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Review

Glutathione transferases as mediators of signaling pathways involved in cell proliferation and cell death

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Glutathione transferases (GSTs) are enzymes that catalyze the conjugation of glutathione (GSH) to a variety of electrophilic substances. Their best known role is as cell housekeepers engaged in the detoxification of xenobiotics. Recently, GSTs have also been shown to act as modulators of signal transduction pathways that control cell proliferation and cell death. Their involvement in cancer cell growth and differentiation, and in the development of resistance to anticancer agents, has made them attractive drug targets. This review is focused on the inhibition of GSTs, in particular GSTP1-1, as a potential therapeutic approach for the treatment of cancer and other diseases associated with aberrant cell proliferation.
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Glutathione transferases (EC 2.5.1.18), also referred to as glutathione *S*-transferases or GSTs, are members of a multigene family of isoenzymes ubiquitously expressed in most living organisms. On the basis of their capacity to bind structurally diverse nonsubstrate ligands, they were initially thought to be 'all-purpose' carrier proteins involved in intracellular transport. It was subsequently shown that these enzymes catalyze the conjugation of glutathione (GSH) to a variety of electrophilic compounds, thus establishing the now widely accepted role of GSTs as cell housekeepers involved in the detoxification of endogenous as well as exogenous substances.[1–3]

Advances in the molecular biology of the GSTs over the past several years have revealed a broader role for these enzymes. Indeed, GSTs have been found to be involved in the biosynthesis and metabolism of prostaglandins,[4] steroids,[5] and leukotrienes;[6] in the management of toxic products of lipid oxidation and *S*-glutathiolated proteins generated by oxidative stress;[7–9] and in the acquisition of resistance to chemotherapeutic agents.[10–12] More recently, several GST isoenzymes have been shown to modulate cell signaling pathways that control cell proliferation and cell death (apoptosis).[13–17] Because of their cytoprotective role and their involvement in the development of resistance to anti-cancer agents, GSTs have become attractive drug targets. This review focuses on the inhibition of GSTP1-1, the most prevalent and ubiquitous non-hepatic isoenzyme, as a potential therapeutic approach for the treatment of cancer.

Cytosolic GSTs

The GSTs encompass three major families of proteins: (1) cytosolic, (2) mitochondrial, and (3) microsomal (also referred to as membrane-associated proteins in eicosanoid and glutathione (MAPEG)), of which the cytosolic GSTs constitute the largest family.[18] On the basis of amino acid sequence similarities, substrate specificity, and immunological cross-reactivity, seven classes of cytosolic GSTs have been identified in mammals.[19–21] These classes are designated by the names of the Greek letters α (*alpha*), μ (*mu*), π (*pi*), σ (*sigma*), θ (*theta*), ω (*omega*), and ζ (*zeta*), and abbreviated in Roman capitals A, M, P, S, T, O, and Z.[22,23]

Most GST classes show a high degree of polymorphism and include several subunits. Each subunit (ca. 199–244 amino acids in length, 22–29 kDa) contains a catalytically independent active site that consists of a GSH-binding site ('G-site') in the amino-terminal domain and a site that binds the hydrophobic substrate ('H-site') in the carboxy-terminal domain. More than a dozen cytosolic GST subunits have been identified in humans. As the functional enzymes are dimeric, and those of the α and μ classes, in addition to homodimers, can also form heterodimers, the number of isoenzymes that can be generated from these subunits is significantly larger (Table 1). The isoenzymes are named according to their class and subunit composition, with each subunit designated by an Arabic numeral (e.g., GSTA1-2 denotes the enzyme composed of subunits 1 and 2 of the α class).[23]

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Abbreviations: AML, acute myeloid leukemia; AP-1, activator protein-1; ASK1, apoptosis signal-regulating kinase 1; BCNU, *N,N′*-bis(2-chloroethyl)-*N*-nitrosourea; CDNB, 1-chloro-2,4-dinitrobenzene; CFU-GM, colony forming unit-granulocyte, monocyte; DXR, doxorubicin; EA, ethacrynic acid; ERK, extracellular signal-regulated kinase; 5-FU, 5-fluorouracil; GSH, glutathione; GST, glutathione *S*-transferase; H_2O_2, hydrogen peroxide; HEL, Haloenol lactone; HFF, human foreskin fibroblast; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MAPEG, membrane-associated proteins in eicosanoid and glutathione; MDS, myelodysplastic syndrome; NCI, National Cancer Institute; NTZ, nitazoxanide; ROS, reactive oxygen species; TLK117, (γ-glutamyl-*S*-(benzyl)cysteinyl-*R*(-)-phenylglycine); TRAF2, TNF receptor-associated factor 2; UV, ultraviolet
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Table 1 Classes of mammalian cytosolic GSTs

