of lithium aluminum hydride was cautiously added. The mixture was stirred at room temperature for 16 hr, and the excess hydride was decomposed by the dropwise addition of 6.5 ml of saturated aqueous sodium potassium tartarate solution. The solid was collected by filtration and washed with 50 ml of ethyl acetate. The combined filtrate and washings were dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Crystallization of the residue from ether–petroleum ether yielded 669 mg (73%) of 5-methoxy-2,4-dimethyl-3-(2-morpholinoethyl)indole, mp 126–128°. The characterization of this substance is given in Table II.

Acknowledgment. The authors are indebted to Messrs. Brancone and Fulmor and their staffs for the microanalyses and spectral data, respectively.

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Glutaryl-*S*-(*p*-bromobenzyl)-L-cysteinylglycine. A Metabolically Stable Inhibitor of Glyoxalase I†

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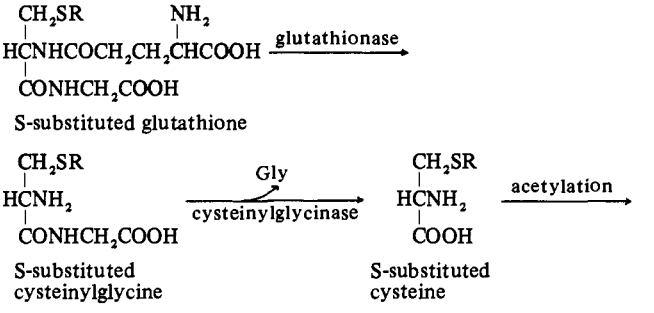
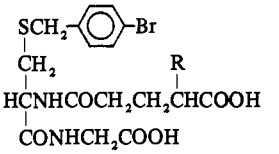
The antineoplastic action of α-ketoaldehydes, including methylglyoxal, has been well documented.[1-3] However, these agents are rapidly metabolized to the corresponding inactive α-hydroxy acids by the glyoxalase enzyme system.[4] These observations prompted our previous suggestion that an appropriate glyoxalase inhibitor in combination with a ketoaldehyde may be an effective means of chemotherapy.[5] Since reduced glutathione is a cofactor in the glyoxalase reaction, S-substituted glutathione derivatives were found to be effective inhibitors of glyoxalase I obtained from yeast.[4-6] Some of these inhibitors exhibited cytotoxic activity against L1210 leukemia and KB cells in tissue culture and also increased the toxicity of methylglyoxal in L1210 cells.[5] The rapid metabolism of S-substituted glutathione derivatives by glutathionase in the mouse rendered these inhibitors inactive when tested *in vivo*.

Glutathione and its S-substituted derivatives are known to be rapidly hydrolyzed in animals by two enzymes, glutathionase (γ-glutamyl transpeptidase) and cysteinylglycinase.[7-9] Glutathionase is responsible for releasing an S-substituted cysteinylglycine from a glutathione derivative; cysteinylglycinase further degrades the cysteinylglycine derivative so formed as illustrated by the degradation of an S-substituted glutathione in Scheme I.

Scheme I. Metabolic Degradation of Glutathione Derivatives



In view of these observations, it became desirable to design a glyoxalase inhibitor that would resist the rapid degradation by the glutathionase enzymes. Compound **1** represents a tripeptide analog of the previously tested *S*-*p*-bromobenzylglutathione (**2a**) in which the γ-glutamyl moiety is replaced by a glutaryl group. The rationale for selecting **1**

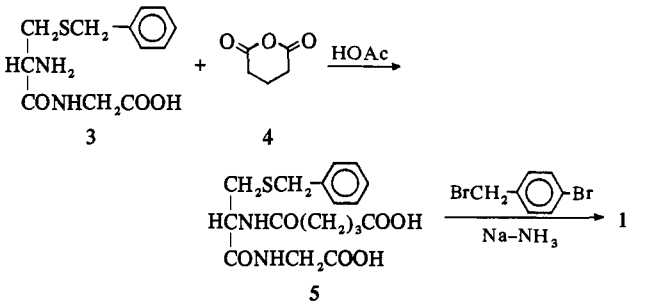


1, R = H
2a, R = NH_2
2b, R = NHAc

was based on the fact that the glutathione derivative **2a** was the most potent inhibitor of yeast glyoxalase I in a series of 40 compounds tested.[6] In addition, the lack of an absolute requirement for the free α-amino group was evidenced by the potent, but decreased, inhibitory activity of the N-acetylated derivative **2b**.[6] Thus, replacement of the α-amine by a hydrogen should result in a glyoxalase inhibitor that cannot be recognized as a γ-glutamyl peptide by the glutathionase enzyme.

