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ORIGINAL ARTICLE

Exploring the inhibitory potential of hormone replacement therapy drugs on glutathione transferase P1-1

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ABSTRACT

Aim: Glutathione transferase P1-1 (GST P1-1) plays a crucial role in the human phase II detoxication system and is implicated in drug resistance in certain cancer cells. Analogs of drugs used in Hormone Replacement Therapy (HRT) have been reported to exhibit inhibition of various GSTs. This study aims to investigate the inhibitory potential of several HRT drugs on GST P1-1 for the possible use of counteracting drug resistance and for other therapeutic purposes.

Materials and Methods: GST P1-1 was expressed and then purified in a single step using Nickel Sepharose affinity chromatography. Synthetic drugs that are used in HRT were screened for inhibition of GST P1-1 using 1-chloro-2,4-dinitrobenzene as the substrate. The IC50 value of the most potent compound was calculated and binding location and formation of bonds to GST P1-1 were identified through docking analysis.

Results: Screening the inhibitory effect of eight synthetic estrogenic drugs reveals that estradiol valerate is the most potent inhibitor, showing 72 ± 4% inhibition of GST P1-1. This effect is followed by estradiol cypionate 53 ± 5%, mestranol 39 ± 4%, and estradiol propionate 35 ± 2%. The most potent compound estradiol valerate has an IC50 value of 30 ± 2 μM. According to docking analysis, it binds to the H-site of the enzyme where the residues Phe9, Arg14, Val36, Trp39, Ile105, Tyr109, Pro203, Asn207, and Gln210 were within 5 Å proximity of the ligand. Estradiol valerate forms Pi-alkyl interactions with Phe9 and Val36, as well as an alkyl interaction with Ile104.

Conclusion: Estradiol valerate is a modest inhibitor for hGST P1-1, however it fits in the area of H-site of the enzyme and forms bonds with critical key residues. Understanding its binding site on the enzyme is critical for designing other inhibitors targeting GSTs or possibility for the potential use as a substrate with other GSTs.

Keywords: Glutathione transferase P1-1, synthetic estrogens, enzyme inhibition, cancer, docking analysis.

INTRODUCTION

Glutathione transferases (GSTs; EC 2.5.1.18) is a diverse and essential enzyme family widely distributed across living organisms. They play multifaceted roles, primarily associated with the detoxication of both endogenously and exogenously produced compounds. These enzymes catalyze the conjugation of glutathione (GSH) to electrophilic regions present on hydrophobic molecules, increasing their solubility for elimination from the organisms [1]. Apart from the canonical detoxication functions, human GSTs catalyze isomerization reactions involved in steroid hormone biosynthesis and participate in intracellular transport by binding small ligands such as: bilirubin, steroids, drugs, etc. Moreover, they play a key point in modulating apoptosis through their influence on c-Jun-N-terminal kinase [2-4].

In mammals, seven classes of cytosolic GSTs (alpha, pi, mu, zeta, sigma, omega, and theta) are found, and several of the classes contain multiple isoenzymes [5]. Each GST exhibits unique tissue distribution and substrate specificity, contributing to the body's overall detoxication capacity and other functions. For instance, alpha, mu and pi are involved in detoxication processes, sigma in prostaglandin synthesis, zeta in tyrosine metabolism, omega in oxidative stress response, and theta in the metabolism of various xenobiotics [1,4]. With the advance of database information and utilization of artificial intelligence, it is predicted that the human GST family is more extensive than previously known, with additional members and unknown functions [6]. The enzymes exist in dimeric forms, with each subunit containing an active site composed of two distinct binding sites known as G-site and H-site. The G-site, where GSH binds to specific amino acid residues, is conserved among the GSTs, whereas the residues in the H-site are variable [7].