Class	Old enzyme designation	New enzyme designation	Subunits
Alpha	GSTα	GSTA	1,2,3,4,5
Mu	GSTμ	GSTM	1,2,3,4,5
Omega	GSTω	GSTO	1,2
Pi	GSTπ	GSTP	1,2
Sigma	GSTσ	GSTS	1
Theta	GSTτ	GSTT	1,2
Zeta	GSTζ	GSTZ	1

Expression of the different classes of GSTs varies among tissues and with developmental stage. For example, α-class GSTs are predominantly expressed in liver, testis, and kidney, and their expression levels are similar in both adult and fetal tissues. In contrast, GSTπ (GSTP1-1), originally isolated from placenta, is found mainly in brain, lung, and heart; its expression in liver decreases during embryonic development, becoming very low in adult tissue.[24–26]

GSTP1-1 and Cancer

Given their cytoprotective role as phase II enzymes involved in the deactivation of harmful electrophilic compounds, much of the earlier work on GSTs was focused on the identification of endogenous as well as exogenous substrates. Among the latter, special attention was given to carcinogens, pesticides, and other environmental pollutants. This effort was followed by studies of the expression level of specific isoenzymes in a variety of cancer cell lines, as this level was deemed to be a potential indicator of cancer development and resistance to anticancer drugs.

The majority of human tumor cell lines, including those selected *in vitro* for resistance to chemotherapeutic agents, overexpress GSTP1-1.[27,28] In fact, GSTP1-1 was found to be the predominant isoenzyme (up to 2.7% of the total cytosolic protein) in all but 2 of 60 tumor cell lines used in the Drug Screening Program of the National Cancer Institute (NCI). Significant quantitative correlations among enzymatic activity, total enzyme protein, and mRNA were shown, particularly in those cell lines selected for resistance to alkylating agents such as melphalan, chlorambucil, cyclophosphamide, BCNU (*N,N'*-bis(2-chloroethyl)-*N*-nitrosourea), and cisplatin. Overexpression of GST α and μ isoenzymes was also observed, but comparable correlations were much less apparent.[29]

A variety of human cancers, including of breast, colon, kidney, lung, and ovarian, usually express high levels of GSTP1-1 compared with the surrounding tissues. Consequently, GSTP1-1 expression has been considered to be a marker for cancer development.[30,31] High expression levels have been associated not only with disease progression but also with drug resistance in patients undergoing chemotherapy.

Many anticancer drugs have been described as being substrates of multiple GSTs. However, and in spite of the alleged broad substrate specificity of the GSTs, specific isoenzymes have shown preferential substrate specificity. For example, GSTT1-1 has the highest activity toward BCNU, which is significant as GSTT1-1 is expressed in the brain, a common target for BCNU treatment.[32] Similarly, α-class isoenzymes are very effective at catalyzing the conjugation of GSH to alkylating agents such as chlorambucil.[33,34] In contrast, GSTP1-1 has a relatively weak affinity for the majority of anticancer drugs, even though its increased expression has been correlated with the development of the multidrug-resistant phenotype. This apparent discrepancy may be explained in terms of the influence of GSTP1-1 on signaling pathways that affect cell survival.

GSTπ (GSTP1-1) and Cell Signaling

In addition to their GSH-conjugating activity, GSTs have been recognized for their ability to bind structurally diverse nonsubstrate ligands such as steroids, heme and bilirubin. More recently, isoenzymes from several GST classes have been shown to associate with members of the mitogen-activated protein kinase (MAPK) pathways involved in cell survival and death signaling. In this non-enzymatic role, GSTs function to sequester the kinase in a complex, thus preventing it from acting on downstream targets. The result of this action is a regulation of pathways that control cell proliferation and apoptotic cell death.

