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Laboratory note

Synthesis and antimicrobial investigation of thiazolinoalkyl-4(1*H*)-pyridones

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Summary — A number of thiazolinoalkyl-4(1*H*)-pyridones have been synthesized using 4-pyrone derivatives with cysteamine HCl, and their antibacterial and antifungal activities have been tested. Their chemical structures have been proved by means of their IR, ^{1}H-NMR, mass spectroscopic data and by elemental analysis. Investigation of antimicrobial activity of compounds was done by tube dilution and disk tecniques 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*). A significant inhibitory effect was recorded for many compounds against *C albicans* (**7a**, **c**, **d**; minimal inhibitory concentration (MIC) = 12.5–25 µg/ml), *S aureus* ATCC 25923 (**4c**, **7a**; MIC = 25 µg/ml), *P aeruginosa* ATCC 27923 (**7a**; MIC = 25 µg/ml), *S faecalis* RSKK 10541 (**4c**; MIC = 25 µg/ml), *C pseudotropicalis* (**4d**, **6d**, **7c**; MIC = 25 µg/ml) and *C stellatoidea* (**4d**; MIC = 25 µg/ml).

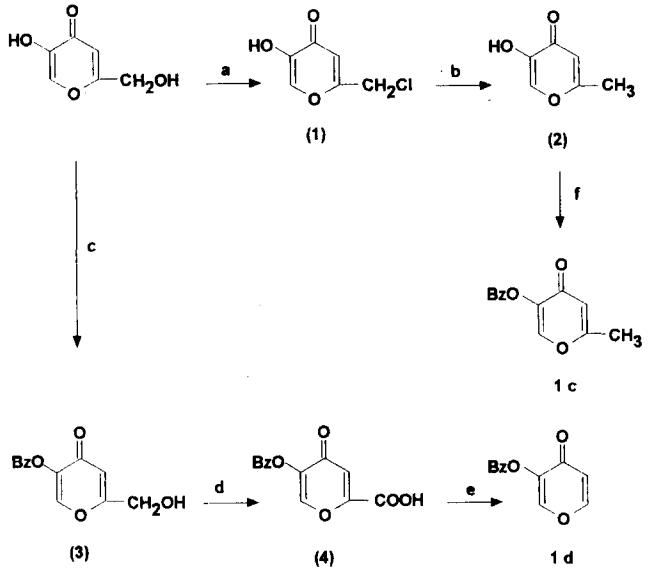
thiazolinomethyl-4(1*H*)-pyridone / thiazolinoethyl-4(1*H*)-pyridone / *in vitro* study / antibacterial agent / antifungal agent

Introduction

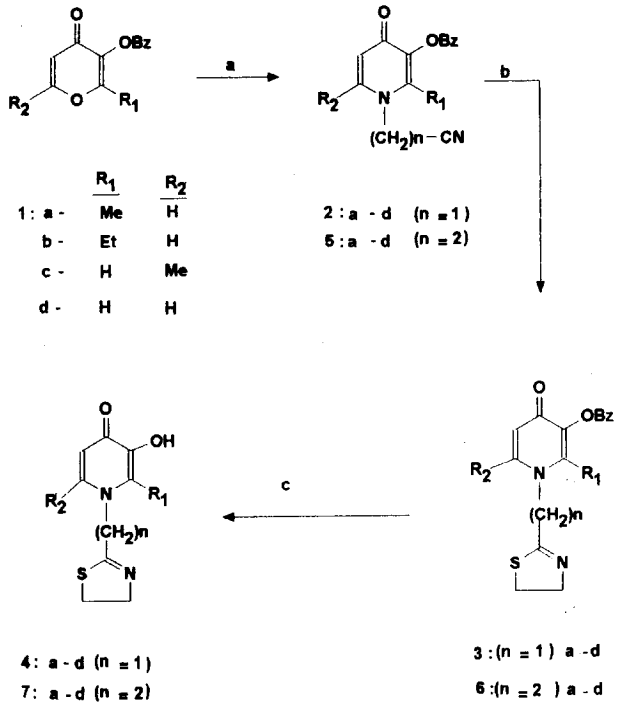
Thiazolines have been associated with various types of microbiological properties, and Badawey *et al* have prepared and tested a number of thiazolopyrimidine derivatives for their antimicrobial effects [1, 2]. In recent years, thiazoline derivatives have also been reported to exhibit a broad spectrum of biological activity [3–10]. Caujolle *et al* explained the microbiological importance of thiazolines and synthesized several new compounds having substituted aryl alkyl thiazoline derivatives [11]. In view of this fact, we have synthesized several derivatives of thiazolinoalkyl-4(1*H*)-pyridone having either an ether functional group or hydroxyl group at position 3, 5 of pyridone ring (schemes 1 and 2) and we now report the anti-microbial activities associated with members of this series.

