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Molecular Docking Analysis of Hydroxy Chalcones and Flavones from Anisaldehyde and Veratraldehyde as EGFR Inhibitors: Predicting Anticancer Potential

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ABSTRACT. This study aimed to investigate the potential of hydroxy chalcone and flavone derivatives as inhibitors of the epidermal growth factor receptor (EGFR) with anticancer properties. Molecular docking simulations were conducted using Autodock Tools 1.5.6 and Discovery Studio visualizer. The EGFR protein structure with the PDB code 1M17 was utilized as the receptor, explicitly targeting the binding pocket. Redocking of the reference ligand erlotinib yielded a binding energy of -7.51 kcal mol^{-1} with an RMSD of 0.54 Å, confirming the accuracy of the docking protocol. The hydroxy chalcone and flavone derivatives exhibited binding energies ranging from -6.50 to -7.67 kcal mol^{-1} when interacting with the EGFR protein. Among the studied compounds, compound 2',5'-dihydroxy-3,4-dimethoxychalcone (1g) displayed the lowest binding energy. Interactions involving amino acids such as Met769, Ala719, Thr766, Lys721, and Glu738 were identified as crucial hydrogen bonding interactions between the ligands and the EGFR protein. These findings suggest that 2',5'-dihydroxy-3,4-dimethoxychalcone holds strong potential as a tyrosine kinase inhibitor, positioning it as a promising candidate for further development as an anticancer agent. The outcomes of the computational analysis conducted through the pkCSM online platform indicated that the chemical 2',5'-dihydroxy-3,4-dimethoxychalcone had favorable pharmacokinetic characteristics and showed low toxicity levels.

Keywords: molecular docking, hydroxy chalcone, flavone, egfr, ADMET

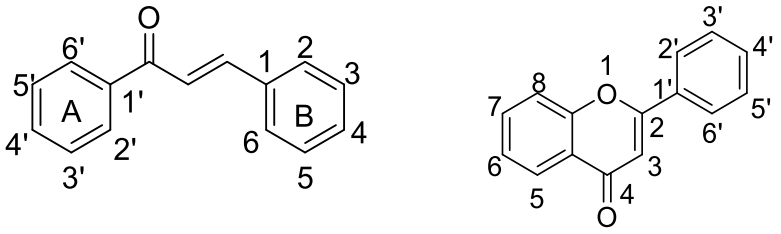
INTRODUCTION

Cancer is a leading cause of death worldwide. In 2020, there were 19.2 million cancer cases (WHO, 2020). Cancer cells attack and destroy surrounding tissues before spreading to other parts of the body (Vadlakonda et al., 2017). Protein tyrosine kinase (PTK) controls cell growth and apoptosis. Alterations in PTK function lead to irregular cell proliferation (Wei et al., 2008). Tyrosine kinase inhibitors offer promising breakthroughs in anti-angiogenesis and innovative cancer therapy models.

Tyrosine kinase inhibitors like the Epidermal Growth Factor Receptor (EGFR) have gained recognition for their essential role as anticancer agents (Al-Anazi et al., 2022; Yewale et al., 2013). EGFR regulates cancer cell survival (Hasibuan et al., 2021), and abnormal EGFR activity contributes to cancer cell formation (Safdari et al., 2015). A potential strategy in cancer treatment is to suppress EGFR kinase activity using small-molecule drugs (Al-Anazi et al., 2022). The critical role of EGFR in cancer proliferation involves blocking its binding sites, making it an effective anticancer therapy (Ciardiello & Tortora, 2003). Tyrosine kinase inhibitors like erlotinib can inhibit EGFR activity by binding to the ATP binding site through hydrogen bonding and disrupting EGFR signaling (Ismail et al., 2016).

Understanding of EGFR inhibitors continues to evolve due to gene mutations. The first generation of inhibitors consists of gefitinib and erlotinib, whereas the second generation includes afatinib and dacomitinib. Osimertinib is classified as a third-generation inhibitor. (Li et al., 2023; Xu et al., 2023). Jeon et al. (2023) are developing fourth-generation EGFR inhibitors based on the 7H-pyrrolo[2,3-d]pyrimidine derivative compounds.

Molecular docking approaches accurately predict ligand-protein interactions and conformations (Golik et al., 2021; Huey et al., 2007). Stable complexes formed indicate that molecular docking predicts the orientation and interactions of compounds with molecules. The binding energy between the target molecule and receptor determines the optimal conformation of the compound (Murthy & Narsaiah, 2019). The AutoDock program can predict and generate 3D structures resulting from ligand-protein interactions (Muthuvel et al., 2018; Sousa et al., 2006).



