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SYNTHESIS AND ACTIVITY OF γ-(L-γ-AZAGLUTAMYL)-S-(*p*-BROMOBENZYL)-L-CYSTEINYLGLYCINE: A METABOLICALLY STABLE INHIBITOR OF GLYOXALASE I

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Abstract: The inhibition of glyoxalase I enzyme to increase cellular levels of methylglyoxal has been developed as a rationale for the production of anticancer agents. Synthesis of a peptidomimetic analog of the previously prepared potent glyoxalase inhibitor, S-(*p*-bromobenzyl)glutathione (PBBG), was accomplished by inserting a urea linkage, NH-CO-NH, to replace the γ-glutamyl peptide bond. Thus, the target compound, γ-(L-γ-azaglutamyl)-S-(*p*-bromobenzyl)-L-cysteinylglycine **6**, was a potent inhibitor of glyoxalase I with almost no loss of activity when compared to PBBG. However, unlike PBBG, **6** was completely resistant to enzymatic degradation by kidney homogenate or by purified γ-glutamyltranspeptidase enzyme. © 1999 Elsevier Science Ltd. All rights reserved.

The powerful carcinostatic activity of α-ketoaldehydes, including the physiological metabolite, methylglyoxal, has been known for several years.[1] However, the use of these agents as antitumor agents has been precluded by their rapid metabolism to the inactive α-hydroxy acids by the glyoxalase enzyme system.[2] Thus, inhibition of glyoxalase I (EC 3.2.1.6) as a rationale for the development of antitumor agents was originally proposed by Vince and Wadd.[3] Since reduced glutathione (GSH) is a cofactor in the glyoxalase reaction, S-substituted glutathione derivatives were found to be effective inhibitors of glyoxalase I.[4] It was found that a nonpolar region exists on the enzyme and plays an important role in the formation of an enzyme-inhibitor complex. The most potent inhibitor,PBBG, gave 920-fold better binding affinity over that of S-methylglutathione.[5] However, two major factors obviating the antitumor activity of this inhibitor are the inability of the charged molecule to penetrate the cell membrane, and rapid degradation by the γ-glutamyltranspeptidase system.[6] Diesterification of the glutathione analog has recently been reported to provide a more resistant form of the inhibitor which delivers the PBBG to the target enzyme.[7] The antitumor activity of the esterified forms of our original inhibitors has confirmed our early proposal, and has prompted us to design peptidomimetic analogs that retain potent inhibition of glyoxalase I and concomitantly resist hydrolysis by γ-glutamyltranspeptidase.

S-(*p*-bromobenzyl)glutathione

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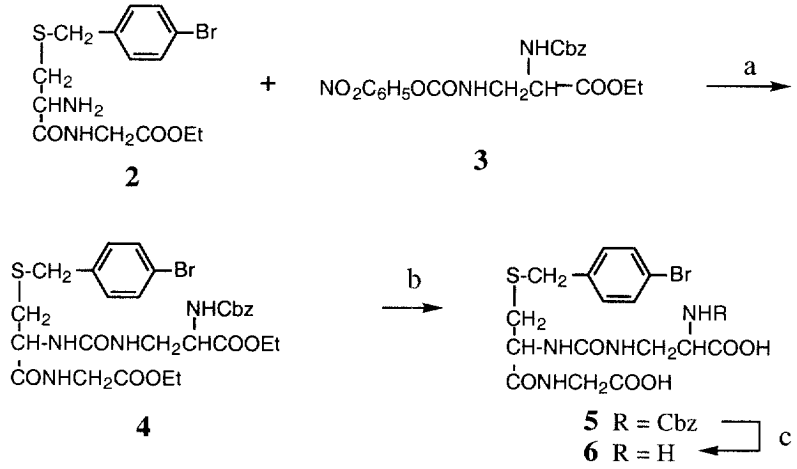
The introduction of a urea linkage, NH-CO-NH, in place of the γ-glutamyl peptide bond was acheived as outlined in Scheme 1. S-(*p*-Bromobenzyl)-L-cysteinylglycine (**1**) was prepared as previously described[8] and converted to the corresponding ethyl ester **2** using thionyl chloride and ethanol. The protected dipeptide **2** was condensed with the carbamate **3**[9] and gave the blocked aza tripeptide **4.** The ester groups were hydrolyzed to the corresponding diacid **5**. Treatment of **5** with HBr/AcOH selectively removed the carbobenzoxy blocking group while leaving the thioether bond intact and gave the title compound, **6**.



Scheme 1: (a) dioxane/chloroform, 40 °C, 24 h; (b) 0.2N NaOH, rt, 4 h; (c) HBr/AcOH, rt, 2 h.

