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Short communication

Synthesis and antimicrobial activity of thiazolinomethyl-2(3*H*)-benzoxazolone derivatives (I)

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Summary — A number of thiazolinoalkyl-2(3*H*)-benzoxazolones have been synthesized by using cyano derivatives of 6-acyl-2(3*H*)-benzoxazolones with cysteamine HCl. Their antibacterial and antifungal activities have been evaluated. The chemical structures have been proved by means of their IR, ^{1}H-NMR and mass spectroscopic data and by elemental analysis. The antimicrobial activity of compounds was investigated by tube dilution and disk techniques using bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* RSKK 10541) and yeast-like fungi *(Candida parapsilosis, C albicans, C pseudotropicalis, C stellatoidea)*. Inhibitory effects were observed for many compounds against *C albicans, eg,* compounds **3b** and **3c** MIC = 25 μg/ml, *C pseudotropicalis, eg,* compound **3b** MIC = 12.5 μg/ml, and *P aeruginosa* and *S faecalis, eg,* compound **3c**, MIC = 25 μg/ml.

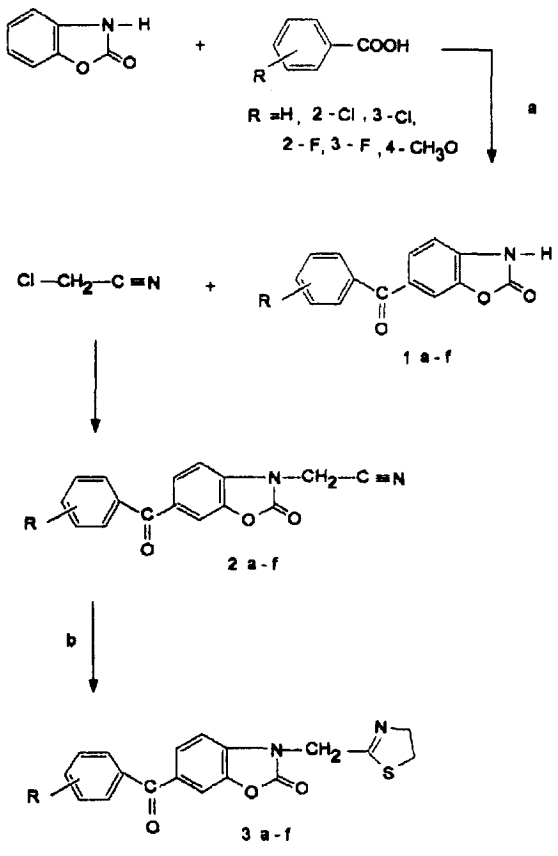
thiazolinomethyl-2(3*H*)-benzoxazolone derivative / antibacterial agent / antifungal agent

Introduction

2(3*H*)-Benzoxazolone derivatives have been associated with various types of biological properties. Lespagnol and coworkers prepared and tested a number of derivatives of 2(3*H*)-benzoxazolones for their anticonvulsive, hypnotic, antipyretic and analgesic properties [1–3]. 2(3*H*)-Benzoxazolone and its 3-methyl, 3-ethyl, and 3-hydroxyethyl derivatives have shown anticonvulsant activity, and antiinflammatory, analgesic and antipyretic activities have been described in 2(3*H*)-benzoxazolinone and its 6-acyl derivatives [4–11]. The pronounced biological activity of many 2(3*H*)-benzoxazolone derivatives and the medicinal value of 5-chloro-2(3*H*)-benzoxazolone [12] prompted the investigation of 3-substituted-2(3*H*)-benzoxazolones. In recent years, benzoxazolones have also been reported to possess antimicrobial and anticholinergic activities [13–19]. On the other hand, thiazolines have been associated with various types of microbiological properties. Badawey *et al* prepared and tested a number of thiazolopyrimidine derivatives for their antimicrobial effects [20, 21]. Caujolle *et al* explained the microbiological importance of thiazolines and synthesized several new substituted aryl alkyl thiazoline derivatives [22, 23]. These observations led us to synthesize a series of 6-acyl-3-thiazolinomethyl-2(3*H*)-benzoxazolones with a view to testing their antibacterial and fungicidal activities.