GST P1-1, also known as GST Pi, is the most extensively studied isoenzyme in humans. Due to its high-level expression in some cancer cells, cause of chemoresistance, and regulatory role in apoptosis, it is of interest in cancer research [8,9]. Consequently, specific and potent inhibitors for GST P1-1 are being pursued by screening large libraries of compounds, including diuretics, antidepressants, antifungals, insecticides, antimalarials, etc [10]. Repurposing of established drugs is an alternative to the design of novel agents, and the pharmacology and safety of estrogens have been studied extensively. The aim of this study was to evaluate the inhibitory potential of various estrogen derivatives on human GST P1-1. The inhibitory effects of these compounds were screened, the IC50 of the most potent inhibitor was calculated, and the binding residues in the enzyme were identified.

MATERIALS and METHODS

Expression and purification of human GST P1-1

The human GST P1-1 gene obtained from a K562 erythroleukemia cDNA library expressing the *GSTP1*A* allele was available in the pKXHP1 plasmid [11]. *Escherichia coli* XL-1 Blue cells were transformed with the plasmid via heat shock and grown on agar plates. A single colony was selected and inoculated in 2YT media, incubated overnight (ON) at 37 °C in a shaking incubator. A new 2TY culture was obtained by addition of 1:1000 of the ON culture, further grown, and induced with isopropyl β-D-thiogalactopyranoside (0.2 mM) when absorbance (OD600) of the culture reached around 0.4. The culture was incubated for an additional 16 hr. Cell pellets obtained by centrifugation (5000 g for 8 min at 4 °C) were resuspended in lysis buffer (10 mM Tris HCl, 1 mM EDTA, 0.2 mM DTT, pH 7.0) supplemented with lysozyme, subjected to sonication (4 cycles of 20 sec), and centrifuged (10000 g, 50 min at 4 °C), resulting in a supernatant fraction [11].

The lysate was applied to a Nickel-Sepharose affinity column (Cytiva), equilibrated with binding/washing buffer (20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4), and thoroughly washed. The matrix-bound GST P1-1 underwent elution with buffer (washing buffer including 300 mM imidazole), and dialyzed (10 mM Tris-HCl buffer, pH 7.8, containing 0.2 mM DTT and 1 mM EDTA).

Enzymatic assay

Enzymatic measurements were performed in quartz cuvettes with the contents 0.1 M phosphate buffer (pH 6.5), 1 mM EDTA, and the substrates 1 mM GSH and 1 mM CDNB (1-chloro-2,4-dinitrobenzene) [12]. The synthetic drugs obtained from MicroSource

Discovery Systems were dissolved in DMSO at 10 mM concentration. The percentage (1% v/v) of solvent present in the assay system has no inhibitory effect on enzyme activity. The inhibitor was added just before CDNB, and initial absorbance change was monitored for 1 min at 340 nm using Shimadzu UV-2501 spectrophotometer.

Docking analysis

Molecular docking studies were perfomed with Autodock Vina, provided in the Chimera Software [13,14]. The crystal structure of human GST P1-1 (PDB: 6GSS) and the ligand (estradiol valerate) were downloaded from the websites (https://www.rcsb.org/ and https://pubchem.ncbi.nlm.nih.gov/) in pdb and sdf formats, respectively. Water molecules, and ligands were removed from the protein structure. Incomplete side chains, hydrogens, and Gasteiger charges were added using using Dock Prep Tools. Similar preparations were carried out for the ligand including minimization and addition of hydrogen and Gasteiger charges. The entire protein was centered, and docking was executed. Results were visualized and analyzed using UCSF Chimera and Discovery Studio Softwares.

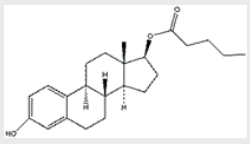
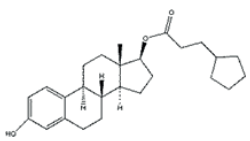
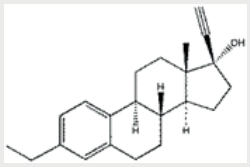
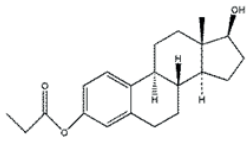
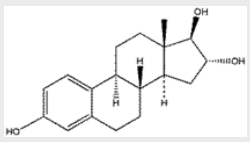
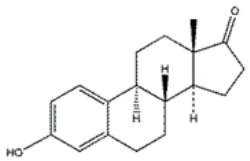
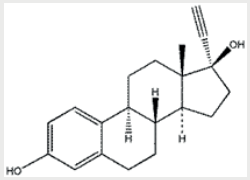
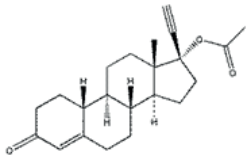
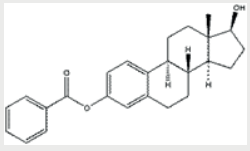
Statistical analysis

