Table I—Effects of Pentostatin and N^6-(Δ^2-Isopentenyl)adenosine on the Proliferation of L-1210 Leukemia Cells in Culture [a]

	Compound Added [b]						
	None	I		II		I plus II	
Time, hr [c]	Cells/ml × 10^{-5}	Cells/ml × 10^{-5}	Inhibition, %	Cells/ml × 10^{-5}	Inhibition, %	Cells/ml × 10^{-5}	Inhibition, %
24	3 ± 0.5 [d]	3 ± 0.5	0	0.8 ± 0	73	0.5 ± 0	83
48	14.5 ± 1.0	14 ± 0.8	3.2	4.5 ± 0.5	69	2.8 ± 0.5	81
72	34 ± 1.0	33.5 ± 1.0	1.5	11.1 ± 1.0	68	4.5 ± 0.5	86
96	42 ± 1.5	41 ± 2.0	2.4	14.2 ± 1.0	66	9 ± 1.0	79
144	50 ± 1.5	48 ± 2.0	4.0	22 ± 1.0	56	7.5 ± 1.0	85

[a] Each T-flask initially contained 2 × 10^5 L-1210 mouse leukemia cells per milliliter (100% viability by trypan blue exclusion) in 5 ml of growth medium (RPMI 1640 plus 10% fetal calf serum). Experiments were conducted using 2 or 3 replicates for each type of determination. [b] Additions (0.1–0.3 ml) of each agent dissolved in RPMI 1640 medium followed by sterile filtration were made at time zero to yield final concentrations of I (2 μg/ml) and/or II (25 μg/ml). [c] Aliquots (0.1–0.2 ml) of each cell suspension were removed aseptically at various time intervals for the following determinations: total cell count using Turk's solution, cell viability (trypan blue exclusion), and HPLC (15, 16). [d] Mean ± *SD*.

II against cultured L-1210 cells (Table I). Although I alone (≤10 μg/ml) does not interfere with L-1210 cellular proliferation, it is capable of enhancing the antileukemic effects of II (25 μg/ml). At an optimal concentration of 5–10 μg/ml, I in combination with II results in almost total cell death (96%) within 24 hr. The few remaining cells have viability values of 40–50%. The most impressive effect of I (2 μg/ml) is its ability to prevent the cytotoxic capacity of II from declining at longer intervals of incubation time, when L-1210 leukemia cells are in the stationary phase of growth. This effect is discernible at concentrations of as low as 0.2 μg/ml[7]. These results suggest that inhibition of N^6-(Δ^2-isopentenyl)-adenosine-aminohydrolase prevents the inactivation of II, thereby enhancing and prolonging its effectiveness as an antitumor drug agent. In other recent studies (15–17), it has been demonstrated that the usefulness of II against L-1210 cells may also be potentiated by controlled release of this nucleoside from a polymeric silicone matrix.

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Arylidenepyruvic Acid Thiosemicarbazone and Thiazoline Derivatives As Potential Antimicrobial Agents

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Abstract □ Two novel series of arylidenepyruvic acid thiosemicarbazone and thiazoline derivatives were synthesized and evaluated as potential antimicrobial agents. These substances did not exhibit any significant antibacterial effects when tested against a variety of microorganisms.

Keyphrases □ Antimicrobial agents, potential—arylidenepyruvic acid thiosemicarbazone and thiazoline derivatives, synthesis, evaluation for antibacterial activity □ Arylidenepyruvic acids—thiosemicarbazones and thiazolines derivatives, synthesis, antimicrobial effects.

The introduction of a thiosemicarbazone moiety to alter the pharmacological activity of a variety of biologically active compounds has been demonstrated recently in several studies from this laboratory (1–5). Continuing such studies, the thiosemicarbazones (II–XIV) derived from various arylidenepyruvic acids and the corresponding thiazolines (XV–XXVII, Scheme I) were synthesized and tested for antimicrobial activity.

RESULTS AND DISCUSSION

Chemistry—The thio compounds (II–XXVII) were prepared as shown in Scheme I. A mixture of pyruvic acid or the properly substituted arylidenepyruvic acid (I), prepared through Claisen condensation of pyruvic acid and various aryl aldehydes (6), and an equivalent amount of 4-substituted 3-thiosemicarbazide was heated under reflux in aqueous acetic acid. The products (II–XIV) which separated on concentrating and cooling the mixtures, were crystallized from ethanol. The reaction of these

