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Short communication

New human Glutathione-S-transferase P1-1 inhibitors and their ligand binding site and GSH complex formation descriptions

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Introduction

Glutathione-S-transferases (GSTs) are a family of dimeric multifunctional enzymes that play an important role in metabolism and detoxification of numerous xenobiotics, electrophilic chemicals (including drugs), environmental carcinogens, and products of oxidative stress in living organisms. GSTs are found in almost all organisms from mammals to plants and even in some prokaryotes. Human GSTs consist of three families: cytosolic GSTs, mitochondrial GSTs, and microsomal GSTs. In mammalian cells cytosolic GSTs are further categorized into seven major classes according to their amino acid sequence: alpha, mu, pi, theta, zeta, omega, and sigma subfamilies, which have been identified in dimeric forms (Mathew et al., 2006).

For the GST-mediated detoxification reactions, a substrate/GSH conjugation (complex formation) is required. GSTs are a family of Phase II detoxification enzymes that catalyze the conjugation of the GSH to a wide variety of electrophilic compounds. GSTs catalyze the conjugation of the reduced form of glutathione (GSH) to electrophilic centers of endogenous and exogenous hydrophobic compounds. For GST-mediated catalytic reactions, the activation of the sulfur atom of the G site bound GSH to the thiolate anion, which is a strong nucleophile, is required in order to perform a GS-substrate conjugate by attacking to the electrophilic center of substrates bound to the H site. Furthermore, the GS-conjugated compounds may be actively extruded from the cell through specialized pumps; principally, the multidrug resistance proteins MRP-1 and MRP-2 (Wu and Dong, 2012).

Human glutathione-S-transferase pi1-1 (hGST P1-1) is a member of the pi class subfamily of cytosolic GST and composed of two homodimer GST P1 subunits. The structural analysis indicates that the hGST P1-1 is a soluble protein comprised of 209 amino acid residues. The crystal structures show that each GST subunit of the protein dimer contains an independent catalytic site composed of two components. The first is a binding site specific for GSH or a closely related homolog (the G site) formed from a conserved group of amino-acid residues in the amino-terminal domain of the polypeptide. The second component is a site that binds the hydrophobic substrate (the H site), which is structurally variable. Amino acid variations of the H-site, among the different GST classes, determine substrate specificity (Wu and Dong, 2012).

It is known that hGST P1-1 participates in a particular role in one of the mechanisms of the development of resistance in cancer cells towards the administration of anticancer agents in chemotherapy. Human GST P1-1 is overexpressed in many cancers and contributes to multidrug resistance by directly conjugating to chemotherapeutic agents including cisplatin, adriamycin, etoposide, thiotepa, and chlorambucil. It is suggested that this resistance is related to high expression of hGST P1-1 in cancers such as breast, lung, colon, pancreas and cervix, thereby contributing to resistance to chemotherapy. Studies have shown that hGST P1-1 levels correlate with resistance to standard chemotherapy and are elevated in biopsies of tumour tissues that have become resistant to therapy after administration of anticancer agents (Wu and Dong, 2012). Consequently, inhibitors of human GST P1-1 catalytic activity remain a potential therapeutic tool in cancer cell resistance to drugs. In order to overcome this resistance specific hGST P1-1 inhibitors are in demand.

Furthermore, a variety of sulfonamido-containing compounds are apparently accommodated by the H-site of GST and found to be the substrates for GSTs. Data given in the literature are clearly shown that GSTs can mediate the enzymatic cleavage of the sulfonamide bond displaying a sulfonamidase activity by catalyzing the GSH-mediated

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hydrolysis of sulfonamide bonds. Groups capable of withdrawing sufficient electron density from the alpha carbon atom to the sulfonyl group are an absolute requirement in this enzymatic process. GST-mediated sulfonamide cleavage results in the formation of the GS-conjugate, the corresponding amine, and sulfur dioxide. The released GS-conjugate may provide a strong inhibitor against intracellular GSTs, thus facilitating cancer chemotherapy (Mathew et al., 2006).

