A New Method for Rapidly Generating Inhibitors of Glyoxalase I inside Tumor Cells Using *S*-(*N*-Aryl-*N*-hydroxycarbamoyl)ethylsulfoxides

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The enediol analogue *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)glutathione is a powerful mechanism-based competitive inhibitor of the anticancer target enzyme glyoxalase I. Nevertheless, this compound exhibits limited toxicity toward tumor cells in vitro because it does not readily diffuse across cell membranes. We describe an efficient method for indirectly delivering the enzyme inhibitor into murine leukemia L1210 cells via acyl interchange between intracellular glutathione and the cell-permeable prodrug *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)ethylsulfoxide. The second-order rate constant for the acyl-interchange reaction in a cell-free system is 1.84 mM^{-1} min^{-1} (100 mM potassium phosphate buffer, 5% ethanol, pH 7.5, 25 °C). Incubation of L1210 cells with the sulfoxide in vitro results in a rapid increase in the intracellular concentration of the glyoxalase I inhibitor ($k_{app} = 1.41 \pm 0.03$ min^{-1} (37 °C)) and inhibition of cell growth ($GI_{50} = 0.5 \pm 0.1$ μM). This represents an improvement in both efficiency and potency over the dialkyl ester prodrug strategy in which the inhibitor is indirectly delivered into tumor cells as the [*glycyl*,*glutamyl*] diethyl or dicyclopentyl esters. The fact that π-glutathione transferase catalyzes the acyl-interchange reaction between GSH and the sulfoxide suggests that the sulfoxide, or related compounds, might exhibit greater selective toxicity toward tumor cells that overexpress the transferase.

Introduction

Glyoxalase I (GlxI) is receiving renewed interest as a possible anticancer target, given its role in removing methylglyoxal from cells and the observation that tumor cells are exceptionally sensitive to the cytotoxic effects of extracellular methylglyoxal.[1−4] The enzyme metabolizes methylglyoxal by catalyzing the conversion of the thiohemiacetal, formed from glutathione (GSH) and methylglyoxal, to *S*-D-lactoylglutathione via an enediol proton-transfer mechanism. Glyoxalase II promotes the subsequent hydrolysis of *S*-D-lactoylglutathione to D-lactate and GSH. Years ago, Vince and Daluge hypothesized that inhibitors of glyoxalase I might function as antitumor agents by inducing elevated levels of intracellular methylglyoxal.[5]

In support of this prediction, Lo and Thornalley demonstrated that the competitive inhibitor, *S*-*p*-bromobenzylglutathione, is toxic to human leukemia 60 cells in vitro, once the inhibitor is delivered into the cells as the lipophilic [*glycyl*,*glutamyl*] diethyl ester.[6] This prodrug strategy relies upon intracellular esterases to catalyze the hydrolysis of the diethyl ester to give the inhibitory diacid. The same laboratory reported that the corresponding dicyclopentyl ester is about twice as potent as the diethyl ester against a range of different human tumor cell lines in culture.[7] We subsequently demonstrated that tight-binding enediol analogues of the GlxI reaction could be delivered as the diethyl esters into murine leukemia L1210 and B16 melanotic melanoma cells in culture, resulting in growth inhibition and cell death.[8] For example, the diethyl ester of one of these enediol analogues (**1a**$(Et)_2$) was found to inhibit the growth of both L1210 and B16 cells, with GI_{50} values of 7 and 15 μM, respectively (Figure 1). Compound **1a**$(Et)_2$ was also found to be much less toxic to nonproliferating splenic lymphocytes, possibly reflecting reduced sensitivity to methylglyoxal and/or reduced chemical stability inside these cells.

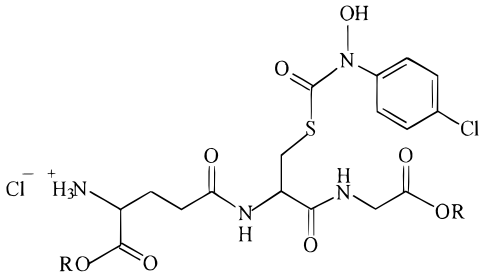

Figure 1. Enediol analogue *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)glutathione (**1a**, R = H) and prodrugs **1a**$(Et)_2$ (R = C_2H_5) and **1a**(cyclopentyl)$_2$ (R = cyclopentyl). Compound **1a** is a tight-binding competitive inhibitor of human GlxI (K_i = 46 nM) and stable analogue of the enediol(ate) intermediate that is thought to form along the reaction pathway of GlxI.[9,10]

