

Research Article

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Some anticancer agents as effective glutathione S-transferase (GST) inhibitors

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Abstract: The objective of this research was to investigate the impact of anthraquinone (AQ) compounds on the activity of the enzyme glutathione S-transferase (GST). The interaction between GST and some AQs (alizarin, purpurin, quinizarin, and dantron) was investigated, and IC_{50} and K_i levels were determined for each compound. The results obtained reveal that these compounds are potent GST inhibitors. K_i values of these compounds against GST were found ranging from 9.133 ± 0.895 to $36.992 \pm 6.194 \mu M$. In the *in vitro* study, purpurin was identified as the most potent AQ against GST. Thereafter, binding mode exploration of purpurin to the enzyme was undertaken to elucidate its mechanism of action. To this end, molecular docking was conducted. According to the docking results, purpurin can bind to the enzyme and form a stable complex. Together with this, binding potential of purpurin was less than the standard ligand. Examination of both the inhibitory activity *in vitro* and molecular docking interactions of these anticancer agents with GST, an enzyme important for detoxification metabolism, led to the identification of important relationships between these compounds and GST. The findings may offer structural direction for developing superior anticancer drugs or powerful GST inhibitors.

Keywords: GSTs, inhibition, anthraquinones, docking

1 Introduction

The enzyme glutathione S-transferase (GST) (EC 2.5.1.18) has a valuable function in the elimination of exogenous and endogenous xenobiotics [1]. Xenobiotic detoxification occurs enzymatically in three distinct stages of metabolism. Phases I and II entail the transfer of lipophilic compounds, whereas in phase

III the detoxification processes are separated into phase I and phase II reactions [2,3]. During Phase I, the cytochrome P450 enzyme system plays a critical role as the primary barrier against foreign substances in the body [4]. In Phase II, conjugation reactions occur among the polar compounds generated by the Phase I reactions. The multifunctional enzyme family takes part in phase II reactions and aids in linking a considerable number of electrophiles with glutathione (GSH). GSH transferases are members of a family of phase II detoxification enzymes. They catalyze the conjugation of endogenous and exogenous sources to less toxic metabolites that are generally easier to eliminate [5]. At this point, polar compounds produced by phase I reactions experience conjugation reactions [6]. Conjugation reactions comprise chemical processes where external substances combine with internal substances within the organism to enhance their excretion from the body. GSTs facilitate the initial stage of mercapturic acid production, which serves as the ultimate water-soluble substance in the detoxification metabolic process. Acids produced in the liver are transported through the bloodstream to the kidneys, where they are excreted in the urine [7].

Anthraquinones (AQs) are the aromatic compounds, which are the largest group of naturally and synthetically occurring quinones. AQs represent a significant compound class with broad applications stemming from their biological properties [8]. Aloe and rhubarb are plants that contain AQs and have been utilized in traditional Chinese medicine for over 4,000 years due to their anti-inflammatory, antioxidant, and antibacterial properties. AQs, whether natural or synthetic, are effective as therapeutic agents, including laxatives and mild tranquilizers. They are also utilized in a variety of commercial applications, such as cosmetics, food, and textiles [9,10]. Although AQs have similar chemical structures, differences in biological activity are attributed to specific functional groups attached to certain positions, such as hydroxyl groups. Carcinogenicity, genotoxicity, and mutagenicity have been reported for some AQs. In addition, some AQs used in this study such as alizarin, purpurin, dantrone, and quinizarin are used as anticancer agents as well [11].

Alizarin has been found to have a strong inhibitory effect on human colon carcinoma and bone tumors [12,13]. Purpurin, an AQ derived from *Rubia* plant roots, exhibits antioxidant

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effects responsible for its antigenotoxic, anticancer, and antimicrobial activities in both *in vivo* and *in vitro* tests.

In a study investigating the anticancer potential of AQs, it was found that treatment with dantrone and quinizarin reduced both highly metastatic melanoma mouse cells and intracellular polyamine levels effectively [14,15]. However, more information is needed on their biological properties. Therefore, this study focuses on the biological impact of particular AQ derivatives (alizarin, purpurin, quinizarin, and dantrone) through their interactions with GST.

