prepare VIII except that (1) the reaction time was 4 hr and (2) the benzene solution of the reaction-mixture residue was washed with water, dried ($MgSO_4$), and treated with activated carbon before the dilution with cyclohexane. The analytical sample (from benzene–cyclohexane) melted at 55°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 227 (22.1), 260 (7.3), 289 (11.8) in ethanol.

Anal. Calcd for $C_{24}H_{25}N_7O_4$: C, 60.62; H, 5.31; N, 20.62. Found: C, 60.53; H, 5.29; N, 20.86.

N-(5-Amino-3-ethyl-3H-*v*-triazolo[4,5-*d*]pyrimidin-7-yl)aspartic Acid (XI).—A mixture of 10.1 g of X, 1 l. of absolute ethanol, and 2.0 g of 5% palladium–charcoal catalyst was treated with hydrogen at atmospheric pressure and room temperature until the calculated quantity of hydrogen had been absorbed. After the catalyst had been removed by filtration and the ethanolic filtrate concentrated to about 150 ml and chilled, a white solid precipitated; 5.16 g, mp 227–230° dec. A second portion of 540 mg (mp 225–235° dec) was obtained by evaporating the ethanol from the filtrate, dissolving the white residue in 1 *N* NaOH, and precipitating the product by acidification. A solution of the combined portions in 100 ml of 1 *N* NaOH was acidified to pH 2 with 6 *N* HCl and refrigerated. The white crystalline XI, which was separated by filtration, washed with water, and dried *in vacuo*, weighed 4.96 g (78%); mp 234–236° dec (cap.); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 256 (16.0), 273 (13.3) in 0.1 *N* HCl; 230 (16.6 sh), 265 (sh), 291 (12.9) in phosphate buffer (pH 7); 230 (16.0 sh), 265 (sh), 291 (12.8) in 0.1 *N* NaOH.

Anal. Calcd for $C_{10}H_{13}N_7O_4$: C, 40.64; H, 4.44; N, 33.21. Found: C, 40.71; H, 4.66; N, 33.14.

N-(5-Amino-3-butyl-3H-*v*-triazolo[4,5-*d*]pyrimidin-7-yl)aspartic acid (IX) was obtained by hydrogenation of VIII by the procedure for XI. The white solid remaining after removal of the catalyst and evaporation of the ethanol was recrystallized twice from 50% aqueous ethanol; yield of IX, 33%; mp 175–180° (cap.); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 256 (16.3), 274 (13.4) in 0.1 *N* HCl; 218 (18.0), 230 (sh), 265 (sh), 290 (13.1) in phosphate buffer; 230 (sh), 265 (sh), 290 (13.3) in 0.1 *N* NaOH.

Anal. Calcd for $C_{12}H_{17}N_7O_4$: C, 44.57; H, 5.36; N, 30.33. Found: C, 44.37; H, 5.36; N, 30.09.

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6-Azauracil Derivatives of Fluoropyruvic Acid[1]

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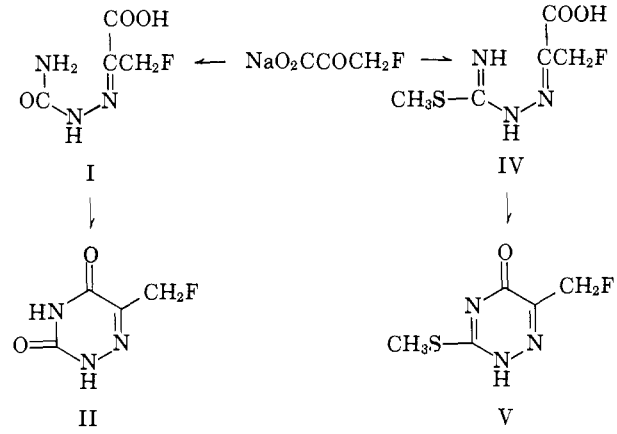
The current interest in 6-azauracil [*as*-triazine-3,5-(2H,4H)-dione] and its derivatives as potential anticancer agents[2,3] and in thiosemicarbazones as antiviral agents[4,5] has prompted the syntheses of the compounds described in this paper.

