data (table II). In their IR spectra compounds **9–16** all showed a strong band at 1624 cm^{-1}, which is more likely assignable to a C=N rather than a C≡N group, and at 760 cm^{-1}, which is a characteristic frequency of the C-S bond. In the ^{1}H-NMR spectra methylene protons appeared as a sharp singlet signal at 4.00–4.16 ppm for $=NCH_2$-, and a triplet signal for SCH_2 at 3.15–3.20 ppm. The protons of aromatic rings were observed at the expected values. The results of microanalysis also confirmed the structures of the compounds.

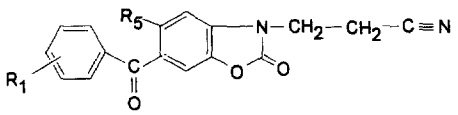
Biological investigation and discussion

Antimicrobial activity

The antimicrobial activity of the prepared compounds was tested against Gram-positive and Gram-negative bacteria and against fungi using the diffusion technique [17] and broth dilution test tube method [18].

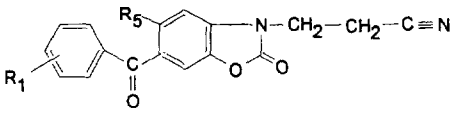
Table I. Structures and chemical data of (**a**) 6-acyl-3-cyanoethyl- and (**b**) 6-acyl-3-thiazolinoethyl-2(3*H*)-benzoxazolone derivatives.

a.



Compound	R_1	R_5	*Yield (%)*[a]	*Mp (° C)*[b]	*Formula*	*Analysis (%)*[c]
1	H	H	70	162–163	$C_{17}H_{12}N_2O_3$	C, H, N
2	H	Cl	68	179–181	$C_{17}H_{11}ClN_2O_3$	C, H, N
3	2-Cl	H	75	170–171	$C_{17}H_{11}ClN_2O_3$	C, H, N
4	3-Cl	H	72	223–224	$C_{17}H_{11}ClN_2O_3$	C, H, N
5	3-F	H	63	191–193	$C_{17}H_{11}FN_2O_3$	C, H, N
6	4-F	H	65	205–206	$C_{17}H_{11}FN_2O_3$	C, H, N
7	4-NO_2	H	79	227–228	$C_{17}H_{11}N_3O_5$	C, H, N
8	4-CH_3O	H	82	203–204	$C_{18}H_{14}N_2O_4$	C, H, N

b.



Compound	R_1	R_5	*Yield (%)*[a]	*Mp (° C)*[b]	*Formula*	*Analysis (%)*[c]
9	H	H	76	185–187	$C_{19}H_{16}N_2O_3S$	C, H, N
10	H	Cl	63	198 (dec)	$C_{19}H_{15}ClN_2O_3S$	C, H, N
11	2-Cl	H	78	195 (dec)	$C_{19}H_{15}ClN_2O_3S$	C, H, N
12	3-Cl	H	70	200 (dec)	$C_{19}H_{15}ClN_2O_3S$	C, H, N
13	3-F	H	74	210 (dec)	$C_{19}H_{15}FN_2O_3S$	C, H, N
14	4-F	H	69	215 (dec)	$C_{19}H_{15}FN_2O_3S$	C, H, N
15	4-NO_2	H	67	218 (dec)	$C_{19}H_{15}N_3O_5S$	C, H, N
16	4-CH_3O	H	79	197 (dec)	$C_{20}H_{18}N_2O_4S$	C, H, N

[a]Yields are of the products obtained from first crystallization. [b]Melting points were determined on a Thomas–Hoover apparatus and are uncorrected. [c]C, H, N analyses were performed by the Scientific and Technical Research Council of Turkey, Gebze, Turkey.

