2-Thienyl *p*-Tolyl Sulfone.—2-Lithiothiophene was formed by metalation of thiophene with *n*-butyllithium.[4a] The yield has been shown to be essentially quantitative.[13] A solution of 2-lithiothiophene from 8.4 g (0.10 mole) of thiophene in ether–pentane–hexane solvent was maintained at room temperature during the addition (50 min) of 70.4 g (0.30 mole) of 2-chloroethyl *p*-toluenesulfonate in 85 ml of ether. The mixture was stirred for 3 hr at room temperature, hydrolyzed with excess water, and extracted with ether in the usual fashion. Distillation of the extracts gave unreacted 2-chloroethyl *p*-toluenesulfonate and thiophene from unreacted 2-lithiothiophene as the only distillable products. The residual red oil was crystallized from acetone with charcoal treatment to yield 8.1 g (34%) of colorless crystals, mp 120–121°.

Anal. Calcd for $C_{11}H_{10}O_2S_2$: C, 55.40; H, 4.24; S, 26.90. Found: C, 55.36, 55.51; H, 4.20, 4.36; S, 26.71, 26.88.

The infrared spectrum (KBr disk) of the sulfone showed strong bands at approximately 7.6 and 8.7 μ which may be assigned[14] to the sulfone group.

Acknowledgment.—The authors would like to express appreciation to the National Cancer Institute for pharmacological testing and for partial financial support under Public Health Service Research Grant No. CA-04068 from the National Cancer Institute.

(13) D. A. Shirley and K. R. Barton, *Tetrahedron*, **22**, 515 (1966).

(14) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, pp 360–361.

Studies on Methylglyoxal Bis(guanylhydrazone)[1] Analogs. V. Methylglyoxal Guanylhydrazone Thiosemicarbazones and Related Compounds[2]

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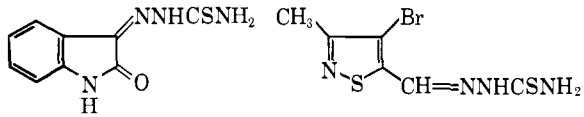
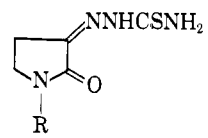
Derivatives of benzaldehyde thiosemicarbazone have been reported to be active tuberculostatic[3] and antiviral[4] agents. Additional work on thiosemicarbazone compounds led to the discovery of antiviral activity of isatin 3-thiosemicarbazone (I) (ITSC) against the pox group of viruses in human and type 2 polio in ERK cells.[5] A number of monocyclic thiosemicarbazones, such as derivatives of nicotinaldehyde, isonicotinaldehyde, and 2- and 3-thenaldehydes, have also shown high antiviral activity.[6] 4-Bromo-3-methylisothiazole-5-carboxaldehyde thiosemicarbazone (II), when given orally, was found to protect mice infected intracerebrally with neurovaccinia.[5] Two thiosemicarbazones derived from substituted pyrrolidine-2,3-diones (III, R = C_2H_5 and CH_2CH_2OH) demonstrated protection against experimental influenza infection in mice.[5]



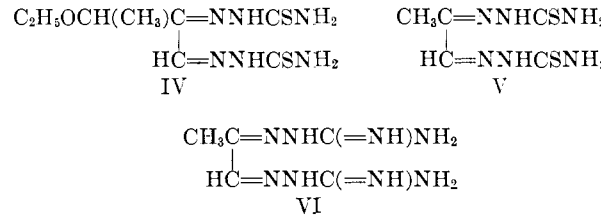
I

II



III

2-Keto-3-ethoxybutyraldehyde (kethoxal) has been shown to have antiviral activity in embryonated eggs infected with PR-8 influenza and Newcastle disease.[7] Its bis(thiosemicarbazone) derivative (IV, KTS) was



IV V VI

found to be very effective when given orally or intraperitoneally to rats bearing the Walker 256 carcinosarcoma and S180.[8] This activity pattern is similar to a closely related compound, methylglyoxal bis(thiosemicarbazone)[9] (V), but *different* from that of the guanylhydrazone analog (VI). The latter compound, the structural modifications of which have been systematically studied in our laboratories for the past 3 years,[10] was found to be active in leukemia L1210 and Ca755 and is considered as one of the few agents clinically effective in the therapy of adult acute myelogenous leukemia.[11]

In view of the antituberculous, antiviral, and antileukemic as well as other antitumor activities demonstrated by the thiosemicarbazone and guanylhydrazone derivatives, and the importance of the methylglyoxal moiety in cell growth,[12] synthesis and biological evaluation of compounds VII and VIII are of pertinent interest.[13]

(1) According to *Chemical Abstracts*, the name for this compound is 1,1'-[(methyl)ethanediylidenedinitrilo]diguanidine.

