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ORIGINAL ARTICLE / ÖZGÜN MAKALE

GRAPHENE QUANTUM DOTS AND ITS DERIVATIVES AS A POTENTIAL INHIBITOR OF GLUTATHIONE *S*-TRANSFERASE PI-1 ISOENZYME: A COMPUTATIONAL STUDY

GLUTATYON S-TRANSFERAZ PI-1 İZOENZİMİNİN POTANSİYEL BİR İNHİBİTÖRÜ OLARAK GRAFEN KUANTUM NOKTALARI VE TÜREVLERİ: HESAPLAMALI BİR ÇALIŞMA

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ABSTRACT

Objective: *Phase II detoxification enzymes called Glutathione S-Transferases (GSTs) protect our bodies from the harmful effects of xenobiotics. The GSTP1 isoenzyme not only detoxifies toxic substances but also contributes to cancer treatment resistance. The earliest and most potent GST inhibitor is ethacrynic acid (EA). This work compares graphene quantum dots (GQDs) with EA that has been shown to be beneficial in anticancer investigations, using molecular docking analysis in order to offer the idea of a possible inhibitor of GSTP1.*
Material and Method: *The density functional theory (DFT) method was applied to theoretical calculations on the GQDs compound using Gaussview 5.0 software. The application Gaussian 09 was used to refine the geometry. Calculations of molecular electrostatic potential (MEP) were used to identify the compounds' reactive sites. PyRx Tools and AutoDock Vina software were used to conduct molecular docking studies between the optimized EA and the GQDs molecule with GSTP1. The receptor-ligand interactions were visualized using Discover Studio Visualizer 4.0.*
Result and Discussion: *GQDs were found to interact with the H Site residues of GSTP1, as in EA. However, their electrophilicity was much lower than EA. We think that they can be GSTP1 inhibitors by increasing their electrophilicity with surface modifications.*
Keywords: *Density functional theory, glutathione S-transferase, graphene quantum dots, molecular docking*

ÖZ

Amaç: *Glutatyon S-Transferazlar (GST'ler) adı verilen Faz II detoksifikasyon enzimleri vücudumuzu ksenobiyotiklerin zararlı etkilerinden korur. GSTP1 izoenzimi yalnızca toksik maddeleri detoksifiye etmekle kalmaz, aynı zamanda kanser tedavisi direncine de katkıda bulunur. En erken ve en etkili GST inhibitörü etakrinik asittir (EA). Bu çalışma, GSTP1'in olası bir inhibitörü fikrini sunmak için moleküler yerleştirme analizini kullanarak antikanser araştırmalarında faydalı olduğu gösterilen EA ile grafen kuantum noktalarını (GQD'ler) karşılaştırmaktadır.*
Gereç ve Yöntem: *Gaussview 5.0 yazılımı kullanılarak, yoğunluk fonksiyonel teorisi (DFT) yöntemi GQDs bileşiği üzerindeki teorik hesaplamalara uygulandı. Geometriyi iyileştirmek için Gaussian 09 uygulaması kullanıldı. Bileşiklerin reaktif bölgelerini belirlemek için moleküler elektrostatik potansiyel (MEP) hesaplamaları kullanıldı. Optimize edilmiş EA ve GQDs molekülü ile GSTP1*

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arasında moleküler yerleştirme çalışmaları yürütmek için PyRx Tools ve AutoDock Vina yazılımı kullanıldı. Reseptör-ligand etkileşimleri Discover Studio Visualizer 4.0 kullanılarak görselleştirildi.
Sonuç ve Tartışma: *GQD'lar, EA'da olduğu gibi GSTP1'in H Site rezidüleriyle etkileşimde olduğu bulundu. Ancak, elektrofiliklik kapasitesi EA'ya göre çok daha düşüktü. Yüzey modifikasyonları ile elektrofilikliği artırılarak, GSTP1 inhibitörü olabileceği düşüncesindeyiz.*
Anahtar Kelimeler: *Glutatyon S-transferaz, grafen kuantum noktaları, moleküler kenetlenme, yoğunluk fonksiyonel teorisi*

INTRODUCTION

With their multifunctional and broad-spectrum substrate specificity, Glutathione *S*-transferases (GSTs), found in many human tissues and organs, play a defensive role in organisms exposed to harmful substances [1,2]. To carry out this function, GSTs catalyze the thiol (-SH) group's nucleophilic attack on electrophilic substrates. This attack originates from the cysteine residue (Cys) of glutathione (GSH). Therefore, this conjugation reaction facilitates the synthesis of water-soluble conjugates that are less harmful and can be expelled from cells and tissues than the initial toxic molecule [3].