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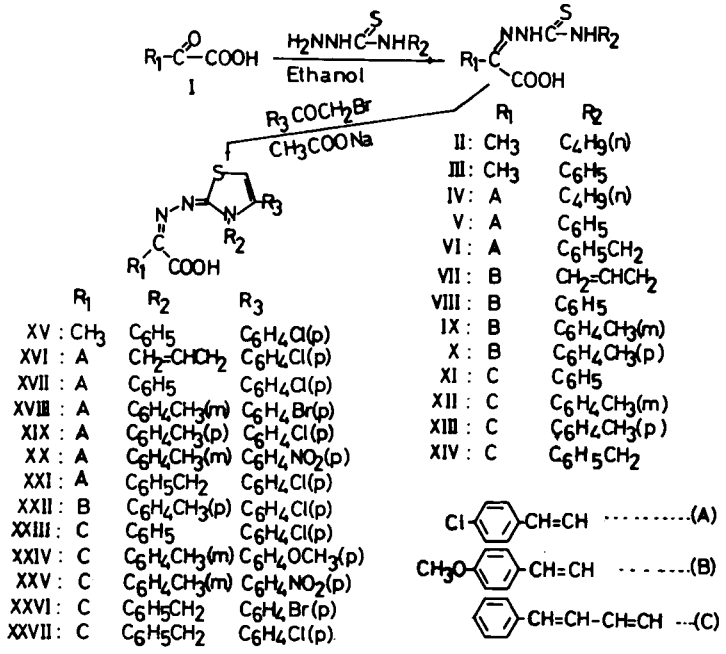
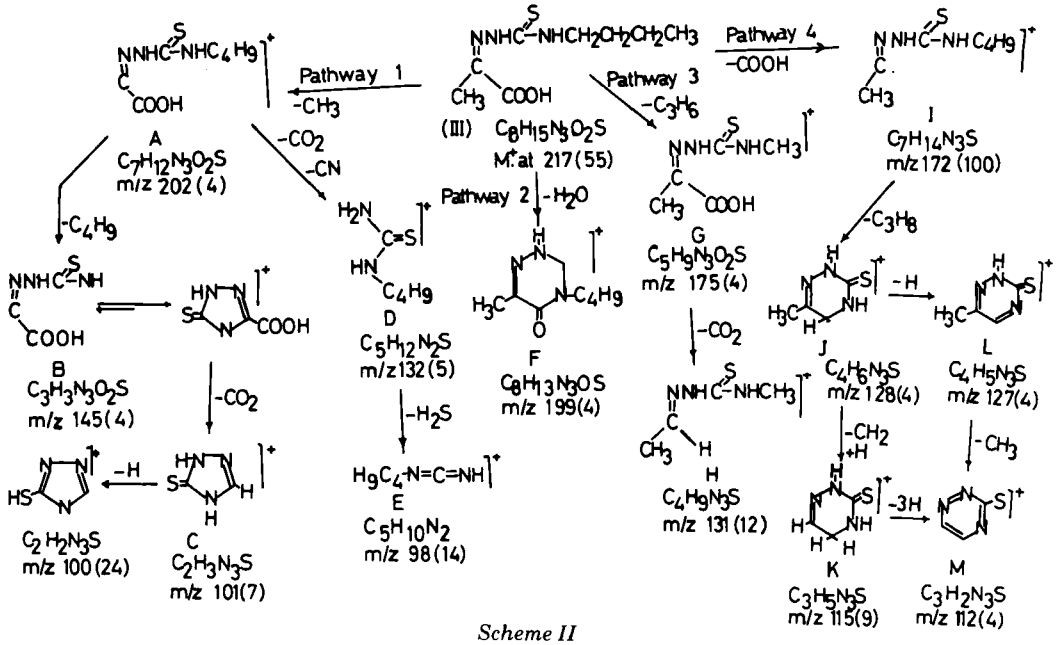


Scheme I



Scheme II

thiosemicarbazones with phenacyl bromide or the appropriately substituted phenacyl bromide and sodium acetate under reflux in ethanol yielded the arylidenepyruvic acid thiazoline derivatives (XV–XXVII).

The structure of the products was confirmed by IR and, for some representative examples, by ^{1}H-NMR spectral data. The IR spectra of the thiosemicarbazones (II–XIV) showed the bands characterizing the OH, NH, C=O, C=N, and NCS groups. Compounds XV–XXVII, on the other hand, lacked the NH absorption while showing absorption for C=O and C=N groups. The ^{1}H-NMR spectrum of III showed the CH_3 protons as a singlet at 2.21, the aromatic protons as a multiplet between 7.1 and 7.9, and the two NH and COOH protons as two singlets at 10.92 and 11.24 ppm, respectively. The spectrum of V showed the aromatic and the unsaturated side-chain protons as a multiplet between 7.0 and 8.1, while the protons of the COOH and the two NH functions appeared as singlets (disappearing on deuteration) at 10.92, 12.15, and 12.8 ppm, respectively. The spectra of the thiazolines XV and XXIV lacked the NH protons and showed the triazoline proton at 6.79 ppm.

