(5) J. S. Zweig and N. Castagnoli, Jr., *J. Med. Chem.*, **17**, 747 (1974); P. Jacob, III, and N. Castagnoli, Jr., unpublished data.
(6) L. L. Butcher, *J. Neural. Transm.*, **37**, 189 (1975).
(7) F. A. B. Aldous, B. C. Barrass, K. Brewster, D. A. Buxton, D. M. Green, R. M. Pinder, P. Rich, M. Skeels, and K. J. Tutt, *J. Med. Chem.*, **17**, 1100 (1974).
(8) A. T. Shulgin, *J. Pharm. Pharmacol.*, **25**, 271 (1973); F. Benington, R. D. Morin, J. Beaton, J. R. Smythies, and R. J. Bradley, *Nature (London), New Biol.*, **242**, 185 (1975).
(9) S. B. Matin, P. S. Callery, J. S. Zweig, A. O'Brien, R. Rapoport, and N. Castagnoli, Jr., *J. Med. Chem.*, **17**, 877 (1974).
(10) N. P. McGraw, P. S. Callery, and N. Castagnoli, Jr., *J. Med. Chem.*, **20**, 185 (1977).
(11) J. Gal, L. Gruenke, and N. Castagnoli, Jr., *J. Med. Chem.*, **18**, 683 (1975); R. Weinkam, J. Gal, P. Callery, and N. Castagnoli, Jr., *Anal. Chem.*, **48**, 203 (1976).
(12) D. E. Nichols and A. T. Shulgin, *J. Pharm. Sci.*, **65**, 1554 (1976).
(13) R. T. Standridge, H. G. Howell, J. A. Gylys, R. A. Partyka, and A. T. Shulgin, *J. Med. Chem.*, **19**, 1400 (1976).
(14) C. F. Barfknecht, D. E. Nichols, and W. J. Dunn, *J. Med. Chem.*, **18**, 208 (1975).
(15) A. Quilico, *Gazz. Chim. Ital.*, **57**, 793 (1927).
(16) E. H. Huntress and F. H. Carten, *J. Am. Chem. Soc.*, **62**, 603 (1940).
(17) C. M. Suter and H. L. Hansen, *J. Am. Chem. Soc.*, **55**, 2080 (1933).
(18) L. Field and F. A. Grunwald, *J. Org. Chem.*, **16**, 946 (1951).
(19) R. Adams and C. S. Marvel, "Organic Syntheses", Collect. Vol. I, Wiley, New York, N.Y., 1941, p 504.
(20) K. Fries, H. Koch, and H. Stukenbrock, *Justus Liebigs Ann. Chem.*, **468**, 162 (1929).
(21) H. Burton and E. Hoggarth, *J. Chem. Soc.*, 14 (1945).
(22) A. Kiss, E. Vinkler, and E. Csetneky, *Acta Phys. Chem.*, **2**, 192 (1949); *Chem. Abstr.*, 44, 5706*g* (1966).
(23) D. E. Nichols, C. F. Barfknecht, and D. B. Rusterholz, *J. Med. Chem.*, **16**, 480 (1973).
(24) S. B. Matin, M. Rowland, and N. Castagnoli, Jr., *J. Pharm. Sci.*, **62**, 821 (1973).
(25) Although O- and S-demethylations of **6c** and **9c** would lead to thiohydroquinones, subsequent two-electron oxidations of these species to quinoid species are unlikely in view of the high energy of the carbon–sulfur π bond: J. B. Hendrickson, D. J. Cram, and G. S. Hammond, "Organic Chemistry", 3rd ed, McGraw-Hill, New York, N.Y., 1970, pp 790–792.