The three classifications of GSTs found in human cells and tissues, based on their primary structures and amino acid sequences, structural features, enzymatic properties, antibody reactions, chemical affinities, and behaviors, are categorized as cytosolic, microsomal, and mitochondrial GSTs. There are 11 distinct GST isoenzymes known to be present in humans: cytosolic GSTs have seven isoenzymes, including GST Alpha (GSTA), GST Mu (GSTM), GST Pi (GSTP), GST Sigma (GSTS), GST Theta (GSTT), GST Zeta (GSTZ), and GST Omega (GSTO); microsomal GSTs have three isoenzymes, MGST1, MGST2, and MGST3, which are membrane-bound proteins involved in eicosanoid and glutathione metabolism, part of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG); and mitochondrial GST has one member, GST Kappa (GSTK) [2,4].

The coding gene of GSTP, the most comprehensive, most studied and even the first isoenzyme studied in this enzyme family, is located on chromosome 11 and consists of seven exons. Two identical dimer subunits, each with 210 amino acids and two binding sites, typically make up human GSTP. The G-Site, which binds GSH, and the H-Site, which binds the electrophilic substrate, are two examples of these binding positions [5,6]. To enhance catalytic activity, GSTP can selectively bind to GSH or GSH analogs through the G-site, thereby facilitating the interaction between GST amino acid residues and GSH sulfhydryl groups and ubiquitous electrophilic substances at the H-sites [7].

To defend cells against oxidative stressors like reactive oxygen species (ROS), GSH and other related metabolic enzymes are essential. Redox (oxidation and reduction) processes offer this defense [5,8,9]. Research investigating the activities of antioxidant enzymes γ-Glutamyl Cysteine Synthase (γ-GCS) and Glutathione Reductase (GR) in tumors and peripheral normal tissues revealed that the GSTP1 isoenzyme has a strong positive connection with these antioxidant enzymes. Accordingly, by catalyzing glutathione's interaction with intracellular ROS and/or other electrophiles, GSTP1 may contribute to the control of cellular redox status, enhancing tumor cells' antioxidant capacity and assisting them in fending against externally harmful stimuli [10]. GSTP1, in particular, is involved in the intracellular transport of a wide range of substances, including bile acids, thyroid hormone, and steroid hormones, which are lipophilic and do not function as substrates for conjugation reactions, along with detoxifying reactive metabolites, GSTP1 in particular, which has the ability to bind non-enzymatic ligands. Furthermore, through their roles in protein-protein interactions, a number of kinases, including c-Jun N-terminal kinase (JNK), Mitogen-Activated Protein Kinase (MAP-Kinase), Protein Kinase C (PKC) and Protein Kinase A (PKA), and Epidermal Growth Factor Receptor (EGFR), directly alter the activities of these proteins. These kinases regulate a variety of cellular processes, such as cell proliferation, apoptosis, autophagy, and inflammation [11]. For instance, when GSTP1 is overexpressed or when it develops a reaction against ROS, interacts with and inhibits c-Jun N-terminal kinase-1 (JNK-1), which is crucial for the apoptotic pathway and cell proliferation, when it is overexpressed or when it develops a reaction against ROS. In this instance, GSTP1 seems to play a "non-enzymatic" antiapoptotic role in addition to influencing a cellular redox status [6,7]. Many malignancies, including those of the gastrointestinal tract [12], lung [4,13], breast [1,14], pancreatic [15], liver [16], ovary [17], lymphoma [18], and melanoma

[8], exhibit overexpression of GSTP1. Tumor cells also use GSTP1 to create a complex with GSH and chemotherapeutic drugs, which aids in detoxification and prevents apoptosis in malignant tissues or cells. P glycoprotein 1 (P-gp 1), which is encoded by the MDR1 (Multidrug Resistance) gene, causes an increase in their expression in tumor tissues. They collaborate with multidrug resistance proteins (MRP), which belong to the ATP-binding cassette (ABC) carrier protein family, to promote drug resistance [12,14,19,20].

For all of these reasons, scientists are looking for potent inhibitors of GSTP1 because they believe it is a good target for overcoming treatment resistance in cancer [6,21,22]. Accordingly, GSTP1 inhibitors are classified into two classes, α and β, based on their GSH conjugates formed with GSH and unsaturated carbonyl derivatives. It is known that GSTs catalyze the chemical conjugation of ethacrynic acid (EA) ([2,3-Dichloro-4(2-methylene-butyryl)phenoxy] acetic acid), a diuretic derived from α and β unsaturated carbonyl derivatives, to GSH [23]. Through covalent binding, EA and the EA-GSH conjugate are both strong reversible inhibitors of GSTs, especially those of the GSTA, GSTM, and GSTP classes [23,24]. Early investigations on EA's inhibitory action revealed that it significantly enhanced the toxicity of chlorambucil in resistant cells and increased the sensitivity of human leukemia, lymphoma, and myeloma cells to doxorubicin and nitrogen mustards [25]. EA has also been demonstrated to enhance the cytotoxicity of several alkylating drugs, including melphalan, carmustine, and mitomycin C, and to have anti-proliferative effects on tumor cells [26]. Despite all of these benefits, EA's low specificity, lack of isoenzyme specificity, and clinically significant diuretic side effects have restricted its usage as a GST inhibitor target [27-29]. To lessen the aforementioned negative effects, researchers have either synthesized several EA analogs [23,26] or have been investigating and testing various GST inhibitors [30].