5-Fluoromethyl-6-azauracil [6-fluoromethyl-*as*-triazine-3,5(2H,4H)-dione] (II) was prepared by the ring closure of the known fluoropyruvic acid semicarbazone



(I).[6] The classical base-catalyzed ring closure of α-keto acid semicarbazones[7] to 3,5-dione-*as*-triazines failed, apparently due to the lability of the α-imino fluorine. Finally ring closure to II was accomplished by the method recently described by Heidelberger and Dipple.[8] Since fluoropyruvic acid 3-thiosemicarbazone (III) could not be cyclized to the *as*-triazine, fluoropyruvic acid 3-methylisothiosemicarbazone (IV) was used. Cyclization of IV to 3-(methylthio)-6-fluoromethyl-*as*-triazin-5(2H)-one (V) was effected in boiling water.[9] The infrared[10] and ultraviolet spectra[11] of this compound are similar to that of the known 3-(methylthio)-*as*-triazin-5(2H)-one, indicating the structure shown (V). The ease of cyclization of the semicarbazones I and IV indicates that they both have the *syn* configuration.

These compounds have been submitted to the National Institutes of Health, Cancer Chemotherapy National Screening Center, for anticancer and antiviral screening. Antiviral screening of I based on its ability to inhibit or prevent cytopathic effect (CPE) in tissue culture as found by the Viral Chemotherapy Section, Drug Evaluation Branch, CCNSC, National Cancer Institute, is given in Table I.

TABLE I

Virus system	Results[a]
Columbia SK	±
Vaccinia	+
Lymphocytes choriomeningitis	−

[a] ± = 25–50% CPE (cytopathic effect), + = 0–50%, and − = 50–100%. Negative results were obtained when I was tested in the *in vivo* Columbia SK virus system.

Experimental Section[12]

Fluoropyruvic Acid Semicarbazone (I).[6]—To a solution of 5.65 g (0.051 mole) of semicarbazide hydrochloride in 35 ml of water

(1) Supported largely by the Research Grant CA 08095-02 from the National Cancer Institute, Public Health Service.

(2) R. Lee Clark, Jr., "Cancer Chemotherapy," Charles C Thomas, Publisher, Springfield, Ill., 1961, pp 35–36.

(3) J. Skoda, *Progr. Nucleic Acid Res.*, **3**, 197 (1963).

(4) C. H. Stuart-Harris and L. Dickinson, "The Background to Chemotherapy of Virus Diseases," Charles C Thomas, Publisher, Springfield, Ill., 1964, pp 65–70.

(5) R. L. Thompson, *Advan. Chemotherapy*, **1**, 106 (1964).

(6) P. V. Nair and H. Basch, *J. Org. Chem.*, **23**, 137 (1958). The authors reported the melting point and analysis of I; however, since the preparation was not given, it is included in this paper.

(7) J. Gut, *Advan. Heterocyclic Chem.*, **1**, 204 (1963).

(8) C. Heidelberger and A. Dipple, Abstracts, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, p 13D.

(9) The facile cyclization of glyoxylic acid 3-methylisothiosemicarbazone has been reported by (a) A. R. Restivo and F. A. Dondzila, *J. Org. Chem.*, **27**, 2281 (1962); (b) P. K. Chang and T. L. V. Ulbricht, *J. Am. Chem. Soc.*, **80**, 976 (1958); and (c) E. Cattelain, *Bull. Soc. Chim. France*, **11**, 256 (1944).

(10) M. Horak and J. Gut, *Collection Czech. Chem. Commun.*, **28**, 3392 (1963).

(11) J. Jonas and J. Gut, *ibid.*, **27**, 1886 (1962).

(12) Melting points were determined using a Kofler hot stage. Ultraviolet absorption spectra were recorded by a Bausch and Lomb Spectronic 505 spectrophotometer. Infrared spectra were determined on a Perkin-Elmer Infracord spectrophotometer. Analyses were performed by Micro-Tech Laboratories, Skokie, Ill.

was added 3.65 g (0.028 mole) of sodium fluoropyruvate.[13] On cooling overnight at 0° the desired compound crystallized, was removed by filtration, and washed with cold water, yielding 3.55 g (76%) of I. The product turned brown at *ca.* 185°, melting at 206–207° dec; lit.[6] turns brown at 198–200° and decomposes at 205°.