Based on the information provided, this study aims to ascertain the inhibitory effects of several AQs on the GST enzyme. In addition, the relationship between the compounds showed the strongest inhibition effect and GST enzyme activity was determined to use molecular docking analysis. In this study, the primary goal was to examine the potential for GST enzyme inhibitors to undergo structural modifications and pharmacological research advancement.

2 Materials and methods

2.1 Materials

All chemical ingredients were procured from Merck and the enzyme GST (human placenta GST, 2.5.1.18) used was obtained from Sigma-Aldrich (Sternheim, Germany). All chemicals utilized in the investigation were of analytic purity.

2.2 GST activity assay

The compounds' inhibition activities of the GST enzyme were assessed using the same methods as in earlier studies conducted [16]. Briefly, the activity of GST was assayed at 25°C with the substrate 1-chloro-2,4-dinitrobenzene (CDNB). Phosphate buffer (pH 6.5), 20 mM GSH, and 25 mM CDNB were used as the reconstitution medium for enzyme activity.

In enzyme research, an enzyme unit is the amount of enzyme that catalyzes the conversion of 1 μmol of substrate to product in 1 min. Absorbance changes at 340 nm for 1 min were measured with a spectrophotometer. The GST enzyme activity was measured spectrophotometrically at 25°C using reduced GSH and CDNB as substrates.

2.3 Enzyme inhibition studies

The inhibition efficiency of four AQ compounds on the GST was examined. These compounds are both naturally and synthetically available and were widely used and investigated for their various biological properties [17–19]. A UV/Vis spectrophotometer was used for enzyme inhibition studies. For this purpose, the absorbance of the control sample was measured without the tested compound and designated as 100% activity. The activities of additional samples, prepared using various inhibitor concentrations, were quantified by measuring their absorbances. In this context, the reduction activity indicated the inhibition potential of a sample, and enzyme activity-concentration plots were plotted for each tested substance. IC_{50} values were determined by employing the graphical equation to calculate the concentration of inhibitor required for a 50% decrease in enzyme activity. K_i values were determined through the use of Lineweaver–Burk plots generated from the analysis of five distinct substrate concentrations. Inhibitors were added to the reaction mixture at two different concentrations and all experimental measurements were repeated three times to determine the K_i constants.

2.4 Molecular docking

The human GST crystal structure was obtained from the RCSB protein data bank (PDB) (<https://www.rcsb.org/>). The human GST structure with a PDB code of 2GSS has a 1.9 Å resolution and an inhibitor, ethacrynic acid, is complexed in it [20].

Table 1: Values for IC_{50} , K_i , and inhibition types of AQ compounds on GST enzyme activity

	IC_{50} (μM)	R^2	K_i (μM)	Inhibition types
Alizarin	43.268 ± 8.684	0.992	29.588 ± 4.365	Noncompetitive
Purpurin	16.635 ± 3.247	0.992	9.133 ± 0.895	Competitive
Quinizarin	31.203 ± 7.589	0.992	20.018 ± 3.287	Noncompetitive
Dantron	40.310 ± 11.024	0.990	36.992 ± 6.194	Noncompetitive

Molecular docking of the most active hydroxyanthraquinone derivative in the *in vitro* study, purpurin, was performed by using AutoDock Vina as described in previous studies and compared to the bound ligand [21,22]. The docking analysis results were viewed using Biovia Discovery Studio and subsequently scrutinized.

2.5 Statistical analysis

All procedures were carried out a minimum of three times. The data were analyzed utilizing SPSS 25.0 software (SPSS Inc., Chicago, IL, USA). ANOVA was used to compare the analysis of variance at the lowest significance level ($p < 0.05$).

3 Results and discussion

3.1 GST enzyme inhibition

Inhibition effect was determined by kinetic study for alizarin, purpurin, quinizarin, and dantron on GST activity.

