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In Silico Approach of Predicting The Interaction of Epigallocatechin-3 Gallate and Chlorogenic Acid on E47 and THOC5 in Macrophage Differentiation

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ABSTRACT. *Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia and glucose intolerance. Vascular hyperglycemia triggers the release of inflammatory mediators, which can increase monocyte differentiation into macrophages. Compounds such as epigallocatechin-3 gallate (EGCG) from green tea and chlorogenic acid (CGA) from green coffee are known to have anti-inflammatory effects. This study aimed to analyze the effect of EGCG and CGA on macrophage differentiation markers by assessing their binding activity with THOC5 and E47 proteins. An in silico assessment was conducted using metformin, which is widely used as a first-line antidiabetic drug, as the control ligand. Pharmacokinetic and drug-likeliness predictions were carried out. Additionally, molecular docking and molecular dynamics analysis were performed on the active residues of the proteins to assess the binding affinity and complex stability. The results obtained demonstrate that the binding affinity energy of EGCG and CGA to THOC5 and E47 was more favorable than that of metformin, yet their bioavailability value was poor. Molecular dynamics simulation showed that EGCG had a more stable complex compared to CGA and metformin. In conclusion, EGCG and CGA could potentially be effective as anti-inflammatory agents in diabetes mellitus.*

Keywords: *diabetes mellitus, epigallocatechin-3-gallate, chlorogenic acid, macrophage differentiation, molecular docking*

INTRODUCTION

Diabetes mellitus (DM) is a progressive metabolic disorder characterized by chronic hyperglycemia and impaired glucose homeostasis, with type 2 diabetes mellitus (T2DM) accounting for over 90% of global diabetes cases [1,2]. Chronic hyperglycemia drives vascular dysfunction through direct and indirect mechanisms, including oxidative stress, inflammatory pathway activation, and immune cell stimulation, which collectively contribute to micro- and macroangiopathies [3]. Atherosclerosis, a leading complication of DM, is responsible for more than 50% of diabetes-related mortality, underscoring the urgency of addressing hyperglycemia-induced vascular damage [4,5]. Macrophages play a central role in chronic inflammation in diabetes and its vascular complications. Monocytes differentiate into macrophages that promote inflammatory responses through lipid uptake and cytokine secretion [6]. Consequently, targeting macrophage differentiation and activity represents a promising therapeutic strategy to mitigate diabetes-related vascular alterations.

Emerging evidence highlights the interplay between genetic predisposition and metabolic dysregulation. Single-nucleotide polymorphisms (SNPs) in genes such as *THOC5* have been linked to increased risks of vascular pathologies [7]. THOC5 regulates the nuclear export of *Id3* mRNA, encoding a helix-loop-helix (HLH) protein that dimerizes with E47 to suppress the cell cycle inhibitor p21. This THOC5-E47-p21 axis is critical for macrophage proliferation and differentiation, positioning THOC5 and E47 as pivotal molecular targets for modulating inflammatory responses [8,9]. Pharmacological inhibition of these proteins could attenuate macrophage-driven inflammation, yet limited studies have explored small-molecule interventions targeting this pathway.

Current diabetes management prioritizes glycemic control, with metformin remaining the first-line therapy. Metformin is known to have various beneficial effects, which are primarily mediated through the AMP-activated protein kinase (AMPK) signaling [10]. Previous studies have shown that metformin can modulate macrophage activity and suppress pro-inflammatory responses by this pathway [11].

Metformin has also been reported to alter cell cycle regulation, including p21 expression, via AMPK/mTOR pathways [12]. However, its direct effects on macrophage differentiation remain unclear. Other antidiabetic agents have been reported to modulate macrophage activity. Thiazolidinediones, such as pioglitazone, inhibit macrophage proliferation through peroxisome proliferator-activated receptor gamma (PPARγ) activation, while sodium-glucose cotransporter-2 (SGLT2) inhibitors have been shown to terminate inflammatory activation of tissue macrophages, recruitment of monocytes from the bloodstream, and macrophage polarization towards the M1 phenotype [13,14]. These findings highlight the importance of targeting macrophage-related pathways in diabetes management. Recent interest has shifted toward natural compounds with dual hypoglycemic and anti-inflammatory properties. Epigallocatechin-3-gallate (EGCG), a major polyphenol in green tea (*Camellia sinensis*), and chlorogenic acid (CGA), abundant in green coffee (*Coffea canephora*), exhibit strong antioxidant and anti-inflammatory activities [15–17]. EGCG suppresses monocyte chemotaxis by downregulating monocyte chemoattractant protein-1 (MCP-1) expression, while CGA modulates cytokine signaling in macrophages [15, 17]. Both compounds have demonstrated atheroprotective effects by inhibiting CD36 and PPARγ, receptors implicated in ox-LDL uptake and foam cell formation [18]. Despite these advances, their influence on THOC5 and E47—key upstream regulators of p21 and macrophage differentiation—remains unexplored.