The compounds which showed inhibition zones are recorded in table III and were further evaluated for their minimal inhibitory concentrations (MICs) against the other test organism using the broth dilution technique. As revealed by the results, compounds **9–12** were almost inactive against all bacteria and fungi. Most of the compounds showed good activity against bacteria. Compounds **13** and **15** exhibited strong activity against *Staphylococcus aureus* ATCC 25923 (MIC values of 8.4 and 16.9 μg/mL respectively) and **15** and **16** showed activity against *Bacillus subtilis* ATCC 1633 (MICs of 4.2 and 16.9 μg/mL). A fluoride, nitro or methoxy functional group at the 3- and 4-positions of the aromatic ring resulted in potentiation of the antimicrobial activity associated with broad spectrum properties.

Experimental protocols

Chemistry

The melting points were determined in open glass capillaries on a Thomas–Hoover apparatus and are uncorrected. The infrared spectra were recorded on a Perkin-Elmer Model 457 IR

Table II. Spectral data of 6-acyl-3-cyanoethyl- and 6-acyl-3-thiazolinoethyl-2(3*H*)-benzoxazolones.

Compound	*IR (cm^{-1})*[a]				*^{1}H-NMR (ppm)*[b]				
		Lactam	*Aromatic ketone*						
	-C≡N	*C=O*	*C=O*	*C=N*	*-C-S*	*-CH_2- (s)*	*Ar H (m)*	*S-CH_2 (t)*	*=N-CH_2 (t)*
1	2240	1775	1645			5.15	7.00–7.85		
2	2240	1780	1645			5.20	7.10–7.70		
3	2240	1775	1650			5.20	7.05–7.70		
4	2240	1775	1645			5.20	7.10–7.80		
5	2240	1780	1650			5.20	7.00–7.75		
6	2240	1775	1645			5.15	7.05–7.80		
7	2240	1775	1645			5.15	7.10–7.85		
8	2240	1780	1650			5.20	7.15–7.80		
9		1780	1645	1620	7.62	5.20	7.10–7.80	3.05	4.25
10		1780	1650	1624	7.60	5.15	7.05–7.70	3.10	4.20
11		1775	1645	1624	7.62	5.20	7.00–7.70	3.10	4.25
12		1780	1650	1624	7.62	5.15	7.10–7.80	3.10	4.25
13		1775	1645	1620	7.60	5.20	7.15–7.85	3.05	4.20
14		1775	1645	1620	7.62	5.15	7.10–7.80	3.05	4.20
15		1775	1645	1624	7.62	5.20	7.05–7.80	3.05	4.25
16		1780	1650	1620	7.60	5.15	7.00–7.80	3.10	4.20

[a]IR spectra were determined on a Perkin-Elmer Model 457 IR in KBr pellets. [b] ^{1}H–NMR spectra were charted on a Brucker 80: s: singlet; t: triplet; m: multiplet; using tetramethylsilane as the internal standard and $CDCl_3$ as solvent.

Table III. Biological activity of 6-acyl-3-thiazolinoethyl-2(3*H*)-benzoxazolone derivatives.

Compound	*MIC (μg/mL) (growth inhibition zone size)**							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
9	135 (–)	135 (–)	135 (–)	135 (–)	6.75 (+)	135 (–)	135 (–)	135 (–)
10	135 (–)	135 (–)	135 (–)	135 (–)	135 (–)	135 (–)	135 (–)	67.5 (+)
11	135 (–)	135 (–)	67.5 (+)	33.7 (+)	135 (–)	135 (–)	135 (–)	135 (–)
12	135 (–)	33.7 (++)	135 (–)	67.5 (–)	135 (–)	135 (–)	135 (–)	135(–)
13	135 (–)	8.4 (++++)	135 (–)	33.7 (++)	135 (–)	135 (–)	135 (–)	135 (–)
14	135 (–)	67.5 (+)	135 (–)	33.7 (++)	135 (–)	135 (–)	135 (–)	135 (–)
15	67.5 (+)	16.9 (+++)	67.5 (+)	4.2 (++++)	33.7 (++)	67.5 (+)	135 (–)	135 (–)
16	135 (–)	33.7 (++)	135 (–)	16.9 (+++)	135 (–)	135 (–)	135 (–)	67.5 (–)

a: *Streptococcus faecalis* ATC 29212, b: *Staphylococcus aureus* ATCC 25923, c: *Pseudomonas aeruginosa* ATCC 27853, d: *Bacillus subtilis* ATCC 1633, e: *Candida stellatoidea*, f: *C parapsilosis*, g: *Tichosporon*, h: *C albicans*. *0–5 mm (–), 6–8 mm (+), 9–11 mm (++), 12–15 mm (+++), >15 mm (++++).