Nevertheless, this prodrug strategy has at least two limitations.[8] First, the diffusion of the diethyl esters into cells is a relatively slow process. The apparent first-order rate constant for diffusion of **1a**$(Et)_2$ into L1210 cells in vitro is only about 0.10 min^{-1} at 37 °C, possibly reflecting the fact that **1a**$(Et)_2$ is a cationic species under physiological conditions. Second, the diethyl ester prodrugs undergo rapid esterase-catalyzed hydrolysis in the

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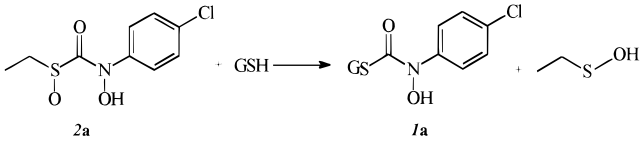
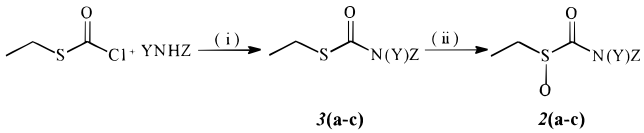
Scheme 1. Acyl-Interchange Reaction between Glutathione (GSH) and *S*-(*N*-*p*-Chlorophenyl-*N*-hydroxycarbamoyl)ethylsulfoxide (**2a**) To Give Enediol Analogue **1a**



*2***a** *1***a**

Scheme 2. Synthetic Route to the Acylating Reagents Shown in Table 1[a]



***3*(a-c)** ***2*(a-c)**

a: Y = OH; Z = C_6H_4Cl

b: Y = OH; Z = CH_3

c: Y = CH_3; Z = C_6H_4Cl

[a] Reagents and conditions: (i) K_2CO_3/Et_2O; (ii) for **3a**,**b**, *m*-chloroperbenzoic acid was used; for **3c**, Oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2HSO_4$) was used.

plasma of most inbred strains of laboratory mice, complicating efficacy studies using murine models.

Here we show that enediol analogue **1a** can be rapidly generated inside L1210 cells via an acyl-interchange reaction between intracellular GSH and the cell-permeable prodrug *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)ethylsulfoxide (**2a**), Scheme 1. This prodrug strategy has important advantages over the dialkyl ester prodrug strategy.

Chemistry

The dicyclopentyl ester of enediol analogue **1a** was obtained by acid-catalyzed esterification of **1a** in cyclopentanol/HCl. The synthetic route to the acylating reagents used with GSH to produce different enediol analogues is outlined in Scheme 2. Oxidation of thioesters **3a**−**c** to the corresponding sulfoxides **2a**−**c** followed published methods.[11,12] *N*-(*p*-Chlorophenyl)hydroxylamine was prepared by reduction of *p*-chloronitrobenzene with hydrazine hydrate in the presence of 5% Rh on carbon.[13]

Results and Discussion

Chemical Kinetics. The studies described in this report were originally inspired by the observation that intracellular sulfoxidation of herbicidal *N*,*N*-di-*n*-propylcarbamate thioalkyl esters promotes their catabolism by facilitating acyl interchange with GSH.[11] We reasoned that sulfoxides of *N*-aryl/alkyl-*N*-hydroxycarbamate thioethyl esters might also undergo rapid acyl interchange with GSH, providing an efficient method for generating enediol analogue inhibitors of glyoxalase I inside tumor cells. Indeed, sulfoxides **2a**,**b** are at least 10^6-fold more reactive than the thioester **3a** toward GSH, on the basis of a comparison of the second-order rate constants for the acyl-interchange reactions, Table 1. The N−OH group inductively activates the sulfoxides for acyl interchange, as the rate constant for **2a** exceeds that of the corresponding N−CH_3 derivative **2c** by 13-fold. Ultraviolet and NMR spectroscopy confirmed the products of the interchange reactions to be the corresponding enediol analogues.