Advances in computational biology offer robust tools for predicting ligand-protein interactions and pharmacokinetic profiles. Molecular docking and dynamics simulations enable the evaluation of binding affinities, complex stability, and conformational changes, while tools like SwissADME assess drug-likeness and bioavailability [19, 20]. Such in silico approaches are particularly valuable for screening natural compounds, which often face challenges in oral bioavailability and metabolic stability [19, 21]. Previous studies have employed these methods to investigate EGCG and CGA interactions with lipid metabolism targets, yet their binding mechanisms with THOC5 and E47 remain uncharacterized [22].

This study aims to elucidate the potential of EGCG and CGA as modulators of macrophage differentiation by targeting THOC5 and E47. We hypothesize that these compounds exhibit favorable binding to THOC5 and E47, thereby suppressing macrophage differentiation. By integrating molecular docking, dynamics, and pharmacokinetic analyses, this study provides insights into how natural polyphenols modulate macrophage-related pathways at the molecular level. The findings may inform the development of adjuvant therapies targeting inflammation in diabetes, complementing existing glycemic control strategies.

RESEARCH METHODS

Pharmacokinetic Predictions

The pharmacokinetic profiles and drug-likeness of EGCG, CGA, and metformin were evaluated using SwissADME (http://www.swissadme.ch/) [19]. Canonical SMILES data were submitted to predict absorption, distribution, metabolism, and excretion (ADME) properties. Lipinski's Rule of Five (molecular weight ≤500 Da, hydrogen-bond acceptors ≤10, hydrogen-bond donors ≤5, LogP ≤5) was applied to assess oral bioavailability potential [23]. Gastrointestinal (GI) absorption and bioavailability scores were computed to examine suitability for oral administration. Compounds violating two or more Lipinski criteria were flagged as having suboptimal drug-like properties [24].

Protein and Ligand Preparations

The three-dimensional (3D) structures of THOC5 and E47 proteins were obtained from publicly accessible databases. The predictive structure of THOC5 (UniProt ID: Q13769) was modeled using the AlphaFold Protein Structure Database (AF-Q13769-F1) (https://alphafold.ebi.ac.uk) [25]. The experimentally resolved crystal structure of E47 (PDB ID: 2YPB) was retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org) [26]. Both receptor structures were preprocessed using Discovery Studio 2021 (BIOVIA, San Diego, CA, USA) to remove water molecules, co-crystallized ligands, and heteroatoms, followed by energy minimization to optimize hydrogen bonding networks and correct structural anomalies [27].

Following structure preparation, binding site identification was performed. The binding site of THOC5 was predicted using PrankWeb (https://prankweb.cz/), which identified key residues within the putative active pocket, including CYS403, PRO406, GLY407, HIS409, GLY543, LEU544, GLY546, ASN549, ASN570, and ILE576 [28]. Although the crystal structure of E47 is available, binding site prediction was performed to identify potential ligand-binding regions that may not be apparent from the static structure alone, particularly for small-molecule

interactions that differ from native DNA-binding interfaces [29]. For E47, the DNA-binding cavity was identified using a 3D footprint analysis , which mapped residues critical for DNA recognition (THR192, ASN193, ARG195, GLU196, ARG199, and GLN200) [30].

The ligands—epigallocatechin-3-gallate (EGCG; PubChem CID: 65064), chlorogenic acid (CGA; PubChem CID: 1794427), and metformin (PubChem CID: 4091)—were downloaded in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) [31]. Metformin was selected as a reference ligand due to its role as a first-line therapy for diabetes and its documented effects on metabolic and inflammatory pathways. Although metformin is not known to target THOC5 or E47 directly, it serves as a clinically relevant comparator for evaluating the binding potential of EGCG and CGA. Canonical SMILES data were extracted for pharmacokinetic analysis. Ligand preparation involved energy minimization using the universal force field (UFF) in Open Babel 3.1.1 integrated in PyRx 0.8, to ensure stable conformations and correct bond geometries [32, 33]. The minimized ligands were converted into PyRx-compatible formats for subsequent docking studies.

Molecular Docking

Molecular docking was performed using AutoDock Vina 1.1.2 integrated in PyRx 0.8 to evaluate the binding affinities and interaction modes of EGCG, CGA, and metformin with THOC5 and E47 [34]. The receptors were treated as rigid macromolecules, while ligands were treated as fully flexible. The docking grid for THOC5 was centered at E47 was centered at (x = 21.7570, y = 3.6050, z = −1.1107), while the grid for E47 was centered at (x = −10.8258, y = −34.1708, z = −6.4418). Dimensions were set to 20 Å × 20 Å × 20 Å to ensure adequate coverage of the predicted binding sites and surrounding residues. Exhaustiveness was set to 50 to ensure robust sampling of ligand conformations. Post-docking, the complexes were visualized in Discovery Studio 2021 to analyze hydrogen bonds, hydrophobic interactions, and binding poses [27].