Research on nanotechnology has grown in importance in the treatment of cancer. An example of this is graphene quantum dots (GQDs), a subclass of quantum dots (QDs) that are made from graphene sheets and have characteristics similar to both graphene and carbon dots. Because of these characteristics, GQDs are now increasingly frequently utilized in fields such as energy storage, drug delivery, and sensing and imaging. Furthermore, the ability to intentionally alter their properties by heteroatom doping accounts for their utilization in a variety of cutting-edge applications. They have unique qualities as a result [31]. Nanocomposites incorporating graphene have started to emerge as viable carriers for medicinal applications in research [32]. GQDs can enter cells and interact with the proteins and genetic material there. They alter the nucleus and cytoplasm of the cell, which eventually results in cytotoxic effects [33,34]. After a sequence of metabolic events from the mitochondria to the cytoplasm, exposure to graphene and graphene-based nanomaterials increases intracellular Reactive Oxygen Species (ROS), such as the superoxide radical generated by the cell's mitochondria, following a sequence of metabolic events from the mitochondria to the cytoplasm [35,36]. Furthermore, some research has attempted to combine GQDs with antibodies specific to cancer cells or integrate them into a carrier system to lessen their cytotoxicity in healthy cells [37]. Furthermore, because of their small size, high surface area, superior conductivity, and optical qualities, GQDs-a significant research topic in the field of nanotechnology-have a wide range of potential applications such as energy storage, sensors, and biomedical applications. This is due to their unique physical and chemical properties as well as their biological activity [38,39]. On the other hand, little is known about their possible characteristics in relation to cancer medication resistance.

In light of this, the purpose of this study was to compare the inhibitory potential of GQDs with ethacrynic acid using molecular docking analysis. Additionally, the study aimed to provide theoretical information that the molecule can also contribute to resistance against certain factors through various surface modifications. This was based on literature studies where analyses were conducted and the molecule's current use as a drug carrier. The potential results were supported by density functional theory (DFT) calculations.

MATERIAL AND METHOD

Density Functional Theory (DFT) Calculations

The GQDs molecule, whose molecular geometry was retrieved from the PubChem database and the model specified in Ref.[40], was subjected to theoretical calculations using the density functional theory (DFT) method [41] utilizing Gaussview 5.0 software [42]. The Gaussian 09 software was used to make geometric optimizations in the ground state in order to produce a high-quality theoretical approach [43]. The LanL2DZ (Los Alamos National Laboratory 2 double-ζ) basis set and the B3LYP (Becke's three-parameter hybrid exchange correlation function) functional were employed in these optimizations [44,45]. Then, in order to determine the reactive sites of the molecules and investigate their electrophilic and nucleophilic fields, molecular electrostatic potential (MEP) calculations were also carried out.

Molecular Docking Calculations

Molecular docking studies between the optimized Ethacrynic Acid and GQDs compound and GSTP1 protein (retrieved from the Protein Data Bank, PDB ID: 2GSS [25]) were performed via PyRx Tools [46] using AutoDock Vina software [47]. UCSF Chimera 1.18 software was used to remove water molecules and other heteroatoms from the protein prior to the docking process [48]. Using PyRx Tools, binding energies (kJ/mol) were computed as vina scores to assess the materials' affinity for binding to the protein. Software called Discover Studio Visualizer 4.0 was used to visualize receptor-ligand interactions [49]. The purpose of these analyses was to understand how the materials might interact with biological targets.

RESULT AND DISCUSSION

The binding affinities of ethacrynic acid and GQDs to GSTP1 protein are shown in Table 1. The result with the lowest binding affinity was ethacrynic acid. The reference ligand had a comparatively poor interaction with the GSTP1 protein, as seen by the maximum binding affinity value of -6.5 kcal/mol. With binding affinity values ranging from -11.6 to -10.9, GQDs show the strongest interaction. This indicates that GQDs and the GSTP1 protein have a high potential for binding. Furthermore, the stability of the reference ligand's binding sites is indicated by the fact that ethacrynic acid's consistently low RMSD (Root Mean Square Deviation) values, which range from 0 to 20 units. It is evident that the values of GQDs span a broad range from 0 to 30. This demonstrates the possibility of both stable and changing binding locations (Table 1).