All measurements were made in triplicate and each point in the figures was given with standard deviation of the mean value. The IC50 value was calculated using the regression analysis program of GraphPad Prism 4.0. The IC50 represents the concentration that exhibits 50% of remaining enzyme activity.

RESULTS

In this study, the inhibition effect of synthetic estrogen derivatives used in Hormone Replacement Theraphy (HRT) on human GST P1-1 are studied. A total of eight compounds, distinguished by different side chains were screened in a concentration of 33 µM. Among the results, estradiol valerate exhibited the highest potency, significantly reducing enzyme activity by 72 ± 4% at the tested concentration. The other compounds showed moderate inhibition, estradiol cypionate at 53 ± 5%, mestranol at 39 ± 4%, and estradiol propionate at 35 ± 2%, respectively. However, estriol, estrone, ethinyl estradiol and estradiol benzoate showed no inhibition (Table 1).

Table 1. Screening the inhibition of estrogen derivatives with human GST P1-1. Table shows percentage of inhibition of GST P1-1 in the presence of 33 µM synthetic drugs assayed with CDNB substrate

Compound	Structure	Inhibition (%)
estradiol valerate		72 ± 4
estradiol cypionate		53 ± 5
mestranol		39 ± 4
estradiol propionate		35 ± 2
estriol		no inhibition
estrone		no inhibition
ethinylestradiol		no inhibition
norethindrone acetate		no inhibition
estradiol benzoate		no inhibition

Estradiol valerate was the most promising compound for further analysis. Inhibition analysis with varying concentrations of estradiol valerate, with saturated levels of GSH and CDNB, revealed

an IC50 value of 30 ± 2 μM (Figure 1). However, due to solubility limitations in measurements and turbidity observed at higher concentrations in spectrophotometer cuvettes, further kinetic experiments to elucidate the precise mechanism of binding and inhibition kinetics were impeded. Therefore, binding analysis of estradiol valerate docking to GST P1-1 was conducted using computer software.

The analysis, utilizing Autodock Vina software with the structures of estradiol valerate and GST P1-1, aimed to elucidate the ligand binding location on the enzyme and interactions with amino acid residues. Ten different docking analysis results were obtained, and the one with the lowest energy, scoring -8.4 kcal/mol, was selected. The docking studies showed that the ligand, estradiol valerate fits the H-site region of human GST P1-1. Amino acid residues within the range of 5 Å of the ligand included Phe9, Arg14, Val36, Trp39, Ile105, Tyr109, Pro203, Asn207, and Gln210 (Figure 2). Notably, estradiol valerate formed π-alkyl interactions with Phe9 and Val36, and an alkyl interaction with Ile105 (Figure 3).

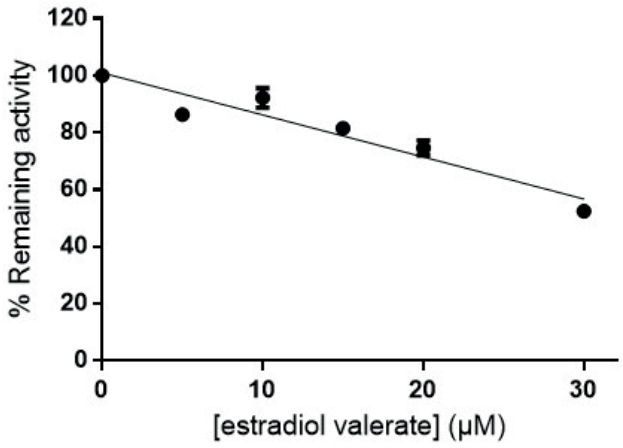


Figure 1. Inhibition profile of estradiol valerate for GST P1-1 with the substrate CDNB.