Molecular Dyamics

Molecular dynamics (MD) simulations were conducted using YASARA 19.12.14 to validate the stability of ligand-protein complexes identified in docking studies [35]. The AMBER14 force field was employed under physiological conditions: 310 K temperature, 1 bar pressure, 0.9% NaCl concentration, pH 7.4, and 0.997 g/mL water density [36]. Each system was solvated in a cubic water box with a 10 Å buffer margin, and counterions were added to neutralize charges. Energy minimization was performed using steepest descent and simulated annealing algorithms to eliminate steric clashes.

Simulations ran for 50 ns with a time step of 2 fs. Trajectories were saved every 100 ps for analysis. Stability metrics—Root-Mean-Square Deviation (RMSD) of Ca atoms, backbone atoms (RMSD-Bb), and ligand movement (RMSD-LigMove)—were calculated to assess conformational fluctuations. A threshold of ≤3 Å was used to define stable complexes [37]. Root-Mean-Square Fluctuation (RMSF) of DNA-binding residues in E47 provided insights into local flexibility and interaction rigidity. All analyses were performed using YASARA's built-in tools.

RESULTS AND DISCUSSIONS

Bioactive Predictions with Swiss-ADME

Pharmacokinetic evaluation is a crucial procedure in drug-development studies to obtain compounds with high biological activity and low toxicity, as well as to consider the access and concentration levels of therapeutic targets [19]. The prediction of ADME characteristics in this study was analyzed using five parameters of Lipinski's Rule, along with the addition of GI absorption and bioavailability score, considering that green tea and green coffee are commonly

Table 1. Pharmacokinetic prediction of the compounds.

Name	MW	HBA	HBD	Log P	MR	Violation	GI Absorption	Bioavailability
Metformin	129.16	2.00	3	-0.56	36.93	0	High	0.55
EGCG	458.37	-0.44	11	8	112.06	2	Low	0.17
CGA	354.31	-1.05	9	6	83.50	1	Low	0.11

Note: MW: molecular weight (Da); HBA: hydrogen bond acceptors; HBD: hydrogen bond donors; LogP: lipophilicity; MR: molar refractivity (cm^3/mol); GI absorption: gastrointestinal absorption.

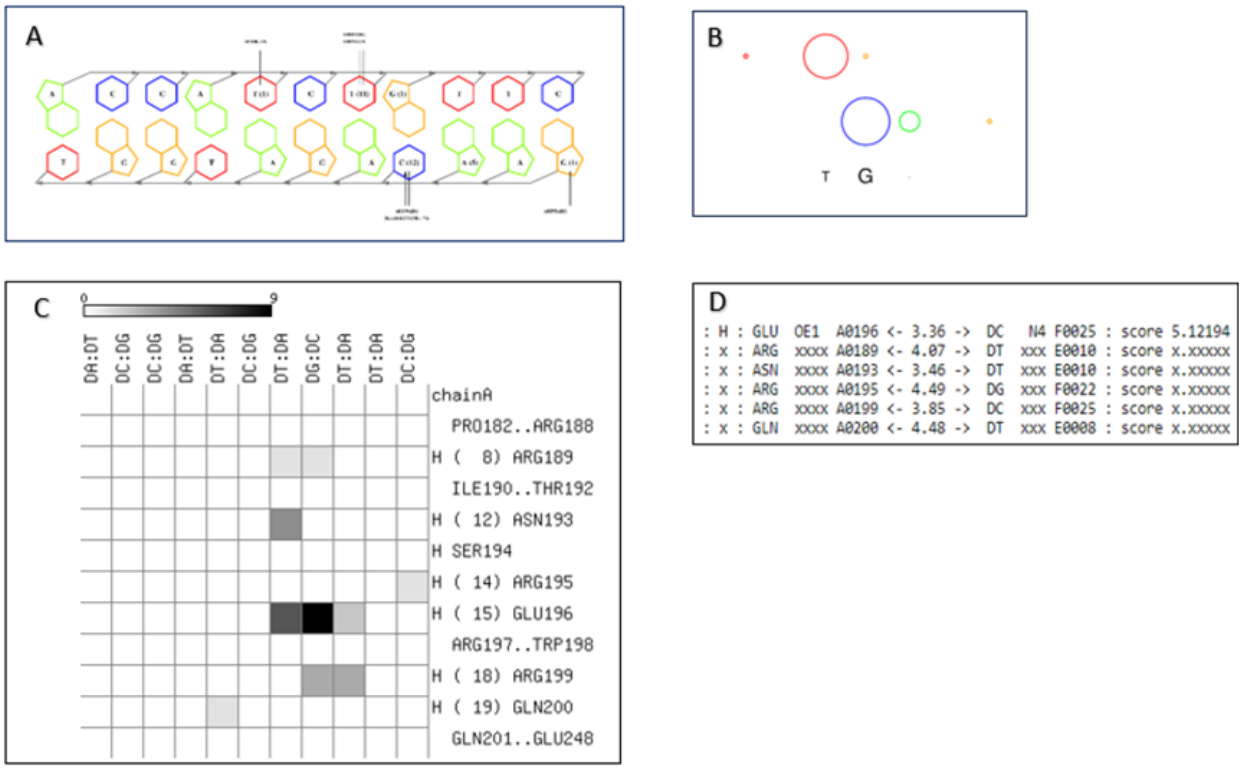

Figure 1. A) Interface graph, B) Footprint logo, C) Interface matrix, D) Atomic interactions.