spectrophotometer using samples in KBr discs. ^{1}H-NMR spectra were measured on a Perkin-Elmer R32 at 90 MHz using tetramethyl silane as the internal standard and $CDCl_3$ [7, 28]. Analyses indicated by elemental symbols were within ±0.4% of the theoretical values and were performed by the Scientific and Technical Research Council of Turkey (Gebze, Turkey).

6-Acyl-2(3H)-benzoxazolones

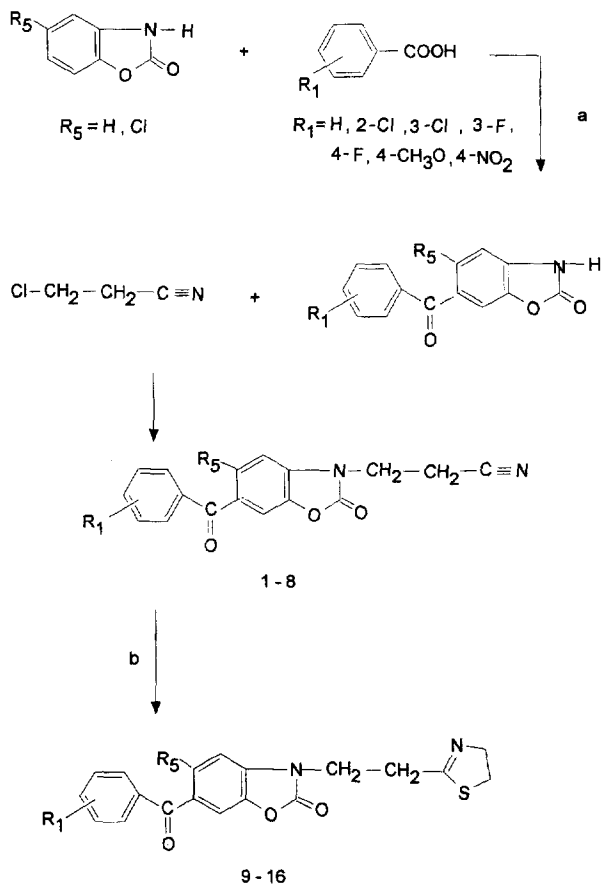
These were prepared by treating 2(3*H*)-benzoxazolone and the appropriate carboxylic acid with polyphosphoric (PPA) according to literature procedures [3].

Sodium salt of 6-acyl-2(3H)-benzoxazolones

The sodium salts of the 6-acyl-2(3*H*)-benzoxazolones were prepared by dissolving the appropriate 6-acyl-2(3*H*)-benzoxazolone in a solution of sodium ethoxide.

*General procedure for preparation of 6-acyl-3-cyanoethyl-2(3H)-benzoxazolones **1–8***

A solution of a sodium salt of a 6-acyl-2(3*H*)-benzoxazolone (0.1 mol) in ethanol and chloropropionitrile (0.3 mol) was heated under reflux for 6 h. The reaction mixture was evaporated to dryness. The residue was dissolved in cold water



Scheme 1.

and extracted with chloroform. The organic phase was dried with Na_2SO_4, filtered and evaporated to dryness. Recrystallization from methanol gave pure 6-acyl-3-cyanoethyl-2(3*H*)-benzoxazolone derivatives (tables I, II).

General procedure for preparation of thiazoline derivatives of 6-acyl-2(3H)-benzoxazolones ***9–16***

Cyanoethyl derivatives of 2(3*H*)-benzoxazolones (0.1 mol) and cysteamine HCl (0.1 mol) were dissolved in ethanol (50 mL). The reaction mixture was refluxed for 6–8 h under an N_2 atmosphere then evaporated to dryness. The residue was dissolved in ice-water and extracted with chloroform, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was recrystallized from isopropanol (tables I, II).