Chemistry

Scheme 1 shows the results of the reaction of 1-cyanoalkyl-4(1*H*)-pyridone derivatives **2a–d** and **5a–d** with cysteamine HCl yielding the anticipated thiazolinoalkyl-4(1*H*)-pyridones **3a–d**, **6a–d**. Acid-catalyzed removal of the benzyl-protecting group furnished the desired thiazolinoalkyl-4(1*H*)-pyridone. Susceptibility of the free bases to air oxidation necessitated prepa-



Scheme 1. (a) $SOCl_2$; (b) Zn/HCl; (c, f) BzCl; (d) JR; (e) NMP.



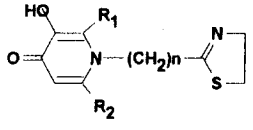
Scheme 2. (a) Aminoacetonitrile/pyridine; (b) cysteamine HCl/EtOH; (c) 20% HCl.

ration of the corresponding hydrochloride salts for storage and biological testing.

Since a convenient route to 4-pyridones (**1a–d**) is the condensation of 4-pyrones with primary amines [12], commercially available kojic acid, maltol and ethyl maltol were selected as starting materials for the synthesis of a series of 4-pyridone analogs (scheme 2). Condensation of the benzyl ether of the starting material with the aminoacetonitrile afforded the desired pyridones. Difficulties with the Jones' oxidation of the hydroxymethyl group of the compound necessitated conversion to the pyronecarboxylic acid, benzyl kojic acid, prior to the insertion reaction. Pyronecarboxylic acid could be converted to **1d** by heating in *N*-methylpyrrolidone (NMP) and caused thermal decarboxylation to afford benzyloxy-4-pyrone.

The basic structures of these compounds were confirmed by IR and ^{1}H-NMR spectral data (table I). In addition the mass spectra of the compounds revealed molecular ion peaks. The reaction products were assigned structures that were in accord with their spectroscopic and chemical behaviour. Thus all of these compounds showed a strong band at 1624 cm^{-1} in their IR spectra which is assignable to a C=N group rather than to a -C≡N group, and at 762 cm^{-1}, which is characteristic of a C-S bond. The methylene group protons in the ^{1}H-NMR spectra of all compounds appeared as a sharp singlet signal at 3.70–3.80 ppm for -N-CH_2-, a triplet signal at 4.00–4.18 ppm for =N-CH_2 and a triplet for S-CH_2 at 3.10–3.20 ppm. Characteristic doublet peaks of pyridone were observed at aromatic field. The results of microanalysis also confirm the structure of the compounds.

Table I. Structures and chemical data of pyridinomethyl/ethyl thiazoline derivatives.