Synthesis and Evaluation of α-Hydroxythiol Esters as Antitumor Agents and Glyoxalase I Inhibitors[1]

Stan S. Hall,* Lidia M. Doweyko, Arthur M. Doweyko, and Judith S. Ryan Zilenovski

Carl A. Olson Laboratories, Department of Chemistry, Rutgers University, Newark, New Jersey 07102.
Received January 31, 1977

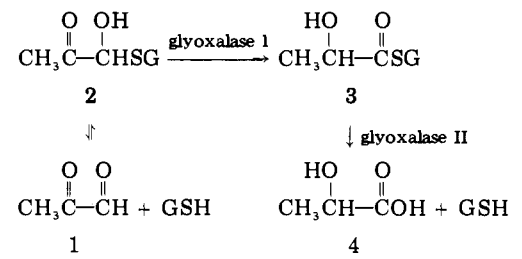
Synthesis of a series of α-hydroxythiol esters made available, for the first time, product-like molecules that were evaluated as inhibitors of the enzyme glyoxalase I and as potential antitumor agents. All the α-hydroxythiol esters tested were competitive inhibitors of the enzyme, albeit weak; however, the relative $[I]_{50}$ values suggested information about the active site. Antileukemic activity in L1210 lymphoid leukemia indicated no significant activity by these α-hydroxythiol esters.

The glyoxalase system, which consists of glyoxalase I [*S*-lactoylglutathione methylglyoxal lyase (isomerizing); E.C. 4.4.1.5], the coenzyme glutathione (GSH), and glyoxalase II (*S*-2-hydroxyacylglutathione hydrolase; E.C. 3.1.2.6), converts methylglyoxal (**1**) to lactic acid (**4**) (see Scheme I).

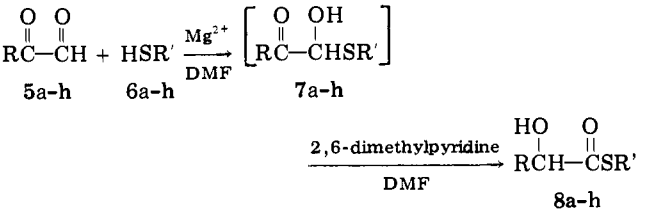
It has been suggested that the glyoxalase enzyme system, which is found widely distributed in cells of all forms of life,[2,3] may be involved in the regulation of cell growth by maintaining a proper concentration of one of the substrate components methylglyoxal (**1**).[4,5] The deficiency of this ketoaldehyde **1**[6] and the unusually high concentration of lactic acid[7] in cancer cells imply that such cells, having lost the ability to maintain a proper concentration of methylglyoxal, continue to proliferate at an unchecked rate. Selective inhibition of glyoxalase I that may result in a buildup of methylglyoxal (**1**) again in these cells might provide an effective means of chemotherapy.

Numerous studies, testing this hypothesis, have been carried out using substrate-related molecules. The carcinostatic activity of α-ketoaldehydes, including methylglyoxal (**1**), is known[8] but not effective since these agents are rapidly metabolized to the corresponding α-hydroxy acids by the glyoxalase enzyme system.[4] *S*-Alkyl derivatives and related compounds of the coenzyme glutathione (GSH) cause potent competitive inhibition of glyoxalase I[4,9-11] and cytotoxic activity against L1210 leukemia and KB cells in tissue culture.[12] However, the rapid degradation of S-substituted glutathione derivatives by glutathionase and cysteinylglycinase renders many of these inhibitors ineffective when tested in vivo.[13,14] Consequently attempts have been made to find degradation resistant analogues.[13]

Scheme I



Scheme II



The discovery in this laboratory of a convenient method to prepare α-hydroxythiol esters[15] made available a unique series of compounds that should be competitive inhibitors of the glyoxalase system and might be effective anticancer

Table I. Synthesis and Inhibition Studies of α-Hydroxythiol Esters

No.	R	R′	% yield[a]	K_i[b]	$[I]_{s0}$[c]
8a	CH_3	$(CH_2)_3CH_3$	41	12.9	16.5
8b	CH_3	$CH_2C_6H_5$	56	5.5	7.1
8c	C_6H_5	$(CH_2)_3CH_3$	79	29.6	37.8
8d	C_6H_5	$CH_2C_6H_5$	63	6.3	8.1
8e	C_6H_5	CH_2COOCH_3	42	8.2	10.5
8f	C_6H_5	$CH_2COO(CH_2)_3CH_3$	80	34.7	44.5
8g[d]	C_6H_5	$CH_2COOC_8H_{17}$	76	27.3	35.0
8h	C_6H_5	$(CH_2)_2COOCH_3$	67	38.6	49.6