Protein-ligand interaction analysis is one of the key computations in the molecular docking analysis approach. The binding location of EA in the three-dimensional GSTP1 protein crystal structure is displayed in Fig. 1a. The structural order is shown by color representations of the protein's helical, beta-sheet, and alpha-helix structures (green and yellow, respectively). The red dashed circle indicates the binding pocket where EA is placed. This position implies that EA has a potent ability to bind to the protein's active site. The specific interactions between the EA molecule and the amino acid residues in the binding pocket are depicted in two dimensions in Fig. 1b. Conventional hydrogen bonds, carbon-hydrogen bonds, π-π stacking, and van der Waals interactions are present. Through π-π interactions and traditional hydrogen bonding, aromatic amino acids like TYR (tyrosine) and PHE (phenylalanine) greatly enhance the binding stability specifically. Additionally, residues like ARG, GLY, ILE, and ASN are stabilized by van der Waals forces and are compatible with the binding site of EA. Fig. 1c shows a superficial visualization of the donor and acceptor sites of hydrogen bonds in the binding pocket. The hydrogen bond donor sites, represented in magenta, and the acceptor sites, shown in green, indicate strong hydrogen bond interactions that support the positioning of EA in the binding site. These bonds allow EA to be stably positioned in the binding site. The binding pocket's hydrophobicity map is displayed in Figure 1d. The binding site's apolarity and the strong hydrophobic interactions that EA has with those sites are represented by the dark patches. The parts that are open to polar interactions are indicated by the blue areas, which represent the hydrophilic portions. EA's flexibility and stability in the binding mechanism are enhanced by its capacity to engage with both polar and apolar locations in the binding site.

Table 1. Binding affinities of ethacrynic acid and GQDs to GSTP1 protein

	Ligand Model	Binding Affinity (kcal/mol)	RMSD* (ub)**	RMSD (lb)***
Ethacrynic Acid	1	-6.5	0	0
	2	-6.4	3.753	2.634
	3	-6.4	19.939	16.96
	4	-6.2	6.597	1.528
	5	-6.2	6.541	2.213
	6	-6.0	19.56	16.424
	7	-5.9	6.508	2.548
	8	-5.6	20.461	17.167
	9	-5.6	2.743	1.881
GQDs	1	-11.6	0	0
	2	-11.4	5.403	0.781
	3	-11.4	30.149	25.314
	4	-11.3	4.208	0.695
	5	-11.2	28.941	24.49
	6	-11.0	2.775	2.645
	7	-11.0	28.984	25.11
	8	-10.9	29.891	25.296
	9	-10.9	4.341	2.615

*:RMSD (Root Mean Square Deviation) values; **: ub (upper bound) and ***: lb (lower bound)

According to Oakley et al.'s earlier crystallographic structure elucidation work, ethacrynic acid interacts with the GSTP1 protein's Tyr7, Phe8, Val10, Arg13, Trp38, Ile104, Tyr108, and Gly205 residues [25]. In our work, EA interacts with the GSTP1 residues Val35, Gly205, Arg13, Gly12, Ile104, Asn204, Pro9, and Trp38 through van der Waals interactins, with Val10 through π-π stacking; with Phe8 through Pi-sigma interactions,; and with Tyr108 and Tyr7 residues through conventional hydrogen bonding, much like in the study of Oekley et al. The reference study stated that the inhibitor was situated in a hydrophobic pocket that was bordered by the aliphatic moieties of Arg13, Val35, Ile104, and Tyr108 as well as the side chains of Tyr7, Phe8, Pro9, and Val10. The loop portion of the inhibitor was positioned between the aromatic side chains of Tyr108 and Phe8 [25]. Additionally, our investigation showed that EA interacts with the binding sites through strong hydrophobic interactions. The findings of our docking analysis can be considered reliable based on data from the literature.