GSTπ was among the first isoenzymes found to inhibit c-Jun N-terminal kinase (JNK) through direct protein–protein interaction.[13] JNK is a MAP kinase involved in stress response, apoptosis, inflammation, and cellular differentiation and proliferation. Ultraviolet (UV) radiation, protein synthesis inhibitors, and a variety of stress stimuli can activate JNK that, in turn, phosphorylates c-Jun, a component of the activator protein-1 (AP-1) transcription factor. This activation leads to induction of AP-1-dependent target genes involved in cell proliferation and cell death.[35]

Studies of low basal JNK activity in normally growing, non-stressed 3T3-4A mouse embryonic fibroblasts led to the identification of GSTπ as a c-Jun–JNK complex-associated protein that selectively inhibited the phosphorylation of c-Jun by JNK. Isoenzymes of the α and μ classes were also shown to associate with the c-Jun–JNK complex *in vitro*, but showed weaker JNK inhibitory activity. UV irradiation or treatment of the cells with hydrogen peroxide (H_2O_2) caused an increase in JNK activity and the appearance of a high-molecular-weight (ca. 97 kDa) oligomeric form of GSTπ, showing that GSTπ inhibition of JNK is due primarily to their association, and that this association is disrupted under conditions that induce oligomerization of GSTπ (Figure 1).[13] Treatment of GSTπ with H_2O_2 is known to inactivate the enzyme by promoting the formation of intra- and/or inter-subunit disulfide bonds involving Cys-47 and Cys-101.[36]

The above findings, combined with the involvement of JNK in apoptosis and the observation that GSTπ (i.e., GSTP1-1) is often highly expressed in tumor tissues, suggested a possible role of this isoenzyme in apoptosis resistance during anticancer therapy. To test this hypothesis, apoptosis was induced in a neoplastic T-cell line (Jurkat) by treatment with H_2O_2 or etoposide, and changes in GSTP1-1 levels were followed by western blotting. Apoptosis was observed after treatment of the cells with H_2O_2, and it was paralleled by the appearance of a dimeric form (ca. 46 kDa) of GSTπ and the intensification of its monomeric form (ca. 21.5 kDa). These findings suggest

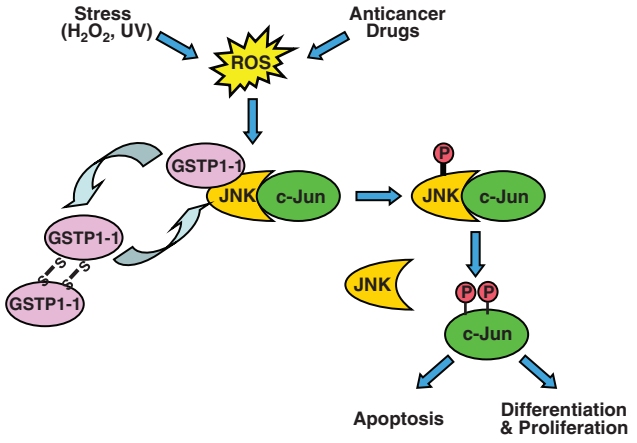

Figure 1 Role of GSTP1-1 in cell signaling

a partial inactivation of GSTP1-1 by dimerization involving disulfide bond formation between Cys-47 from two different subunits, and also by intrasubunit disulfide bond formation between Cys-47 and Cys-101. This inactivation was associated with an increased K_m^{GSH} and a decreased specific activity toward 1-chloro-2,4-dinitrobenzene (CDNB).[37]

The initial studies of JNK activity in non-stressed and stressed 3T3-4A mouse fibroblasts led to the conclusion that it was the monomeric form of GSTπ that inhibited JNK. Subsequent molecular dynamic calculations on the three-dimensional structure of GSTπ, free and bound to an inhibitor that blocks its ability to inhibit JNK-jun activation, identified four putative domains involved in the interaction between GSTπ and the c-Jun–JNK complex. The potential interaction site implicated in this analysis was found to be distal to the GST subunit dimerization domain (involving Cys-47 and Cys-101), suggesting that JNK may actually interact *in vivo* with homodimeric GSTP1-1.[38] This hypothesis would seem to be supported by a recent study showing the dissociation constant of homodimeric GSTP1-1 to be in the subnanomolar range ($K_d < 1$ nM), making it unlikely that the monomeric form would exist in any significant amount at the concentrations commonly used for assay measurement.[39]