[a] Isolated pure by column chromatography. [b] K_i values (mM) were determined from a Lineweaver-Burke treatment of inhibition data at 16.36 mM methylglyoxal and 1.42–7.09 mM GSH. [c] The $[I]_{s0}$ is defined as the millimolar concentration of inhibitor required to inhibit the enzymatic reaction 50% and was calculated using the expression $[I]_{s0} = K_i(K_m + [S]_0)/K_m$ for the substrate component concentrations of 16.36 mM methylglyoxal and 2.84 mM GSH. [d] The thiol was prepared from an isomeric mixture of octanol and isooctyl alcohol.

agents. This new synthetic procedure provided the opportunity to evaluate, for the first time, product-like structures.

Synthesis. A series of α-hydroxythiol esters was prepared as shown in Scheme II. Equilibration of the glyoxal **5** with the thiol **6** was carried out in the presence of Mg^{2+} (0.5 equiv) in DMF solvent. The resulting α-ketohemithiol acetal **7**, which is presumably chelated to the Mg^{2+}, is subsequently converted to the corresponding α-hydroxythiol ester **8** by the addition of 2,6-dimethylpyridine (1 equiv). The mechanism of this reaction has been previously discussed.[1,15] Increased isolated yields were realized by the presence of trace amounts of hydroquinone that retarded oxidation of the thiols to dimers and by the use of the sterically hindered base, 2,6-dimethylpyridine, rather than pyridine or triethylamine, which catalyzes the hydrolysis of the α-hydroxythiol esters during work-up. The isolated yields of the α-hydroxythiol esters synthesized by this procedure are listed in Table I.

Enzyme Inhibition Studies. The usual assay procedure[16] for glyoxalase I that monitors α-hydroxythiol ester **3** concentration at 240 nm could not be used for this study because of the interference from the α-hydroxythiol ester inhibitor at this wavelength. Consequently, the assay method chosen for this study, which was originally designed for crude cellular extracts,[17] measures the concentration of unreacted methylglyoxal as a monitor of enzyme activity. Aliquots of the reaction mixture were quenched in known volumes of semicarbazide solutions that resulted in the quantitative conversion of methylglyoxal to the corresponding bis(semicarbazone). The optical density of these solutions at 286 nm afforded the concentration of the bis(semicarbazone). To prevent precipitation of the α-hydroxythiol esters, all assay solutions contained DMF (3.6%) as a cosolvent. Initial velocities of the glyoxalase I reaction were measured in the presence and absence of a suitable concentration of α-hydroxythiol esters **8a–h** for a series of substrate concentrations. The dissociation constants of the enzyme inhibitor complex (K_i) obtained from double reciprocal plots[18] are tabulated in Table I. The concentration of inhibitor required to inhibit the enzymatic reaction 50% was then calculated for the substrate component concentrations of 16.36 mM methylglyoxal and 2.84 mM

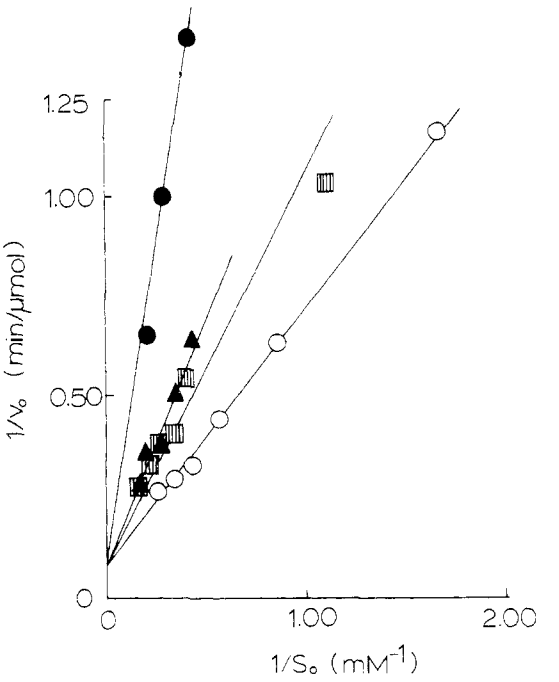


Figure 1. Representative double reciprocal plots showing competitive inhibition of glyoxalase I. V_0 is the initial velocity and S_0 is the initial substrate concentration of **2**: (○—○) no inhibitor present; (▲—▲) 5.6 mM **8b**; (▥—▥) 16.7 mM **8c**; (●—●) 8.4 mM **8e**.