The three-dimensional binding of GQDs to the GSTP1 protein is depicted in Fig. 2a. Here, the colors green, yellow, and orange stand in for the protein's alpha-helix and beta-sheet structures. The GQDs molecule is situated in the protein's active site and in the binding location indicated by a red dashed circle. This suggests that GQDs are firmly attached to the binding pocket specific to the protein, and this association might be biologically significant. The GQDs molecules' interactions with the GSTP1 protein are shown in two dimensions in Fig. 2b. The key amino acid residues in the GQD binding regions, as well as the types of interactions and their distances, are described in detail here. Thus, van der Waals contacts are formed by residues Gly205, Asn204, Tyr108, Tyr7, Gly12, and Arg13; π-alkyl interactions by residues Val10, Val35, Trp38, and Ile104; and π-π stacking interactions by Phe8. We can better understand the strength and manner in which GQDs molecules attach to GSTP1 because the residues form hydrogen bonds, hydrophobic contacts, and π-π interactions with GQDs. The hydrogen bond donor (magenta) and acceptor (green) sites in the GSTP1 binding area are seen on the surface in Fig. 2c. GQDs form strong hydrogen bonds when they bind to these areas, and these interactions make the binding region more stable. The proper positioning and interaction of GQDs in the binding area suggest possible pathways that could influence the protein's biological functions. Lastly, the binding site's hydrophilic and hydrophobic regions are displayed in Fig. 2d. Whereas the blue color denotes the hydrophilic regions, the brown hydrophobic regions show that the GQDs molecules forms strong connections with these regions. This suggests that GQDs interact with both polar and apolar regions of the protein to help it bind and secure a stable place in the binding pocket. Our research is the first to

compare the potential inhibitory effects of GQDs based on EA, showing that the residues to which GQDs and EA bind GSTP1 are quite comparable. Furthermore, the strength of the connection is highlighted by the fact that interactions such as hydrogen bonds, hydrophobic interactions, van der Waals, and π-π stacking interactions are responsible for the contact with these residues in both the reference study [25] and our docking investigation. It should be noted, nevertheless, that GQDs exhibit van der Waals interactions with the GSTP1 protein's Gln51 residue, but EA does not. Furthermore, our data indicate that GQDs, such as EA, bind to the hydrophilic and hydrophobic domains of GSTP1, demonstrating that GQDs help the protein bind by interacting with both polar and apolar areas and achieve stability in the binding pocket.