(a) (b)

Figure 1. General structure (**a**) chalcone, (**b**) flavone

Erlotinib is one of the most commonly used EGFR inhibitors, particularly in the treatment of cancers with EGFR gene mutations, such as non-small cell lung cancer and pancreatic cancer (Sun et al., 2022). The evaluation of a compound's activity as an anticancer drug can be determined by its capacity to suppress the activity of EGFR. (Mphahlele et al., 2018; Prajapati & Reddy, 2017; Syahri et al., 2020). Interactions between compounds and proteins primarily involve hydrogen bonding. Therefore, bioactive compounds must possess hydrogen bond acceptor groups capable of interacting with amino acid residues in the EGFR protein. Previous research has shown that derivatives of 3,4,5-trimethoxy- and 3,4-dimethoxychalcones can form hydrogen bonds with EGFR proteins (Danova et al., 2023).

Chalcones are important flavonoid compounds and intermediates in organic synthesis, including heterocyclic compounds such as flavones, flavanols, and flavanones. Chalcones and flavonoids have been extensively developed for the synthesis of their derivatives and evaluation of their pharmacological activities (Díaz et al., 2017; dos Santos et al., 2019; Fikroh et al., 2020; Kristanti et al., 2020; Kulkarni et al., 2013; Simon et al., 2015; Suma et al., 2019; Torrenegra et al., 2016; Yun et al., 2016). The chemical structure of chalcones affects their anticancer activity. Saturated Cα and Cβ bonds play a crucial role in anticancer activity (Zenger et al., 2015). Hydroxy and methoxy groups on the aromatic ring have been observed to influence the cytotoxicity exhibited against cancer cells (Dona et al., 2019; Suwito et al., 2015). Research by Anwar et al. indicates that hydroxy groups on the A ring can potentially act as anticancer agents. In addition to predicting ligand-protein interactions, computational chemistry enables the prediction of molecular chemical properties, pharmacokinetic properties (ADME), and compound toxicity levels. This study aims to evaluate the influence of hydroxy and methoxy groups on chalcone and flavonoid compounds on their biological activity through molecular docking. This research will investigate the complex structures of hydroxychalcones and flavonoids in the active site of the EGFR protein. Furthermore, this study will assess the pharmacokinetic properties and toxicity levels of potential drug candidates.

EXPERIMENTAL SECTION

Material

The crystal structure of the EGFR kinase domain (PDB: 1M17) was obtained from the Protein Data Bank (PDB) (Gunasekaran et al., 2023; Stamos et al., 2002). For this study, the computational analysis was performed using an ASUS system with an Intel Core i3-7020U CPU @ 2.30 GHz and 8.00 GB RAM. The operating system utilized was 64-bit OS Windows 10 Home. A series of hydroxy chalcone and flavone derivatives (**Figure 2**) were employed as experimental ligands.

Preparation of protein receptor and ligand

The X-ray crystallographic structure of the protein EGFR bound to 4-Anilinoquinazoline (AQ4) was acquired from the Protein Data Bank (PDB). The receptor molecules were prepared using Chimera 1.13 software. The UCSF Chimera program was used to complete the missing atoms in the EGFR protein's 3D structure, and the modified structure was saved in PDB format. The modified configuration was stored in PDB format. In the Chimera software, a uniform orientation was chosen and subsequently reversed for all molecular entities. Subsequently, the selected molecules were deleted, and the resulting structure was saved in PDB format. To obtain the ligand, first select the native ligand AQ4, then choose 'invert all molecules.' Subsequently, the selected molecules should be removed and the ligand outcomes ought to be preserved in mol^2 format.

Molecular docking study

The redocking process was assessed using the native ligand AQ4 (erlotinib) as the reference ligand with AutoDock Tools 1.5.6. The docking parameters used by the genetic algorithm were set to default values with 40 GA runs. The number of points in the xyz dimension used was 40, with the coordinates of the central grid point in the maps being 22.908, -0.226, 53.420. A successful redocking process was considered when the Root Mean Square Deviation (RMSD) value was below 2 Å (Huey et al., 2007). Once the redocking method was validated, the sixteen molecules were docked onto the protein EGFR. The AutoDock scoring function was utilized to predict the binding affinity between the ligands and the receptor.