Chemistry. *S*-Benzyl-L-cysteinylglycine (**3**)[10] was condensed with glutaric anhydride (**4**) in glacial acetic acid (Scheme II) and gave glutaryl-*S*-benzyl-L-cysteinylglycine

Scheme II



(**5**) in good yield. Removal of the benzyl group from **5** followed by condensation with *p*-bromobenzyl bromide in liquid ammonia and sodium gave the desired product **1**.

Biological Results. The inhibitor concentrations required for 50% inhibition of the glyoxalase I reaction, using 1.25 m*M* methylglyoxal and 0.217 m*M* glutathione as substrates, are summarized in Table I. The tenfold increase in inhibition by the *p*-bromo analog **1** compared with the benzyl derivative **5** is consistent with our previous observation[6] with glutathione derivatives that a *p*-bromo group greatly enhances binding to the enzyme. The inactivity of S-substituted cysteinylglycines such as **3** illustrates the contribu-

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‡Undergraduate research participant.

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Table I. Inhibition of Glyoxalase I

Compd	I_{50}[a]
1	0.300 ± 0.05
3	No inhibition
5	3.10 ± 0.1
6	0.100 ± 0.002

[a] The I_{50} is defined as the millimolar concentration of inhibitor required to inhibit the enzymatic reaction 50% at 1.25 m*M* methylglyoxal and 0.217 m*M* glutathione.

tion of the glutaryl moiety to binding. Also, a comparison of **5** and *S*-benzylglutathione (**6**) demonstrates a further contribution to binding by the α-amino group of **6**. However, even with the removal of the α-amino group, relatively potent inhibitors can be prepared by increasing the binding strength elsewhere in the molecule. The *p*-bromobenzyl group in compound **1** represents such a case.

Because of the excellent separation of metabolic products on thin-layer chromatograms, *S*-benzylglutathione (**6**) was used as a reference in studying the effect of kidney homogenate glutathionase on **1**. Results of the hydrolysis indicate that **6** is significantly degraded after 15 min of incubation with kidney homogenate and degradation is essentially complete after 1 hr. The end product being formed by the action of homogenate on **6** is *S*-benzylcysteine. This was determined by spotting a solution of *S*-benzylcysteine on the plate along with the various timed homogenate mixtures. At zero time a yellow spot appeared at R_f 0.168 which corresponded to glycylglycine (which was added as a γ-glutamyl acceptor), and a purple spot appeared at R_f 0.520 which was identified as **6**. Two additional spots at R_f 0.315 and 0.403 were found to be due to kidney homogenate. After 15 min, the incubation mixture showed spots corresponding to glycylglycine (R_f 0.168), **6** (R_f 0.520), *S*-benzylcysteine (R_f 0.684), and an additional spot at R_f 0.074 which was not identified but is most probably the glycylglycine–glutamic acid condensation product formed from the glutathionase reaction. The 1-hr incubation aliquot indicated a complete conversion of **6** to *S*-benzylcysteine. No intermediate product could be detected in any of the aliquots. When compound **1** was incubated with kidney homogenate, examination of timed aliquots by the same chromatographic method gave no indication of degradation. Even after 2 hr of incubation, only two spots were detected corresponding to **1** (R_f 0.403) and glycylglycine.

In a second experiment, time aliquots from the homogenates containing either **6** or **1** were added to the glyoxalase reaction in sufficient quantity so that the final concentration of inhibitor resulted in 50% inhibition of the glyoxalase reaction. By this method it was observed that only 33% of **6** remained after 15 min and no inhibition of the glyoxalase reaction was detected after 45 min. Preheated homogenate (100° for 1 min) had no effect on the *S*-benzylglutathione (**6**). In the case of compound **1**, incubation with homogenate for 2 hr gave no detectable loss in the ability of the aliquots to inhibit the glyoxalase reaction. These results are consistent with the chromatographic assays.

Results of this study reveal that **1** is a relatively good inhibitor of the yeast glyoxalase I enzyme and also that this compound is resistant to the action of glutathionase. Since degradation by glutathionase may play an important part in the inactivation of previously tested glyoxalase inhibitors in test animals, compounds related to **1** may be useful in enhancing the antitumor activity of α-ketoaldehydes. We have recently isolated glyoxalase from mouse lymphoid leukemia cells. Preliminary testing indicates that compounds related to **1** demonstrate significant binding to the tumor enzyme.# Replacement of the *p*-bromobenzyl moiety with a potential irreversible binding group is being explored.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. The ir and nmr spectra of new compounds were consistent with their structures. The elemental analyses (C, H, N) were performed by M-H-W Laboratories, Garden City, Mich., and are within ±0.4% of the theoretical value.