Compound	R_1	R_2	*n*	*Yield (%)*[a]	*Melting point*[b]	*Formula*	*Anal (%)*[c]
4a	CH_3	H	1	88.2	265 (dec)	$C_{10}H_{12}N_2O_2S$·2HCl	C, H, N
4b	C_2H_5	H	1	80.3	258 (dec)	$C_{11}H_{14}N_2O_2S$·2HCl	C, H, N
4c	H	CH_3	1	75.2	248 (dec)	$C_{10}H_{12}N_2O_2S$·2HCl	C, H, N
4d	H	H	1	70.8	273 (dec)	$C_9H_{10}N_2O_2S$· 2HCl	C, H, N
7a	CH_3	H	2	88.4	287 (dec)	$C_{11}H_{14}N_2O_2S$·2HCl	C, H, N
7b	C_2H_5	H	2	85.6	290 (dec)	$C_{12}H_{16}N_2O_2S$·2HCl	C, H, N
7c	H	CH_3	2	69.2	296 (dec)	$C_{11}H_{14}N_2O_2S$·2HCl	C, H, N
7d	H	H	2	67.6	289 (dec)	$C_{10}H_{12}N_2O_2S$·2HCl	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 Butterworth Laboratories Ltd (UK).

Table II. Biological activity of thiazolinoalkyl-4(1*H*)-pyridone derivatives.

Compound	*MIC (μg/ml)*							
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*
3a	75+	75+	100–	75+	100–	100–	100–	100–
3b	100–	100–	75+	100–	75+	75+	100–	100–
3c	75+	75+	75+	75+	100–	100–	100–	100–
3d	100–	100–	100–	100–	75+	75+	50++	75+
4a	50++	50++	75+	50++	75+	50++	75+	75+
4b	75++	75+	100–	100–	100–	100–	75+	75+
4c	50+	25+++	50++	25+++	50++	50++	75+	75+
4d	75+	75+	50++	50++	50++	25+++	25+++	25+++
6a	100–	100–	75+	75+	75+	100–	100–	100–
6b	100–	100–	100–	100–	100–	100–	100–	100–
6c	75+	75+	75+	100–	100–	100–	100–	75+
6d	100–	75+	75+	75+	75+	50++	75+	100–
7a	50++	25+++	25+++	50++	50++	12.5++++	25+++	50++
7b	50++	75+	75+	50++	100–	75+	75+	75+
7c	100–	100–	75+	75+	50++	25+++	25+++	50++
7d	75+	50++	50++	50++	50++	25+++	50++	50++

[a]*Escherichia coli* ATCC 25922; [b]*Staphylococcus aureus* ATCC 25923; [c]*Pseudomonas aeruginosa* ATCC 27923; [d]*Staphylococcus faecalis* RSKK 10541; [e]*Candida parapsilosis*; [f]*C albicans*; [g]*C pseudotropicalis*; [h]*C stellatoidea*. Growth inhibition zone size: 0–5 mm (–), 6–8 mm (+), 9–11 mm (++), 12–15 mm (+++), > 15 mm (++++).

Biological investigation and discussion

The antimicrobial activity of the prepared compounds was tested against Gram-positive and Gram-negative bacteria and against fungi using the diffusion technique [13] and broth dilution test-tube method [14].

The compounds that showed inhibition zones are recorded in table II and were further evaluated for their minimal inhibitory concentrations (MICs) against the other test organisms using the broth dilution technique. Compound **6b** was inactive against all bacteria and fungi. Most of compounds showed slight to moderate activity against all microorganisms. In this series of compounds, it seems that compounds **3d**, **3b** lacked activity against the Gram-positive and Gram-negative bacteria but the compound **3d** was active against the test fungi. Similar activity was recorded for **3b**, **3d** and **7c**. Only one compound **7a** (3-hydroxy-2-methyl-1-thiazolinoethyl-4(1*H*)-pyridone) was active against *Candida albicans* (MIC 12.5 μg/ml). Compound **4a–d** and **7a–d** exhibited strong activity against all microorgnaisms while the hydroxyl functional group on the 4-pyridone ring resulted in 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 457 IR spectrophotometer using samples in potassium bromide disks. ^{1}H-NMR spectra were measured on a Perkin–Elmer R.32 90 MHz using tetramethylsilane as the internal standard and $CDCl_3$ (7.28). Mass spectra were obtained from V6 16 F mass spectrometer with V6 data system 2000. Analyses indicated by elemental symbols were within ± 0.4% of the theoretical values and were performed by Butterworth UK.