glutathione (Table I). In addition, double reciprocal plots also established that each α-hydroxythiol ester **8a–h** was a competitive inhibitor of glyoxalase I (Figure 1). The K_m and V_{max} values for glyoxalase I were determined to be 8.26 mM and 12.8 μmol/min, respectively.

These competitive inhibitors presumably bind at the active site of glyoxalase I and of these the most effective were **8b** and **8d**. Since both methyl- and phenylglyoxal are substrates for the enzyme, the significant common structural feature of **8b** and **8d** is the benzyl R′ group. The effective binding of the benzyl group suggests that there is an important hydrophobic binding region at the glyoxalase I active site normally occupied by glutathione that accommodates the benzyl group exceedingly well.

Biological Results. The α-hydroxythiol esters **8a–f** and **8h** were evaluated in the L1210 lymphoid leukemia and assays were performed according to the specifications of Drug Evaluation Branch, National Cancer Institute, Bethesda, Md. 20014. The tests were made on BDF_1 mice. The compounds, as emulsions in saline, saline with Tween 80, or Klucel (hydroxypropylcellulose, HPC), were given as intraperitoneal injections 24 or 48 h after tumor implantation using multiple dose assays and a three- or six-mouse assay procedure. When injections were repeated the interval was every fourth day to a maximum of three injections. Control animals were similarly treated except the injections contained no test compound. Antitumor activity was evaluated with respect to the mean survival time in days of the treated animals over that of the controls. These results are summarized in Table II. No significant antileukemic activity was found in these α-hydroxythiol esters. Failure of these compounds in the in vivo studies, in spite of their rather effective competitive inhibition of glyoxalase I, may be primarily due to the ease of hydrolysis of α-hydroxythiol esters.

Experimental Section

Infrared spectra were determined in CCl_4 with a Beckman Model IR-10 infrared recording spectrophotometer. Ultraviolet

Table II. L1210[a] Screening Data on α-Hydroxythiol Esters

No.	Vehicle[b]	Day of first inj[c]	No. of inj[d]	O.D.[e]	Toxicity[f]	Tumor evaln,[g] test/control	L1210 (T/C) %[h]
8a	Tween 80	2	2	100	3/3	10.0/10.1	99
8b	Tween 80	2	2	200	3/3	10.3/10.5	98
8c	Saline	1	1	100	6/6	9.8/9.3	105
8d	Saline	1	3	200	6/6	10.7/10.6	100
8e	Klucel	1	3	200	6/6	10.5/10.1	103
8f	Tween 80	1	3	100	6/6	10.2/9.7	105
8h[i]	Klucel	1	1	400	6/6	9.8/9.3	105

[a] Standard inoculum of 10^5 L1210 lymphoid leukemia cells implanted intraperitoneally (ip) on day 0 in BDF_1 mice. [b] Compounds injected ip as suspensions in saline, saline with Tween 80, or Klucel (hydroxypropylcellulose, HPC). [c] Day of first injection after cell implantation. [d] Number of injections every fourth day at same dosage level. [e] Optimum dose in mg/kg for ip drug administration that provided maximum (T/C) %. The multiple dose levels assayed were 400, 200, and 100 mg/kg. [f] Number of survivors of total number of animals started on test. Recorded on day 5 in survival systems as a measure of drug toxicity. [g] Mean survival time of animals in days. [h] (T/C) % is the ratio of mean survival time of treated animals to control animals expressed as percent. In general, an increase in survival of treated animals as compared to control animals resulting in a (T/C) % of 125 or more is required for further testing. [i] 8g was not tested.