Chemistry

6-Acyl derivatives of 2(3*H*)-benzoxazolone were prepared by reacting 2(3*H*)-benzoxazolone with aromatic acids in the presence of polyphosphoric acid. Scheme 1 shows the results of the reaction of the 6-acyl-1-cyanoalkyl-2(3*H*)-benzoxazolone derivatives (**2a–f**) with cysteamine HCl yielding the anticipated thiazolinomethyl-2(3*H*)-benzoxazolone derivatives (**3a–d**) (table I). The basic structures of these compounds were confirmed by IR and ^{1}H-NMR spectral data (table II). The reaction products were assigned a structure which is in accord with their spectroscopic and chemical behaviour. In their IR spectra, all of these compounds (**3a–d**) showed a strong band at 1624 cm^{-1}, which is more assignable to a C=N rather than to a C≡N, and another at 762 cm^{-1}, which is a characteristic peak of C-S bond. Aromatic ketones have C=O stretching bands in the 1650–1670 cm^{-1}



Scheme 1. a: polyphosphoric acid; b: cysteamine·HCl.

region. Lactams have also been observed between 1770 and 1780 cm^{-1}. In the ^1H-NMR spectra methylene protons appeared as a sharp singlet signal at 3.70–3.80 ppm for $-NCH_2-$, a triplet signal at 4.00–4.16 ppm for $=NCH_2$ and triplet for $S-CH_2$ at 3.10–3.20 ppm. The protons of aromatic rings were observed at the expected values. The results of microanalysis also confirm 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 [24] and broth dilution test-tube method [25].

The compounds that 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 **3e** and **3f** were inactive against all bacteria and fungi. Most of compounds have shown good activity against fungi. Compounds **3b** and **3c** exhibited strong activity against all microorganisms while the chloride functional group at 2 and 3 positions of aromatic ring resulted in potentiation of the antimicrobial activity associated with broad spectrum properties.

Table I. Structures and chemical data of 6-acyl-3-thiazolinomethyl-2(3*H*)-benzoxazolone derivatives.

Compound	*R*	*Yield (%)*[a]	*Melting point*	*Formula*	*Analysis (%)*
2a	H	82	154–155	$C_{16}H_{10}N_2O_3$	C, H, N
2b	2-Cl	75	161–163	$C_{16}H_9ClN_2O_3$	C, H, N
2c	3-Cl	79	169–171	$C_{16}H_9ClN_2O_3$	C, H, N
2d	2-F	81	156–158	$C_{16}H_9FN_2O_3$	C, H, N
2e	3-F	83	146–147	$C_{16}H_9N_2O_3$	C, H, N
2f	4-CH_3O	77	155–157	$C_{17}H_{12}N_2O_4$	C, H, N
3a	H	65	149–152	$C_{18}H_{14}N_2O_3S$	C, H, N
3b	2-Cl	69	155–158	$C_{18}H_{13}ClN_2O_3S$	C, H, N
3c	3-Cl	72	159–161	$C_{18}H_{13}ClN_2O_3S$	C, H, N
3d	2-F	75	148–150	$C_{18}H_{13}FN_2O_3S$	C, H, N
3e	3-F	79	145–148	$C_{18}H_{13}FN_2O_3S$	C, H, N
3f	4-CH_3O	82	162–165	$C_{19}H_{16}N_2O_4S$	C, H, N

[a]Yields are of the products obtained from first crystallization.

Table II. Spectral data of 6-acyl-3-cyanomethyl-2(3*H*)-benzoxazolones **2a–f** and **3a–f**.