consumed via the oral route. Compounds that fulfill these parameters are more likely to have favorable drug potential. The results of pharmacokinetic and bioavailability are presented in Table 1. The analysis with the Lipinski Rule concluded that EGCG did not meet the criteria of a drug (violation: 2). Metformin, used as a control, showed high GI absorption, whereas EGCG and CGA exhibited low GI absorption. The bioavailability values of EGCG and CGA are relatively low (score ≤0.55).

Based on these results, EGCG and CGA may not be optimal as therapeutic agents in their native form due to limited bioavailability. Accordingly, further optimization through advanced drug delivery systems and chemical modifications is necessary to improve their pharmacokinetic properties and therapeutic potential. Lipid-based formulations and surface modification (such as PEGylation) have been widely explored to enhance stability and absorption. Methylation of hydroxyl groups has been reported to increase lipophilicity, thereby enhancing membrane permeability and systemic exposure [38]. Similarly, glycosylation may increase solubility and facilitate intestinal absorption through carrier-mediated transport mechanisms [39]. Prodrug approaches, such as esterification, may increase membrane permeability and enable enzymatic conversion into active compounds after absorption [40]. In addition, lipophilic derivatization through acylation has been shown to improve cellular uptake of polyphenolic compounds [41]. These strategies highlight the potential of chemical modification to improve the bioavailability and drug-likeness of EGCG and CGA.

DNA-Binding Target Prediction

To analyze the interaction of protein–DNA complexes, a computational approach was carried out using DNA footprinting [30]. Formation of a complex involves direct or indirect readout in DNA recognition by proteins, and if identified, allows reliable prediction of DNA-binding sites. Based on the DNA-binding protein database, the alignment of the E47 protein covering the entire interface (6/6) of the HLH DNA motif with a coverage value of 100% was further analyzed. This sequence is coded as 2ypa_A complex formed from protein chain A in the PDB entry 2ypa, with an expectation value of 3e-22 and an interface signature RNRERQ. The interface residue numbers were aligned with the protein sequence in the database, consisting of A0192: 11, A0193: 12, A0195: 14, A0196: 15, A0199: 18, and A0200: 19. Accordingly, six residues were identified as the DNA-binding interface of E47: THR192, ASN193, ARG195, GLU196, ARG199, and GLN200. Figure 1 shows a graphical representation summarizing the interactions between proteins and double-stranded DNA segments at the interface.

The interface graph provides a detailed visualization of the contacts between protein residues and nucleotides responsible for specific DNA recognition. Each nucleotide is represented by uppercase (A, T, G, C) in hexagon-shaped lines, with the number of side-chain heavy atom contacts

Table 2. Binding affinity and interactions of amino acid residues with ligands.

Macromolecule	Ligand	Binding affinity (kcal/mol)	Hydrogen bonds	Hydrophobic contacts
THOC5	Metformin	-4.2	GLN 458, PRO 453	PRO 457
	EGCG	-7.3	GLY 407, ASP 408, PRO 440	HIS 409, LEU 442, LYS 412, THR 413, GLN 419
	CGA	-7.0	ASP 408, LYS 412, GLY 410, LYS 411	LEU 442, HIS 451, LYS 454
E47	Metformin	-3.3	ASN193	-
	EGCG	-5.3	THR192, ARG195	ARG189
	CGA	-5.2	ARG195, ARG197	GLU196, ASN193

within 4.5 Å indicated. Notably, no indirect readout bases or inferred complementary bases marked as solid bases or lowercase letters were observed in the PDB coordinates, indicating that the interaction between E47 and DNA is dominated by direct readout mechanisms. The footprint diagram further illustrates nitrogen bases as circles with diameters proportional to the number of side-chain contacts, with both DNA strands plotted separately. Consistent with this, no filled circles indicating indirect readout bases were detected. The interface matrix shows contacts between side-chain atoms (vertical axis) and nucleotide bases (horizontal axis), where the most contacted base pairs are highlighted in darker shades. At this interface, the highest number of contacts was observed at GLU196 (DG: DC), with a total of six atomic interactions contributing to the readout mechanism. The secondary structure of each interface residue is indicated by one-letter codes ('H', 'E', 'T', 'C') alongside their sequence positions.

Taken together, these findings confirm that E47 recognizes DNA primarily through direct readout interactions, where specific contacts between amino acid side chains and nucleotide bases determine DNA binding specificity. In general, protein–DNA recognition may involve both direct and indirect readout mechanisms that could occur either alone or simultaneously. However, in this case, only direct interactions were observed. This suggests a relatively specific and well-defined binding interface, although such interactions may still be influenced by structural complexity, including the absence of a dedicated force field for protein–DNA interactions and the potential involvement of water molecules at the interface [42].