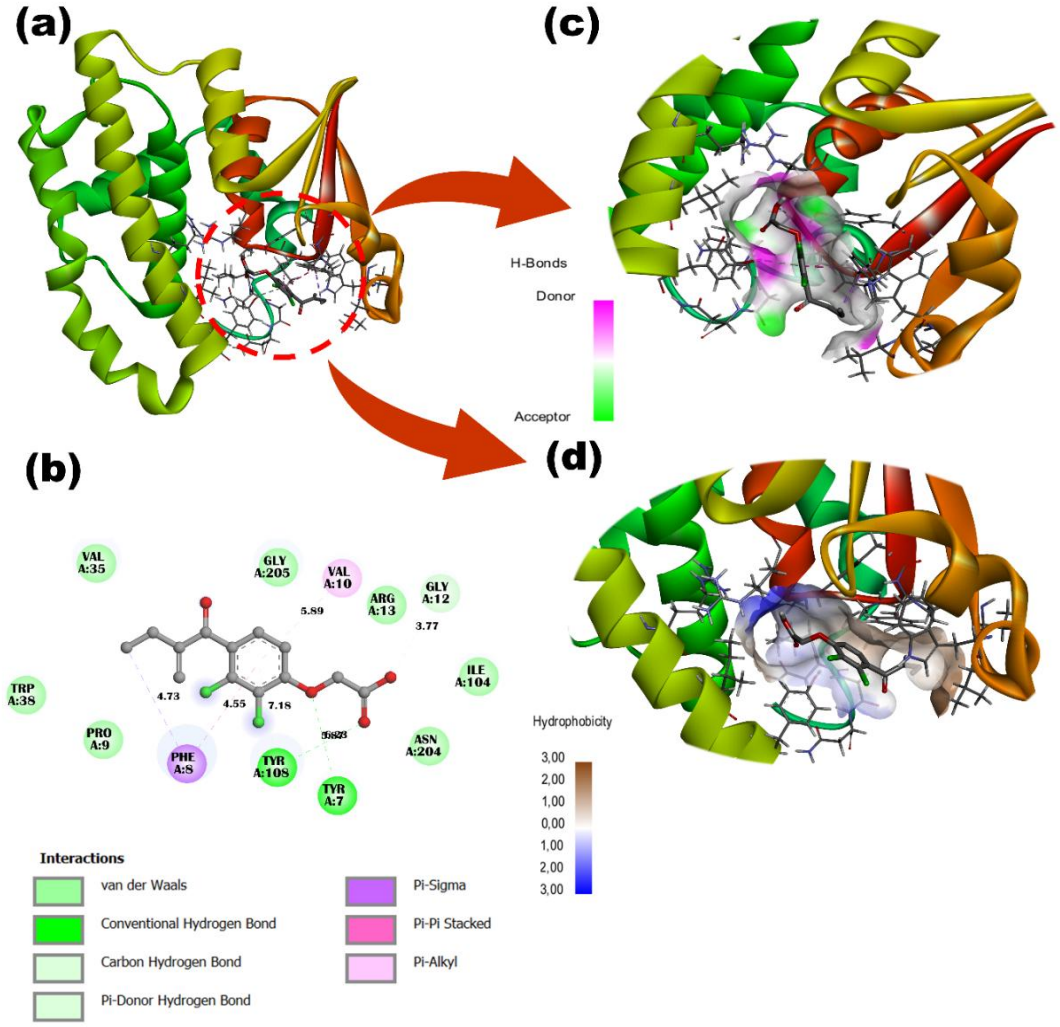

Figure 1. (a) A three-dimensional illustration that shows how the EA molecule binds to the GSTP1 active site and the interaction sites (red dashed circle); **(b)** a two-dimensional interaction map that displays the binding pocket residues that interact with EA; **(c)** a surface visualization of the GSTP1 binding pocket; **(d)** a map of the GSTP1 binding site's hydrophobicity, which shows the hydrophilic (blue) and hydrophobic (brown) areas surrounding the EA molecule

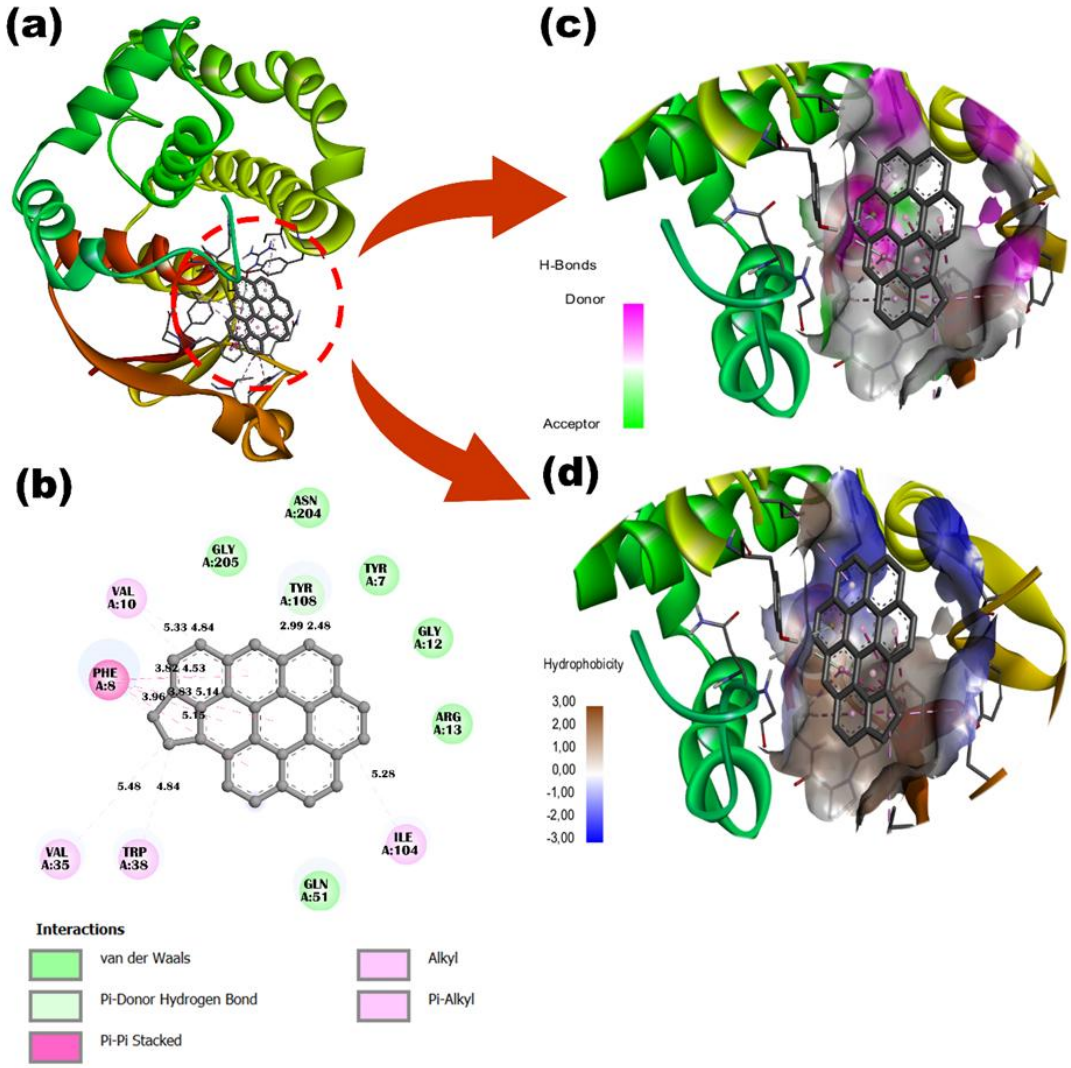

Figure 2. (a) A three-dimensional illustration of the GQDs molecule's binding to the GSTP1 active site and interaction sites (red dashed circle); **(b)** A two-dimensional interaction map showing the residues in the binding pocket interacting with the GQDs; **(c)** A visualization of the surface of a GSP1 binding pocket; **(d)** A map of the hydrophobicity of the GQDs molecule, showing the hydrophilic (blue) and hydrophobic (brown) regions