The modified GSH analog **6** was assayed for its ability to inhibit yeast glyoxalase I enzyme in 0.05M phosphate buffer, pH 6.6 , 30 °C. The dissociation constant (K_i) of the enzyme inhibitor complex was obtained from double reciprocal plots. The substrate for the glyoxalase enzyme is the hemimercaptal, $CH_3COCH(OH)SG$, which forms spontaneously upon the addition of GSH and methylglyoxal. The concentrations of hemimercaptal for each determination were calculated using the dissociation constant, 3.1 x 10^{-3}M previously determined for the equilibrium reaction.[5] It has been shown previously that PBBG is a competitive inhibitor of glyoxalase I demonstrating that GSH, the hemimercaptal substrate, and the S-substituted GSH analogs can bind to the active center of the enzyme.[8] A comparison of analog **6** with PBBG revealed that almost no loss of binding activity occurred by the substitution of NH for the CH_2 group in the glutamic acid moiety. The competitive binding of **6** (K_i = 15.5 ± 3.1 μM) corresponds to that of PBBG (K_i = 9.14 ± 1.3 μM). The K_m for the glyoxalase substrate was 0.50 mM in each experiment.

Excellent separation of metabolic products on cellulose thin-layer plates developed with butanol:acetic acid:water (6:2:1) and sprayed with ninhydrin was obtained using PBBG as a reference in studying the effect of kidney homogenate γ-glutamyltranspeptidase on compound **6**. Results of the hydrolysis, illustrated in Figure 1, indicate that PBBG is significantly degraded after 15 min of incubation with the kidney homogenate. Lane 2 shows PBBG (high R_f spot) and the added acceptor, glycylglycine (low R_f) at zero time (solvent front

corresponds to top of diagram). Lane 3 illustrates 15 min of incubation and shows a new high R_f spot corresponding to complete degredation to the dipeptide, S-(*p*-bromobenzyl)cysteinylglycine (shown in lane 1). The new low R_f spot corresponds to γ-glutamylglycylglycine, the other expected product from the transpeptidase reaction. Lane 4 represents compound **6** at zero incubation time, while lane 5 shows that **6** is resistant to degradation even after 2 h of incubation. A similar experiment using isolated enzyme gave the same results (Figure 2). Thus, incubation of PBBG with purified γ-glutamyltranspeptidase shows complete hydrolysis after 15 min (lane 2). Compound **6** incubation results are shown in lane 3 (zero time) and lane 4 (2 h) demonstrating complete resistance to the purified enzyme even after two hours of incubation.

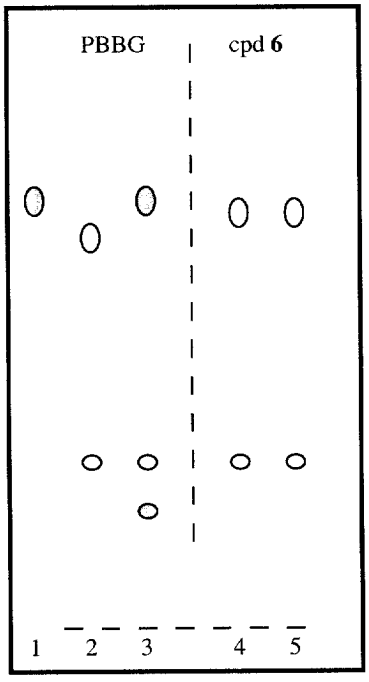


Figure 1. Thin- layer chromatagram of incubation aliquots containing PBBG or cpd **6** with mouse kidney homogenate. See text for details

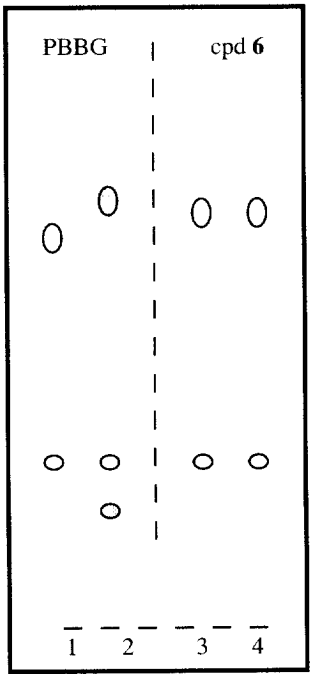


Figure 2. Thin-layer chromatogram of incubation aliquots cotaining PBBG or cpd **6** with γ–glutamyltranspeptidase. See text for details

This study demonstrated that the peptidomimetic glutathione analog **6** is an excellent inhibitor of the glyoxalase I enzyme. The substitution of the urea bond for the peptide moiety on the γ-glutamyl moiety had almost no effect on binding to the enzyme. At the same time, compound **6** was completely resistant to γ-glutamyltranspeptidase in kidney homogenates and purified enzyme. Since degradation by γ-glutamyltranspeptidase may play an important role in the inactivation of previously tested glyoxalase inhibitors in vivo, compounds based on **6** may be useful in enhancing the antitumor activity of α-ketoaldehydes such as methylglyoxal. We are presently preparing a series of esterified derivitives of **6** for such studies.

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