the genetic material is occurring with time and that badly damaged sperm are being eliminated cannot now be discarded.

Sperm inactivation is another possible explanation for the decrease in frequency of mutation but it is rather difficult to show since the housefly is polyspermic.[3] However, **2** does not induce sperm inactivation in *Bracon*, even at doses greater than those needed to induce dominant lethal mutations in 100% of the sperm,[8] but with **11**, cytological examination established that the eggs were fertilized with the sperm from treated males.[3]

The data also suggest that mutagenic effectiveness is not necessarily directly related to the number of aziridinyl groups since compounds such as **5** and **7** were nearly as effective as **2**, and **8** was more active.

When the nonheteroaromatic compounds **9**, **10**, and **11** were tested for recovery, the number of recovered dominant lethal mutations did not decrease after storage for 7 days.

Experimental Section[9,10]

Melting points were taken with a Thomas-Hoover apparatus and are corrected. Pmr spectra were obtained in $CDCl_3$ with $(CH_3)_4Si$ as an internal reference on a Varian A-60A spectrometer. The ir spectra were measured in KBr or CsI pellets on a Cary Model 14 spectrophotometer. All spectral correlations were as expected.[11] Elemental analyses were performed by Huffman Laboratories, Inc., Wheatride, Colo.

Aziridinyllithium.—The azyridinyllithium (AzLi) was generated at room temperature *in situ*.[5] The aziridine (Az) was diluted tenfold with solvent (either C_6H_6 or Et_2O), and drops of MeLi[12] (about 1.6 *M* in Et_2O) were added rapidly. Because of the low boiling point of Az, a 10% molar excess was used. All work with Li derivatives was done in flame-dried glassware under N_2.

2-Chloro-4,6-bis(1-aziridinyl)pyrimidine (5).[6]—A solution of 9 g of 2,4,6-trichloropyrimidine in 45 ml of C_6H_6 was added dropwise to a solution of 11 ml of Az and 14 ml of Et_3N in 100 ml of C_6H_6 (in an ice bath). The ice bath was removed, and the solution was stirred for 4 hr. Charcoal was added to the mixture, and the suspension was filtered through Super Cel. The solution was concentrated *in vacuo* to 30 ml, and several portions of C_6H_{14} were added; the crystals were allowed to grow between additions; yield 73%. The product was recrystallized from C_6H_{14}; mp 97° dec. *Anal.* ($C_8H_9ClN_4$) C, H, Cl, N.

2,4,6-Tris(1-aziridinyl)pyrimidine (6).[6] **Method A.**—Direct conversion of 2,4,6-trichloropyrimidine with AzLi yielded a mixture of partially substituted pyrimidine plus some trisaziridinyl product. Pmr data indicated the presence of both monoaziridinyldichloro isomers and **5**. Column chromatography on alumina did effect a separation, but the overall yield was poor. Compound **5** was therefore used as a starting material to improve the yield.

Method B.—AzLi (40 mmoles) was generated in 30 ml of C_6H_6. 2,4-Bis(1-aziridinyl)-6-chloropyrimidine (7.0 g) was added slowly (about 10 min) as a solid. After 3 hr of stirring, 1 ml of H_2O and some charcoal were added to the mixture and the material was filtered. The solution was evaporated to near dryness, and 30 ml of C_6H_{14} was added. The mixture was then triturated and the supernatant C_6H_{14} was discarded. The residue was taken up in hot C_6H_{14}, charcoal was added, and the solution was allowed to cool after filtration. The compound was recrystallized from C_6H_{14}; yield 63%; mp 132–133° dec. *Anal.* ($C_{10}H_{13}N_5$) C, H, N.

2,6-Bis(1-aziridinyl)pyridine (7).—The compound was prepared in the same manner as **6**, method B, except that the quantity of AzLi was adjusted for the 2 equiv of Br to be replaced on 2,6-dibromopyridine. The product was recrystallized from C_6H_{14}; yield 65%; mp 98–100°. *Anal.* ($C_9H_{11}N_3$) C, H, N.

2,6-Bis(1-aziridinyl)pyrazine (8).—AzLi and 2,6-dichloropyrazine, when allowed to react by method B, produced the desired product in good yield. However, the reaction was somewhat more exothermic than the others reported; thus, the addition of the dichloro compound was slower. The product was recrystallized from C_6H_{14}; yield 75%; mp 57.5–59°. *Anal.* ($C_8H_{10}N_3$) C, H, N.

