Notes

Antitumor Activity of *S*-(*p*-Bromobenzyl)glutathione Diesters *in Vitro*: A Structure−Activity Study

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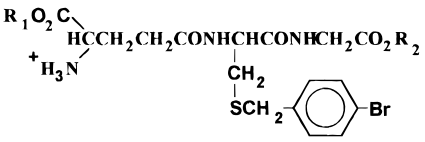
S-(*p*-Bromobenzyl)glutathione is a competitive inhibitor of human glyoxalase I which is part of the cytosolic glyoxalase system. It may be delivered into the cytosol of cells by diesterification wherein it is deesterified by cytosolic nonspecific esterases. *S*-(*p*-Bromobenzyl)glutathione diesters had antitumor activity *in vitro* and *in vivo*. The inhibition of human leukemia 60 cell growth *in vitro* by a series of alkyl and cycloalkyl diesters of *S*-(*p*-bromobenzyl)glutathione was investigated. For *n*-alkyl diesters, the *n*-propyl diester was the most potent derivative with a median growth inhibitory concentration GC_{50} value of 7.77 ± 0.01 μM (N = 18). The most potent derivative was *S*-(*p*-bromobenzyl)glutathione cyclopentyl diester which had a GC_{50} value of 4.23 ± 0.01 μM (N = 21) and also had antitumor activity *in vivo*.

Introduction

Inhibition of glyoxalase I (EC 3.2.1.6) was proposed as a novel strategy for the development of antitumor agents by Vince and Wadd in 1969.[1] The consequent accumulation of the physiological α-oxoaldehyde metabolite methylglyoxal was expected to lead to antiproliferative activity which had previously been found for high concentrations of exogenous methylglyoxal.[2] The poor efficacy of methylglyoxal was attributed to rapid detoxification by the glyoxalase system. Glutathione *S*-conjugates were evaluated as competitive inhibitors of glyoxalase I;[3] *S*-(*p*-bromobenzyl)glutathione (Chart 1) was the most potent analogue studied[3] and had an inhibition constant K_i value of 0.08 μM[4]. *S*-(*p*-Bromobenzyl)glutathione was not a potent antitumor agent, however, which has now been attributed to the poor delivery of this glutathione *S*-conjugate into cells to reach the glyoxalase I enzyme target in the cytosol of cells and extracellular degradation by γ-glutamyl transferase.[5]

S-(*p*-Bromobenzyl)glutathione ethyl diester (**1**) was developed as a prodrug vehicle for the delivery of *S*-(*p*-bromobenzyl)glutathione into cells. Unlike the ethyl monoester derivative, it inhibited the growth of human leukemia 60 (HL-60) cells *in vitro*.[5] Since then, diesterification has also been found to be a much more potent strategy than monoesterification for the delivery of reduced glutathione into cells.[6] Incubation of HL-60 cells with *S*-(*p*-bromobenzyl)glutathione cyclopentyl diester lead to the delivery of diester into the cell cytosol wherein it was hydrolyzed sequentially to *S*-(*p*-bromobenzyl)glutathione cyclopentyl monoester and *S*-(*p*-bromobenzyl)glutathione. There was a subsequent increase in the concentration of methylglyoxal and induction of apoptosis.[7] In this report, we describe a structure−activity study of the antileukemia activity of diester derivatives of the glyoxalase I inhibitor *S*-(*p*-bromobenzyl)glutathione *in vitro*.

Chart 1. Ester Derivatives of *S*-(*p*-Bromobenzyl)-glutathione[a]



[a] *S*-(*p*-Bromobenzyl)glutathione $R_1 = R_2 = H$; *S*-(*p*-bromobenzyl)glutathione monoester $R_1 = H$, R_2 = alkyl, cycloalkyl; *S*-(*p*-bromobenzyl)glutathione diester $R_1 = R_2$ = alkyl, cycloalkyl.

Chemistry

S-(*p*-Bromobenzyl)glutathione diesters were synthesized by acid-catalyzed esterification of *S*-(*p*-bromobenzyl)glutathione in the appropriate alcohol.[7] The esterified product was contaminated with the corresponding monoester derivative. *S*-(*p*-Bromobenzyl)glutathione diesters were purified by hydrophilic interaction column chromatography using Dowex-1 anion exchange resin in the formate form with methanol as eluent: the diester was eluted shortly after the void volume, well-resolved from the monoester derivative. A series of *n*-alkyl diester derivatives, ethyl, *n*-propyl, *n*-butyl, and *n*-pentyl diesters; isopropyl diester; and cyclopentyl and cyclohexyl diesters were prepared; corresponding monoesters were also isolated and characterized. This series of diesters was chosen following findings of the delivery of cysteine into tissues *in vivo* by ester derivatives where cyclopentyl and cyclohexyl esters were found to be the most effective prodrugs.[8]