All AQs utilized in the research significantly impeded the activity of the GST enzyme at micromolar levels, as demonstrated in Table 1. The K_i values and types of inhibition for all compounds were determined and are presented in Table 1.

For these AQs, inhibitory concentrations (IC_{50} values) up to 50% inhibition were calculated using regression analysis plots as shown in Figure 1. IC_{50} values were obtained ranging from 16.635 ± 3.247 to $43.268 \pm 8.684 \mu M$ as shown in Table 1. *In vitro* studies revealed that GST was effectively inhibited by these compounds. To comprehend the nature of GST inhibition, kinetic experiments were conducted using varying concentrations of compounds. Each concentration of inhibitors had corresponding changes to the concentrations of GSH substrates. Lineweaver–Burk plots were generated by graphing $1/v$ vs $1/(GSH)$ for various concentrations of the compounds screened in Figure 2. The K_i values and inhibition modes of the compounds tested in the study are shown in Table 1. According to the table, K_i values ranged from 9.133 ± 0.895 to $36.992 \pm 6.194 \mu M$ (Table 1). Purpurin showed the strongest inhibition effect with IC_{50} value of $16.635 \pm 3.247 \mu M$ and K_i value of $9.133 \pm 0.895 \mu M$.

Although all compounds strongly inhibited the enzyme at the micro molar level; alizarin, dantron and quinizarin