Table 1. Second-Order Rate Constants (k_2) for Acyl-Interchange Reactions between Glutathione and Selected Carbamoyl Esters[a]

$CH_3CH_2XC(O)N(Y)Z + GSH \rightarrow GSC(O)N(Y)Z + CH_3CH_2XH$

carbamoyl ester	substituent X	substituent Y	substituent Z	k_2 (mM^{-1} min^{-1})
3a	S	OH	C_6H_4Cl	$\sim 3.5 \times 10^{-7}$ [b]
2a	S(O)	OH	C_6H_4Cl	1.84 ± 0.07[c]
2b	S(O)	OH	CH_3	0.59 ± 0.02[c]
2c	S(O)	CH_3	C_6H_4Cl	0.14 ± 0.01[c]

[a] Conditions: potassium phosphate buffer (0.1 M), pH 7.5, 5% ethanol, 25 °C. [b] Calculated from the initial rate of appearance of product (~0.1% reaction) in the presence of a 15-fold excess of glutathione, monitored by reverse-phase high-performance liquid chromatography. [c] Calculated from the first-order rate of loss of reactants under pseudo-first-order conditions (≥10-fold excess of glutathione), monitored spectrophotometrically.

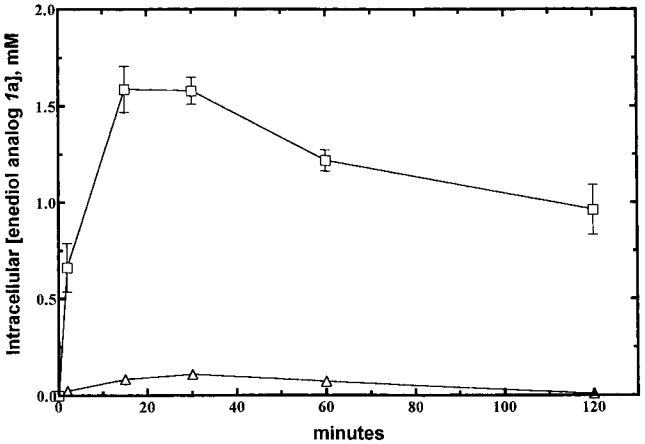


Figure 2. Time-dependent change in the intracellular concentration of *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)glutathione (**1a**) in L1210 cells incubated in the presence of 0.19 mM *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)ethylsulfoxide (**2a**) (□) and in the presence of 0.01 mM **2a** (△). Error bars represent standard deviations for quadruplicate determinations. Conditions: RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 37 °C.

Cell Permeability Studies. Thus, enediol analogue should rapidly form inside tumor cells incubated in the presence of sulfoxide, provided that the sulfoxide can diffuse across the cell membrane and intracellular GSH is free to react with the sulfoxide. Indeed, incubation of L1210 cells in the presence of 0.19 mM **2a** results in the rapid appearance of intracellular enediol analogue **1a**, Figure 2. Compound **1a** was isolated from cell extracts by reverse-phase C_{18} column chromatography and quantitated by interpolation from standard curves. The maximum concentration of **1a** (1.6 mM) observed after about 20 min is close to the reported concentration of GSH in L1210 cells (1.9 mM),[14] indicating that most of the intracellular GSH is free to react with **2a.** Moreover, the maximum intracellular concentration of enediol analogue exceeds the extracellular concentration of the sulfoxide by 8-fold, indicating that the rate constant for efflux of the enediol analogue is much less than that for influx of the sulfoxide. Similarly, incubation of the cells with 0.01 mM sulfoxide for 30 min results in a 13-fold higher concentration of intracellular **1a**, Figure 2. Thus, the enediol analogue can be concentrated inside tumor cells under conditions where the

Table 2. Apparent First-Order Rate Constants for Delivery of Enediol Analogue **1a** into L1210 Cells via Different Prodrugs and Comparative Toxicities of the Prodrugs to L1210 Cells[a]

prodrug	rate constant (min^{-1})[b]	GI_{50} (μM)[c]	TGI (μM)[c]	LC_{50} (μM)[c]
2a	1.41 ± 0.03	0.5 ± 0.1	3.9 ± 0.4	13 ± 8
1a(cyclopentyl)$_2$	0.59 ± 0.01	3.4 ± 0.1	5.3 ± 0.1	8.3 ± 1.0
1a(Et)$_2$	0.10 ± 0.01[d]	7 ± 3[d]	27 ± 10[d]	54 ± 5[d]

[a] The mean and standard deviations for the cell toxicity parameters for each prodrug were calculated from three different experiments carried out on different days. Conditions: RPMI 1640/10% fetal calf serum, 37 °C. [b] Derived from the slopes of plots of initial rates of appearance of the inhibitory diacid **2** inside L1210 cells versus the extracellular concentration of prodrug. [c] Defined in the legend to Figure 4. [d] From ref 8.