(2) This investigation was supported by the Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, National Institutes of Health, Public Health Service, Contract PH-43-65-94.

(3) (a) G. Domagk, *Naturwissenschaften*, **33**, 315 (1946); (b) R. Behnisch, F. Mietzsch, and H. Schmidt, *Angew. Chem.*, **60**, 113 (1948); (c) N. P. Buu-Hoï, M. Welsch, G. Dechamps, H. L. Bihan, F. Binon, and N. D. Xuong, *J. Org. Chem.*, **18**, 121 (1953).

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(5) *Cf.* H. E. Whipple, Ed., *Ann. N. Y. Acad. Sci.*, **130**, 71 (1965).

(6) R. L. Thompson, S. A. Minton, Jr., J. E. Officer, and G. H. Hitchings, *J. Immunol.*, **70**, 229 (1953).

(7) (a) G. E. Underwood and S. D. Weed, *Proc. Soc. Exptl. Biol. Med.*, **93**, 421 (1956); (b) W. F. McLimans, G. E. Underwood, E. A. Slater, E. V. Davis, and R. A. Siem, *J. Immunol.*, **78**, 104 (1957).

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(9) (a) C. Neuberg, and M. Kobel, *Biochem. Z.*, **188**, 215 (1927). (b) Testing information provided by CCNSC.

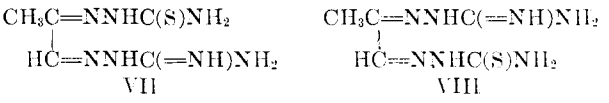
(10) (a) E. G. Podrebarac, W. H. Nyberg, F. A. French, and C. C. Cheng, *J. Med. Chem.*, **6**, 283 (1963); (b) F. Baiocchi, C. C. Cheng, W. J. Haggerty, Jr., L. R. Lewis, T. K. Liao, W. H. Nyberg, D. E. O'Brien, and E. G. Podrebarac, *ibid.*, **6**, 431 (1963); (c) E. G. Podrebarac and C. C. Cheng, *ibid.*, **7**, 806 (1964); (d) T. K. Liao, F. Baiocchi, and C. C. Cheng, *J. Org. Chem.*, **30**, 560 (1965).

(11) (a) B. L. Freedlander and F. A. French, *Cancer Res.*, **18**, 360 (1958); (b) E. J. Freireich and E. Frei, III, *Proc. Am. Assoc. Cancer Res.*, **3**, 319 (1962); (c) E. J. Freireich, E. Frei, III, and M. Karon, *Cancer Chemotherapy Rept.*, **No. 16**, 183 (1962).

(12) A. Szent-Györgyi [*Science*, **149**, 34 (1965)] recently stated that substances which promote ("promine") or retard ("retine") cell growth may provide keys to fundamental problems of cellular biology. Further study with the isolated "retine" indicated that methylglyoxal, combined in some fashion with a nitrogen-containing moiety, is the chief constituent.

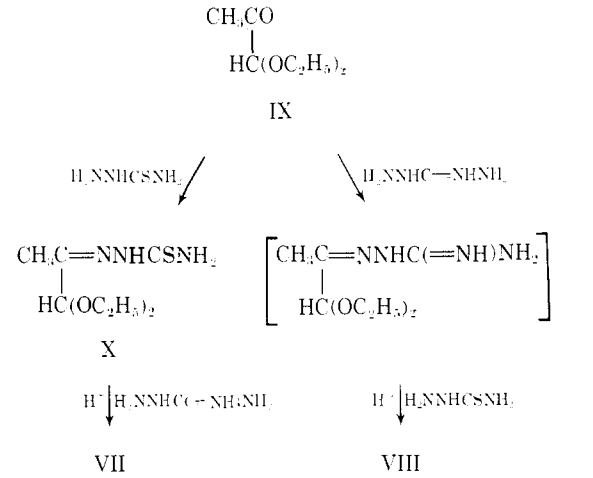
(13) According to Thompson, *et al.*,[6] most thiosemicarbazones possessing high antiviral activity are those in which the thiosemicarbazone group, $=NNHCSNH_2$, is separated by *two* carbon atoms from a N or S atom. This statement has since been substantiated by Furst[14] based on chelation studies. Our structure–activity study of the guanylhydrazone derivatives in antileukemic screenings indicated that a similar relationship is also one of the fundamental requirements in the $=NNHC(=NH)NH_2$ series.