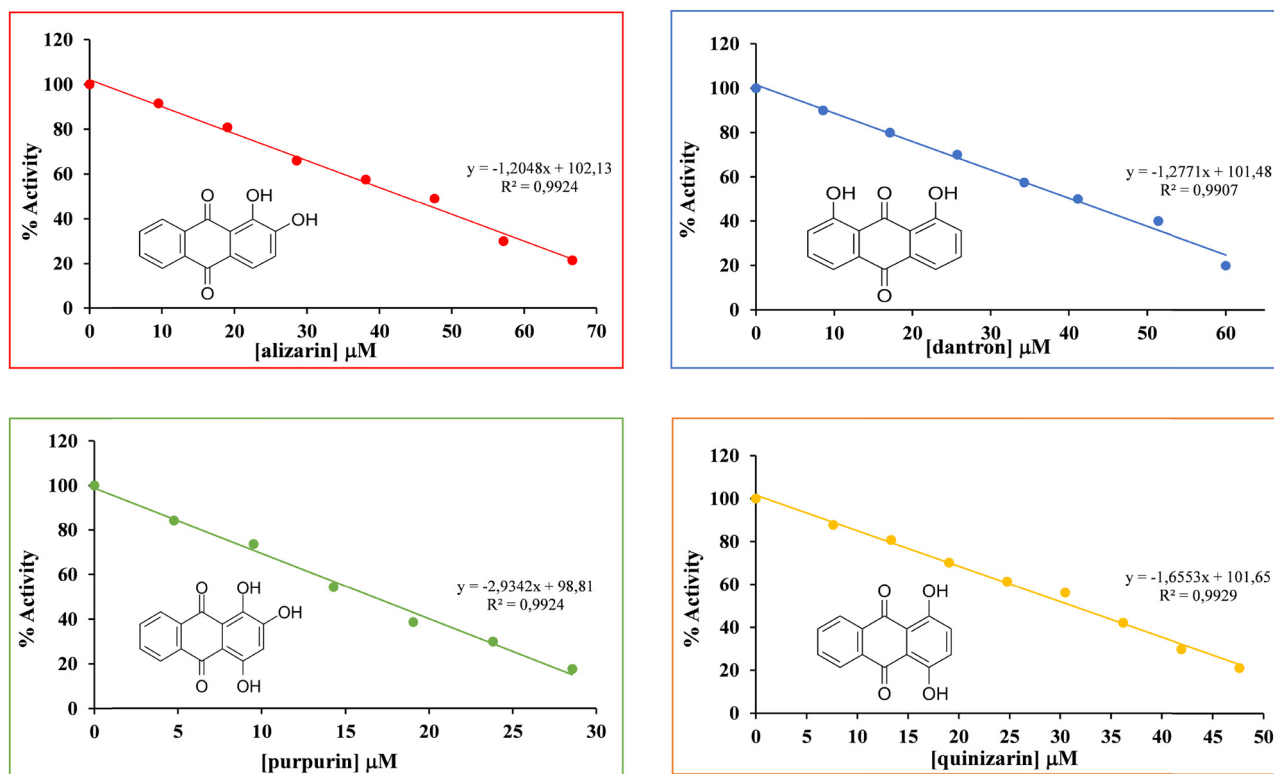


Figure 1: Enzyme activity and inhibitor concentration graphs for IC_{50} calculation and chemical structures of used AQs including alizarin, dantron, purpurin, and quinizarin.

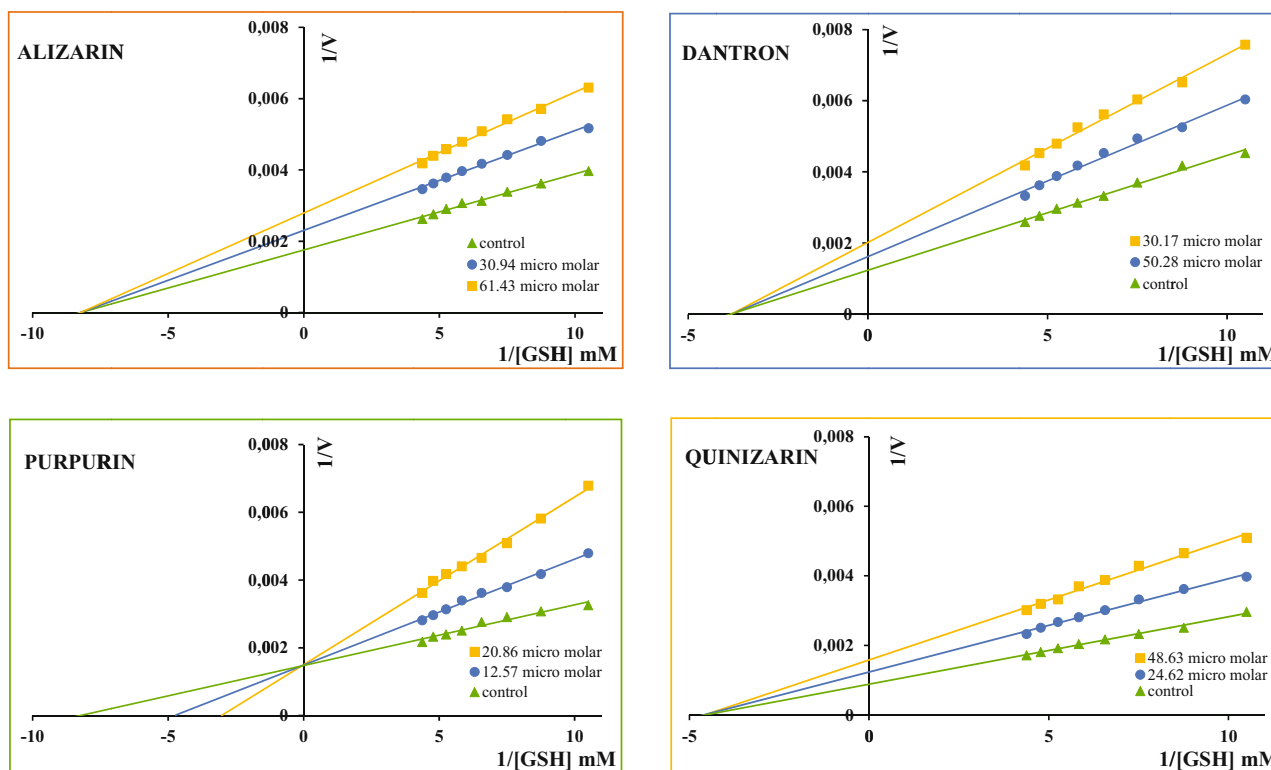


Figure 2: For alizarin, dantron, purpurin, and quinizarin, K_i values were calculated and inhibition modes determined using Lineweaver–Burk plots.