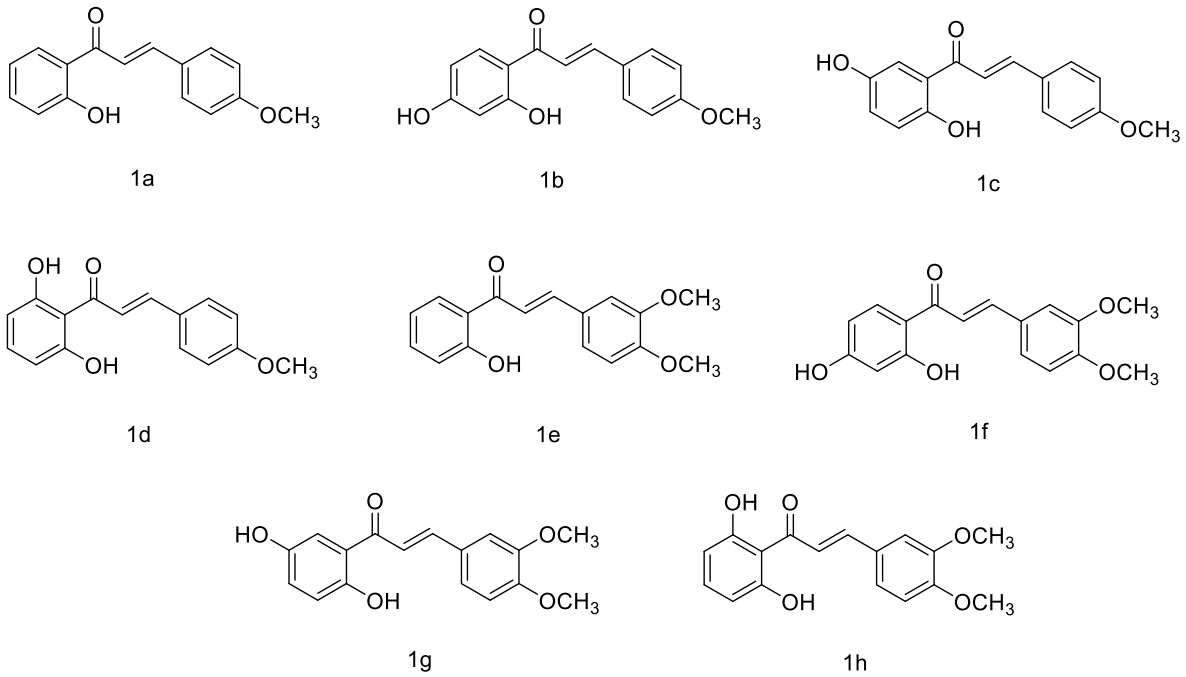


Figure 2. Chemical chalcone structures in the research. (1a) 2'-hydroxy-4-methoxychalcone, (1b) 2',4'-dihydroxy-4-methoxychalcone, (1c) 2',5'-dihydroxy-4-methoxychalcone, (1d) 2',6'-dihydroxy-4-methoxychalcone, (1e) 2'-hydroxy-3,4-dimethoxychalcone, (1f) 2',4'-dihydroxy-3,4-dimethoxychalcone, (1g) 2',5'-dihydroxy-3,4-dimethoxychalcone, (1h) 2',6'-dihydroxy-3,4-dimethoxychalcone.