The mass spectrum of III, as a representative example, showed the molecular ion peak at *m/z* 217 (Scheme II). This indicated that the molecule had undergone cleavage of a methyl group from the pyruvic acid portion to give ion *A* at *m/z* 202, (pathway 1). Ion *A* eliminated a butyl group forming the triazole ion *B*, at *m/z* 145, which eliminated carbon dioxide to give ion *C* at *m/z* 101. In an alternate way, ion *A* was found to eliminate carbon dioxide and a cyanide function to form the butylthiourea ion *D*, at *m/z* 132, which lost hydrogen sulfide to yield the carbodiimide ion *E* at *m/z* 98. In pathway 2, the molecule lost water to produce the triazine ion *F*, at *m/z* 199, while in pathway 3, the successive loss of propane and carbon dioxide gave ions *G* and *H* at *m/z* 175 and 131, respectively. The loss of a carboxylic function from the main product III (pathway 4) gave ion *I*, as the base peak, at *m/z* 172. This ion on losing propane gave ion *J*, at *m/z* 128, which on elimination of methylene and acceptance of hydrogen gave the triazine ion *K* at *m/z* 115. The loss of hydrogen from ion *J* followed by cleavage of the methyl group produced ions *L* and *M* at *m/z* 127 and 112, respectively.

Antimicrobial Screening—Using the serial dilution method in nutrient agar[1], all the products in concentrations of 100, 10, and 1 μg/ml were found to be inactive against *Escherichia coli* (NCTC 10418), *Klebsiella aerogenes* A, *Pseudomonas aeruginosa* (NCTC 10662), *Serratia mar-*

[1] The tests were performed in accordance with the protocol of antimicrobial screening of the Chemotherapeutic Research Centre, Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey, RH3 7AJ, United Kingdom.

Table I—Synthesized Thiosemicarbazones (II–XIV) and Thiazolines (XV–XXVII)

Compound	Yield, %	Melting Point	Molecular Formula	Analysis, %[a] C	H	N
II	92	197°	$C_8H_{15}N_3O_2S$	44.23 43.87	6.96 7.21	19.35 19.63
III	93	191–192°	$C_{10}H_{11}N_3O_2S$	50.63 50.74	4.67 4.77	17.72 17.93
IV	93	173–174°	$C_{15}H_{18}ClN_3O_2S$	53.02 53.30	5.30 5.40	12.37 12.72
V	91	185–186°	$C_{17}H_{14}ClN_3O_2S$	56.74 57.05	3.89 4.02	11.68 11.87
VI	95	194° (dec.)	$C_{18}H_{16}ClN_3O_2S$	57.83 57.73	4.28 4.34	11.24 11.47
VII	91	131–132°	$C_{15}H_{17}N_3O_3S$	56.42 56.69	5.37 5.53	13.16 13.01
VIII	94	183–184°	$C_{18}H_{17}N_3O_3S$	60.84 60.64	4.82 4.62	11.83 11.56
IX	93	254–255°	$C_{19}H_{19}N_3O_3S$	61.78 62.10	5.19 5.40	11.38 11.28
X	95	217–218°	$C_{19}H_{19}N_3O_3S$	61.78 61.72	5.19 5.48	11.38 11.62
XI	91	198° (dec.)	$C_{19}H_{17}N_3O_2S$	64.95 65.19	4.88 4.81	11.96 11.38
XII	93	168–169°	$C_{20}H_{19}N_3O_2S$	65.74 65.38	5.24 5.47	11.50 11.65
XIII	92	187–188°	$C_{20}H_{19}N_3O_2S$	65.74 65.38	5.24 5.10	11.50 11.69
XIV	95	204°	$C_{20}H_{19}N_3O_2S$	65.74 65.61	5.24 5.31	11.59 11.63
XV	75	148–149°	$C_{18}H_{13}ClN_3O_2S$	58.24 58.12	3.50 3.87	11.33 11.28
XVI	68	179–181°	$C_{22}H_{17}Cl_2N_3O_2S$	57.64 57.44	3.71 4.10	9.17 9.19
XVII	68	197°	$C_{25}H_{17}Cl_2N_3O_2S$	60.72 60.51	3.44 3.78	8.50 8.85
XVIII	68	251–253°	$C_{25}H_{19}BrClN_3O_2S$	56.47 56.53	3.43 3.57	7.60 7.89
XIX	68	193–195°	$C_{26}H_{19}Cl_2N_3O_2S$	61.41 61.23	3.74 3.71	8.26 8.11
XX	64	179–180°	$C_{26}H_{19}ClN_4O_4S$	60.17 60.46	3.60 3.93	10.80 10.56
XXI	72	207–209°	$C_{26}H_{19}Cl_2N_3O_2S$	61.41 61.20	3.74 3.83	8.26 8.05
XXII	70	181–183°	$C_{27}H_{22}ClN_3O_3S$	64.34 64.12	4.36 4.54	8.34 8.19
XXIII	69	220°	$C_{27}H_{20}ClN_3O_2S$	66.73 66.57	4.12 4.38	8.56 8.54
XXIV	72	118–120°	$C_{29}H_{25}N_3O_3S$	70.29 70.53	5.90 5.72	8.48 8.64
XXV	65	212–214°	$C_{28}H_{22}N_4O_4S$	65.87 64.68	4.34 4.37	10.98 10.67
XXVI	70	198–199°	$C_{28}H_{22}BrN_3O_2S$	61.76 61.43	4.04 4.36	7.72 7.33
XXVII	70	195–196°	$C_{28}H_{22}ClN_3O_2S$	67.26 67.17	4.40 4.62	8.41 8.65