Compounds **1**, **2**, **3**, **4**, **9**, **10**, and **11** were prepared by methods described in the literature[4,5] or purchased from commerical sources. Compound **12** was prepared by the method of Heine, *et al.*[13]

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(9) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values.

(10) Mention of a proprietary product does not constitute a recommendation or an endorsement of this product by the U. S. Department of Agriculture.

(11) The ir assignments for azirdine were based on the paper by H. L. Spell, *Anal. Chem.*, **39**, 185 (1967).

(12) MeLi solution from Foote Mineral Co., was used directly.

Potential Antineoplastics. V. Synthesis of Ethyl 2,3-Dioxobutyrate 2-Arylhydrazono-3-thiosemicarbazones

H. G. GARG AND R. A. SHARMA

Department of Chemistry, University of Roorkee, Roorkee, India

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Ever since the initial observation made by Brockman, *et al.*,[1] that 2-formylpyridine thiosemicarbazone possessed mild but definite antileukemic activity, chemists have tried to prepare several classes of thiosemicarbazones,[2-4] but virtually none of these appeared to be a clinically useful drug. In spite of this unpromising background we synthesized a few ethyl 2,3-dioxobutyrate 2-arylhydrazono-3-thiosemicarbazones, because the incorporation of an arylazo moiety in several cases resulted in the enhancement of the potency of candiate drugs.[5] Characteristics of the new ethyl 2,3-dioxobutyrate 2-arylhydrazono-3-thiosemicarbazones are summarized in Table I.

The synthetic route in all cases involved the coupling of aryldiazonium salts with ethyl 3-oxobutyrate 3-thiosemicarbazone.[6]

Biological Activity.—Shown in Table II are the data for antitumor activity against L-1210 lymphoid leukemia. From this primary screening it appears that the level of toxicity varied greatly in this group. Ethyl 2-(2,5-dimethylphenyl)hydrazono-, 2-(3,5-dimethylphenyl)hydrazono-, 2-(2,5-dimethoxyphenyl)hydrazono-, and 2-(2-methoxyphenyl)hydrozono-2,3-dioxobutyrate 3-thiosemicarbazones are somewhat more potent than other members in this series.

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(5) R. E. Harmon, F. E. Dutton, and H. D. Warren, *ibid.*, **11**, 627 (1968).

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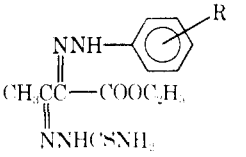
TABLE I

CHARACTERISTICS OF ETHYL 2,3-DIOXOBUTYRATE 2-ARYLHYDRAZONO-3-THIOSEMICARBAZONES



No.	R	Mp, °C	Yield, %	Color[a]	Formula	Analyses[b]
1	H	241–242	65	ON	$C_{13}H_{17}N_5O_2S$	N, S
2	2-NO_2	112–113	60	OYF	$C_{13}H_{16}N_6O_4S$	N, S
3	2-Br	210–212	70	YN	$C_{13}H_{16}BrN_5O_2S$	N, Br
4	2-Me	165–166	75	YN	$C_{14}H_{19}N_5O_2S$	N, S
5	4-Me	227–228	75	OYN	$C_{14}H_{19}N_5O_2S$	N, S
6	2-Et	201–202	70	YN	$C_{15}H_{21}N_5O_2S$	N, S
7	2-MeO	202–203	72	YN	$C_{14}H_{19}N_5O_3S$	N, S
8	4-EtO	204–205	75	ORN	$C_{15}H_{21}N_5O_3S$	N, S
9	2,3-Cl_2	154–155	80	YN	$C_{13}H_{15}Cl_2N_5O_2S$	N, Cl
10	2,4-Cl_2	135–136	75	OP	$C_{13}H_{15}Cl_2N_5O_2S$	N, Cl
11	3,5-Cl_2	133–134	75	YF	$C_{13}H_{15}Cl_2N_5O_2S$	N, Cl
12	2,3-Me_2	144–145	70	YN	$C_{15}H_{21}N_5O_2S$	N, S
13	2,4-Me_2	215–216	65	OYN	$C_{15}H_{21}N_5O_2S$	N, S
14	2,5-Me_2	206–207	65	RBuN	$C_{15}H_{21}N_5O_2S$	N, S
15	2,6-Me_2	183–184	70	ON	$C_{15}H_{21}N_5O_2S$	N, S
16	3,4-Me_2	212–213	75	ON	$C_{15}H_{21}N_5O_2S$	N, S
17	3,5-Me_2	192–193	74	RN	$C_{15}H_{21}N_5O_2S$	N, S
18	2,5-$(MeO)_2$	97–98	65	RBuN	$C_{15}H_{21}N_5O_4S$	N, S
19	2-Cl-6-Me	190–191	78	YN	$C_{14}H_{18}ClN_5O_2S$	N, Cl
20	4-Cl-2,5-$(MeO)_2$	178–179	60	ON	$C_{15}H_{20}ClN_5O_4S$	S, Cl