Glutaryl-*S*-benzyl-L-cysteinylglycine (5). To a solution of 2.34 g (20.5 mmol) of glutaric anhydride (**4**) in 25 ml of glacial HOAc was added 5.00 g (18.7 mmol) of *S*-benzyl-L-cysteinylglycine (**3**).[10] The solution was allowed to stand at room temperature for 1 hr and the solvent was removed *in vacuo* at 50°. The amber syrup was crystallized from water and gave white solid (5.67 g), mp 114–116°. Recrystallization from water gave **5** as a pure product: yield 4.69 g (66%); mp 123–125°. *Anal.* ($C_{17}H_{22}N_2O_6S$) C, H, N.

Glutaryl-*S*-(*p*-bromobenzyl)-L-cysteinylglycine (1). To 1.00 g (2.62 mmol) of **5** in a 100-ml round-bottom flask submerged in an acetone–Dry Ice bath was added approximately 30 ml of liquid ammonia. After solution was complete, 180 mg of sodium (just enough to produce a lasting dark purple color) was added to the rapidly stirred solution followed by addition of 675 mg (2.70 mmol) of α,*p*-dibromotoluene. The mixture was stirred for 30 min (in which time the dibromotoluene had dissolved), and 318 mg (6.00 mmol) of ammonium chloride was added. The ammonia was allowed to evaporate and the solid residue was dissolved in 15 ml of cold water. The aqueous mixture was immediately filtered through a Celite pad and acidified at 0° with 6 *N* HCl. A white gummy precipitate formed which solidified on standing: yield 1.03 g; mp 160–163°. Recrystallization from EtOH–H_2O gave the analytical product **1** as a white powder: 896 mg; mp 171–172°. *Anal.* ($C_{17}H_{21}N_2O_6BrS$) C, H, N.

Glyoxalase Inhibition. A 40% commercial solution of methylglyoxal was distilled using reduced pressure from a water aspirator. The water distilled first followed by the pale greenish methylglyoxal. Both fractions were collected in the same flask submerged in an ice bath. A 1-ml fraction of the distillate was diluted 250 times with water and passed through 50 ml of Amberlite IRA-400 resin (carbonate form) to remove acidic components. The solution of methylglyoxal was standardized by the method of Friedman.[11] Yeast glyoxalase I was obtained from Sigma (2000 μg/ml) and was diluted to 20 μg/ml with a solution of 30% glycerin containing 0.1% bovine serum albumin. The enzymatic reaction was performed as previously described[6] using final concentrations of 1.25 m*M* methylglyoxal and 0.217 m*M* reduced glutathione. The concentration of inhibitor required for 50% inhibition was obtained from a plot of V_0/V_i *vs.* [I] where V_0 = initial velocity of the uninhibited enzymatic reaction and V_i = initial velocity of the inhibited reaction at various inhibitor concentrations [I].

Preparation of Kidney Homogenate. A male BDF_1 mouse (20–25 g) was killed by cervical dislocation and the kidneys were removed and immediately homogenized with 5 ml of cold 0.01 *M* Tris buffer, pH 8.04. The homogenate was centrifuged at 4° at 2000 g (4000 rpm) at 4° for 15 min. The supernatant was dialyzed overnight in 0.01 *M* Tris buffer, pH 8.04 at 4°.

Hydrolysis of *S*-Benzylglutathione (6). Stock solutions of *S*-benzylglutathione (6.0 m*M*), glycylglycine (12 m*M*), and magnesium acetate (12 m*M*) were prepared using 0.01 *M* Tris buffer, pH 8.0. Several tubes were prepared by placing 0.5 ml of *S*-benzylglutathione, 0.5 ml of glycylglycine, 0.5 ml of magnesium acetate, 1.4 ml of 0.01 *M* Tris buffer, and 0.1 ml of dialyzed homogenate in each tube. The tubes were allowed to incubate at 25° and at selected time intervals of 0, 15, 30, 45, 60, and 120 min the enzymatic hydrolysis was terminated by immersing a tube in boiling water for 1 min. The tubes were centrifuged for 5 min at 3000 rpm (1085 g) to remove the protein precipitate. The clear supernatant was spotted on cellulose tlc plates (Eastman No. 6065) and the plates were sprayed with a ninhydrin solution (0.2 g of ninhydrin, 50 ml of butanol, and 50 ml of acetone) and warmed in a drying oven until the spots appeared. The same supernatant was added to the glyoxalase reaction in order to determine the concentration of unhydrolyzed **6**. The zero time supernatant mixture showed that no loss of **6** occurred during the work-up.

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Synthesis of 3-(Aminoalkyl)-1-[(5-nitrofurfurylidene)amino]hydantoins

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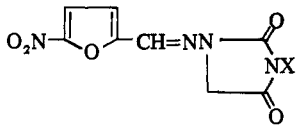
The success of 1-[(5-nitrofurfurylidene)amino]hydantoin (Ia)[1,†] as an antibacterial agent has prompted the synthesis of analogs substituted in the 3 position with aminoalkyl groups (Ib). *In vitro* screening data against three organisms are presented.