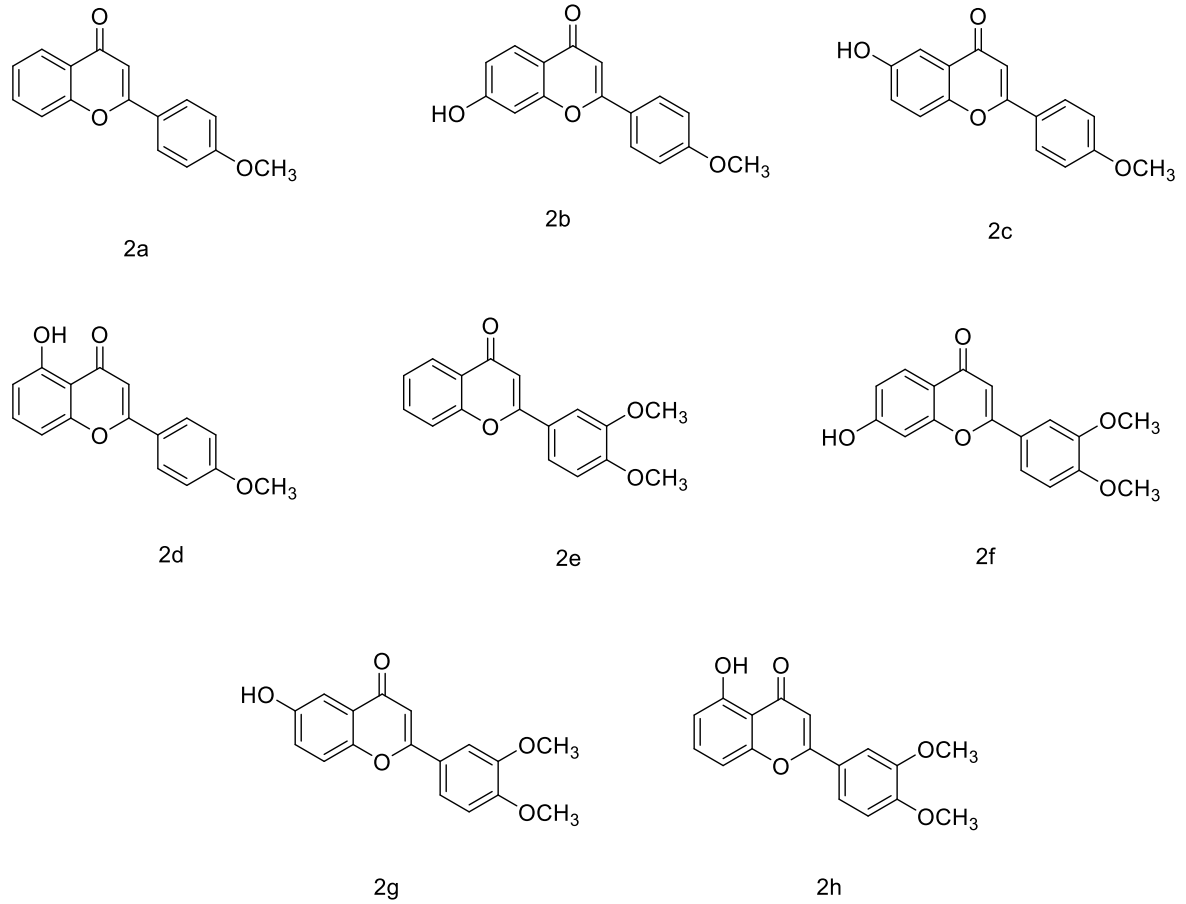


Figure 3. Chemical flavone structures in the research. (2a) 4'-methoxyflavone, (2b) 7-hydroxy-4'-dimethoxyflavone, (2c) 6-hydroxy- 4'-methoxyflavone, (2d) 5-hydroxy- 4'-methoxyflavone, (2e) 3',4'-dimethoxyflavone, (2f) 7-hydroxy-3',4'-dimethoxyflavone, (2g) 6-hydroxy-3',4'-dimethoxyflavone, (2h) 5-hydroxy-3',4'-dimethoxyflavone.

The confirmation with the lowest binding energy was deemed the most favourable for the ligand. The Discovery Studio visualizer program was employed to observe the interaction between ligands and amino acid residues.

ADMET Prediction and Physicochemical Properties

This study conducted ADMET prediction and assessed various physicochemical properties, including Molecular Weight (MW), the logarithm of the octanol/water partition coefficient (Log P), Polar Surface Area (PSA), Hydrogen Bond Donors (HBD), and Hydrogen Bond Acceptors (HBA). We employed the pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/prediction) for these predictions. The same tool was also used to assess pharmacokinetic properties related to absorption, distribution, metabolism, and excretion (ADME). To evaluate the oral toxicity, specifically LD50, following the globally harmonized system (GHS) guidelines, we employed the Protox online tool (https://tox-new.charite.de/protox_II).

RESULTS AND DISCUSSION

Redocking Analysis

EGFR plays a pivotal role in various physiological processes, including epithelial tissue formation, homeostasis, and tumor cell evolution (Sigismund et al., 2018). Due to its significant role in cell biology, EGFR has been pinpointed as a potential target in the development of anticancer drugs aiming to inhibit cancer cell proliferation. In the context of this research, the focus was placed on the EGFR protein with the PDB code 1M17 (Danova et al., 2023; Suma et al., 2019).