The interaction between GSTπ and JNK has also been established *in vivo.* Compared with wild-type mice (GSTP1/P2$^{(+/+)}$), a significant increase in constitutive JNK activity was found in the liver and lung of transgenic mice in which both the *GSTP1* and *GSTP2* genes were deleted (GSTP1/P2$^{(-/-)}$). The increase in JNK activity was accompanied by a significant increase (eightfold) in AP-1 DNA-binding activity. This study not only shows the role of GSTπ as a direct inhibitor of JNK *in vivo,* but also its role in regulating the constitutive expression of specific downstream molecular targets of the JNK signaling pathway.[40]

GSTP1-1 has also been reported to associate with TNF receptor-associated factor 2 (TRAF2), a member of the TNF receptor-associated factor protein family. Overexpression of GSTP1-1 was found to inhibit TRAF2-induced activation of both JNK and p38-MAPK. GSTP1-1 also attenuated TRAF2-enhanced autophosphorylation of apoptosis signal-regulating kinase 1 (ASK1) and inhibited TRAF2–ASK1-induced apoptosis by suppressing the interaction of TRAF2 and ASK1.[41]

Most anticancer agents induce cell death through activation of the MAPK pathways, in particular those involving JNK and p38-MAPK. The role of GSTP1-1 as an endogenous inhibitor of JNK activation has direct relevance to the GSTP1-1-overexpressing phenotypes of many drug-resistant tumors. Indeed, elevated expression of GSTP1-1 during drug treatment can alter the balance of regulation of signaling pathways that influence cell proliferation and apoptosis, thereby conferring on tumor cells the ability to escape death.[42] This process may provide an explanation for the numerous examples of drug resistance that link GSTP1-1 overexpression with agents that are either poor substrates for this enzyme or not substrates at all.

GSTP1-1 as a Therapeutic Target

Because of its high expression in different tumors and its dual role as an enzyme involved in the deactivation of anticancer agents and as an inhibitor of signaling pathways leading to apoptosis, GSTP1-1 has emerged as a promising cancer therapeutic target.

Many compounds have been described in the literature as being GSTP1-1 inhibitors, including GSH analogs, GSH conjugates, small organic molecules, and natural products.[43–46] Their potency and degree of specificity toward this particular isoenzyme, however, vary considerably. A summary of the most relevant inhibitors is given in the following sections.

GSH Analogs

Modification of the GSH backbone has been one of the successful strategies used in the design of GST inhibitors. This approach takes advantage of the inherent affinity of GSH for the GSTs and aims at building specificity by exploiting subtle structural differences among the isoenzymes.[47,48] The highly conserved and selective G-site in GSTs, however, has made it difficult to achieve isoenzyme specificity without concomitant loss of binding affinity. The γ-glutamyl residue of GSH, on the other hand, has proven to be absolutely critical for binding, whereas changes in the glycine and cysteine residues are tolerated, provided they maintain the appropriate balance of charge and hydrophilicity.[49]

A systematic evaluation of GSH analogs containing substituents at both the glycine α-carbon and the cysteine thiol group identified the tripeptide TLK117 (γ-glutamyl-*S*-(benzyl)cysteinyl-*R*(-)-phenylglycine) as a potent and selective inhibitor of GSTP1-1 ($K_i = 0.4\ \mu$M). The binding affinity of TLK117 for the G-site of GSTP1-1 is greater than that of GSH itself, and its selectivity for GSTP1-1 is over 50-fold larger compared with isoenzymes of the α and μ classes.[50,51]

Although the presence of both carboxylic acid groups is vital for enzyme inhibition, cellular uptake experiments showed that TLK117 did not enter the cells to any significant extent. To circumvent this problem, the carboxylic acid groups were esterified to give TLK199, the diethyl ester prodrug of TLK117 (Figure 2). Similar to what has been observed with GSH diethyl ester,[52] TLK199 is taken up by cells and rapidly hydrolyzed to the phenylglycine monoethyl ester, whose intracellular concentration rises to levels that are significantly higher than those found after treatment of the cells with the