Molecular Docking Simulation

Molecular docking analysis of EGCG and CGA with target proteins (THOC5 and E47) is presented in Table 2. The results obtained, including binding affinity values and hydrogen or hydrophobic interactions, were evaluated by comparison with metformin. Metformin is widely used as a first-line therapy in diabetes mellitus with minimal hypoglycemic effect. Its inclusion as a reference compound provides a clinically relevant benchmark given its established role in modulating metabolic and inflammatory mechanisms. Although the direct interaction of metformin with THOC5 or E47 has not been documented, a study by Molnar [43] reported that metformin inhibits p21 expression induced by high glucose through the AMPK/mTOR pathway. This effect is mediated by decreased AMPK activation, affecting downstream mTOR signaling. However, the inhibition of the mTOR pathway did not adversely affect p21 expression, indicating that metformin regulates p21 upstream.

Based on the docking results, the binding affinities of EGCG and CGA to target proteins were more negative than those of metformin, indicating stronger and more favorable binding. EGCG exhibited most negative binding affinity toward THOC5 (−7.3 kcal/mol), while for E47, CGA (−5.2 kcal/mol) showed the lowest value, suggesting relatively higher stability compared to the standard drug. The interaction profiles between macromolecules and ligands are illustrated in Figures 2 and 3.

These findings demonstrate that EGCG and CGA have better potential to bind THOC5 and E47 than metformin. THOC5 and E47 not only function upstream of p21 signaling but also modulate cellular proliferation. For THOC5, several interacting residues identified in the docking analysis overlapped with the predicted active site, including GLY407, HIS409, and ASN549. These residues are located within the binding pocket and may contribute to mRNA processing and downstream inflammatory pathways, suggesting that ligand binding may interfere with protein function. Meanwhile, E47 plays a fundamental role