Results and Discussion

S-(*p*-Bromobenzyl)glutathione diesters were evaluated for antileukemia activity by determining the median growth inhibitory concentration (GC_{50}) and median toxic concentration (TC_{50}) values with HL-60 cells *in vitro*. For the *n*-alkyl series of *S*-(*p*-bromoben-

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zyl)glutathione diesters, the propyl diester had the lowest GC_{50} and TC_{50} values. The cyclopentyl diester, however, was the most potent of the *S*-(*p*-bromobenzyl)-glutathione diesters evaluated with a GC_{50} value of 4.23 $\pm$ 0.01 μM ($N = 21$) and a TC_{50} value of 8.86 $\pm$ 0.01 μM ($N = 21$). The antitumor activity of *S*-(*p*-bromobenzyl)-glutathione diesters was not limited to activity against HL-60 cells. For the National Cancer Institute panel of leukemia, non-small lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer cell lines, *S*-(*p*-bromobenzyl)glutathione ethyl diester gave GC_{50} values in the range 7−20 μM,[11] and *S*-(*p*-bromobenzyl)glutathione cyclopentyl diester had antitumor activity *in vivo* against murine adenocarcinoma 15A.[7] Incubation of HL-60 cells with *S*-(*p*-bromobenzyl)glutathione cyclopentyl diester lead to the delivery of diester into the cell cytosol wherein it was hydrolyzed to *S*-(*p*-bromobenzyl)glutathione cyclopentyl monoester and *S*-(*p*-bromobenzyl)-glutathione.[7]

S-(*p*-Bromobenzyl)glutathione and *S*-(*p*-bromobenzyl)-glutathione monoesters were inactive (GC_{50} and TC_{50} values >300 μM) although some monoester derivatives were potent inhibitors of human glyoxalase I: the inhibitor constant K_i value of *S*-(*p*-bromobenzyl)glutathione ethyl monoester was 2.36 μM.[9] In contrast, *S*-(*p*-bromobenzyl)glutathione ethyl diester was a poor inhibitor of human glyoxalase I and had a K_i value of 235 μM.[9] This is consistent with the diesterification of *S*-(*p*-bromobenzyl)glutathione being important for delivery into the cell cytosol, *cf.* the delivery of GSH into the cytosol of cells by GSH diester, and also important to confer resistance to degradation by γ-glutamyl transpeptidase on the external surface of the plasma membrane of cells.[6]

Precedents for maximal pharmacological response of the *n*-propyl and cyclopentyl diesters were in a series of *n*-alkyl esters of ibuprofen,[10] and the delivery of cysteine into rat lung tissue by cysteine esters.[8] This ester structure−activity effect is probably due to a balance of factors influential on the ability of *S*-(*p*-bromobenzyl)glutathione diesters to deliver *S*-(*p*-bromobenzyl)glutathione into cells: (i) the ability of the ester groups to be resistant to cleavage by plasma and extracellular plasma membrane-bound nonspecific esterases, and (ii) the susceptibility of the ester groups to hydrolysis by nonspecific esterase in the cytosol of the target cells.

Conclusion

S-(*p*-Bromobenzyl)glutathione diesters, glyoxalase I inhibitor prodrugs, have antitumor activity *in vitro* where a limited ester structure−activity study suggested that *n*-propyl and cyclopentyl diesters have potent antitumor activity. Their prospective mechanism of action is different from current clinical antitumor agents,[7,11] and they are good candidates for further pharmacological evaluation.

Experimental Section

Trypan blue, reduced glutathione, Dowex 1 (chloride form), and dimethylsulfoxide were purchased from Sigma Chemical Co. Ltd. (Poole, Dorset, U.K.). *p*-Bromobenzyl bromide, hydrogen chloride gas, trifluoroacetic acid, dimethyl sulfoxide-d_6, and alcohols used were purchased from Aldrich Chemical Co. Ltd. (Poole, Dorset, U.K.). Tissue culture medium RPMI 1640 and fetal calf serum were purchased from Gibco Europe Ltd. (Paisley, Scotland). Methanol (HPLC grade) was purchased from Rathburn Chemicals Ltd. (Walkerburn, Scotland). Silica gel 60 F_{234} TLC plates were purchased from BDH Chemicals (Poole, Dorset, U.K.). *S*-(*p*-Bromobenzyl)glutathione was synthesized by modification of the the method of Vince *et al.*[3,7] NMR spectra were recorded on a Jeol ex270 MHz NMR spectrometer. FAB mass spectra were recorded on a Kratos MS50 FAB mass spectrometer with compounds dissolved in a glycerol matrix. *S*-(*p*-Bromobenzyl)glutathione ethyl diester was synthesized as previously described.[7]