Ia, X = H
b, X = $(CH_2)_nNRR'$

Chemistry. The most direct approach to the preparation of these analogs would be treatment of the sodium salt of nitrofurantoin with aminoalkyl halides. Unfortunately, this method is not applicable because of the sensitivity of nitrofurans to alkaline reagents. For this reason four different approaches were developed. The method used for each compound is designated in Table I.

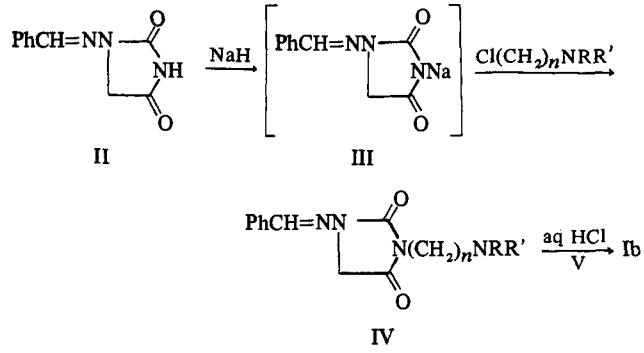
In method A, the sodium salt (III) of 1-benzylideneaminohydantoin (II)[2] was treated with the appropriate aminoalkyl chloride. The benzylidene group was then removed by acid hydrolysis and replaced by the 5-nitrofurfurylidene group by treatment with 5-nitro-2-furaldehyde (V) as shown in Scheme I.

Most of the aminoalkyl halides used in method A either were commercially available or have been reported in the literature. The preparation of 3-chlorobutyl-*N*,*N*-dimethylamine, the intermediate for **28**, and 3-chloropropyl-*N*-methyl-*N*-isopropylamine, the intermediate for **6**, is described in the Experimental Section.

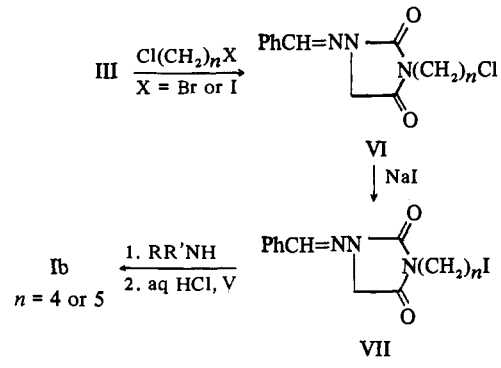
When the aminoalkyl side chain contains four or five carbon atoms (n = 4 or 5, Ib), method A cannot be used because the required chlorobutyl- or chloropentylamines cyclize rapidly upon neutralization of their salts to form quaternary salts which are too stable to function as alkylating agents. Therefore, method B (Scheme II) was used for the preparation of those analogs in which n = 4 or 5. Compound III was treated with an alkyl chlorobromide or chloroiodide. With the former the product was the chloroalkyl intermediate VI; this proved unreactive toward most amines and was therefore converted to the iodo compound VII with sodium iodide. When an alkyl chloroiodide was used the product was a mixture of chloro- and iodoalkyl intermediates (the latter formed by exchange of VI with the sodium iodide liberated). Treatment of the mixture with more sodium iodide completed the conversion to VII. Reaction of VII with various amines followed by hydrolysis and treatment with V then gave the desired products.

Two of the compounds (**34** and **35**) were made by heating II with the appropriate vinylpyridine in pyridine solution, followed by removal of the benzylidene group, and treatment with V as shown in Scheme III (method C).

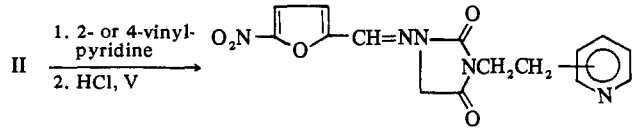
Scheme I



Scheme II



Scheme III



34, 35

The primary aminoalkyl analogs (R = R′ = H, formula Ib) were prepared by reduction of the appropriate nitriles (method D, Scheme IV). These nitriles (VIII), in all cases except one, were made by reaction of III with ω-halonitriles; for the trimethylene compound acrylonitrile was used. The most effective conditions found for the reduction were Raney nickel catalyst in acetic anhydride solution with sodium acetate.[3] The acetylated amines IX, which were formed in good yields, were then hydrolyzed and treated with V which reacted preferentially with the ring amino group. This point was determined by treatment of **36**

†The Norwich Pharmacal Company's registered trademarks are Furadantin and Macrodantin; the generic name is nitrofurantoin.