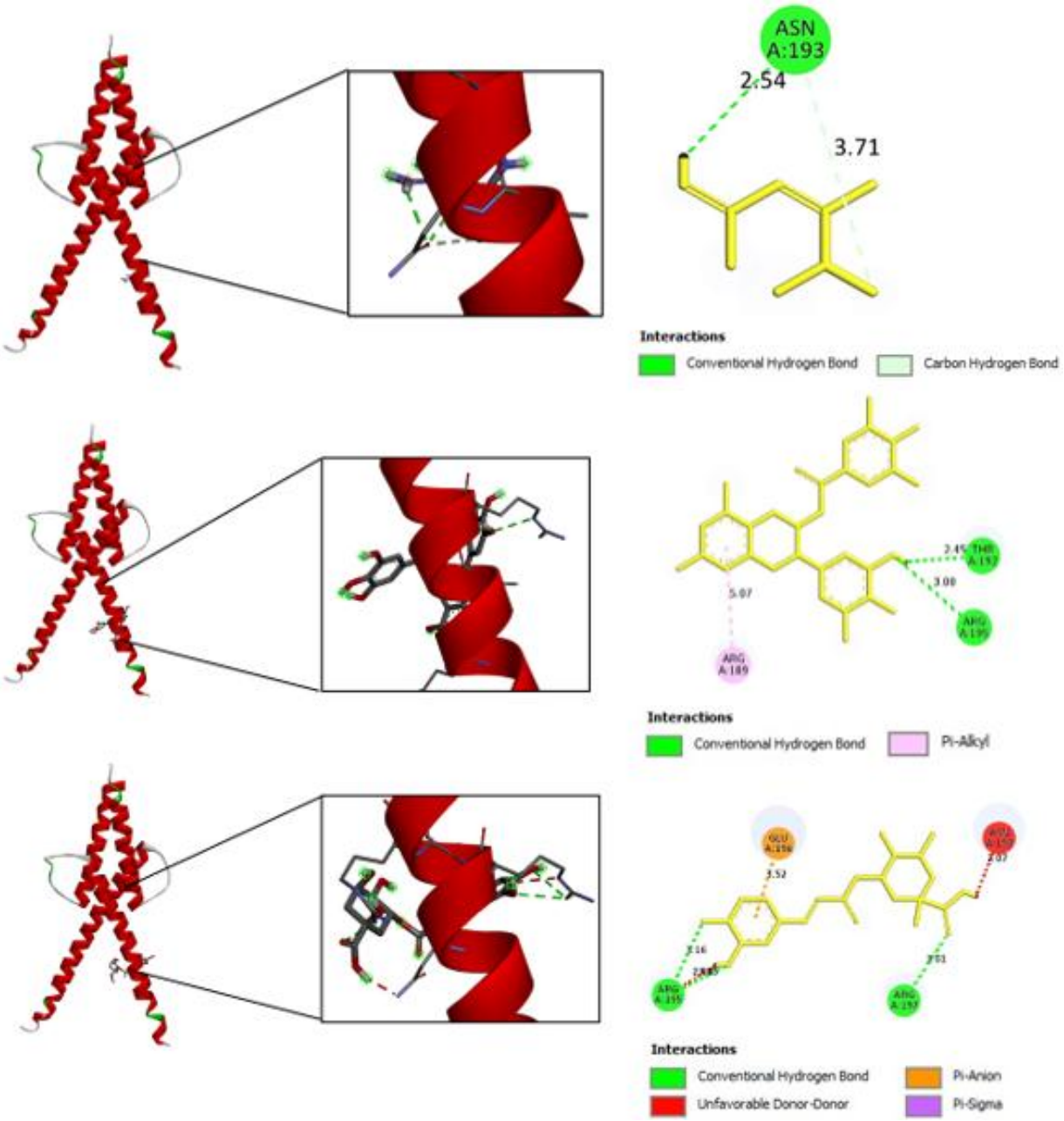

Figure 2. 3D and 2D interaction of amino acid residue of E47 with: a) Metformin, b) EGCG, c) CGA.

in cell regulation due to its ability to bind specific DNA sites, particularly within the bHLH domain associated with p21, which are critical for DNA recognition and transcriptional activity. Interaction analysis revealed that EGCG and CGA bind to residues within the DNA-binding site of E47, including THR192, ASN193, ARG195, and GLU196. The involvement of these residues indicates that ligand binding occurs directly at the functional site, potentially interfering with E47–DNA interactions and altering transcriptional regulation related to macrophage differentiation.

Notably, the interacting residues of EGCG and CGA differed from those of metformin, with no shared interacting residues. Collectively, these findings indicate that such differences in binding interactions may contribute to the modulation of macrophage-related pathways through alternative molecular mechanisms compared to conventional antidiabetic drugs. EGCG and CGA are therefore expected to inhibit the activity of THOC5 and E47 and enhance regulatory control over p21 transcription. Compared to small molecules targeting other regions of transcription factors, targeting DNA-binding ligands has several advantages, including (i) the ability to regulate only a subset of genes controlled by the transcription factor, (ii) the ability to specify target sequences through "programmable" functional groups, and (iii) tractable binding sites compared to the often amorphous nature of protein-protein interfaces [44].

Ligand–receptor interactions in this study were primarily characterized by hydrogen bonds and hydrophobic interactions within the active sites of THOC5 and E47. The formation of hydrogen bonds with key residues contributes to binding stability and proper ligand positioning within the