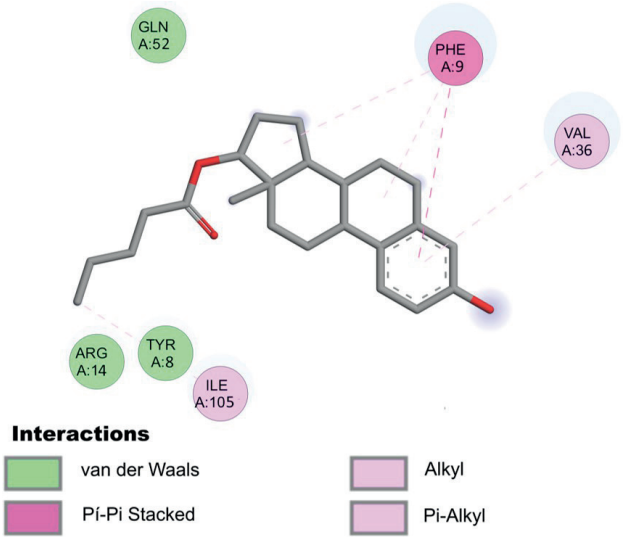


Figure 3. 2D view of interactions of estradiol valerate with GST P1-1.

DISCUSSION

In cancer research, GST P1-1 holds significant importance, particularly in understanding and overcoming drug resistance. Increased activity of

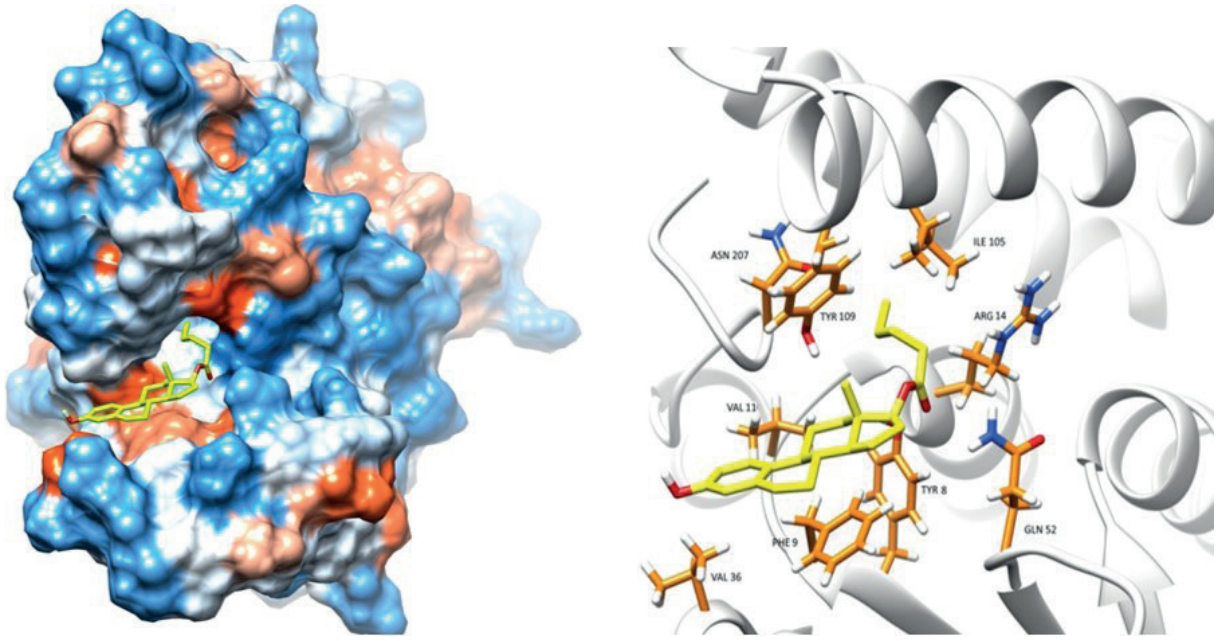


Figure 2. 3D structure of docking analysis. **(left)** Surface of GST P1-1 colored according to electrostatic potential with estradiol valerate (yellow). **(right)** Residues of amino acids within 5 Å proximity of estradiol valerate.