[a] Bu, brown; F, fibers; N, needles; O, orange; P, plates; R, red; Y, yellow. [b] All analytical results were within ±0.4% of the theoretical values.

TABLE II

SCREENING RESULTS OF ETHYL 2,3-DIOXOBUTYRATE 2-ARYLHYDROZONO-3-THIOSEMICARBAZONES AGAINST L-1210 LYMPHOID LEUKEMIA[a]

No.[b]	Survivors[c]	Animal weight diff (T-C)	Average evaluation T'est	Control	T/C[d], %
1	6/6	−0.1	8.3	8.8	94
2	6/6	−1.2	8.3	9.6	8.6
3	6/6	−1.3	8.3	8.8	94
4	0/6	−0.8	0.0	9.6	
5	5/6	−1.3	8.8	9.4	93
6	0/6	−0.8	0.0	9.6	
7	2/6	−2.8	9.0	8.8	102
8	6/6	−2.4	9.3	9.6	96
9	0/6	−0.8	0.0	9.6	
10	0/6	−0.4	0.0	9.4	
11	4/6	−4.0	8.3	9.6	86
12	0/6	−0.8	0.0	9.6	
13	0/6	−0.4	0.0	9.4	
14	2/6	−3.3	10.0	9.4	106
15	0/6	−0.4	0.0	9.4	
16	0/6	−0.4	0.0	9.4	
17	6/6	−0.7	9.7	9.4	103
18	5/6	−3.0	9.0	8.8	102
19	1/6	−0.7	9.0	9.4	96
20	0/6	−0.8	0.0	9.6	

[a] Host, BDF_1 mice; vehicle, carboxymethylcellulose, acetone, saline; route, intraperitoneal; parameter, mean survival time 30 days; tissue, ascitic fluid; level, 10^6 cells. [b] Numbers correspond to those in Table I. [c] All doses were 400 mg/kg. [d] Ratio of mean survival time of test animals (T) to control animals (c).

Experimental Section

All melting points were determined using a Kofler hot-stage type apparatus and are uncorrected.

Ethyl 3-Oxobutyrate 3-Thiosemicarbazone.—Thiosemicarbazide·HCl (2.56 g, 0.02 mole) in H_2O (15 ml) was shaken with ethyl 3-oxobutyrate (2.60 g, 0.02 mole) in the presence of NaOAc (2.0 g). The thiosemicarbazone separated out after 0.5 hr, was collected, washed well with H_2O, and recrystallized (C_6H_6): yield 1.93 g (95%), mp 93–94°. *Anal.* ($C_7H_{13}N_3O_2S$) N, S.

Ethyl 2,3-Dioxobutyrate -2-(5-Chloro-2,4-dimethoxyphenyl)-hydrazono-3-thiosemicarbazone.—5-Chloro-2,4-dimethoxyaniline (1.88 g, 0.01 mole) was dissolved in 3 *N* HCl (3 ml) and cooled to 0°. $NaNO_2$ (0.7 g, 0.01 mole) dissolved in H_2O (10 ml) was gradually added and the diazonium salt solution so obtained was filtered into a well-cooled, stirred mixture of NaOAc (2.5 g) and ethyl 3-oxobutyrate 3-thiosemicarbazone (2.03 g, 0.01 mole) containing EtOH (20 ml). The product thus precipitated was collected, washed well with H_2O, and recrystallized from EtOH (3.0 g, 75%) as deep red needles, mp 234–235°. *Anal.* ($C_{15}H_{20}ClN_5O_4S$), Cl, N.

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