The redocking procedure allows researchers to gain deeper insights into binding energy and the native ligand conformation when interacting with EGFR. Consequently, redocking serves as a validation tool to evaluate the accuracy and reliability of the molecular docking method applied in this study, ensuring that the adopted approach is appropriate.

The Chimera program was utilized in the experimental series to model the EGFR receptor and the native ligand, erlotinib. The insertion of erlotinib into the EGFR model facilitated the visualization of the native ligand binding pose with the protein. **Figure 4** depicts the AQ4 structure overlapping before and after the docking procedure. Based on the redocking results, erlotinib exhibited a binding energy of -7.51 kcal/mol and an RMSD value of 0.54 Å. A lower RMSD value suggests that the docked ligand is positioned closely to the protein target binding site, similar to the native ligand (Huey et al., 2007). The binding energy parameter highlights the quality of interaction between the ligand and its protein receptor, with a lower value indicating a more stable interaction. These data solidify the reliability and accuracy of the Autodock program used in this research.

Furthermore, redocking analysis results identified that erlotinib, the native ligand, interacts with the amino acid residues Met769 and Cys773 at the EGFR binding site. The outcome was consistent with earlier research by Sanduja et al. (2020), Suma et al. (2019), and Gundogdu (2023), which reported hydrogen bonding interactions between the ligand and the Amide nitrogen of Met769 and the oxygen of Cys773. **Figure 5** illustrates these interactions, providing further evidence of a binding pattern consistent with previous studies. Specifically, MET769 and CYS773 were found to form conventional hydrogen bonds with erlotinib, with bond lengths of 1.78 and 1.91Å, respectively.

Docking study of hydroxychalcone and flavone derivates

Molecular docking is vital in forecasting how binding mechanisms and ligand affinities work within protein structures, thereby simplifying the analysis of ligand-protein interactions. In this study, we explore the interactions between EGFR and chalcones (1a-1h) and flavones (2a-2h), particularly in terms of binding energy. All compounds were docked into the EGFR protein at the same site as the reference ligand AQ4. Table 1 presents the binding affinity ranges of chalcones and flavones to EGFR, ranging from -6.23 to -7.67 kcal/mol. These values were then compared to molecular docking data with the EGFR inhibitor, erlotinib, which has an energy of -7.51 kcal/mol.

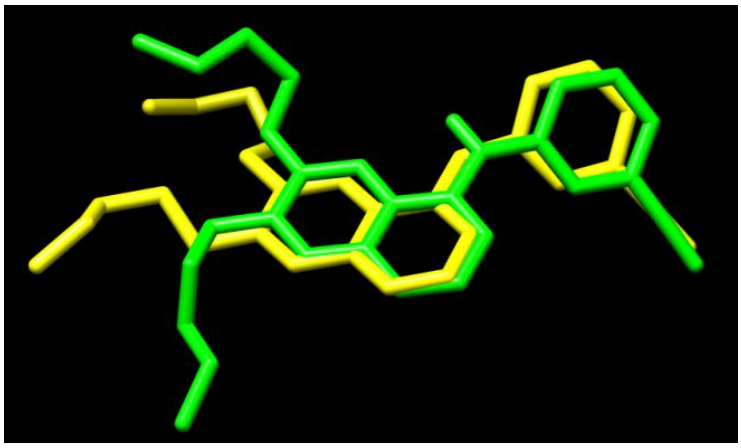
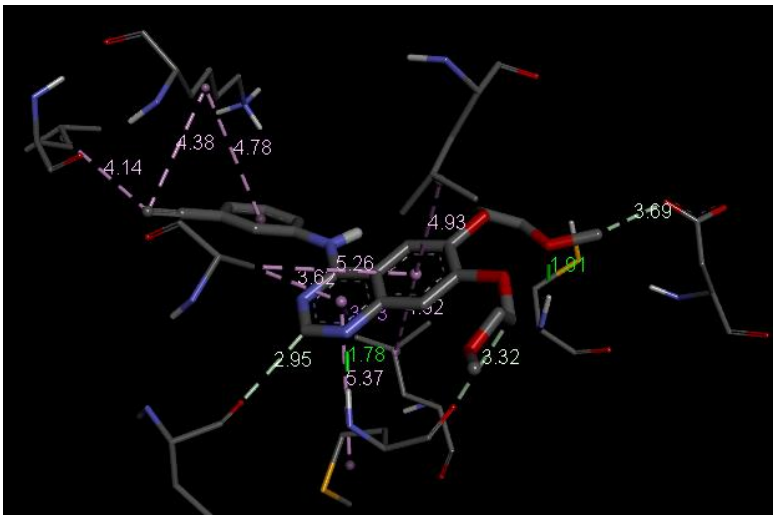
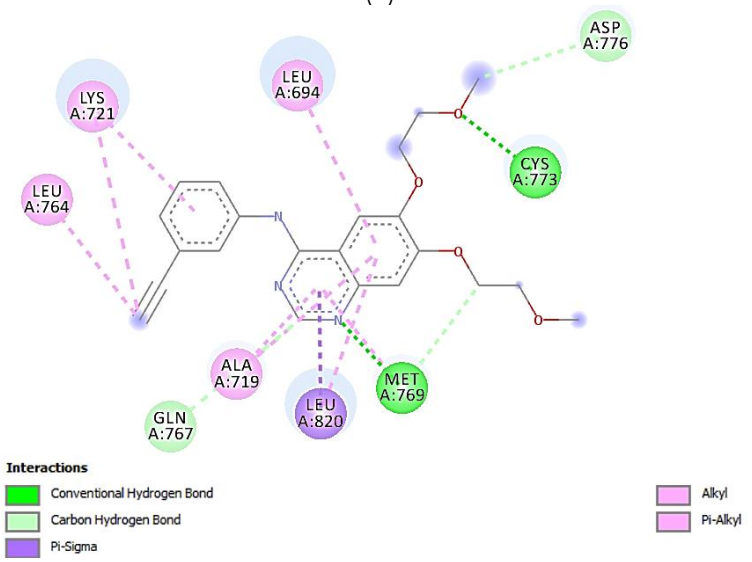