GST P1-1 leads to drug inactivation, contributing to resistance against drugs such as chlorambucil, brostallicin, and cyclophosphamide [15,16]. This inactivation might be accompanied by modulating apoptosis through its roles in signaling pathways and transport systems [17]. Based on these premises, several research techniques are being used to suppress GST P1-1 and enhance the efficacy of drugs in chemotheraphy through using specific and potent inhibitors, produgs and antisense cDNA [18,19].

Hormone replacement therapy remains a primary approach for managing menopausal symptoms caused by decreased estrogen levels, involving the administration of synthetic drugs to alleviate symptoms and restore hormonal balance. However, concerns have been raised regarding the potential risk of breast cancer associated with the use of these drugs [20]. Intriguingly, various compounds used in HRT have been described in research papers for their inhibitory effects on various GST from different classes [10,21]. The most potent inhibitors identified in the literature are ethacrynic acid, its analogs, Telintra compounds (TLK117, TLK199), NBDHEX, tin compounds (tributyltin bromide, triphenyltin chloride), and merbromine, all have IC50 values at concentrations <5 μM [10,22-24]. In this study, we identified estradiol valerate as the most potent compound, with an IC50 of 30 ± 2 μM, making it a modest inhibitor (Figure 1).

In humans, all GSTs share a similar protein fold with less than approximately 30% of the amino acid composition preserved among the different classes. The diversity of amino acids in the active H-site enables the binding of a wide array of compounds, including drugs, hormones, enviromental pollutants, inhibitors, substrates, etc., attributing to various functions. According to data obtained from crystal structures of GST P1-1, Tyr8, Phe9, Arg14, Val36, Trp39, Tyr109, Asn207, Gly208 are the amino acids involved in the H-site of GST P1-1. These residues line the active site cleft and play a crucial role in binding and catalyzing the conjugation reactions between glutathione and electrophilic substrates [25]. According our docking results, estradiol valerate forms π-alkyl interactions with Phe9 and Val36, and an alkyl interaction with Ile105 (Fig 3). The contact with Ile105 is noteworthy since it is the signature residue of the *GSTP1*A* allele, the most frequently occurring variant [26]. Residue 105 in the *GSTP1*B* allele is Val. Additionally Phe9, Ile105, Tyr109 and Gln210 are found within 5Å proximity of the ligand with other residues (Figure 2). The docking location of estradiol valerate indicates that the ligand fits in the area of H-site of the protein. Inhibitors binding to the H-site may also act as substrates provided that they contain electrophilic groups. The steroid nucleus of the estrogen derivatives studied here do not contain double bonds that can undergo a GST-catalyzed double-bond isomerization like 5-pregnen-3,20-dione or 5-androsten-3,17-dione, as demonstrated in our previous studies of GSTs from human and other mammalian sources as well as in the mosquito *Anopheles gambiae* [3,27,28]. However, it is well established that GSTs may display esterolytic activities [29] and recent studies demonstrate activity of GST P1-1 with fluorescein esters [30]. Therefore, estradiol valerate and other estrogen derivatives may also be worth testing as substrates of the members of the GSTome.

In conclusion, synthetic estrogen drugs that we studied have minor inhibition effect on human GST P1-1. Even the most potent estradiol valerate is a modest inhibitor. However, the knowledge about its binding location and formation of bonds with the critical residues might assist in the design new inhibitors or substrates targeting GSTs.

Author contribution

Study conception and design: YM and BM; data collection: YM; analysis and interpretation of results: YM and BM; draft manuscript preparation: YM and BM. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval

No ethical approval is required for this study.

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Conflict of interest

The authors declare that there is no conflict of interest.

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