spectra were determined in ethanol with a Cary Model 14 recording spectrophotometer equipped with a thermostated cell compartment. The ^1H NMR spectra were determined in CCl_4 at 60 MHz using a Varian Associates Model A-60 NMR spectrometer. The chemical shifts are expressed in δ values (parts per million) relative to a Me_4Si internal standard. The mass spectra were obtained with a Consolidated Electronics Corp. Model 110-21B mass spectrometer. All pH measurements were determined with a Radiometer/Copenhagen Model PHM62 pH meter equipped with a Radiometer combination Model GK2301C single electrode. Analyses of the elements indicated by the symbols were within ±0.4% of the calculated values for all new compounds. All syntheses were performed under a static prepurified nitrogen atmosphere. Purification of the product by column chromatography was accomplished on 60–200 mesh silica gel (Grace, grade 62) by elution with petroleum ether, benzene, and diethyl ether and appropriate intermediate mixtures thereof. *N,N*-Dimethylformamide (DMF) and 2,6-dimethylpyridine were distilled just prior to use. The thiols (Evans Chemetics, Inc.) were used without further purification.

Phenylglyoxal monohydrate (Aldrich Chemical Co., 97%) was purified by three recrystallizations from CH_2Cl_2–acetone (4:1) yielding colorless needles: mp 88–90 °C.[19] Methylglyoxal hydrate (25 mL, Aldrich Chemical Co., 40% aqueous solution), used in the syntheses, was partitioned between 50 mL of diethyl ether–methylene chloride (1:1) and 25 mL of water saturated with NaCl, KCl, and Na_2CO_3. After separation, the aqueous phase was extracted with three 25-mL portions of Et_2O–CH_2Cl_2 (1:1). The combined organic phase was dried (Na_2SO_4), filtered, and stored at 0 °C. As needed, the solvent of an aliquot of this solution was removed in vacuo yielding a pale yellow viscous oil shown to be a 1:1 mixture of the mono- and dihydrate of methylglyoxal.[1]

Glyoxalase I (grade IV from yeast, solution in 50% glycerol–0.4 M $(NH_4)_2SO_4$–0.002 M KH_2PO_4, pH 6.5) is available from Sigma Chemical Co. The activity of this solution was 1000 units/mL (1.59 mg of protein/mL and 630 units/mg of protein). One unit will convert 1.0 μmol of substrate to product/min at pH 6.6 at 25 °C. A fresh GSH solution (Fisher Scientific Co.) was prepared daily using distilled water.

General Synthesis. *S*-Butyl Thiollactate (8a). To a solution of 1.48 g (15 mmol) of methylglyoxal hydrate and 1.61 g (7.5 mmol) of $Mg(OAc)_2{\cdot}4H_2O$, in 16 mL of DMF that had stirred for 10 min, was added a solution of 2.70 g (30 mmol) of *n*-butylthiol and 55 mg (0.5 mmol) of hydroquinone in 2 mL of DMF. After 1 h at 25 °C, a solution of 1.60 g (15 mmol) of 2,6-dimethylpyridine in 2 mL of DMF was added to the reaction mixture. Then after an additional 1 h of stirring, the reaction mixture was partitioned between a cold mixture of 50 mL of diethyl ether and 25 mL of water saturated with NaCl and KCl. After separation, the aqueous phase was extracted with two 25-mL portions of ether. The combined organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo at water aspiration pressure and then at vacuum pump pressure (lyophilized) to remove the DMF. Filtration column chromatography yielded 0.99 g (41%) of **8a** as a pale yellow viscous oil: IR 3625, 3550, 1680 cm^{-1}; UV λ max 235 nm (ε 3310); NMR δ 0.97 (3 H, perturbed t), 1.44 (3 H, d, J = 6 Hz) superimposed on 1.2–1.8 (4 H, m), 2.89 (2 H, t, J = 7 Hz), 3.70 (1 H, br, exchangeable with D_2O), 4.30 (1 H, q, J = 7 Hz); MS m/e (rel intensity) 162 (M^+, 5), 145 (4), 134 (36), 117 (16), 105 (14), 90 (39), 57 (28), 56 (84), 45 (100). Anal. ($C_7H_{14}O_2S$) C, H.