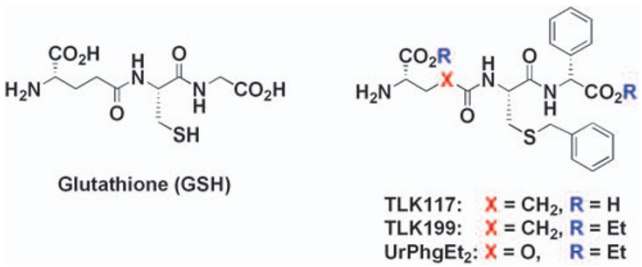

Figure 2 Structures of GSH and GSH analogs that inhibit GSTP1-1

monoester itself. High levels of the monoester, in turn, provide the cells with a means of producing TLK117 over a period of time.[53]

Given the high levels of expression of GSTP1-1 in neoplastic cells and its putative involvement in the deactivation of chemotherapeutic agents, combinations of TLK199 with several of these agents were tested in cancer cell lines overexpressing GSTP1-1 to determine whether TLK199 would act as a chemosensitizer. TLK199 potentiated chlorambucil toxicity by ca. twofold in HT4-1 cells (a subclone of the HT-29 human colon adenocarcinoma cell line made sensitive to ethacrynic acid), but the effect was less pronounced in a SKOV-3 (human ovarian carcinoma) cell line. TLK199 produced a slight increase in sensitivity to adriamycin in both cell lines, but had no effect on the sensitivity to mitomycin C.[53]

The potentiating effects of TLK199 observed in tumor cells were deemed not large enough to pursue in a clinical setting, particularly in the absence of toxicological information for different combination regimens. Instead, its effect on the sensitization of normal cells to cytotoxics, especially in the case of bone marrow, which is often the site of dose-limiting toxicity, was examined.

TLK199 was shown to act as a small-molecule myeloproliferative agent. It increased circulating white blood cells in normal mice and accelerated neutrophil recovery in rats and mice after 5-fluorouracil (5-FU)-induced neutropenia. It also stimulated bone marrow progenitor (colony-forming unit-granulocyte, monocyte (CFU-GM)) proliferation in normal mice, an effect that required GSTπ, as mice with a GSTπ-null genotype (GST$\pi^{(-/-)}$) did not respond to TLK199.[14,54] These activities may be explained in terms of the ability of TLK199 (i.e., TLK117) to disrupt the interaction between GSTP1-1 and JNK and, as a result, modulate kinase pathways that affect cell proliferation and differentiation. Treatment of cells with TLK199 efficiently decreased GSTP1-1-mediated inhibition of JNK and led to a twofold increase in basal JNK activity in non-stressed cells.[13] A human promyelocytic leukemia cell line (HL-60) made resistant to TLK199 by chronic exposure to the drug showed elevated activities of JNK1 and extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2), which allowed these cells to proliferate under stress conditions that induced apoptosis in wild-type cells.[14] These and other studies suggest that the myeloproliferative effects observed with TLK199 are dependent upon both GSTP1-1 expression and JNK activity, and that disruption of the GSTP1-1/JNK interaction by treatment with TLK199 results in normal bone marrow progenitor cell proliferation through a pathway other than those affected by standard cytokines. TLK199 (ezatiostat hydrochloride; Telintra, Patheon, Inc., Mississauga, Ontario, Canada) is currently being tested for the treatment of myelodysplastic syndrome (MDS), a bone marrow neoplastic disease characterized by an ineffective production or dysplasia of myeloid blood cells and a risk of transformation into acute myeloid leukemia (AML).[55,56]

GSTP1-1 is polymorphic. In humans, the *GSTP1* gene has been mapped to chromosome 11q13, and four allelic variants have been described: *GSTP1*A* (wild-type Ile 105, Ala 114), *GSTP1*B* (Val 105, Ala 114), *GSTP1*C* (Val 105, Val 114), and *GSTP1*D* (Ile 105, Val 114).[57–59] These allelic variants have been associated with different susceptibility and clinical outcomes in several diseases, including cancer. Recently, the effects of inducible expression of wild-type *GSTP1*A* and mutant *GSTP1*C* haplotypes on cell proliferation and apoptosis in NIH-3T3 fibroblasts were examined. GSTP1-1 protected cells from apoptosis induced by treatment with H_2O_2; however, no differences between these two haplotypes could be observed using measurements of apoptosis.[60] TLK117 was designed for efficient inhibition of the most abundant allelic variant, *GSTP1*A*, but it also competitively inhibits *GSTP1*B* with similar potency.[61] Its activity against the other two allelic variants has not been determined.