Figure 4. The overlapping structure of AQ4 (erlotinib) before docking (yellow) and after docking (green).



(a)



(b)

Figure 5. The visualization of hydrogen bonding between Erlotinib active site of EGFR (**a**) 3D visualization and (**b**) 2D visualization.

Table 1. Docking results for compounds and erlotinib with EGFR

Entry	compounds	Binding Energy (kcal/mol)	Hydrogen Bond	RMSD (Å)
1	1a	-6.91	MET769, LYS721, ASP831	0,08
2	1b	-6,81	MET769, LYS721	1,50
3	1c	-7.66	MET769, LYS721, GLU738, THR766	0,12
4	1d	-7.11	MET769, LYS721, GLU738, ALA719	0,16
5	1e	-6.75	MET769, LYS721	1,47
6	1f	-6.50	MET769, LYS721	1,62
7	1g	-7.67	MET769, LYS721, GLU738, THR766, ALA719	0,15
8	1h	-7.11	MET769, LYS721, THR766, ASP831	1,35
9	2a	-7.14	Met769, LYS721	1,70
10	2b	-6,58	MET769, LYS721	0.09
11	2c	-6.98	MET769, LYS721	0,86
12	2d	-6.57	MET769, LYS721	0,31
13	2e	-7.25	MET769, LYS721	0,96
14	2f	-7,18	MET769, LYS721	1,41
15	2g	-7.28	MET769, LYS721	1,22
16	2h	-6.23	MET769, CYS773,THR766, GLN767	0,1
17	AQ4(Erlotinib)	-7.51	Met769, Cys773	0.54

Based on the data in **Table 1**, the binding energies of hydroxychalcone and flavone are within the range of -6.23 to -7.67 kcal/mol. Among all compounds, 1g exhibits the lowest binding energy for chalcones at -7.67 kcal/mol, while for flavones, compound 2g has a binding energy of -7.28 kcal/mol. The difference in binding energy between 1g and 2g can be associated with the structural characteristics of chalcone and flavone compounds. Chalcone molecules have an open molecular structure, allowing for greater conformational flexibility compared to flavones, which possess a closed molecular structure with three cyclic rings, resulting in a more rigid conformation. Limited molecular movement in 2g may restrict its ability to form favorable interaction conformations with the target receptor, resulting in higher binding energy than 1g.