***S*-Benzyl thiollactate (8b):** IR 3625, 3550, 1675 cm^{-1}; UV λ max 233 nm (ε 2225); NMR δ 1.08 (3 H, d, J = 6 Hz), 3.74 (2 H, s), 4.05 (1 H, q, J = 7 Hz), 4.37 (1 H, br, exchangeable with D_2O), 6.96 (5 H, apparent s); MS m/e (rel intensity) 196 (M^+, 21), 124 (11), 91 (100), 45 (71). Anal. ($C_{10}H_{12}O_2S$) C, H.

***S*-Butyl thiolmandelate (8c):** IR 3520, 1670 cm^{-1}; UV λ max 238 nm (ε 5240); NMR δ 0.75 (3 H, perturbed t), 1.0–1.6 (4 H, m), 2.65 (2 H, t, J = 7 Hz), 4.00 (1 H, d, J = 5 Hz, exchangeable with D_2O), 4.92 (1 H, d, J = 5 Hz, collapses to a singlet with D_2O), 7.17 (5 H, apparent s); MS m/e (rel intensity) 224 (M^+, 0.2), 196 (3), 107 (100), 79 (31), 77 (16). Anal. ($C_{12}H_{16}O_2S$) C, H.

***S*-Benzyl thiolmandelate (8d):** IR 3530, 1670 cm^{-1}; UV λ max 238 nm (ε 827); NMR δ 3.48 (1 H, br, exchangeable with D_2O), 3.94 (2 H, s), 4.96 (1 H, s), 7.07 (5 H, apparent s), 7.19 (5 H, apparent s); MS m/e (rel intensity) 167 (38), 124 (50), 107 (62), 91 (100), 79 (88), 77 (55). Anal. ($C_{15}H_{14}O_2S$) C, H.

Methyl mercaptoacetate mandelate (8e): IR 3520, 1745, 1690 cm^{-1}; UV λ sh 234 nm (ε 1770); NMR δ 3.52 (2 H, s), 3.59 (3 H, s), 4.3 (1 H, br, exchangeable with D_2O), 5.17 (1 H, s), 7.36 (5 H, m); MS m/e (rel intensity) 209 (7), 181 (4), 135 (14), 107 (57), 105 (100), 77 (86). Anal. ($C_{11}H_{12}O_4S$) C, H.

Butyl mercaptoacetate mandelate (8f): IR 3520, 1740, 1690 cm^{-1}; UV λ sh 233 nm (ε 6330); NMR δ 0.91 (3 H, perturbed t), 1.1–1.8 (4 H, m), 3.55 (2 H, s), 4.08 (2 H, t, J = 7 Hz), 4.72 (1 H, br, exchangeable with D_2O), 5.22 (1 H, s), 7.41 (5 H, m); MS m/e (rel intensity) 254 (3), 209 (6), 148 (26), 107 (100), 79 (33), 77 (21), 57 (17). Anal. ($C_{14}H_{18}O_4S$) C, H.

Octyl mercaptoacetate mandelate (8g): IR 3530, 1740, 1690 cm^{-1}; UV λ sh 233 nm (ε 2160); NMR δ 0.75–1.0 (9 H, complex m), 1.0–1.8 (6 H, br m), 3.58 (2 H, s), 3.8–4.2 (2 H, br m), 4.3 (1 H, br, exchangeable with D_2O), 5.22 (1 H, br s, sharpens with D_2O), 7.4 (5 H, m); MS m/e (rel intensity) 204 (12), 181 (5), 107 (100), 79 (39), 77 (26), 71 (42), 57 (64), 43 (37). Anal. ($C_{18}H_{26}O_4S$) C, H.

Methyl 3-mercaptopropionate mandelate (8h): IR 3525, 1745, 1675 cm^{-1}; UV λ sh 236 (ε 4454); NMR δ 2.48 (2 H, t, J = 6 Hz), 2.98 (2 H, t, J = 6 Hz), 3.58 (3 H, s), 4.25 (1 H, br, exchangeable with D_2O), 5.14 (1 H, s), 7.38 (5 H, m); MS m/e (rel intensity) 119 (13), 107 (36), 105 (20), 89 (8), 79 (73), 77 (100), 59 (47). Anal. ($C_{12}H_{14}O_4S$) C, H.