5-Fluoromethyl-6-azauracil (II).—A mixture of 1.0 g (6 mmoles) of I, 50 ml of $SOCl_2$, and 1 drop of dry pyridine was refluxed 1 hr (clear solution), allowed to stand overnight at room temperature, and evaporated to 15 ml *in vacuo*. On cooling II crystallized and was removed by filtration through sintered glass and washed with dry ether, yielding 550 mg (63%) after drying at 80° *in vacuo*, mp 127–128°. A portion was recrystallized for analysis from chlorobenzene and dried at 80° *in vacuo*; needles, mp 127.5–129°; $\lambda_{max}^{95\%\ EtOH}$ 265 mμ (log ε 3.78); $\lambda_{min}^{95\%\ EtOH}$ 224 mμ; λ_{max}^{Nujol} 3.05, 3.12, 5.82–6.00, 7.75, 8.17, 9.81, 10.06, 11.53, 12.65, 13.00, 13.50, 13.82 μ.

Anal. Calcd for $C_4H_4FN_3O_2$: C, 33.11; H, 2.78; F, 13.10. Found: C, 33.16; H, 2.89; F, 13.29

Fluoropyruvic Acid 3-Thiosemicarbazone (III).—A solution of 915 mg (10 mmoles) of thiosemicarbazide, 11 ml of 1 *N* HCl, and 640 mg (5 mmoles) of sodium fluoropyruvate[13] was allowed to stand at room temperature for 1 min and then cooled in an ice bath. On cooling 600 mg (67%) of III crystallized as needles, mp 186–189° dec. Recrystallization from water (prolonged heating and standing at room temperature being avoided) gave needles; the product turned orange-red at 150–160° and melted at 185–190° dec; $\lambda_{max}^{95\%\ EtOH}$ 230 mμ (log ε 3.84), 301 mμ (log ε 4.13); $\lambda_{min}^{95\%\ EtOH}$ 217, 240 mμ; λ_{max}^{Nujol} 2.92, 3.05, 3.10, 4.01, 6.00, 6.21, 6.32, 6.92, 7.15, 7.90, 8.02, 8.38, 8.81, 9.55, 9.97, 10.31, 11.75, 12.40, 12.72 μ.

Anal. Calcd for $C_4H_6FN_3O_2S$: C, 26.81; H, 3.38; N, 23.45; S, 17.90. Found: C, 26.70; H, 3.40; N, 23.20, 23.43; S, 17.76.

Fluoropyruvic Acid 3-Methylisothiosemicarbazone (IV).—A solution of 4.66 g (20 mmoles) of 3-S-methylisothiosemicarbazide hydriodide[14] and 1.28 g (10 mmoles) of sodium fluoropyruvate[13] in 30 ml of water was allowed to stand at room temperature for 10 min and then at 2° for 2 days, yielding IV which was dried *in vacuo* at 60°; yield 1.35 g (70%), transition to needles at 140–150°, mp 182–184° dec. Recrystallization from ethyl acetate followed by drying *in vacuo* at 70° did not change the melting point; $\lambda_{max}^{95\%\ EtOH}$ 262 mμ (log ε 4.16); λ_{max}^{Nujol} 3.11, 3.21, 6.01, 6.60, 7.24, 8.25, 8.90, 9.71, 10.20, 11.35, 11.72, 13.15, 13.75, 14.08 μ.

Anal. Calcd for $C_5H_8FN_3O_2S$: C, 31.08; H, 4.17; N, 21.75; S, 16.60. Found: C, 30.95; H, 4.39; N, 21.55; S, 16.35.

3-(Methylthio)-6-fluoromethyl-*as*-triazin-5(2H)-one (V).—A mixture of 320 mg (1.51 mmoles) of IV and 5 ml of water was heated to boiling and cooled (prolonged heating being avoided). On cooling 150 mg (57%) of V was obtained as needles, mp 180–187° dec. Recrystallization of a portion of V from water followed by drying at 70° *in vacuo* raised the melting point to 185–187° dec; $\lambda_{max}^{95\%\ EtOH}$ 235 mμ (log ε 4.26); λ_{max}^{Nujol} 3.15, 3.75, 6.25, 6.31, 6.60, 7.40, 7.61, 7.90, 8.08, 8.88, 9.70, 10.02, 10.40, 10.80, 12.58, 12.86, 13.85 μ.