Table 2: Binding residues of ethacrynic acid and purpurin with GST

Molecule	Interaction residues	Bonding type	Distance (Å)
Ethacrynic acid	Tyr7	Conventional hydrogen bond	2.77
	Phe8	Alkyl	4.21
	Val10	Pi-alkyl	5.19
	Arg13	Conventional hydrogen bond	2.54
	Val35	Alkyl	4.53
	Trp38	Alkyl	4.40
	Tyr108	Pi-pi	5.12
	Tyr108	Pi-sigma	3.53
	Asn204	Conventional hydrogen bond	3.32
	Gly205	Carbon hydrogen bond	2.99
Purpurin	Phe8	Pi-pi	3.84
	Phe8	Pi-pi	4.81
	Ile104	Pi-alkyl	5.50
	Tyr108	Conventional hydrogen bond	2.22
	Tyr108	Pi-pi	5.12
	Tyr108	Pi-pi	5.26
	Gly205	Conventional hydrogen bond	2.85

showed similar values, while purpurin showed a lower inhibition value. This can be explained by the extra hydroxyl group, which makes the purpurin compound more polar, while the three AQ structures have very similar structure and functional group.

Only purpurin exhibited competitive inhibition, while all other compounds exhibited non-competitive inhibition effect. This suggests that purpurin binds to the enzyme's active site, whereas other compounds attach to a separate site outside of the enzyme's active site. In order to evaluate and elucidate the binding mechanism better, molecular docking studies were performed.

In the literature, there are a small number of studies on GST inhibition and inhibitory effects of AQs. The effects of some beta-lactam group antibiotics [23], infectious drugs [24], calcium channel blockers [25], chalcones [26], and benzenesulfonamide derivatives [27] on GST were investigated and it was determined that these compounds strongly reduced the enzyme activity. It has also been shown that some naphthoquinone, benzoquinone, and AQ derivatives strongly reduced the paraoxonase 1 enzyme [28,29], which is noted for its strong antioxidant and anticardiovascular properties in metabolism.

However, there are no current studies on the interaction between AQs, which are used for various therapeutic benefits, and GST, which is an important enzyme in

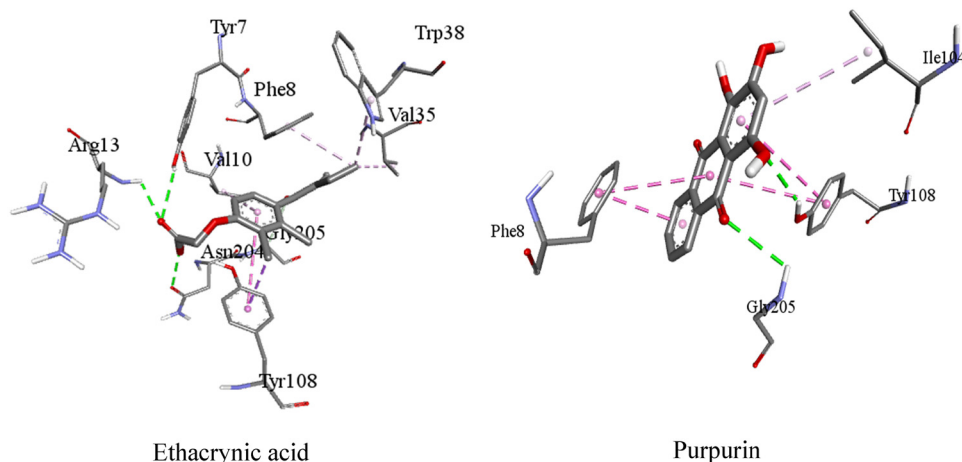


Figure 3: Binding profile of ethacrynic acid and purpurin with GST (hydrogen bonding points are depicted in green).

detoxification metabolism. The study is unique in this respect. According to the general evaluation, treatment with low doses of drugs or herbs containing these AQ structures is recommended due to the high inhibition effect of GST. This is of vital importance in terms of the beneficial and harmful aspects of treatment.