One crucial technique commonly employed for this goal is the development of molecular electrostatic potential (MEP) maps, which are an effective way to view a molecule's electrophilic and nucleophilic regions and assess the contributions of these regions to protein-ligand interactions [50]. By examining the electrophilic and nucleophilic areas of molecules, MEP maps offer crucial information for understanding biological processes and protein-ligand interactions. In this regard, MEP maps acquired for GQDs and EA were assessed. For both compounds, the MEP range is between -0.04 a.u. (red) and 0.04 a.u. (blue) (Fig 3). The red spots on the MEP map of EA (Fig. 3a) are locations that have carbonyl groups and other electrophilic characteristics. These areas may interact with protein regions that form hydrogen bonds or are positively charged. However, as Fig. 1b illustrates, the blue patches in and around the aromatic ring of EA show electrophilic sites that interact with nucleophilic amino acids. Strong and precise binding to the active sites of proteins like GSTP1 is made possible by these characteristics of EA.

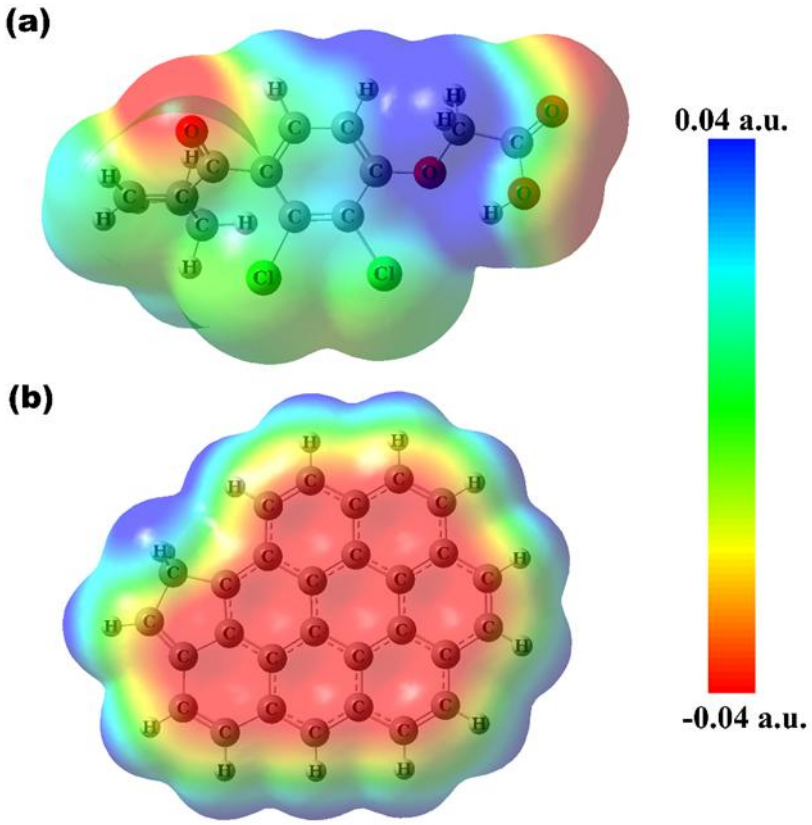

Figure 3. MEP maps of (a) EA and (b) GQDs

The red color in the GQDs' MEP map (Fig. 3b) highlight their nucleophilic characteristics. Examining the map reveals that the red spots represent the graphene structure's double bonds, or aromatic rings; these locations have a greater electron density and are thus nucleophilic. These nucleophilic regions may interact with the positively charged regions of proteins or hydrogen bond donors. The blue colored areas indicate that the carbon atoms are more electrophilic; in particular, the surface-bound sp^2 hybridized carbon atoms can interact with protein nucleophiles. Since GQDs are made entirely of hydrogen and carbon atoms, this highlights the hydrophobic nature of the molecule and raises the possibility of attaching through aromatic interactions—like π-π stacking—instead of polar interactions. This indicates that in biological settings, GQDs can establish potent π-π or π-doped interactions with proteins, particularly amino acids with aromatic rings.