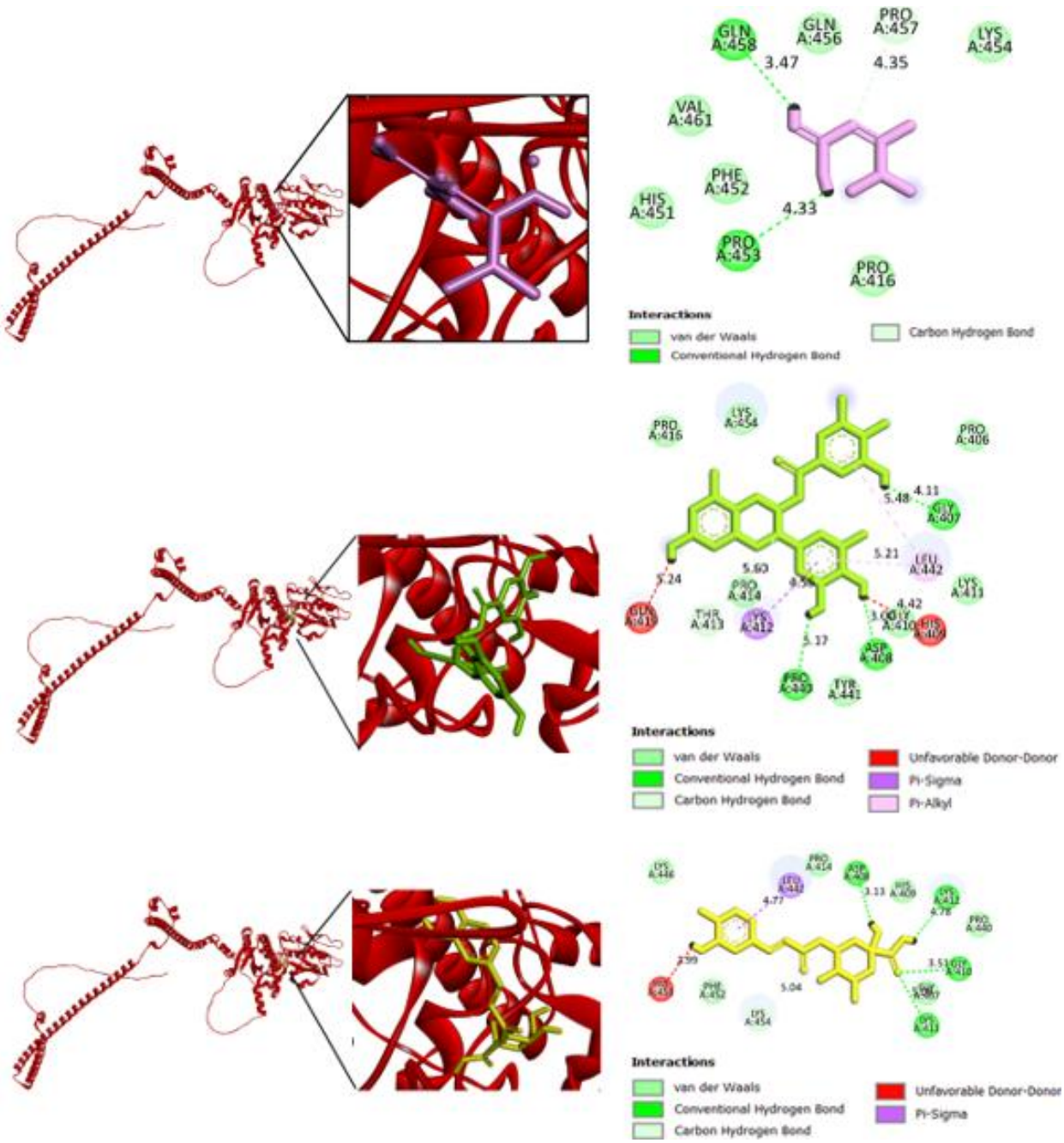

Figure 3. 3D and 2D interaction of amino acid residue of THOC5 with: a) Metformin, b) EGCG, c) CGA.

binding pocket. This binding may induce various conformational changes, such as the movement of loops or domains of the protein, which can affect the functional integrity of the active site [45]. Furthermore, both EGCG and CGA formed multiple hydrogen bonds with shared residues, supporting competitive binding and enhanced stability. Two compounds will be antagonistic or synergistic, depending on the target or pathway with which they interact [46]. However, differences in hydrophobic interactions suggest that these compounds may also engage distinct binding regions to activate other signaling pathways. Accordingly, the combined use of these compounds remains a potential strategy that warrants further investigation.

Molecular Dynamics

Comprehensive prediction of EGCG and CGA interaction with E47 was analyzed using molecular dynamics simulations as shown in Figure 4, including RMSD of the carbon atom (Ca), RMSD of the atom backbone (Bb), RMSD of protein–ligand complex stability (All), and RMSD of ligand movement stability (LigMove). An RMSD value equal to or less than 3 Å is considered favorable, while a value of more than 3 Å is deemed suboptimal for validating the stability of the molecules. The RMSD Ca and Bb values exceeded 3 Å, indicating relatively low structural stability of all simulated complexes. There was no significant difference between the compounds and the control. However, RMSD All showed that