Compound	*IR (cm^{-1})*					*^{1}H-NMR (ppm)*[a]			
	-C≡N	*Lactam C=O*	*Aromatic ketone C=O*	*C=N*	*-C-S*	*-CH_2- (s)*	*Ar H (m)*	*S-CH_2 (t)*	*=N-CH_2 (t)*
2a	2240	1780	1650			5.20	7.10–7.80		
2b	2240	1775	1645			5.15	7.10–7.85		
2c	2240	1775	1650			5.15	7.10–7.75		
2d	2240	1780	1650			5.20	7.00–7.80		
2e	2240	1780	1650			5.20	7.05–7.75		
2f	2240	1775	1645			5.15	7.10–7.70		
3a		1780	1650	1624	760	5.20		3.00	4.20
3b		1775	1645	1624	762	5.15		3.05	4.25
3c		1775	1650	1620	762	5.15		3.05	4.25
3d		1780	1650	1620	760	5.20		3.10	4.25
3e		1780	1650	1624	760	5.20		3.10	4.25
3f		1775	1645	1624	762	5.15		3.05	4.20

[a]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-thiazolinomethyl-2(3*H*)-benzoxazolone derivatives.

	3a	**3b**	**3c**	**3d**	**3e**	**3f**
MIC (μg/ml)						
E coli	100	75	75	100	100	100
S aureus	100	100	50	100	100	100
P aeruginosa	100	100	25	75	100	100
S faecalis	100	75	25	100	75	100
C parapsilosis	75	50	75	100	75	100
C albicans	50	25	25	75	100	100
C pseudotropicalis	50	125	50	100	100	100
C stellatoida	50	50	50	100	100	100
Growth inhibition zone size (mm)						
E coli	0–5	6–8	6–8	0–5	0–5	0–5
S aureus	0–5	0–5	9–11	0–5	0–5	0–5
P aeruginosa	0–5	0–5	9–11	6–8	0–5	0–5
S faecalis	0–5	6–8	9–11	0–5	6–8	0–5
C parapsilosis	6–8	9–11	9–11	0–5	6–8	0–5
C albicans	9–11	12–15	12–15	6–8	0–5	0–5
C pseudotropicalis	9–11	>15	9–11	0–5	0–5	0–5
C stellatoidea	9–11	9–11	9–11	0–5	0–5	0–5

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 spectrophotometer using samples in potassium bromide disks. ^{1}H-NMR spectra were measured on a Perkin Elmer R 32 90 MHz using tetramethyl silane as the internal standard and $CDCl_3$ (7.28 ppm). 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 1a–f

These were prepared by treating 2(3*H*)-benzoxazolone and the appropriate carboxylic acid with polyphosphoric (PPA) according to the literature [5].

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

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

General procedure for 6-acyl-3-cyanomethyl-2(3H)-benzoxazolones 2a–f

A solution of sodium salt of 6-acyl-2(3*H*)-benzoxazolone derivatives (0.1 mol) in ethanol and chloroacetonitrile (0.3 mol)

was heated under reflux for 6 h. The reaction mixture was evaporated to dryness. The residue was dissolved in cold water 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-cyanomethyl-2(3*H*)-benzoxazolone derivatives (tables I and II).

General procedure of thiazoline derivatives of 6-acyl-2(3H)-benzoxazolones 3a–f

Cyanomethyl 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 N_2 atmosphere then evaporated to dryness. Residue was dissolved in ice-water and extracted with chloroform, and dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was recrystallized from isopropanol (tables I and II).

Microbiological methods

Test organism and culture media

Staphylococcus aureus ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* RSKK 10541 and *Escherichia coli* ATCC 25922 were cultivated in nutrient agar and nutrient broth (Mueller-Hinton), while *Candida parapsilosis, C albicans, C pseudotropicalis* and *C stellaoidea* were grown in Sabouraud Dextrose Broth (DIFCO). All cultures without identification number of source are from the collection of University of Hacettepe, Medical Faculty, Department of Microbiology. 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 disks (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 disk were measured. Propylene glycol, which exhibited no antimicrobial activity against the test organism, was used as a negative control.

Minimal inhibitory concentration (MIC) measurements

The substances dissolved in propylene glycol at 1 mg/ml were diluted in broth in the range 100–0.05 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 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 minimal inhibitory concentrations (MIC, table III).

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