Enzyme Inhibition. A commercial 40% methylglyoxal solution was distilled at 90–100 °C (760 Torr) to remove polymeric material, the acidic material removed by passing the diluted distillate through Dowex 1 (hydroxide form), and the filtrate solution standardized using a semicarbazide method.[17] Glyoxalase I was diluted to 20 units/mL with a solution of 30% glycerol containing 0.1% bovine serum albumin. All enzymatic reactions were performed at 30 °C in 0.1 M phosphate buffer at pH 6.6. For each assay the reaction contained a total volume of 11.0 mL that was 16.36 mM with respect to methylglyoxal and 1.42–7.09 mM with respect to GSH. Sufficient glyoxalase I (200 μL of the

20 units/mL solution) was employed to give an easily measurable rate which was followed by removing 65-μL aliquots at 30-s intervals for the first 5 min and immediately quenching each aliquot in 10.0 mL of 0.1 M phosphate buffer at pH 7.0 containing 0.15 M semicarbazide hydrochloride. After complete conversion of methylglyoxal to the bis(semicarbazone) (ca. 10 h), the initial rate of the enzymatic reaction could be determined by measuring the absorption of the bis(semicarbazone) in these solutions at 286 nm (ϵ 3.2 × 10^4 M^{-1} cm^{-1}). Methylglyoxal (16.36 mM), GSH (1.42–7.09 mM), inhibitor (none or 5.6–16.7 mM) in 400 μL of DMF, and buffer (10.3–9.9 mL) were allowed to equilibrate at 30 °C for 5 min to allow time for the formation of the substrate before addition of the enzyme. The initial substrate concentration $[S]_0$ was varied by increasing the GSH concentration from 1.42 to 7.09 mM and calculated at equilibrium from a quadratic equation using the dissociation constant of 3.0 mM.[20] A computer program was used to determine the line of best fit by the method of least squares. The dissociation constants of the enzyme inhibitor complex (K_i) were obtained from double reciprocal plots[18] of $1/V_0$ vs. $1/[S]_0$ and $1/V_i$ vs. $1/[S]_0$. V_0 = initial velocity of the uninhibited enzymatic reaction and V_i = initial velocity of the inhibited reaction at a suitable inhibitor concentration (5.6–16.7 mM). The concentration of inhibitor required to inhibit the enzymatic reaction 50% was calculated using the expression $[I]_{50} = K_i(K_m + [S]_0)/K_m$ for the substrate component concentrations of 16.36 mM methylglyoxal and 2.84 mM glutathione.

Biological Testing. 10^5 L1210 lymphoid leukemia were implanted intraperitoneally into BDF_1 mice on day 0. Ip drug treatment was administered on day 1; day 1, 5, and 9; or day 2 and 6; and evaluated by the mean survival time in days of the treated animals over that of the control. Multiple dose assay levels included 400, 200, and 100 mg/kg and used a three- or six-mouse assay procedure.

Acknowledgment. This investigation was supported primarily by PHS Research Grant No. CA 12984 from the National Cancer Institute and in part by grants from the Research Council (Rutgers University), the National Institutes of Health (Biomedical Sciences Support Grant), and Mrs. Lauretta Horstmann, Bagley, Wis. In addition, the authors thank the Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md., for screening our compounds; and Drs. F. Scheidl and W. Benz, Hoffmann-La Roche Inc., Nutley, N.J., for the microanalyses and mass spectra.