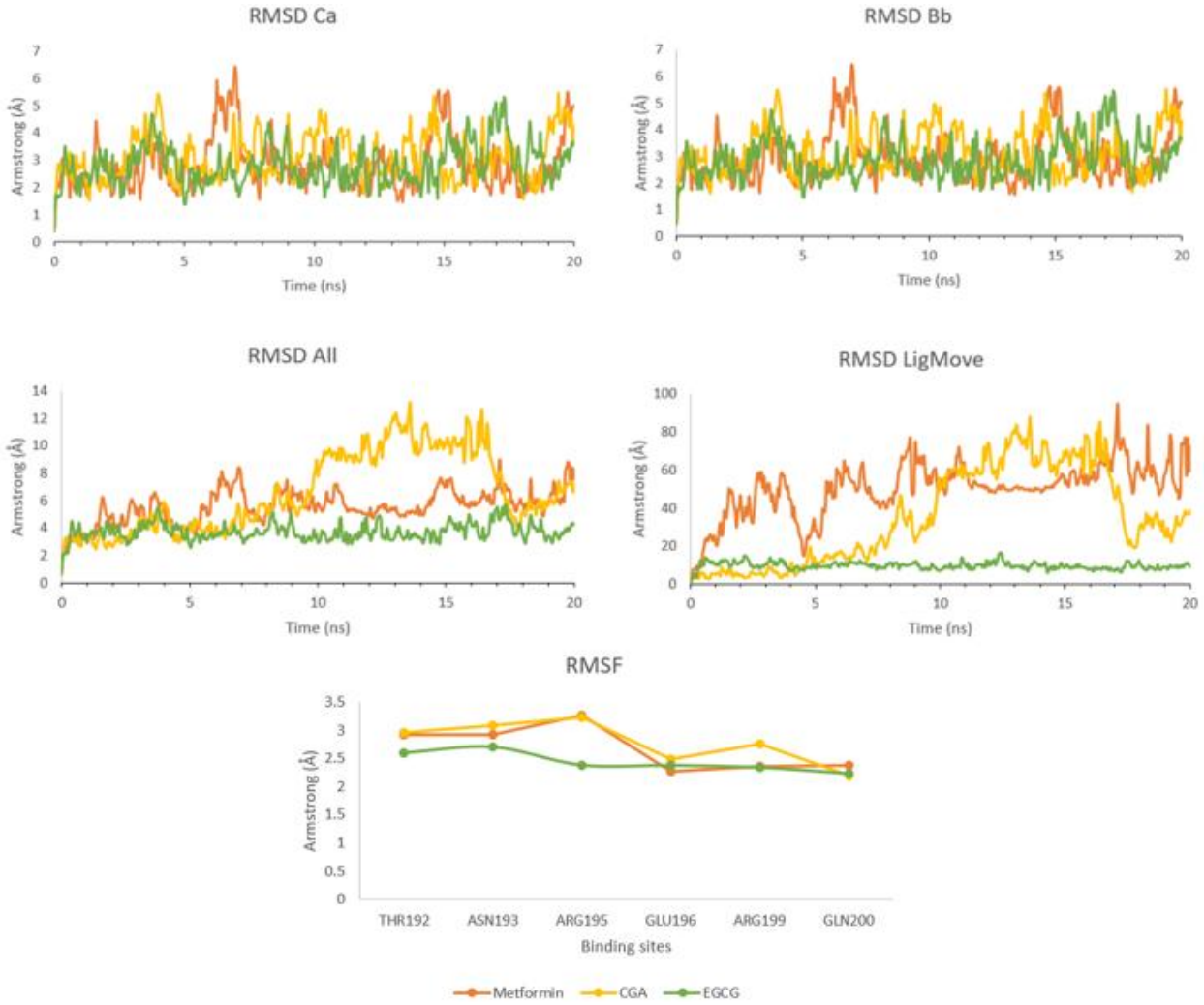

Figure 4. Molecular dynamics of EGCG, CGA, and metformin with E47. RMSD Ca: alpha carbon atom deviation; RMSD Bb: backbone deviation; RMSD All: all-atom complex deviation; RMSD LigMove: ligand movement within the binding site; RMSF: residue-level fluctuation. Lower values indicate greater structural stability.

EGCG and metformin displayed more stable binding conformations with E47 compared to CGA. Consistently, the movement of EGCG tended to remain stable during the 20 ns simulation, as reflected by low RMSD LigMove values and minimal fluctuations. In contrast, CGA showed higher LigMove fluctuations (>60 Å), indicating significant conformational changes during the simulation.

The stability of each residue was further evaluated using RMSF analysis of the DNA-binding site of E47 to identify regions with higher rigidity that contribute to overall molecular motion. Overall, EGCG exhibited the lowest average RMSF values and the least fluctuations compared to CGA and metformin, indicating more stable interactions between amino acid residues and E47. In contrast, CGA and metformin showed higher RMSF values with no significant difference, suggesting less optimal binding to the target residues.

These findings supported the docking results, showing that EGCG exhibits relatively stable binding behavior with lower structural fluctuations compared to CGA and metformin. The high RMSD LigMove value observed for CGA (>60 Å) indicates significant conformational fluctuations, which may reflect reduced binding stability. However, this may also be attributed to the intrinsic flexibility, characterized by multiple rotatable bonds and polar functional groups. Such flexibility may allow dynamic repositioning within the binding pocket rather than complete dissociation. Therefore, although CGA demonstrated favorable binding affinity in docking analysis, its stability may require further optimization. Potential strategies include structural modification to reduce molecular flexibility or enhance interactions with key residues, as well as extended molecular dynamics simulations and binding free energy calculations (e.g., MM-PBSA) to better characterize ligand stability and binding strength [47–49].

Nevertheless, this study has several limitations. Proteins in an activated state adopt various conformations depending on the ligand they bind. Consequently, docking with a rigid protein will

only consider a single conformation, potentially leading to false negatives [50]. In the case of the E47 protein, a conformational change that might occur is activation or deactivation through phosphorylation. Due to the highly negative charge of the phosphate group, phosphorylation of a protein can modify its charge, thereby affecting the protein's conformation and functional activity, including the active site. These mechanisms are not accounted for in the present docking approach, as the interactions were evaluated only in the unactivated state. Further studies should conduct multi-ligand docking to explore potential synergistic effects of combined compounds and discover new, unrevealed pathways in drug discovery.

CONCLUSION

This study demonstrates that EGCG and CGA exhibit favorable binding affinity toward THOC5 and E47, suggesting their potential to modulate macrophage differentiation associated with inflammation. Interaction analysis revealed that both compounds bind to key residues within the predicted active sites, indicating possible involvement with transcriptional and post-transcriptional mechanisms. However, limitations such as rigid docking assumptions and the relatively high structural fluctuations during molecular dynamics simulations should be considered. In addition, the low predicted bioavailability highlights the need for further optimization. Future studies should include in vitro and in vivo validation, as well as extended computational analyses, to confirm the molecular mechanisms and therapeutic potential of these compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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