Several additional modifications of the GSH peptide backbone have been explored with varying degrees of success. One approach focused on replacing the γ-Glu-Cys amide linkage by a carbamate group to increase the metabolic stability toward γ-glutamyltranspeptidase (γ-GT or GGT), an enzyme involved in the degradation of GSH. The carbamate analog of TLK117, UrPhg, was identified as a γ-GT-stable, potent and selective GSTP1-1 inhibitor ($K_i = 3$, 16, and 29 μM *versus* GSTP1-1, GSTM1-1, and GSTA1-1). To improve cellular uptake, the diethyl ester of UrPhg (UrPhg-Et_2) was prepared (Figure 2). Treatment of a rat mammary adenocarcinoma cell line (MTLn3) with UrPhg-Et_2 induced GSTP1-1 oligomerization, as evidenced by the appearance of high-molecular-weight ($\geqslant$92 kDa) bands on a western blot. Concurrently with this oligomerization, a transient increase in both JNK and c-jun phosphorylation was also observed.[62,63]

The incorporation of the carbamate linkage together with the replacement of the cysteine residue by serine or aspartic acid, however, provided compounds devoid of GSTP1-1 inhibitory activity. The lack of activity has been attributed to a combination of conformational and electronic effects resulting from these replacements.[64]

A different approach consisted of replacing the cysteinyl CH_2SH group of GSH with a phosphonate ester. This modification resulted in analogs that inhibited human GSTM1-1, GSTA1-1, and GSTP1-1 ($IC_{50} = 4.7$, 15, and 15 μM, respectively, for the di-*n*-butylphosphonate ester) and were also stable against γ-GT. The SAR of these compounds toward GSTP1-1 showed increased inhibitory potency with increased lipophilicity of the phosphono ester group (n-Bu > i-Pr > Et). Cellular uptake experiment using HT29 (colon cancer) and EPG85-257P (gastric cancer) cells indicated that the dicarboxylic acids did not enter the cells. Esterification of the glycine carboxylic group, however, was enough to facilitate cellular uptake, in which the monoester was converted into the more active free acid.[65,66] The effect of

these inhibitors on JNK activation or cell viability remains largely unknown.

GSH Conjugates

Perhaps the most explored strategy for the development of GST inhibitors has been the conjugation of GSH, through its thiol group, to a variety of structural moieties. The rationale for this strategy rests on the observation that GSTs are subject to product inhibition.[67]

Among the GSH conjugates that have been prepared and studied in cells are those involving anthracyclines. A conjugate of GSH and doxorubicin (DXR) through glutaraldehyde (GSH-DXR) was shown to have potent cytotoxicity against and to induce apoptosis in DXR-sensitive (AH66P) and DXR-resistant (AH66DR) rat hepatoma cell lines. The GSH-DXR conjugate appeared to be a non-competitive inhibitor of GSTP1-1; its cytotoxicity was markedly increased when the cells were co-treated with tributyltin acetate, an inhibitor of GSTP1-1. Conversely, enhancement of GSTP1-1 expression in human hepatoblastoma HepG2 cells caused a decrease in GSH-DXR-induced activation of caspase-3. Subsequent studies showed that binding of GSH-DXR to GSTP1-1 not only inhibited its enzymatic activity but also resulted in activation of JNK, although without significant dissociation of the JNK/GSTP1-1 complex.[68–70]

A series of GSH conjugates intended to increase both the binding affinity and GST isoenzyme specificity consists of two molecules of GSH conjugated to a linker moiety. These bivalent inhibitors were designed so that each GSH unit would simultaneously interact with the active site of each of the two GST monomeric units. Several bis-glutathionyl nitrophenyl derivatives bearing linkers of different flexibility and length were shown to be more potent and more selective inhibitors of GSTP1-1 *in vitro* than the corresponding monofunctional parent compound.[71] The effects of these inhibitors on cells, however, have not been described.