References and Notes

(1) Part 3 in the series "Glyoxalase I Enzyme Studies". For part 2 see S. S. Hall, A. M. Doweyko, and F. Jordan, *J. Am. Chem. Soc.*, **98**, 7460 (1976).
(2) K. Lohman, *Biochem. Z.*, **254**, 332 (1932).
(3) A. Szent-Györgyi, "Bioelectronics", Academic Press, New York, N.Y., 1968, pp 63–76.
(4) R. Vince and W. B. Wadd, *Biochem. Biophys. Res. Commun.*, **35**, 593 (1969).
(5) A. Szent-Györgyi, *Science*, **161**, 988 (1968).
(6) K. F. Lewis, E. H. Majane, and S. Weinhouse, *Cancer Res.*, **19**, 97 (1959).
(7) B. Issekutz, "The Chemotherapy of Cancer", Akademiai Kiado, Budapest, Hungary, 1969, pp 17–19.
(8) F. A. French and B. L. Freedlander, *Cancer Res.*, **18**, 172 (1958).
(9) R. Vince, S. Daluge, and W. B. Wadd, *J. Med. Chem.*, **14**, 402 (1971).
(10) W. O. Kermack and N. A. Matheson, *Biochem. J.*, **65**, 48 (1957).
(11) E. E. Cliffe and S. G. Waley, *Biochem. J.*, **79**, 475 (1960).
(12) R. Vince and S. Daluge, *J. Med. Chem.*, **14**, 35 (1971).
(13) (a) R. Vince, M. Wolf, and C. Sanford, *J. Med. Chem.*, **16**, 951 (1973); (b) P. A. Lyon and R. Vince, *ibid.*, **20**, 77 (1977).
(14) (a) F. Binkley, *J. Biol. Chem.*, **236**, 1075 (1961); (b) J. J. Clapp and L. Young, *Biochem. J.*, **118**, 765 (1970); (c) T. Suga, H. Kumaoka, and M. Akagi, *J. Biochem.*, **60**, 133 (1966).
(15) S. S. Hall and A. Poet, *Tetrahedron Lett.*, 2867 (1970).
(16) E. Racker, *J. Biol. Chem.*, **190**, 685 (1951).
(17) N. M. Alexander and J. L. Boyer, *Anal. Biochem.*, **41**, 29 (1971).
(18) H. Lineweaver and D. Burke, *J. Am. Chem. Soc.*, **56**, 658 (1934).
(19) J. Hine and G. F. Koser, *J. Org. Chem.*, **36**, 3591 (1971).
(20) D. L. Vander Jagt, L.-P. B. Han, and C. H. Lehman, *Biochemistry*, 11, 3735 (1972).

Potential Antitumor Agents. 25. Azalogues of the 4′-(9-Acridinylamino)methanesulfonanilides

William A. Denny, Graham J. Atwell, and Bruce F. Cain*

New Zealand Cancer Society, Experimental Chemotherapy Laboratory, Auckland, New Zealand. *Received February 23, 1977*

Tumor inhibition produced by isomeric, nitro-substituted 4′-(9-acridinylamino)methanesulfonanilide analogues of low base strength (pK_a = 4.79–5.72) might result from in vivo reduction to the corresponding, higher pK_a (7.15–9.80), tumor active amines. The aza analogues, –N═ in place of –C(NO_2)═, have been prepared as nonclassical bioisosteres and screened in the L1210 system. Significant L1210 inhibition produced by isomeric 3- and 4-azalogues, of similar base strength to the corresponding nitro-substituted derivatives, demonstrates that weakly basic analogues can provide biologic activity when there is no prospect of in vivo reduction to more strongly basic products. Obligatory reduction of nitro function, for biologic activity, need not be postulated in this drug series.

Certain nitro group substituted 4′-(9-acridinylamino)-methanesulfonanilide (AMSA, **1**) congeners are significantly tumor inhibitory in L1210 screening assays[1,2] with the 3-nitro isomer (**13**, Table I) proving particularly effective. The powerfully electron-withdrawing nitro function markedly lowers acridine base strength and from the pK_a values of the L1210 active isomers (**12**, **13**) it can be calculated that less than 2% of these compounds would be ionized at physiologic pH (7.4). Additionally, the 3,6-dinitro-substituted AMSA agent (pK_a = 3.68), which would be less than 0.03% ionized at pH 7.4, is also convincingly L1210 active.[3] It has usually been considered that the cation of chemotherapeutic acridines provides observed biologic activity.[4] More highly ionized acridines normally prove either more efficacious or more dose po-