The H Site, one of GSTP1's two binding sites, is often where electrophilic substrates bind to provide their inactivating effects [5-7]. The hexyl moiety of the S-hexyl-GSH complex crystal structure is similar to the location where EA, an inhibitor of GSTs, attaches to the enzyme's H site [51]. Reinemer et al. state that residues Tyr7, Phe8, Val10, Val35, Tyr108, and Gly205 are present in the electrophile-binding site H-Site. Ji et al., based on crystallization findings using S-hexylglutathione or 9-(6-glutathionyl)-10-hydroxy-9,10-dihydrophenanthrene, include the additional residues Arg13 and Asn204 in this area [51,52]. Nevertheless, certain chemicals have also been shown to bind to the residues of the active site, or G Site, and inhibit the enzyme. For instance, TER-117 (g-glutamyl-(S-benzyl)cysteinyl-D-phenylglycine) is the most selective hGST P1-1 inhibitor, according to Oakley and Bello et al. (1997). According to their research, the GSH conjugate bound to hGST P1-1 and the binding of TER-117 to GSTP1 had many structural similarities to that of EA. However, they also noted that this inhibitor differs from EA in that the phenyl ring in the TER-117 structure attaches to the G-site of GSTP [53]. In addition, another study highlighted that Ezatiostat (TLK199), also known as Telintra, inhibits the enzyme by forming significant contacts with amino acid residues in the G Site region of the enzyme, including Tyr7, Lys44, Arg13, and Ser11 [54]. Nevertheless, there is no biological effects or molecular support

for these ideas. It is our belief that both compounds interact with GSTP1 from its active site and alter the enzyme's activity rather than directly inhibiting it. Although GQDs also demonstrated binding and interaction with the GSTP1 protein's H Site residues, as evident in the literature, our results suggest that the core of the GQDs has greater nucleophilic characteristics when the MEP map is analyzed. It is possible to argue that the surface is more electrophilic than the interior. Given that the GSTP1 isoenzyme's surface is more electrophilic than its center, it makes sense that GQDs have attached to the H Site.

Nonetheless, our investigations and data from the literature have shown that EA has potent electrophilic qualities as a GSTP1 inhibitor. Therefore, we think that by making GQDs more electrophilic through various and biocompatible surface modifications, an alternative to EA as a GSTP1 inhibitor can be created. The goal of choosing biocompatible modifications to surface is to minimize toxicity while delivering the desired inhibitory effects. To improve the electrophilic qualities of the GQD structure, heteroatoms like phosphorus (P), boron (B), and nitrogen (N) can be added [53]. For instance, hydrothermal techniques can be used to create N-doped GQDs (N-GQDs), and this process is biocompatible [31,40]. The addition of oxygen-containing compounds, such as carboxyl or carbonyl groups, to the surface of GQDs can improve their interaction with GSTP1 by increasing the GQDs' electrophilia [55]. GQDs can be coated with biomolecules like protein nanofibers [56] or folic acid [57,58] to improve their electrophilic qualities and give them targeting capability. Because it improves the ability to target cells, this strategy is especially well-suited for cancer treatment. Furthermore, the surface electron density of GQDs can be altered by electrochemically adding active groups, and biocompatible outcomes can be obtained [55]. Without a doubt, in vitro and later in vivo experimental research should be conducted to examine the roles of GQDs and biocompatible surface modifications that will increase their electrophilia in preventing drug resistance.

In conclusion, our research has demonstrated for the first time whether GQDs, which are crucial in physics, chemistry, and biology research, can potentially inhibit the GSTP1 isoenzyme. Therefore, our study followed the same methodology as the first studies on the topic in the literature. Using the docking method, we first demonstrated the molecular interaction between EA and GSTP1 and provided a standardization. Then, we compared GQDs with this standard and examined their potential as a GSTP1 inhibitor. As shown in the computational analyses performed in our study, it can be said that GQDs bind to the H Site of the enzyme, interact strongly with similar residues in the H Site of the enzyme like EA, and therefore they can be an effective inhibitor like EA. However, since the electrophilic properties of GQDs are lower than EA, we think that this inhibitory effect should be shown with evidence-based experimental studies by increasing their electrophilicity with biocompatible surface modifications.

AUTHOR CONTRIBUTIONS

Concept: M.K.; Design: M.K., B.B.; Control: M.K., B.B.; Sources: M.K., B.B.; Materials: M.K., B.B.; Data Collection and/or Processing: M.K., B.B.; Analysis and/or Interpretation: M.K., B.B.; Literature Review: M.K., B.B.; Manuscript Writing: M.K., B.B.; Critical Review: M.K., B.B.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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