Small-Molecule Inhibitors

Ethacrynic acid (EA, Figure 3), a compound originally used as a diuretic, is probably the most extensively studied GST inhibitor. It is a potent and reversible inhibitor of GSTP1-1, but it also inhibits isoenzymes of the α and μ classes with similar or even higher potency.[72] EA has been suggested to induce apoptosis in some cancer cell lines, which can be interpreted to be a consequence of GSTP1-1 inhibition. However, this activity may also be because of inhibition of GSTs of the α and μ classes, as these isoenzymes also modulate cell signaling pathways involved in apoptosis. Furthermore, some cancer cell lines have been reported to undergo necrosis, rather than apoptosis, after treatment with EA.[73,74] Thus, the cellular effects of EA are most likely the result of a combination of GST inhibition and other mechanisms of cell death.

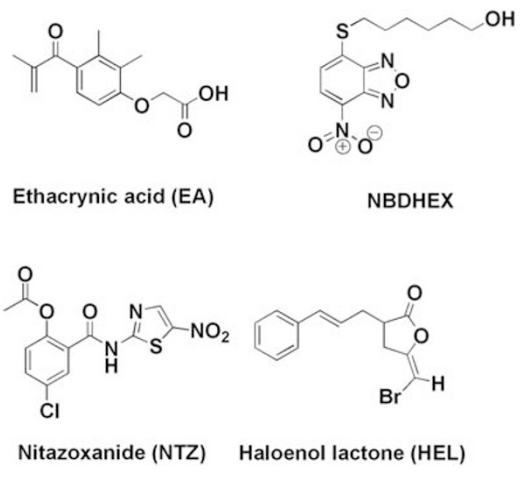

Figure 3 Representative small-molecule GSTP1-1 inhibitors

7-Nitro-2,1,3-benzoxadiazoles have recently been reported to be potent and selective GST inhibitors. The 6-mercaptohexanol derivative, NBDHEX (Figure 3), showed the strongest activity against GSTM2-2 ($IC_{50} = 0.01\ \mu M$) and GSTP1-1 ($IC_{50} = 0.8\ \mu M$), and a much weaker inhibition of GSTA1-1 ($IC_{50} = 25\ \mu M$). The compound was cytotoxic against several cancer cell lines (K562, human myeloid leukemia; HepG2, human hepatic carcinoma; CCRF-CEM, human T-lymphoblastic leukemia, and GLC-4, human small cell lung carcinoma) with CC_{50} values close to the IC_{50} value obtained for inhibition of GSTP1-1. Mechanistically, NBDHEX was found to act as a suicide inhibitor of GSTP1-1; it forms a sigma-complex intermediate with GSH that is tightly stabilized at the active site of the enzyme, thus thwarting its catalytic activity. It was also observed that binding of the GSH–NBDHEX complex to GSTP1-1 resulted in the dissociation of the enzyme from JNK. This process triggered a reactive oxygen species (ROS)-independent activation of the c-Jun–JNK-mediated signal transduction pathway that led to apoptosis in the two leukemic cell lines. A ROS-mediated apoptosis involving the p38-MAPK pathway was also observed in the K562 cell line. Involvement of c-Jun–JNK in NBDHEX-induced apoptosis was confirmed by pre-treatment of the cells with a specific JNK inhibitor, which suppressed apoptosis.[75–77]

NBDHEX was also cytotoxic against two human melanoma cell lines (Me501 and A375). Apoptosis was observed in both cell lines, although at different times after the addition of the compound. JNK activity was required for NBDHEX to trigger apoptosis, confirming that the JNK signaling pathway is an important therapeutic target for this type of tumor. NBDHEX was also effective *in vivo*, in which it inhibited tumor growth by 63 and 70% in the A375 and Me501 melanoma models, respectively.[78]

NBDHEX showed low toxicity *in vivo* after intraperitoneal administration to male BDF1 mice. Interestingly, a slight increase in white cells, particularly neutrophils, was observed after treatment with NBDHEX.[76]

Nitazoxanide (NTZ; Figure 3), a member of the thiazolide class of broad-spectrum anti-protozoan drugs, was shown to inhibit GSTP1-1 and induce apoptosis in a colon cancer cell line (Caco-2) and in non-transformed human foreskin fibroblasts (HFFs). This effect was more pronounced in the Caco-2 cell line than in the less sensitive HFF cells and correlated with the expression level of GSTP1-1.[79] Remarkably, the apoptotic effect of these compounds occurred at concentrations below those required for anti-parasitic activity.