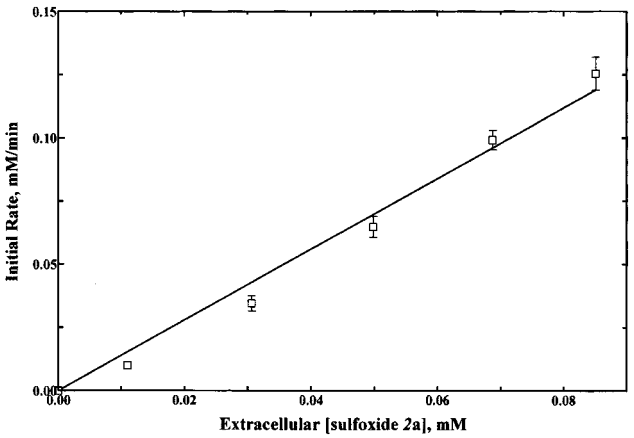


Figure 3. Initial rates of appearance of intracellular *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)glutathione (**1a**) in L1210 cells as a function of the extracellular concentration of sulfoxide **2a**. Error bars represent standard deviations for triplicate determinations. Conditions: RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 37 °C.

extracellular concentration of sulfoxide is low. The explanation for the decrease in concentration of enediol analogue after 20 min is not known but could involve several factors including enzymatic degradation and/or export of the enediol analogue from the cells by the GSH-conjugate export pump that is present in L1210 cells.[15]

The sulfoxide prodrug strategy is more efficient than the dialkyl ester prodrug strategy at delivering enediol analogue **1a** into L1210 cells. The apparent first-order rate constant for formation of **1a** in L1210 cells incubated with sulfoxide is 14-fold larger than that with the diethyl ester prodrug **1a**(Et)$_2$ and 2-fold larger than that with the dicyclopentyl ester **1a**(cyclopentyl)$_2$, Table 2. These rate constants were derived from the slopes of plots of the initial rates of appearance of **1a** inside the cells versus extracellular [prodrug], e.g., Figure 3.

Cytotoxicity Studies. Sulfoxide **2a** is both cytostatic and cytotoxic to L1210 cells in tissue culture, as reflected in the GI_{50} and LC_{50} values obtained by interpolation from the dose−response curve of Figure 4. We believe that this most likely reflects rapid reaction of intracellular sulfoxide with GSH to give enediol analogue, which induces elevated levels of methylglyoxal in the L1210 cells by inhibiting glyoxalase I. The toxicity of the sulfoxide is unlikely to be due to depletion of intracellular GSH, because the total concentration of GSH in L1210 cells is large in comparison to the value of the growth inhibition parameter (GI_{50}). As previously