3D interactions between compounds 1g and 2g and the receptor were visualized using the Discovery Studio Visualizer program. The interactions of compounds 1g and 2g with amino acid residues are depicted in **Figure 6**. This figure illustrates these compounds' specific interactions and binding poses within the receptor binding site. These interactions provide insights into hydrogen bonding contributions to ligand stability and affinity with the receptor. Amino acid residue MET769 forms a conventional hydrogen bond with compound 1g with a bond length of 1.99 Å. In addition to MET769, conventional hydrogen bond interactions with compound 1g include ALA719, THR766, LYS721, and GLU738 with bond lengths of 3.02 Å, 2.14 Å, 2.90 Å, and 1.90 Å, respectively. Compound 2g interacts with amino acid residues MET769 and LYS721 with bond lengths of 1.91 Å and 2.20 Å, respectively. This result indicates that compounds 1g and 2g exhibit stable interactions with the receptor. These interactions further confirm the accurate molecular binding positions, as the redocking results support.

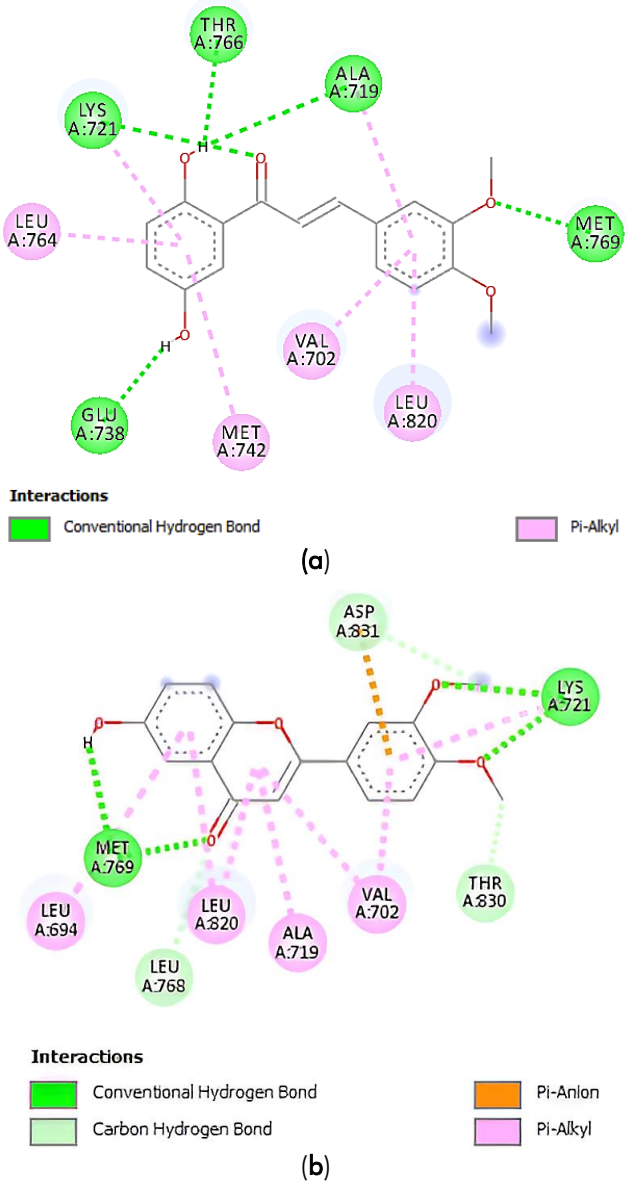


Figure 6. Interactions residue amino acid of compound (a) 1g and (b) 2g.

Compound 1c, with two hydroxy and one methoxy substituent, has a binding energy of -7.55 kcal/mol. This binding energy is slightly higher than compound 1g with two hydroxy and two methoxy substituents. Meanwhile, compound 1e, with one hydroxy and two methoxy substituents, has a binding energy of -6.75 kcal/mol. This binding energy is higher than that of both 1e and 1f. Based on the structures of chalcones 1c, 1e, and 1g, hydroxy and methoxy groups can lower the binding energy.

In flavones, the presence of methoxy and hydroxy groups significantly affects binding energy. Compound 2c, with one hydroxy and one methoxy substituent, has a binding energy of -6.98 kcal/mol. This binding energy is higher than compound 2g, which has one hydroxy and two methoxy substituents. Compound 2e, with two methoxy groups, exhibits a binding energy of -7.25 kcal/mol. The binding energy of 2e is lower than that of 2c but higher than that of 2g. The presence of methoxy and hydroxy groups in flavones 2c, 2e, and 2g structures can reduce binding energy. Lower binding energy indicates better molecular activity (Gundogdu, 2023). Compared to chalcone compounds, flavon has a higher binding energy, indicating lower anticancer activity than chalcone. Previous research also suggests that chalcone compounds are more active than flavon (Bano et al., 2013; Cabrera et al., 2007).