Table 1. Physicochemical and Biological Properties of *S*-(*p*-Bromobenzyl)glutathione Diesters

compound	R_1,R_2	M+1	mp (°C)	yield (%)	GC_{50} (μM)	*N*	TC_{50} (μM)	*N*
1	Et	532 & 534	107−109	35	8.3 ± 0.1	12	16.5 ± 0.1	27
2	*n*-Pr	560 & 562	118−120	70	7.8 ± 0.1	18	10.3 ± 0.3	18
3	*n*-Bu	588 & 590	128−130	75	18.5 ± 1.5	21	26.3 ± 1.0	21
4	*n*-Pt	616 & 618	133−135	66	22.2 ± 2.5	12	24.9 ± 1.3	12
5	*i*-Pr	560 & 562	120−122	13	19.6 ± 0.1	12	20.9 ± 1.1	12
6	cPt	612 & 614	136−138	71	4.2 ± 0.1	21	8.9 ± 0.1	21
7	cHx	640 & 642	121−123	60	29.2 ± 1.2	21	33.1 ± 4.7	21

***S*-(*p*-Bromobenzyl)glutathione cyclopentyl diester (6).** *S*-(*p*-Bromobenzyl)glutathione cyclopentyl diester was prepared and purified by methods similar to those described for the corresponding ethyl diester except the acid-catalyzed esterification of *S*-(*p*-bromobenzyl)glutathione was performed in cyclopentanol for 7 days at room temperature. It was characterized by melting point, FAB mass spectrometry (Table 1), ^{1}H and ^{13}C NMR spectroscopy, TLC, and elemental CHN analysis: ^{1}H NMR (DMSO-d_6) δ 8.54 (t, 1H, $J = 5.86$ Hz, glycyl NH), 8.23 (d, 1H, $J = 7.81$ Hz, cysteinyl NH), 7.49 (d, 2H, $J =$ 8.43 Hz, benzyl *m*-H), 7.28 (d, 2H, $J = 8.43$ Hz, benzyl *o*-H), 5.06 (m, 2H, cyclopentyl 1-H), 4.54 (m, 1H, cysteinyl 2-H), 3.76 (s, 2H, benzyl 1-H and d, 2H, $J = 5.86$ Hz, glycyl 2-H), 3.41 (t, 1H, $J = 5.35$ Hz, γ-glutamyl 1-H), 2.74 (q, 1H, $J = 4.94$ Hz and −12.03 Hz, cysteinyl 3A-H), 2.52 (q, 1H, $J = 4.94$ Hz and −12.03 Hz, cysteinyl 3B-H), 2.25 (t, 2H, $J = 8.12$ Hz, γ-glutamyl 4-H), 1.6−1.9 (m, 10H, γ-glutamyl 3-H and cyclopentyl-2,3,4,5-H); ^{13}C NMR (DMSO-d_6) δ 174.5 (γ-glutamyl C-5), 172.3 (γ-glutamyl C-1), 171.1 (cysteinyl C-1), 169.4 (glycyl C-1), 138.1 (phenyl C-4), 131.4 (phenyl C-3,5), 131.3 (phenyl C-2,6), 120.1 (phenyl C-1), 77.4 (cyclopentyl(γ-glutamyl) C-1), 77.2 (cyclopentyl(glycyl) C-1), 53.5 (cysteinyl C-2), 51.8 (γ-glutamyl C-2), 41.3 (glycyl C-2), 34.5 (cysteinyl C-3), 33.2 (benzyl C-1), 32.4 (cyclopentyl(γ-glutamyl) C-2,5), 32.2 (cyclopentyl(glycyl) C-2,5), 31.7 (γ-glutamyl C-4), 29.8 (γ-glutamyl C-3), 23.4 (cyclopentyl(γ-glutamyl) C-3,4 & cyclopentyl(glycyl) C-3,4). The TLC R_f value on silica gel was 0.90 (mobile phase: chloroform, methanol, acetic acid, 8:1:1). Anal. ($C_{27}H_{40}N_3O_7SBr$) C, H, N, for *S*-(*p*-bromobenzyl)glutathione cyclopentyl diester·H_2O.

Other *S*-(*p*-bromobenzyl)glutathione diesters were prepared, purified, and characterized similarly.

Evaluation of Antileukemia Activity of *S*-(*p*-Bromobenzyl)glutathione Diesters *in Vitro*. HL-60 cells were incubated at 37 °C in RPMI 1640 media containing 10% fetal calf serum under an atmosphere of 5% CO_2 in air, 100% humidity.[7] Cells were seeded at an initial density of 5×10^4/mL and incubated with 0.5−300 μM *S*-(*p*-bromobenzyl)glutathione diester for 2 days.[7] A stock solution of *S*-(*p*-bromobenzyl)-glutathione diester was prepared in dimethyl sulfoxide and diluted into the growth medium such that the final concentration of dimethyl sulfoxide did not exceed 5 mM, a concentration which did not induce differentiation or toxicity in HL-60 cells. Cell viability was judged by the ability of cells to exclude trypan blue. GC_{50} and TC_{50} values were determined by logistic regression of viable cell number and cytotoxicity data on diester concentration, respectively. Nonlinear regression was performed using the ENZFITTER program (Biosoft, Cambridge, U.K.).

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