2-Chloromethyl-5-hydroxy-4-pyrone ***1***

Kojic acid (142 g, 1 mol) was dissolved in thionyl chloride (237 g, 2 mol), followed by stirring for 2 h at room temperature. The yellow solid was filtered, washed with cold petroleum ether 60–70°C. Recrystallization from water gave light-yellow crystals (115 g, 72%), mp 146–147°C.

3-Benzyloxy-2-methyl-4-pyrone ***1a***

Maltol (126 g, 1 mol) was dissolved in methanol (800 ml) and a solution of NaOH (80 g, 2 mol) in 10 ml water was added. The mixture was added benzyl chloride (253.2 g, 2 mol) and heated under reflux for 8 h, then evaporated to dryness and extracted with dichloromethane, washed with 5% NaOH and H_2O, dried (Na_2SO_4) and evaporated. Recrystallization of the resulting tan solid from ether provided **1a**, white needles (197.2 g, 91%), mp 48°C.

2-Methyl-5-hydroxy-4-pyrone **2**
Compound **1** (20 g, 0.12 mol) was suspended in water (500 ml). The temperature of the reaction mixture was raised to 50°C. Zinc dust (16 g, 0.24 mol) was added and stirred at 70°C, for 0.5 h. Conc HCl (13.6 g, 3 mol) was added dropwise followed by stirring for 4 h at 70–80°C. The solution was filtered, poured into the ice-water and extracted with dichloromethane, dried (Na_2SO_4) and evaporated to dryness. Recrystallization of the resulting yellow solid from isopropanol provided **2** as light-yellow needles (10.2 g, 64.7%), mp 125–127°C.

5-Benzyloxy-2-hydroxymethyl-4-pyrone **3**
Anhydrous K_2CO_3 (276.2 g, 2 mol) was suspended in a solution of kojic acid (142 g, 1 mol) and benzyl chloride (253.2 g, 2 mol) in DMF (700 ml). The temperature of the reaction mixture was raised to 110°C and maintained for 3 h. The dark reaction mixture was allowed to cool, poured into ice-water (100 ml) and extracted with dichloromethane. The organic phase was washed with water, dried (Na_2SO_4) and evaporated. Recrystallization of the resulting solid from $CHCl_3$ provided compound **3** as white needles (125 g, 54%), mp 129–130°C (lit [15] mp 131–133°C).

5-Benzyloxy-4-pyrone-2-carboxylic acid **4**
Compound **3** (10 g, 43.2 mmol) was dissolved in acetone (500 ml), cooled in an ice-bath and Jones' reagent was added (25 ml). The organic material was removed by filtration and the filtrate evaporated to dryness. Pure compound **4** (8.89 g, 89%) was obtained by recrystallization from MeOH, mp 194–196°C (lit [16], mp 196°C).

5-Benzyloxy-4-pyrone ***1d***
Benzylcomenic acid (10 g, 40.6 mmol) was dissolved in *N*-methyl pyrrolidone and refluxed overnight. After cooling the reaction mixture, 25 ml DMF was added and evaporated the solvent to give a residue, which was extracted with dichloromethane, washed with 5% NaOH and H_2O, dried, filtered and evaporated off the solvent. The residue was treated with charcoal in EtOH and the resulting yellow solid was recrystallized from toluene (4.68 g, 56%), mp 85–86°C.

General procedure for cyanomethyl-4-(1H)-pyridone derivatives ***2a–d***
A solution of 4-pyrone derivatives (5 mmol) in pyridine (30 ml) and aminoacetonitrile HCl (10 mmol) was heated under reflux for 20 h. The reaction mixture was dissolved in cold water and extracted with dichloromethane. Organic phase was dried with Na_2SO_4, filtered and evaporated to dryness. Recrystallization from iso-propanol gave pure 4-pyridone derivatives.