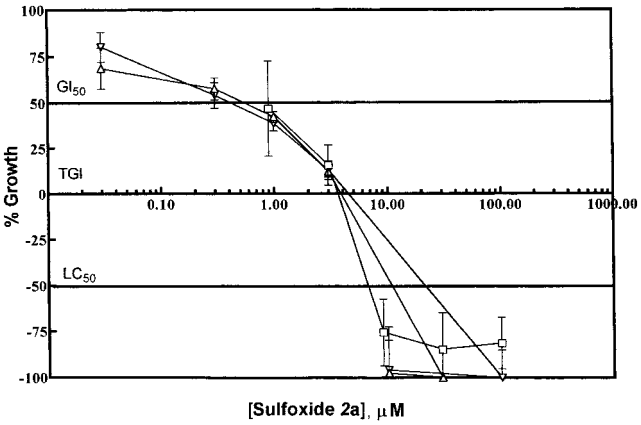


Figure 4. Growth inhibition of L1210 cells by *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)ethylsulfoxide (**2a**) (RPMI 1640/10% fetal calf serum, 37 °C, 48 h). Percentage growth (PG) was calculated from the following relationships: When $(\rho_{test} - \rho_{tzero}) \geq 0$, $PG = 100 \times (\rho_{test} - \rho_{tzero})/(\rho_{ctrl} - \rho_{tzero})$, where ρ_{tzero} = cell density prior to exposure to drug, ρ_{test} = cell density after 48 h exposure to drug, ρ_{ctrl} = cell density after 48 h with no exposure to drug. When $(\rho_{test} - \rho_{tzero}) < 0$, $PG = 100 \times (\rho_{test} - \rho_{tzero})/\rho_{tzero}$. Different symbols correspond to experiments carried out on different days. Error bars represent standard deviations for triplicate determinations. The toxicity parameters were obtained by interpolation from the dose−response curve and are defined as follows: GI_{50}, concentration producing 50% growth inhibition in comparison to no-drug controls; TGI, concentration producing 100% growth inhibition; LC_{50}, concentration producing 50% cell killing.

noted, the minimum concentration of GSH in the L1210 cells used in this study is 1.6 mM. Therefore, the concentration of GSH will be reduced by less than 1% in the presence of a sulfoxide concentration corresponding to the GI_{50} (0.5 μM), assuming that the maximum achievable concentration of enediol analogue inside the cells will be about 10-fold greater than that of the extracellular sulfoxide. In principle, the toxicity of the sulfoxide might arise from acylation of critical sulfhydryl-containing proteins inside the cell. However, this possibility seems less likely, given that the concentration of free GSH inside the cells is orders of magnitude higher than that of protein-associated sulfhydryl groups. Therefore, the sulfoxide is much more likely to react with GSH than with intracellular proteins. Importantly, the GI_{50} value for the sulfoxide is significantly lower than those of the diethyl ester **1a**(Et)$_2$ and dicyclopentyl ester **1a**(cyclopentyl)$_2$, Table 2. This can be explained by the greater rate at which the sulfoxide enters the cells in comparison to the dialkyl esters.

Substrate Properties with GSH Transferase. *N*,*N*-Di-*n*-propyl ethylsulfoxide has been reported to be a substrate for the GSH transferase in plant cells.[11] This prompted us to examine the kinetic properties of sulfoxide **2a** with the π isozyme of GSH transferase from human placenta. Multidrug-resistant tumors of the colon, lung, and breast commonly overexpress the π isozyme of GSH transferase.[16] Therefore, resistant tumors might be exceptionally sensitive to sulfoxides such as **2a**, provided that the sulfoxides are catalytically converted to the inhibitory enediol analogue.

Sulfoxide **2a** proved to be a poor substrate for the transferase from human placenta with an estimated k_{cat}/K_m value of 54 mM^{-1} min^{-1}. This value was derived from the first-order rates of loss of **2a** (ΔOD_{260}) in the

presence and absence of known amounts of transferase: conditions of 0.09 mM potassium phosphate buffer (pH 7.5), 5% ethanol, 0.1 mM GSH (saturating concentration), 0.005 mM **2a**, 25 °C. This represents a rate enhancement of about 30-fold, on the basis of a comparison with the second-order rate constant for the nonenzymic acyl-interchange reaction (Table 1). Thus, transferase activity is unlikely to have a major influence on the steady-state concentration of enediol analogue derived from **2a** in tumor cells containing high concentrations of GSH (∼2 mM) and low levels of transferase activity. Nevertheless, this observation emphasizes the potential importance of using the transferase to generate enediol inhibitors of glyoxalase I inside tumor cells.

Serum Stability. Finally, sulfoxide **2a** exhibits reasonable stability in serum samples obtained from DBA/2 mice, routinely used to evaluate the in vivo efficacy of drugs against L1210 cells. The first-order rate constant for loss of sulfoxide in serum samples from these mice is 0.06 ± 0.01 min^{-1} ($T_{1/2} = 13 \pm 2$ min), $n = 3$. This compares with a half-life of less that 30 s for **2**$(Et)_2$, due to the high levels of esterase activity in plasma.[8] The stability of the sulfoxide, together with its rapid rate of diffusion into L1210 cells, should optimize the chances of delivering toxic levels of drug to L1210 cells implanted in tumor-bearing mice.

Conclusions

Acyl interchange between intracellular GSH and substituted (*N*-hydroxycarbamoyl)alkylsulfoxides is a new prodrug strategy for indirectly delivering mechanism-based competitive inhibitors of glyoxalase I into tumor cells. In principle, this method could be expanded to include any S-conjugate of GSH. Sulfoxide prodrugs offer advantages over dialkyl ester prodrugs in terms of increased rates of intracellular delivery, a corresponding increase in potency, and the potential to undergo GSH transferase-catalyzed conversion to the inhibitory enediol analogues.