Microbiological methods

Test organism and culture media

S aureus ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* ATCC 29212 and *B subtilis* ATCC 1633 were cultivated in nutrient agar and nutrient broth (Mueller-Hinton Broth Oxoid, Basingstoke, Hants, UK), while *Candida parapsilosis*, *C albicans*, *C stellatoidea* and *Tichosporon* were grown in Sabouraud Dextrose Broth (DIFCO, Detroit, MI, USA). All cultures without an identification number of the source are from the collection of the Department of Microbiology, University of Hacettepe, Faculty Medical.

Antibacterial and antifungal screening was carried out using two different methods.

Inhibition zone measurements. The compounds were dissolved in propylene glycol at a concentration of 1 µg/mL. The agar medium (Nutrient agar for bacteria and Sabouraud agar for fungi) was inoculated with 1 mL of 24-h-old culture of the test organism. Filter paper discs (5 mm diameter) saturated with the solution of the test compounds (100 µg/mL) were placed on the agar. After an incubation period of 36 h, the zones of inhibition around the disc were measured. Propylene glycol, which exhibited no antimicrobial activity against the test organism, was used as a negative control.

Minimal inhibitory concentration (MIC) measurement. The substances dissolved in propylene glycol at 1 mg/mL were diluted in broth in the range 270–0.55 µg/mL. Inocula were prepared from well-growing overnight cultures of each test organism such that the final inoculum size was ca 10^6 cells/mL. The tubes were then inoculated with 0.1 mL of inoculum and incubated at 37 °C for 24 h for bacteria and 48 h for fungi. All results are presented as µg/mL and the lowest concentration of the antimicrobial agent that resulted in the complete inhibition of the visible growth of the microorganisms represents the MIC (table III).

References

1 Erol DD, Yuluğ N (1994) *Eur J Med Chem* 29, 893–897
2 Erol DD, Yuluğ N (1995) *Eur J Med Chem* 30, 521–524
3 Erol DD, Rosen A, Erdoğan H, Yuluğ N (1989) Arzneim Forsch 39, 851–853
4 Varma RS, Nobles WL (1973) *J Pharm Sci* 62, 140–143
5 Varma RS, Imam SA (1973) *Indian J Microbiol* 13, 43–47
6 Erol DD, Erdoğan H, Yuluğ N (1989) *J Pharm Belg* 44, 334–338
7 Anıl K, Sengupta U, Umesh C (1978) *Indian J Pharm Sci* 40, 197–199
8 McFarland JW, Hower HL, Conover LH, Lynch JE, Austin WC, Morgan DH (1970) *J Med Chem* 13, 113–119
9 Smee DF, Alaghamandan HA, Jin A, Sharma BSS, Jolley WB (1990) *Antiviral Res* 13, 91–94
10 Badawey ES, Rida SM, Hazza AA, Fahmy HTY, Gohar YM (1993) *Eur J Med Chem* 28, 97–101
11 Badawey ES, Rida SM, Hazza AA, Fahmy HTY, Gohar YM (1993) *Eur J Med Chem* 28, 91–96
12 Bilgin AA, Erol DD, İstanbullu İ (1987) *J Fac Pharm* 7, 57–64
13 Caujolle R, Favrot JD, Loiseau PR (1991) *Pharm Acta Helv* 66, 237–240
14 Ghoneim KM, Osman AN, Said M, El-Kersh TA (1988) *Pharm Acta Helv* 43, 88–92
15 Sircar JC, Suto MJ, Scott ME, Dong MK, Gilbertsen RB (1986) *J Med Chem* 29, 1804–1806
16 Caujolle R, Baziard-Mouysset G, Faurot JD et al (1993) *Eur J Med Chem* 28, 29–35
17 Varma RS, Imam SA (1975) *Defence Sci J* 25, 67–69
18 Jones RN, Barry AL, Gaven TL, Washington JA (1985) *Manual of Clinical Microbiology* (Lenette EH, Ballows A, Hausle WJ, Shadomy HJ, eds) American Society for Microbiology, Washington, DC, 4th edition, 972–977