[a] Calc. over found.

cescene (US32), *Staphylococcus aureus* Oxford, and *Candida albicans* (W97). Such inactivity, despite the antifungal and antimicrobial properties reported for arylidenepyruvic acid (7) and a variety of thiosemicarbazones (8–13), has been attributed to the bulk of the thiosemicarbazone moieties and the electronic changes caused by the presence of these groups on the α-carbon of the various arylidenepyruvic acids used. In the thiazoline derivatives (XV–XXVII), in which the 3- and 4-substituents were selected to fulfill the maximum requirement for hydrophobic π, electronic δ, and steric E_s factors in accordance with Topliss (14), the inactivity was assumed to be due to the bulky arylidenepyruvic acid hydrazone chains which, through intramolecular association with the remainder of the molecule, have hindered the permeability of tested compounds.

EXPERIMENTAL[2]

Substituted Thiosemicarbazone Arylidenepyruvic Acid Derivatives (II–XIV)—Equimolar amounts of pyruvic acid or the appropriately substituted arylidenepyruvic acid (I) (6) and the selected substituted thiosemicarbazides were heated under reflux in 80% aqueous acetic acid for 15–20 min. The mixtures were partially concentrated *in vacuo*, cooled, and the separated products recrystallized from ethanol. The yield and physical data of the products are recorded in Table I. IR (mineral oil): 3520–3420 (OH), 3360–3200 (NH), 1750–1690 (C=O), 1660–1580 (C=N and C=C), 1550–1515, 1315–1240, 1175–1157, and 970–930 cm^{-1} (NCS amide I, II, III, and IV bands, respectively). ^{1}H-NMR (III, DMSO-d_6): δ 2.21 (s, 3, CH_3), 7.1–7.9 (m, 5, Ar-H), and 10.92 and 11.24 ppm (2s, broad, 3, 2 × NH + COOH, disappearing on deuteration). ^{1}H-NMR (V, DMSO-d_6): δ 7.0–7.3 (m, 2, ethylene-H), 7.68 (s, 5, Ar-H), 7.5–8.1 (m, 4, Ar-H), and 10.9, 12.15, and 12.8 ppm (3s, 3, 2 × NH + COOH, disappearing on deuteration). Mass spectrum for III: *m/z* (relative abundance %) M^+ at 217 (65), 202 (4), 199 (4), 174 (22), 172 (100), 171 (4), 145 (4), 144 (5), 132 (5), 131 (12), 130 (20), 129 (4), 128 (4), 127 (4), 117 (4), 116 (59), 115 (9), 112 (4), 101 (7), 100 (24), 98 (14), 96 (31), 89 (13), 88 (59), 75 (19), 74 (11), 73 (14), 72 (61), and 70 (13).

Arylidenepyruvic Acid (3,4-Disubstituted 4-Thiazolin-2-ylidene)hydrazones (XV–XXVII)—An equimolar amount of the thiosemicarbazone derivatives (II–XIV) and phenacyl bromide or substituted phenacyl bromide and sodium acetate was heated under reflux in ethanol for 2–4 hr. The mixture was cooled, diluted with water, and the product removed by filtration. Recrystallization from ethanol or 90% aqueous ethanol gave XV–XXVII. The yields and physical constants of the synthesized thiazolines are recorded in Table I. IR (mineral oil): 3400–3200 (OH, associated), 1740–1660 (C=O), and 1620–1570 cm^{-1} (C=N and C=C).

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[2] Melting points were determined in open capillaries and are uncorrected. IR spectra were measured on a Beckmann 4210 IR Spectrophotometer. ^{1}H-NMR spectra were determined on Varian A60, while the mass spectra were recorded on EAI.MS-50.