Experimental Section

Synthetic methods are outlined in Scheme 2. NMR spectra were taken on a GE QE-300 NMR spectrometer. Mass spectral data were obtained at the Midwest Center for Mass Spectrometry, University of Nebraska−Lincoln. Elemental analyses were obtained at Atlantic Microlabs, Inc., Norcross, GA, and are within ±0.4% of the calculated values unless otherwise indicated. Glutathione transferase was purchased from Sigma Chemical Co., and excess glutathione was removed by filtration through a Centricon filter.

***S*-(*p*-Chlorophenyl-*N*-hydroxycarbamoyl)glutathione Dicyclopentyl Ester (2a(cyclopentyl)$_2$).** This compound was prepared by acid-catalyzed esterification of **2a**, prepared as previously described,[8] in cyclopentanol/HCl (2.3 N) (2 days, room temperature). The orange-colored oil was dissolved in a minimum of 40% ethanol in water and decolorized by stirring with activated charcoal overnight. The charcoal was removed by filtration through Celite and the filtrate brought to dryness in vacuo to give the HCl salt of the dicyclopentyl ester as a colorless oil. Yield > 90%. The product was estimated to be greater than 98% pure by reverse-phase C_{18} HPLC, using methanol/water (55:45) containing 0.25% acetic acid as an eluting solvent: 300 MHz ^{1}H NMR (D_2O, DSS) δ 1.5−2.0 (16 H, m, cyclopentyl-CH_2), 2.21 (2H, m, Glu-$C_\beta H_2$), 2.54 (2H, m, Glu-C_γ H_2), 3.20 (1H, q, Cys-$C_\beta H_a$, J = 7.8, 14.5 Hz), 3.42 (1H, q, Cys-$C_\beta H_b$, J = 5.1, 14.5 Hz), 4.01 (2H, s, Gly-CH_2), 4.08 (1H, t, Glu-C_αH, J = 6.0 Hz), 4.67 (1H, m, Cys-C_αH), 5.18 (2H, m, cyclopentyl-CH), 7.3−7.5 (4H, m, aromatic-H); HR FAB-MS consistent with the molecular formula of **2a**(cyclopentyl)$_2$ ($C_{27}H_{38}N_4O_8SCl$). Anal. **2**(cyclopentyl)$_2$(HCl salt)·$3H_2O$ ($C_{27}H_{44}N_4O_{11}SCl_2$): C calcd 46.14, found 45.51; H calcd 6.26, found 6.14; N calcd 7.97, found 7.17.

***N*-Hydroxy-*N*-*p*-chlorophenylcarbamate Thioethyl Ester (3a).** A solution of thioethyl chloroformate (2.46 g, 20 mmol) in diethyl ether (10 mL) was added dropwise to a ice-cold stirring mixture of *N*-*p*-chlorophenylhydroxylamine (2.83 g, 20 mmol), K_2CO_3 (1.36 g, 10 mmol), diethyl ether (40 mL), and water (2 mL) over a period of 10 min. The reaction mixture was allowed to come to room temperature and followed to completion by TLC (∼180 min). The ether layer was removed, washed once with 5% aqueous HCl and water, and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo and the residue recrystallized from benzene/petroleum ether to give the final product as transparent needles: yield 83%; mp 119 °C dec; IR (KBr) 1600, 1490, 1400, 1340, 1070, 820 cm^{-1}; 300 MHz ^{1}H NMR ($CDCl_3$, TMS) δ 1.32 (t, J = 7.5 Hz, methyl-H_3), 2.90 (q, J = 7.5 Hz, methylene-H_2), 6.68 (bs, OH), 7.34 (d, J = 8.7 Hz, *p*-chlorophenyl ring meta-H_2), 7.52 (d, J = 8.7 Hz, *p*-chlorophenyl ring ortho-H_2). Anal. ($C_9H_{10}NO_2SCl$): C; H calcd 4.32, found 4.29; N calcd 6.05, found 6.00.