3-Benzyloxy-2-methyl-1-cyanomethyl-4(1H)-pyridone ***2a***
Mp 210°C (yield 86%); IR ν cm^{-1}: 2240 -C=N, 2900, 1620. ^{1}H-NMR δ (ppm): 2.24 (s, 3H, CH_3), 5.5 (s, 2H, $-CH_2-$), 5.25 (s, 2H, $-CH_2O$), 6.25 (d, 1H), 7.38 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 254. Anal $C_{15}H_{14}N_2O_2$ (C, H, N).

3-Benzyloxy-2-ethyl-1-cyanomethyl-4(1H)-pyridone ***2b***. Mp 147–148°C (yield 76%); IR ν cm^{-1}: 2240 -C≡N, 2900, 1620. ^{1}H-NMR δ (ppm): 1.10 (t, 3H, CH_2-CH_3), 2.5–2.82 (q, 2H, $-CH_2-CH_3$), 5.15 (s, 2H, $-CH_2-$), 5.30 (s, 2H, $-CH_2-O$), 6.30 (d, 1H), 7.40, m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) M^+ 268. Anal $C_{16}H_{16}N_2O_2$ (C, H, N).

5-Benzyloxy-2-methyl-1-cyanomethyl-4(1H)-pyridone ***2c***. Mp 187–189°C (yield 76%); IR ν cm^{-1}: 2240 -C≡N, 2920, 1600. ^{1}H-NMR δ (ppm): 2.35 (s, 3H, CH_3), 4.98 (s, 2H, $-CH_2-$), 5.20 (s, 2H, $-CH_2-O$), 6.20 (d, 1H), 7.42 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 254. Anal $C_{15}H_{14}N_2O_2$ (C, H, N).

3-Benzyloxy-1-cyanomethy-4(1H)-pyridone ***2d***. Mp 151–153°C (yield 72%); IR ν cm^{-1}: 2240 -C≡N, 2910, 1639. ^{1}H-NMR δ (ppm): 4.99 (s, 2H, $-CH_2-$), 5.15 (s, 2H, $-CH_2-O$), 6.22 (d, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (d, 2H). Mass spectrum (70 eV) m^+ 240. Anal $C_{14}H_{12}N_2O_2$ (C, H, N).

General procedure for cyanoethyl-4(1H)-pyridone derivatives ***5a–d***
A solution of 4-pyrone derivatives (1 mol) in pyridine (50 ml) and 3-aminopropionitrile fumarate (2 mol) was heated under reflux for 14 h. The reaction mixture was evaporated to dryness. Residue was dissolved in cold water and extracted with dichloromethane. Organic phase was dried with Na_2SO_4, filtered and evaporated to dryness. Recrystallization from ethanol gave pure 4-pyridone derivatives.

3-Benzyloxy-2-methyl-1-cyanoethyl-4(1H)-pyridone ***5a***. Mp 235°C (yield 73%); IR ν cm^{-1}: 2240 -C≡N, 2900, 1620. ^{1}H-NMR δ (ppm): 2.01 (s, 3H, CH_3), 2.40 (t, 2H, $-CH_2-$); 4.50 (t, 2H, $-CH_2-$, N), 5.20 (s, 2H, $-CH_2-O$), 6.20, (d, 1H), 7.44 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 268. Anal $C_{16}H_{16}N_2O_2$ (C, H, N).

3-Benzyloxy-2-ethyl-1-cyanoethyl-4(1H)-pyridone ***5b***. Mp 210°C (yield 81%); IR ν cm^{-1}: 2240 -C≡N, 2900, 1620. ^{1}H-NMR δ (ppm): 1.10 (t, 3H, $-CH_2-CH_3$), 2.50–2.75 (q, 4H, $-CH_2-CH_3$), 4.50 (t, 2H, $-CH_2-$), 5.25 (s, 2H, $-CH_2-O$), 6.30 (d, 1H), 7.44 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 282. Anal $C_{17}H_{18}N_2O_2$ (C, H, N).