Haloenol lactones (HELs; Figure 3) have been found to be isoenzyme selective and active site-directed inhibitors of

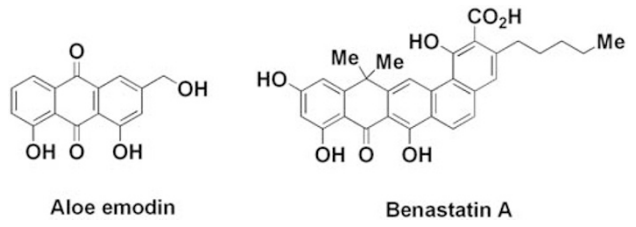

Figure 4 Examples of natural products that inhibit GSTP1-1

GSTs. Preincubation of a representative compound with murine α, μ, or π GST isoenzymes resulted in a time-dependent inhibition that was highly selective for GSTπ. The enzymatic activity could not be restored after extensive dialysis, suggesting that the compound binds covalently at or near the active site of the enzyme, presumably by reaction with the thiol group of Cys-47. The effect of this inhibition on JNK activation or cell viability has not been described; however, the compound potentiated cisplatin-induced cytotoxicity in both cisplatin-sensitive (UOK130) and cisplatin-resistant (UOK_{CR}) human kidney tumor cell lines that overexpress GSTπ.[80–82]

Natural Product Inhibitors

Aloe-emodin (Figure 4), an anthraquinone present in aloe vera leaves, has been shown to induce apoptosis in human hepatocellular carcinoma cell lines. The apoptotic effect is apparently mediated by oxidative stress and sustained JNK activation resulting, at least in part, from GSTP1-1 oxidation and subsequent dissociation from the JNK/GSTP1-1 complex.[83]

Benastatins are aromatic polyketides isolated from culture broths of *Streptomyces* spp. and reported to inhibit human GSTP1-1. Benastatins A and B were found to be competitive inhibitors of GSTs, with $K_i = 5.0$ and 3.7 μM, respectively. Benastatin A (Figure 4) induced apoptosis in a colon 26 cancer cell line in which the dominant isoenzyme is GSTπ. However, flow cytometry analysis revealed that benastatin A blocked the cell cycle at the G1/G0 phase, suggesting that its apoptotic effect on this cell line may not be due solely to inhibition of GST. Benastatins C and D also inhibited GSTπ and stimulated murine lymphocyte blastogenesis *in vitro*.[84,85]

Several other natural products, including flavonoids, plant polyphenols, and alkaloids, have been claimed to inhibit GSTP1-1. However, this inhibition is often not specific, as most of these compounds also inhibit other GST isoenzymes with comparable potency. Thus, their effect on cancer cells, particularly as chemomodulators in cases of GST over-expression, is most likely the result of several mechanisms operating simultaneously.

Conclusions

Studies performed in recent years have revealed a new role for several GST isoenzymes. In addition to their well-established GSH-conjugating enzymatic activity, GSTs of the α, π, and μ classes have been shown to modulate signaling pathways that control cell proliferation, cell differentiation, and cell death by interacting with important signaling proteins in a non-enzymatic way.

Among the different GST isoenzymes, GSTP1-1 has received the most attention because it is usually over-expressed in cancer cells and has been associated with the development of tumor resistance to anticancer drugs. In its newly identified role, GSTP1-1 acts as a repressor of JNK and other protein kinases involved in stress response, cell proliferation, and apoptosis. Although the particular details of these interactions are still being elucidated, there is enough evidence to suggest that GSTP1-1 inhibitors may be useful therapeutic agents for the treatment of cancer and other diseases associated with aberrant cell proliferation.

Conflict of interest

The author is employed by, and has financial holdings in, Telik, Inc.

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