Anal. Calcd for $C_5H_6FN_3OS$: C, 34.28; H, 3.45; N, 23.99; S, 18.31. Found: C, 34.54; H, 3.26; N, 24.08; S, 18.35.

(13) Commercially available.

(14) M. Freund and Th. Paradus, *Chem. Ber.*, **34**, 3110 (1901).

The Synthesis and Antitumor Activity of Several Thiosemicarbazones Related to Kethoxal Bis(thiosemicarbazone)[1]

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A recent report from our laboratory[2] described the synthesis, physical properties, and antitumor activity of a series of α-ketoaldehyde bis(thiosemicarbazones). The most active compounds were shown to be 2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone)[3] (I) and 2-keto-3-ethoxybutyraldehyde bis(N^4-methylthiosemicarbazone)[3] (II). Both compounds were found to have high antitumor activity *in vivo* and *in vitro*. Several closely related compounds including 2-keto-3-ethoxybutyraldehyde bis(N^4-dimethylthiosemicarbazone) showed little or no *in vivo* activity.

We have now prepared several additional compounds closely related to I and II in order to explore further the effects of structure on antitumor activity, several of which do have high biological activity. We wish to report here the synthesis of these compounds and some preliminary biological data.

$$\begin{array}{l} R_1C{=}NNHCSNR_2 \\ | \\ CH{=}NNHCSNR_2 \end{array}$$

I, R_1 = $C_2H_5OCH(CH_3)$–; R_2 = H_2
II, R_1 = $C_2H_5OCH(CH_3)$–; R_2 = $H(CH_3)$
III, R_1 = $CH_3OCH(CH_3)$–; R_2 = H_2
IV, R_1 = $CH_3OC_2H_4OCH(CH_3)$–; R_2 = H_2
V, R_1 = $CH_3CO_2CH(CH_3)$–; R_2 = H_2
VI, R_1 = CH_3; R_2 = $(CH_3)_2$

Chemistry.—The new compounds which were prepared are listed in Table I together with their abbreviations, formulas, and physical and chemical properties. The compounds were prepared by treating the appropriate α-ketoaldehyde with thiosemicarbazide or N^4-dimethylthiosemicarbazide in 2–5% acetic acid solutions. The reactions were straightforward and no complications were experienced.

Three of the new compounds, namely, III, IV, and V, involve modifications of the 3-ethoxy group of the highly active kethoxal bis(thiosemicarbazone) (I). Compound VI was prepared to complete the series of pyruvaldehyde derivatives previously reported.[2]

Biological Results.—The antitumor activity of III, IV, and V is compared with that of I in Table II. The compounds were tested against a nitrogen mustard resistant variant of the Walker 256 carcinosarcoma implanted in Sprague–Dawley male rats, under conditions previously described.[2] VI was not available in sufficient amounts to conduct animal studies on its activity, but *in vitro* tests[4] indicated that this compound was considerably less active than any of the other compounds described here.

Experiment A of Table II shows that 2-keto-3-acetoxybutyraldehyde bis(thiosemicarbazone) (V) when given orally to rats had the least activity of any of the compounds tested by this route, although its toxicity was as great as any of the others. However, when V was given intraperitoneally (expt B) much higher activity was obtained. The low oral activity of this compound may be due to its poor absorption from the intestinal tract or to the splitting of the ester group.

The most active compound of this series was 2-keto-3-methoxybutyraldehyde bis(thiosemicarbazone) (III) as indicated in expt C and D. Because of its high oral activity III was also tested by the intraperitoneal route.

(1) Reported in part at the 149th National Meeting of the American Chemical Society, Detroit, Mich., April 5–9, 1965, Abstracts, p 13N.

(2) H. G. Petering, H. H. Buskirk, and G. E. Underwood, *Cancer Res.*, **24**, 367 (1964).

(3) 2-Keto-3-ethoxybutyraldehyde has been designated kethoxal. Kethoxal bis(thiosemicarbazone) is generally abbreviated as KTS.

(4) These studies were made using a modification of the method described by T. Arai and M. Suzuki [*J. Antibiot.* (Tokyo), **A9**, 169 (1956)]. Further details are now being prepared for publication.