(14) A. Furst, "Chemistry of Chelation in Cancer," Charles C Thomas, Springfield, Ill., 1963, p 67.



VII VIII

Pyruvaldehyde diethyl acetal (IX), the key intermediate for the synthesis of VII and VIII, was prepared from pyruvaldehyde according to the method of Braude and Evans.[15] Addition of thiosemicarbazide to an ethanol solution of IX readily formed pyruvaldehyde diethylacetal thiosemicarbazone (X), which reacted with an equivalent amount of aminoguanidine in the presence of hydrochloric acid to yield VII. When compound IX was first allowed to react with aminoguanidine followed by reaction with thiosemicarbazide in the presence of acid, the other isomer (VIII) was obtained.



IX

X

VII VIII

A semicarbazone analog of VII, pyruvaldehyde 1-guanylhydrazone 2-semicarbazone, was prepared from IX in a manner similar to that described for the preparation of VII.

Preliminary antitumor screening results[16] of VII and VIII with doses ranging between 15–480 mg/kg in BDF_1 mice indicated that replacement of either imino group by sulfur in VI results in the loss of activity against leukemia L1210.

Experimental Section[17]

Pyruvaldehyde Diethyl Acetal Thiosemicarbazone (X).—To a solution of 29.2 g (0.2 mole) of pyruvaldehyde diethyl acetal[15] (IX) in 250 ml of absolute ethanol was added, in one portion, 18.2 g (0.2 mole) of thiosemicarbazide. The reaction mixture was stirred for 15 hr at room temperature. The resulting solid was collected by filtration and washed with absolute ethanol. The product, weighing 31 g (71% yield), was satisfactory for use in the following experiment. For purposes of characterization a small portion was recrystallized from a mixture of ethanol and water to give a product which melted at 128–130°, resolidified, then slowly decomposed above 160°; $\lambda_{max}^{pH\ 1}$ 301 mμ (ε 14,200); $\lambda_{max}^{pH\ 11}$ 234 mμ (ε 8100), 267 mμ (ε 21,200).

Anal. Calcd $C_8H_{17}N_3O_2S$: C, 43.8; H, 7.81; N, 19.2 Found: C, 43.7; H, 7.81; N, 19.4.

Pyruvaldehyde 1-Guanylhydrazone 2-Thiosemicarbazone Hydrochloride (VII).—A solution of aminoguanidine hydrochloride [prepared by the addition of excess HCl to a suspension of 13.6 g (0.1 mole) of aminoguanidine bicarbonate in 150 ml of water] was added to a suspension of 22 g (0.1 mole) of X in 300 ml of ethanol. The mixture was stirred at room temperature for 2 hr after which time the insoluble material was removed by filtration and the filtrate was refrigerated overnight. The solid which separated was collected and the filtrate was concentrated at room temperature. Absolute ethanol (about 5 vol) was added to the concentrate and the solution was cooled overnight. In this manner a second crop of product was obtained. The combined solids were washed with absolute ethanol and dried at 56° (0.01 mm) for 15 hr to give 17 g (69% yield) of product: mp 223–224° dec, $\lambda_{max}^{pH\ 1}$ 308 mμ (ε 47,700), $\lambda_{max}^{pH\ 10}$ 332 mμ (ε 44,800).

Anal. Calcd for $C_5H_{11}N_7S \cdot HCl \cdot 0.5H_2O$: C, 24.3; H, 5.31; Cl, 14.4; N, 39.7; S, 13.0. Found: C, 24.3; H, 5.60; Cl, 14.6; N, 40.0; S, 12.9.