5-Benzyloxy-2-methyl-1-cyanoethyl-4(1H)-pyridone ***5c***. Mp 195°C (yield 65%); IR ν cm^{-1}: 2240 -C≡N, 2900, 1620. ^{1}H-NMR δ (ppm): 2.30 (s, 3H, $-CH_3$), 2.40 (t, 2H), 4.50 (t, 2H), 5.20 (s, 2h), 6.35 (d, 1H), 7.45 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 268. Anal $C_{16}H_{16}N_2O_2$ (C, H, N).

3-Benzyloxy-1-cyanoethyl-4(1H)-pyridone ***5d***. Mp 210°C (yield 60%); IR ν cm^{-1}: 2240 -C≡N, 2900, 1620. ^{1}H-NMR δ (ppm): 2.50 (t, 2H), 4.60 (t, 2H), 5.20 (s, 2H), 6.20 (d, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (s, 2H). Mass spectrum (70 eV) m^+ 254. Anal $C_{15}H_{14}N_2O_2$ (C, H, N).

General procedure for thiazoline derivatives of 4-pyridone
Cyanomethyl and cyanoethyl-4-pyridone derivatives (0.1 mol) and cysteamine HCl (0.1 mol) were dissolved in ethanol (30 ml). The reaction mixture was to reflux for 6–8 h under an N_2 atmosphere then evaporated to dryness and extracted with DCM, washed with water and dried (Na_2SO_4), filtered and evaporated to dryness. The crude product was purified by silica-gel column chromatography with 5:1 $CHCl_3$/MeOH.

3-Benzyloxy-2-methyl-1-thiazolinomethyl-4(1H)-pyridone ***3a***. Mp 165°C (yield 80.5%); IR ν cm^{-1}: 1620, 742. ^{1}H-NMR δ (ppm): 2.20 (s, 3H, CH_3), 3.10 (t, 2H, S-CH_2), 4.20 (t, 2H, =N-CH_2), 5.20 (s, 2H), 5.5 (s, 2H), 6.25 (d, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 314. Anal $C_{17}H_{18}N_2O_2$ (C, H, N).

3-Benzyloxy-2-ethyl-1-thiazolinomethyl-4(1H)-pyridone ***3b***. Mp 168°C (yield 72.3%); IR ν cm^{-1}: 1620, 742. ^{1}H-NMR δ (ppm): 1.15 (t, 3H, $-CH_3$), 2.65 (q, 2H, $-CH_2-$), 3.15 (t, 2H, S-CH_2-), 4.25 (t, 2H, =N-CH_2-), 5.10 (s, 2H), 5.30 (s, 2H), 6.30 (d, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 328. Anal $C_{18}H_{20}N_2O_2$ (C, H, N).

5-Benzyloxy-2-methyl-1-thiazolinomethyl-4(1H)-pyridone ***3c***. Mp 159°C (yield 68%); IR ν cm^{-1}: 1620, 745. ^{1}H-NMR δ (ppm): 2.30 (s, 3H, CH_3), 3.10 (t, 2H, S-CH_2), 4.18 (t, 2H, =N-

CH_2), 4.98 (s, 2H), 5.20 (s, 2H), 6.20 (d, 1H), 7.42 (m, 5H, Ar*H*), 7.70 (s, 1H). Mass spectrum (70 eV) m^+ 314. Anal $C_{17}H_{18}N_2O_2$ (C, H, N).

5-Benzyloxy-1-thiazolinomethyl-4(1H)-pyridone ***3d***. Mp 178°C (yield 68%); IR ν cm^{-1}: 1620, 742. ^{1}H-NMR δ (ppm): 3.10 (t, 2H, S-CH_2), 4.20 (t, 2H, =N-CH_2), 4.95 (s, 2H), 5.15 (s, 2H), 6.22 (s, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (d, 2H). Mass spectrum (70 eV) m^+ 300. Anal $C_{16}H_{16}N_2O_2$ (C, H, N).