3.2 Molecular docking

Molecular docking was conducted to determine the binding mode of the highly potent hydroxyanthraquinone, purpurin, to GST (PDB code: 2GSS). Molecular docking of the complexed inhibitor in the structure utilized, ethacrynic acid, was first undertaken to validate the docking process. Ethacrynic acid interacted with three conventional hydrogen bonds (Tyr7, Arg13, and Asn204), a carbon-hydrogen bond (Gly205), and other interactions (Phe8, Val10, Val35, Trp38, and Tyr108) (Table 2, Figure 3). In a previous crystallographic structural elucidation, ethacrynic acid had interactions with Tyr7, Phe8, Val10, Arg13, Trp38, Ile104, Tyr108, and Gly205 [20]. All these interactions except the one with Ile104 were detected in the docking analysis (Table 2, Figure 3).

Purpurin interacted with GST effectively. However, its interaction strength was weaker than ethacrynic acid. Purpurin interacted with GST via two standard hydrogen bonds (Tyr108 and Gly205) and five additional interactions (Phe8(2), Ile104, and Tyr108(2)) as presented in Table 2 and Figure 3. All the interaction points detected in this study were observed in previous crystallographic structural analysis [20].

In a previous computational study, ethacrynic acid linked two hydrogen bonds at Tyr7 and Tyr108 residues with GST [27]. A GST inhibitor had also three hydrogen

bonds at Tyr7, Arg13, and Leu58 residues as reported in a previous study that included docking [25]. Similarly, various GST inhibitor ligands had various interactions with the interaction residues in this study [24,25,27]. In short, the binding mechanism of ethacrynic acid to GST that was detected in this study was in line with previous experimental and computational studies. The level of assurance by the previous studies showed that the docking process would be reliable.

Computational studies reported similar interaction with these residues [24,25,27]. Furthermore, purpurin exhibited a similar binding mode with ethacrynic acid but with weaker strength (Table 2, Figure 3). Interactions of purpurin at all the residues except Ile104 were also observed in the interaction of ethacrynic acid. The interaction at Ile104 was observed in a previous experimental structural elucidation [20]. Hence, the interactions observed fit with previous experimental and computational studies as well as the interaction of ethacrynic acid detected in this study. On the other hand, the binding energy of purpurin (−7.4 kcal/mol) was slightly lower than that of ethacrynic acid (−6.8 kcal/mol). Therefore, it is expected to exhibit slightly greater affinity for the enzyme.

4 Conclusion

In recent years, the investigation of various enzymes and pharmaceutical activities with toxicological mechanisms has gained importance for drug design and the evaluation of side effects. In this study, the inhibitory effects of alizarin, purpurin, kinizarin and dantron against GST, one of the leading enzymes with important properties in detoxification

metabolism, were evaluated. In conclusion, all AQs showed strong inhibitory effects on GST. This shows that certain doses of the drugs used may cause a decrease in Phase II GST enzyme activity. Since these beneficial AQs strongly inhibit the GST enzyme, they can lead to serious inefficiency in detoxification reactions. Furthermore, a molecular docking analysis was conducted to examine the binding modes of the most potent compound at the active site of the designated enzyme and establish its correlation with experimental results.

The GST inhibition activity of purpurin determined through the wet-lab study was investigated via molecular docking. According to the docking analysis, purpurin demonstrated the capability to bind to the enzyme. However, its binding capacity was inferior to the conventional inhibitor, ethacrynic acid.

By studying the interaction between certain anticancer agents and GST, this research provides novel methods for evaluating cancer progression. Therefore, the possible side effects of long-term use of these anticancer compounds should be well considered and their dosage should be kept under control. Furthermore, these results may lead to promising cancer drug target discovery. In this context, modifying chemical structures further could enhance comprehension of the structure–activity relationships as well as significant interactions at the target enzyme's active site.

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Ethical approval: The carried out research is not associated with the utilization of either humans or animals.

Data availability statement: The data produced and analyzed in this study can be obtained upon reasonable request from the corresponding author.

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