***N*-Hydroxy-*N*-methylcarbamate Thioethyl Ester (3b).** This compound was prepared by the same general method used in the preparation of **3a**: yield 70%; IR (KBr) 3220, 2920, 1620, 1400, 1265, 1200, 1080, 900 cm^{-1}; 300 MHz ^{1}H NMR ($CDCl_3$, TMS) δ 1.71 (t, J = 7.5 Hz, methyl-H_3), 3.29 (q, J = 7.5 Hz, methylene-H_2), 3.70 (s, methyl-H_3), 7.08 (bs, 1H), HR FAB-MS consistent with $C_4H_9NO_2S$. Anal. ($C_4H_9NO_2S$): C; H calcd 6.67, found 6.72; N calcd 10.37, found 10.27.

***N*-Methyl-*N*-*p*-chlorophenylcarbamate Thioethyl Ester (3c).** This compound was prepared by the same general method used in the preparation of **3a**: yield 82%, mp 60−61 °C; IR (KBr) 2970, 1650, 1580, 1480, 1400, 1345, 1260, 1085, 1010, 840 cm^{-1}; 300 MHz ^{1}H NMR ($CDCl_3$, TMS) δ 1.24 (t, J = 7.2 Hz, methyl-H_3), 2.86 (q, J = 7.2 Hz, methylene-H_2), 3.31 (s, methyl-H_3), 7.19 (d, J = 8.4 Hz, 2H), 7.38 (d, J = 8.8 Hz, 2H); HR FAB-MS consistent with $C_{10}H_{12}NOSCl$. Anal. ($C_{10}H_{12}NOSCl$): C; H calcd 5.23, found 5.28; N calcd 6.10, found 6.01.

***S*-(*N*-Hydroxy-*N*-*p*-chlorophenylcarbamoyl)ethylsulfoxide (2a).** A solution of *m*-chloroperbenzoic acid (2.12 g, 12.3 mmol) in diethyl ether (60 mL) was added dropwise to a ice-cold stirring solution of **3a** (2.85 g, 12.3 mmol) dissolved in diethyl ether (30 mL) over a period of 15 min. The reaction mixture was allowed to come to room temperature and stirred for an additional 30 min. The precipitate was removed from the reaction mixture by filtration, thoroughly washed with diethyl ether, and allowed to dry. The final product is a white powder: yield 96%; mp 143−144 °C dec; IR (KBr) 1700, 1490, 1410, 1350, 1260, 1090, 1000, 800 cm^{-1}; 300 MHz ^{1}H NMR (DMSO-d_6, TMS) δ 1.20 (t, J = 7.5 Hz, methyl-H_3), 7.54 (d, J = 9.2 Hz, 2H), 7.71 (d, J = 9.2 Hz, 2H), 11.69 (bs, OH); HR FAB-MS consistent with $C_9H_{10}NO_3SCl$. Anal. ($C_9H_{10}NO_3SCl$): C; H calcd 4.07, found 4.12; N calcd 5.66, found 5.57.

***S*-(*N*-Hydroxy-*N*-methylcarbamoyl)ethylsulfoxide (2b).** This compound was prepared by the same general method used to prepare **2a**: yield 86%; mp 110−112 °C; IR (KBr) 2700, 1710, 1380, 1190, 1010, 970, 840 cm^{-1}; 300 MHz ^{1}H NMR (DMSO-d_6, TMS) δ 1.16 (t, J = 7.5 Hz, methyl-H_3), 2.80−3.05 (m, methylene-H_2), 3.23 (s, methyl-H_3), 10.71 (s, 1H); HR FAB-MS consistent with $C_4H_9NO_3S$. Anal. ($C_4H_9NO_3S$): C; H calcd 5.96, found 6.03; N calcd 9.27, found 9.21.

***S*-(*N*-Methyl-*N*-*p*-chlorophenylcarbamoyl)ethylsulfoxide (2c).** To an ice-cold stirring solution of **3c** (0.5 g, 2.2 mmol) in methanol (15 mL) was added dropwise over 10 min a solution of Oxone (Aldrich; 0.87 g of $2KHSO_5·KHSO_4·K_2SO_4$, containing 2.8 mmol of $KHSO_5$) in water (25 mL). The resulting slurry was stirred at room temperature for 1.5 h. The mixture was diluted to 80 mL with water and extracted three times with methylene chloride (70 mL). The combined organic layers were washed once with water and once with brine and dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo to give a clear oil as crude product. The