The corresponding sulfate salt was similarly prepared: mp 236–237° dec.

Anal. Calcd for $C_5H_{11}N_7S \cdot 0.5H_2SO_4 \cdot 0.5H_2O$: C, 23.2; H, 5.05; N, 37.8; S, 18.6. Found: C, 23.4; H, 5.02; N, 38.0; S, 18.8.

Pyruvaldehyde 1-Guanylhydrazone 2-Semicarbazone Hemisulfate.—A mixture of 7.5 g (0.1 mole) of semicarbazide and 14.6 g (0.1 mole) of IX in 100 ml of absolute ethanol was stirred at room temperature for 18 hr. There was then added 100 ml of an acidic solution of aminoguanidine sulfate [prepared from 13.6 g (0.1 mole) of aminoguanidine bicarbonate and excess sulfuric acid]. After being stirred overnight at room temperature the solid from the reaction mixture was collected by filtration, yielding 17.5 g (72.0% yield) of a white solid, mp 221–222° dec, $\lambda_{max}^{pH\ 1}$ 283 mμ (ε 33,500), $\lambda_{max}^{pH\ 11}$ 294 mμ (ε 25,500).

Anal. Calcd for $C_5H_{11}N_7O \cdot 0.5H_2SO_4 \cdot 0.5H_2O$: C, 24.7; H, 5.37; N, 40.3. Found: C, 24.7; H, 5.60; N, 40.4.

Pyruvaldehyde 2-Guanylhydrazone 1-Thiosemicarbazone Hydrochloride. (VIII).—To a stirred suspension of 14.6 g (0.1 mole) of IX and 9.0 g of sodium acetate in 200 ml of absolute ethanol was added, at room temperature, 11.0 g (0.1 mole) of aminoguanidine hydrochloride. After being stirred at room temperature for 18 hr, the reddish colored mixture was chilled and there was then added 25 ml of concentrated HCl, 50 ml of water, and finally, 9.1 g (0.1 mole) of thiosemicarbazide. With the addition of acid the reddish color of the reaction mixture disappeared. The mixture was stirred at 0° for 2 hr, then at room temperature for 1 hr, during which time the thiosemicarbazide slowly dissolved and a white solid gradually separated. The product was isolated by filtration. Purification was effected by dissolving in water then filtering to remove a small amount of insoluble material. The filtrate was evaporated at room temperature *in vacuo* to yield the desired product. The product (21.0 g, 82% yield) was dried at 25° (0.05 mm) for 15 hr, then allowed to equilibrate with atmospheric moisture: mp 248° dec, $\lambda_{max}^{pH\ 1}$ 305 mμ (ε 50,500), $\lambda_{max}^{pH\ 11}$ 335 mμ (ε 43,600).

Anal. Calcd for $C_5H_{11}N_7S \cdot HCl \cdot H_2O$: C, 23.5; H, 5.53; N, 38.4. Found: C, 23.6; H, 5.90; N, 38.1.

Acknowledgment.—The authors wish to express their appreciation to Mr. Leland R. Lewis, Mr. John R. Gravatt, and Mrs. Margaret Rounds for the analytical and instrumental measurements.

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(16) Biological testing work was carried out by contract screeners of CCNSC of the National Cancer Institute.

(17) All melting points (corrected) were taken on a Thomas–Hoover melting point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2 spectrophotometer.

Some Congeners and Analogs of Dipyridamole

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Since 2,6-bis(diethanolamino)-4,8-dipiperidinopyrimido[5,4-*d*]pyrimidine (dipyridamole) (Ia, R = R″ = $(HOCH_2CH_2)_2N$; R′ = R‴ = piperidino) is a potent coronary vasodilator,[1] we were prompted to

(1) (a) R. Kadatz, *Arzneimittel-Forsch.*, **9**, 39 (1959); (b) G. Brabner, F. Kaindl, and O. Krampp, *ibid.*, **9**, 45 (1959); (c) T. Hockerts and G. Bogelmann, *ibid.*, **9**, 47 (1959); (d) H. J. Bretschneider, A. Frank, C. Bernard, K. Kochsiek, and F. Scheler, *ibid.*, **9**, 49 (1959); (e) H. Spitzbarth, *ibid.*, **9**, 59 (1959).