The benzoxazoles have been the aim of many researches for many years because they constitute an important class of heterocyclic compounds exhibiting substantial chemotherapeutic activities.The newly synthesized 2-substituted-5-(4-nitrophenylsulfonamido)benzoxazole derivatives, which were screened for the in vitro inhibition of hGST P1-1, exhibited significant inhibitor activities. Among the tested compounds, 2-(4-chlorobenzyl)-5-(4-nitrophenyl-sulfonamido)benzoxazole displayed the most potent inhibitory activity for hGST P1-1 with an IC50 value of 10,2 μM, showing a similar potency with the used reference drug ethacrynic acid (Ertan-Bolelli et al., 2014).

The molecular docking studies performed by using CDocker method is revealed that the new synthesized 2-substituted-5-(4-nitrophenyl-sulfonamido)benzoxazoles are acting as catalytic inhibitors of hGST P1-1 by binding to the H-site and performing conjugates with GSH forming S-(4-nitrophenyl)-GS complex via nucleophilic aromatic substitution reaction (Ertan-Bolelli et al., 2014).

It is reported that the active site residues Tyr7 and Tyr108 play important roles for the activity of hGST P1-1. Different models have been proposed for the activation of GST, all highlighting the key role of active site residues Tyr7 and Tyr108 (Wu and Bong, 2012). In the mechanism for activation of GSH, Tyr7 acts as a general base, promotes proton abstraction from the GSH thiol, and creates a thiolate anion with high nucleophilic reactivity. Additionally, the hydroxyl group of Tyr108 appears to contribute to the catalytic mechanism in the conjugation reaction of GSH with the docked sulfonamide-substituted benzoxazoles (Ertan-Bolelli et al., 2014,).

Moreover, it was found that groups capable of withdrawing sufficient electron density from the alpha carbon atom to the sulfonyl group are an absolute requirement. The electrophilic substructure of the sulfonyl group is solely responsible for activation of the sulfonamide bond toward cleavage. On the other hand, the amine portion has little or no impact on the cleavability of sulfonamide substrates (Ertan-Bolelli et al., 2014).

In conclusion, the newly developed hGSTP1-1 inhibitory active 2-substituted-5-(4-nitrophenylsulfonamido)benzoxazoles are promising lead compound for further in vivo studies to develop a new chemotherapeutic agent for the treatment in MDR cancers. It is accepted that the hGST P1-1 contributes directly to drug resistance in some cell types via their catalytic activity. The development of chemotherapy resistant tumour cells is a significant problem encountered in cancer treatment. The finding of over expression of hGST P1-1 in many cancer tissues as well as in drug resistant cell line, suggests that elevated hGST P1-1 expression may be of direct relevance not only to acquired resistance, but also in natural resistance. hGST P1-1 has been shown to catalyse the conjugation of GSH with the alkylating agents chlorambucil and thiotepa, suggesting that overexpression of hGST P1-1 in cells exposed to these drugs would confer resistance. Elevated cellular levels of hGST P1-1 have been shown to accompany resistance to various common anticancer drugs, and the addition of the GST inhibitors will be restored sensitivity to alkylating agents in drug-resistant cells. Therefore inhibitors of hGST P1-1 catalytic activity remain as a potential therapeutic tool in MDR cancer chemotherapy.

References

Ertan-Bolelli, T., Musdal, Y., Bolelli, K., Yilmaz, S., Aksoy, Y., Yildiz, I., Aki-Yalcin, E., Yalcin, I., 2014. Synthesis, biological evaluation of 2-substituted-5-(4-nitrophenylsulfonamido) benzoxazoles as human GST P1-1 inhibitors and description of the binding site features. ChemMedChem. 9, 984-992.

Mathew, N., Kalyanasundaram, M., Balaraman, K., 2006. Glutathione S-transferase (GST) inhibitors. Expert Opin. Ther. Patents. 16:431-444.

Wu, B. and Dong., D., 2012. Human cytosolic glutathione transferases: structure, function, and drug discovery. Trends in Pharmacological Sciences. 33, 656-668.