product was purified by flash chromatography on a silica gel column (CH_2Cl_2:methanol (40:1)), the solvent was removed from the peak fractions, and the product was recrystallized from CH_2Cl_2/petroleum ether to give the final product as colorless cubic crystals: yield 43%; mp 113−115 °C; IR (KBr) 1680, 1480, 1350, 1245, 1050, 1000, 840 cm^{-1}; 300 MHz ^1H NMR ($CDCl_3$, TMS) δ 1.23 (t, $J = 7.2$ Hz, methyl-H_3), 2.98−3.18 (m, methylene-H_2), 3.42 (s, methyl-H_3), 7.24 (d, $J = 8.4$ Hz, 2H), 7.45 (d, $J = 8.4$ Hz, 2H). Anal. ($C_{10}H_{12}NO_2SCl$): C calcd, 48.88, found 48.62; H calcd 4.89, found 4.86; N calcd 5.70, found 5.62.

Cell Permeability Studies. To suspensions of L1210 cells ($\sim 1.5 \times 10^6$ cells/mL) in RPMI 1640 medium, containing 10% heat-inactivated fetal calf serum, gentamycin (10 μg/mL), and L-glutamate, at 37 °C, were added fixed concentrations of **2a**. Aliquots (1 mL) were removed from the cell suspension as a function of time, overlaid onto 0.4 mL of silicone oil (SF-1250 silicone fluids; General Electric Co., Silicone Products Division, Waterford, NY) contained in an Eppendorf centrifuge tube, and centrifuged at 11000*g* (15 min) at 25 °C. The supernatant and silicone oil were decanted from the cell pellet, and residual oil was removed from the inside of the centrifuge tube with a cotton swab. To the pellet was added 1 mL of 70% ethanol in water, and the suspension was sonicated for 2 min. The denatured protein was sedimented by centrifugation (11000*g*, 15 min), and the clear supernatant was removed. The supernatant was brought to dryness under a stream of nitrogen, and the resulting residue was fractionated by reverse-phase HPLC (Waters μBondapak C_{18}, 0.78 × 30 cm), using a mobile phase composed of methanol:water (1:1) containing 0.25% acetic acid at a flow rate of 2 mL/min. The integrated intensity of the peak corresponding to *S*-(*N*-*p*-chlorophenyl-*N*-hydroxycarbamoyl)glutathione (**1a**) was determined at $\lambda = 260$ nm. Concentrations were extrapolated from standard curves that were linear in the range 0.1−4.0 nmol ($r = 0.997$).

Cytotoxicity Studies. Murine lymphocytic leukemia (L1210, G050141) cells were obtained from the NCI, DCT, Tumor Repository (Frederick, MD) and were maintained in RPMI 1640 medium containing L-glutamate (Gibco BRL, Gaithersburg, MD), supplemented with 10% heat-inactivated fetal calf serum and gentamycin (10 μg/mL), under 37 °C humidified air containing 5% CO_2. The L1210 cells have a doubling time of approximately 14 h. Cells in logarithmic growth were introduced into 96-well tissue culture plates at a density of 5000 cells/well (0.15 mL) in the absence and presence of at least five different concentrations of drug, spanning the IC_{50} concentration (in triplicate). Fresh drug was added to the media every 12 h, to compensate for spontaneous hydrolysis of the drug ($T_{1/2} = 4.5$ h). After a 48-h incubation period, cell densities were determined with the use of a Coulter Counter (Model ZBI, Coulter Electronics). Cell viability was determined by the trypan blue exclusion method.[17]

Stability of Sulfoxide 2a in Mouse Serum. To 0.5-mL portions of serum from DBA/2 mice, at 37 °C, was added sulfoxide **2a** to an initial concentration of approximately 0.8 mM. As a function of time, 0.1-mL aliquots of the incubation mixture were transferred to separate microfuge tubes. The samples were immediately deproteinized by the addition of 70% ethanol (0.9 mL), and the protein precipitate sedimented by centrifugation at 13000*g*. The supernatants were then fractionated by reverse-phase HPLC (Waters μBondapak C_{18}, 0.78 × 30 cm), using a mobile phase composed of methanol:water (1:1) containing 0.25% acetic acid at a flow rate of 2 mL/min. The integrated intensity of the peak corresponding to **2a** was determined at 259 nm. The rate constant for decomposition was calculated from the first-order loss of **2a** as a function of time.

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