3-Benzyloxy-2-methyl-1-thiazolinoethyl-4(1H)-pyridone ***6a***. Mp 178°C (yield 77.2%); IR ν cm^{-1}: 1620, 745. ^{1}H-NMR δ (ppm): 1.90 (s, 3H, CH_3), 2.95 (m, 4H), 3.20 (t, 2H, S-CH_2), 4.18 (t, 2H, =N-CH_2), 5.25 (s, 2H), 6.25 (d, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 328. Anal $C_{18}H_{20}N_2O_2$ (C, H, N).

3-Benzyloxy-2-ethyl-1-thiazolinoethyl-4(1H)-pyridone ***6b***. Mp 163°C (yield 70.5%); IR ν cm^{-1}: 1620, 745. ^{1}H-NMR δ (ppm): 1.10 (t, 3H, -CH_2-CH_3), 2.65 (q, 2H), 2.95 (m, 4H), 3.19 (t, 2H, S-CH_2), 4.20 (t, 2H, =-N-CH_2), 5.30 (s, 2H), 6.30 (d, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 342. Anal $C_{19}H_{22}N_2O_2$ (C, H, N).

5-Benzyloxy-2-methyl-1-thiazolinoethyl-4(1H)-pyridone ***6c***. Mp 158°C (yield 68.7%); IR ν cm^{-1}: 1620, 745. ^{1}H-NMR δ (ppm): 2.05 (s, 3H, CH_3), 2.90 (m, 4H), 3.20 (t, 2H, S-CH_2-), 4.20 (t, 2H, =N-CH_2), 5.25 (s, 2H), 6.30 (d, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (d, 1H). Mass spectrum (70 eV) m^+ 328. Anal $C_{18}H_{20}N_2O_2S$ (C, H, N).

5-Benzyloxy-1-thiazolinoethyl-4(1H)-pyridone ***6d***. Mp 165°C (yield 65.6%); IR ν cm^{-1}: 1620, 745. ^{1}H-NMR δ (ppm): 2.85 (m, 4H), 3.20 (t, 2H, S-CH_2), 4.18 (t, =N-CH_2), 5.20 (s, 2H), 6.60 (d, 1H), 7.40 (m, 5H, Ar*H*), 7.70 (d, 2H). Mass spectrum (70 eV) m^+ 314. Anal $C_{17}H_{18}N_2O_2$ (C, H, N).

General procedure for ***4a–d*** *and* ***7a–d***

Each compound (5 mmol) was heated in 20% aqueous HCl (50 ml) under reflux for 24 h. The reaction mixture was then evaporated to dryness under reduced pressure and the residue recrystallized from EtOH/ether to give an analytical sample. IR, ^{1}H-NMR and mass spectra m/e (m^+ of the free base) were as expected (table I).

Microbiological methods

Test organisms and culture media

Staphylococcus aureus ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus faecalis* RSKK 10541, *Escherichia coli* ATCC 25922 were cultivated in nutrient agar and nutrient broth (Mueller-Hinton), while *Candida parapsilosis*, *C albicans*, *C pseudotropicalis*, *C stellaoidea* were grown in Sabouraud Dextrose Broth (DIFCO). All cultures' identification numbers of source were from the collection of University of Hacettepe, Medical Faculty, Department of Microbiology. Antibacterial and antifungal screening was carried out using 2 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 a solution of the test compound (100 μg/ml) were placed on the agar. After an incubation period of 36 h, the zones of inhibition around the disks were measured. Propylene glycol, which exhibited no antimicrobial activity against the test organisms, was used as a negative control.

Minimal inhibitory concentration (MIC) measurements

The substances were dissolved in propylene glycol at 1 mg/ml and were diluted in the 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 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 II).

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