These results indicate that the presence of methoxy and hydroxy groups in chalcones and flavones can impact their potential as anticancer agents. These functional groups likely contribute to the occurring interactions and enhance binding affinity with the target receptor. This suggests that the appropriate combination of methoxy and hydroxy groups can enhance the molecular interactions of chalcone compounds in inhibiting the target receptor for potential anticancer applications.

Chemical computations can evaluate the properties of compound 1g based on Lipinski's rule of five, a well-known guideline for connecting chemical structure to biological activity. According to this principle, a compound must meet certain parameters, including LogP ≤ 5, HBD (hydrogen bond donor) ≤ 5, HBA (hydrogen bond acceptor) ≤ 10, and MW (molecular weight) ≤ 500, to pass through cell membranes and demonstrate good absorption through passive diffusion in the digestive tract. Moreover, liposolubility (LogP) is a crucial measure in absorption, distribution, metabolism, excretion, and toxicity (ADMET) processes. It serves as a fundamental characteristic for assessing the oral bioavailability of potential therapeutic candidates (Hadda et al., 2013). Compound 1g complies with these rules without any violations, as indicated in **Table 2**.

Based on the information in **Table 2**, compound 1g exhibits optimal characteristics, with an intestinal absorption rate (in humans) reaching 93.83%, indicating a superior absorption efficiency (Chander et al., 2017). Moreover, the log BB value of compound 1g, which is recorded at 0.046, surpasses the threshold of 0.3, suggesting the potential of the compound to penetrate the blood-brain barrier. The metabolic process can be evaluated through its interaction with the cytochrome P450 (CYP) enzyme. In this context, cytochrome CYP2D6 is identified as representative of the cytochrome P450 family. Data from Table 2 confirms that compound 1g neither interferes with nor inhibits the CYP2D6 enzyme activity, indicating that the P450 enzyme family likely metabolizes the compound derivatives.

Table 2. The pkCMS software is utilized to estimate the absorption, distribution, metabolism, and excretion (ADME) features of the compounds that have been developed. Additionally, the ProTox-II tool is employed to assess the toxicity of these compounds.

Properties	Compound 1g
Molecular Weight	300.31
LogP	3.0111
HBA	5
HBD	2
PSA (A^2)	127.601
Intestinal absorption (human) (%)	93.835
Skin permeability (log Kp)	-2.744
VDss (log L/kg)	-0.433
BBB permeability (log BB)	0.046
CYP2D6 substrate	No
CYP2D6 inhibitior	No
Total Clearance (log ml/min/kg)	0.173
Renal OCT2 substrate	No
AMES toxicity	Yes
LD50 (mol/kg)	2.249

Furthermore, the analysis related to the excretion process of the compound is derived based on the Total Clearance parameter and the constant of the Renal Organic Cation Transporter 2 (OCT2) substrate. As illustrated in Table 2, the total clearance value stands at 0.173, serving as an indicator to estimate the excretion rate of the compound. Significantly, compound 1g does not affect the renal OCT2 substrate activity, implying that the compound derivatives do not act as an OCT2 substrate.

Lastly, the Ames Toxicity Test is often employed to assess potential toxicity. As per the predictions stated in **Table 2**, compound 1g exhibits mutagenic characteristics. Additionally, analysis through ProTox-II records the LD50 value of compound 1g at 2249, categorizing it into toxicity class V, indicating a high risk when the compound is ingested.

CONCLUSIONS

Based on simulation results, derivatives of chalcone and flavone have been identified as promising anticancer agents. The presence of hydroxy and methoxy groups has been proven to exert a significant impact on the binding affinity of these compounds to the EGFR protein. Among the compounds studied, compound 1g demonstrated the highest binding affinity with an energy value of -7.67 kcal/mol, indicating a stable interaction with EGFR. These findings highlight the potential of compound 1g as a leading candidate for the development of targeted EGFR anticancer therapies. While the ADMET computational predictions for compound 1g are favorable, it's imperative to note that further experimental and in vivo validation is required to confirm the therapeutic efficacy and safety profile of compound 1g. In-depth investigations in this context will significantly contribute to developing effective and safe EGFR-targeted anticancer drugs.

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