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ORIGINAL ARTICLE / ÖZGÜN MAKALE

EVALUATION OF ZERUMBONE AS AN EGFR TYROSINE KINASE INHIBITOR BY MOLECULAR DOCKING METHOD

MOLEKÜLER YERLEŞTİRME YÖNTEMİYLE ZERUMBONUN EGFR TİROZİN KİNAZ İNHİBİTÖRÜ OLARAK DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *EGFR-TK domain is of great importance in the initiation and progression of various cancer types, especially lung cancer. The existing EGFR-TK inhibitors have numerous side effects, which make them improper to be utilized as cancer therapeutics. In this study, we aimed to analyze the activity of zerumbone as an anticancer agent targeting EGFR by molecular docking approach and to evaluate its activity in comparison with curcumin.*

Material and Method: *MEP and HOMO-LUMO analyses were achieved at B3LYP/6-31G(D,P) level to evaluate electrostatic interactions that affect binding of EGFR with zerumbone and curcumin. Their binding energies were determined by molecular docking and compared with erlotinib as reference ligand.*

Result and Discussion: *Docking studies showed higher bindings (lower binding energy) for curcumin and zerumbone with binding energies -8.0 and -7.6 kcal/mol, respectively, compared to erlotinib (-7.3 kcal/mol). However, there is no significant difference between them. The ΔE energy gap of zerumbone was 5.09 eV which implies that this compound has more stability in comparison with curcumin (ΔE=3.68 eV) and erlotinib (ΔE=4.29eV). Also, zerumbone showed strong hydrogen bond interactions with EGFR, making it candidate as EGFR inhibitor, as did both in curcumin and erlotinib. It was concluded that zerumbone may have potential for inhibitory activity against EGFR-TK.*

Keywords: *Curcumin, epidermal growth factor receptor, lung cancer, molecular docking, zerumbone.*

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ÖZ

Amaç: *EGFR-TK bölgesi, başta akciğer kanseri olmak üzere çeşitli kanser türlerinin başlaması ve ilerlemesinde büyük önem taşımaktadır. Mevcut EGFR-TK inhibitörlerinin çeşitli yan etkilerinin varlığı, onların uygun kanser terapötikleri olarak kullanılmalarını sınırlandırmaktadır. Bu çalışmada, moleküler yerleştirme yaklaşımı ile EGFR'yi hedef alan bir antikanser ajan olarak zerumbonun aktivitesini analiz etmeyi ve aktivitesini kurkumin ile karşılaştırmalı olarak değerlendirmeyi amaçladık.*

Gereç ve Yöntem: *EGFR'nin zerumbon ve kurkumin ile bağlanmasını etkileyen elektrostatik etkileşimleri değerlendirmek için B3LYP/6-31G(D,P) seviyesinde MEP ve HOMO-LUMO analizleri yapıldı. Bağlanma enerjileri moleküler yerleştirme ile belirlendi ve referans ligand olarak erlotinib ile karşılaştırıldı.*

Sonuç ve Tartışma: *Yerleştirme çalışmaları, erlotinib (-7.3 kcal/mol) ile karşılaştırıldığında, sırasıyla -8.0 ve -7.6 kcal/mol bağlanma enerjileriyle kurkumin ve zerumbon için daha yüksek bağlanmalar (düşük bağlanma enerjisi) gösterdi. Ancak aralarında önemli bir fark gözlenmedi. Zerumbonun ΔE enerji aralığı 5.09 eV olarak bulundu. Bu sonuç, bu bileşiğin kurkumin (ΔE=3.68 eV) ve erlotinib ile karşılaştırıldığında (ΔE=4.29 eV) daha fazla kararlılığa sahip olduğu anlamına gelmektedir. Ayrıca, zerumbon EGFR ile güçlü hidrojen bağı etkileşimleri gösterdi ve bu da onun hem kurkumin hem de erlotinib'deki gibi EGFR inhibitörü olarak potansiyeli olduğunu göstermiştir. Zerumbonun EGFR-TK'ye karşı inhibitör aktiviteye sahip olabileceği sonucuna varıldı.*

Anahtar Kelimeler: *Akciğer kanseri, epidermal büyüme faktörü reseptörü, kurkumin, moleküler yerleştirme, zerumbon.*

INTRODUCTION

Lung cancer is one of the main causes of cancer-linked deaths worldwide [1]. Its overall 5-year survival rate is 22.9%, with a median survival time of 12 months [2]. Lung cancer is mainly known to have two groups: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). While NSCLC constitutes 80% of all lung cancers, SCLC accounts for 15% and other cancers for 5%. The most common histological subtypes in NSCLC are adenocarcinoma, squamous carcinoma, and large cell carcinoma [3]. Lung cancer does not show obvious symptoms until it reaches the locally advanced or widespread disease stage.

Dysregulation of epidermal growth factor receptor (EGFR) signaling is often found in lung cancer and EGFR level is associated with advanced stage of the disease and poor prognosis. EGFR overexpression has been demonstrated in 40% to 80% of NSCLC biopsies and connected with shorter survival times of NSCLC patients [4–6].

EGFR belongs to the growth factor receptor tyrosine kinases (TKs) family. EGFR-TKs implicated in key cellular functions including cell proliferation, division, and differentiation as previously described. EGFR activation is concluded with activation of Raf/MEK/Erk, STAT, and P13k/AKT pathways leading to cell survival [7]. Since EGFR-driven signaling participate in lung tumorigenesis and tumor progression, targeting the inhibition of EGFR signaling can be a potential and effective therapeutic strategy for NSCLC.

In the drug development for cancer therapy, EGFR and its signaling components can be employed as targets. One of the therapy categories that targets TKs is tyrosine kinase inhibitors (TKIs- eg: gefitinib, erlotinib, and afatinib) [5,8]. Erlotinib (Tarceva®) from first-generation EGFR-TKIs has been approved by US FDA for the treatment of locally advanced or metastatic NSCLC in 2004 [9]. However, lung cancer treatment is often inefficient as a consequence of the drug resistance, reactions to treatment, drug-drug interactions or non-specific targeting of the anticancer drugs. This causes the need of new and more secure treatments. All the time, alternative medicine has been an intriguing field in medical research. Natural compounds show promise in lung cancer by the reason of the fact that having very few side effects [10]. The use of natural compounds including curcumin, capsaicin, eugenol, zerumbone, anethole, gambogic acid, diosgenin, thymoquinone alone and in combination with chemotherapy to inhibit disease progression have reviewed in several studies [11-13].

Zerumbone is an organic compound extracted from the rhizomes of Zingiber zerumbet Smith and

has been investigated for years [14,15]. Zerumbone is a sesquiterpene phytochemical that contains α, β-unsaturated carbonyl residues with three double bonds, one at the C-2 and the rest at the C-6 and C-9 positions. Recent studies have revealed that zerumbone has anti-cancer activity against many types of cancer (liver, cervix, ovary, lung etc.) by modulating several proteins to induce apoptosis and is considered as a promising therapeutic drug [15-18]. Despite potent anti-proliferative, anti-metastatic and pro-apoptotic properties against some types of cancer, exact molecular mechanism of zerumbone has not been fully understood [19].

The detection of specific cancer treatments is based on virtual techniques. Molecular docking is one of the best virtual screening techniques for targeting and processing multiple molecules participating the signaling pathway. In the present study, we aimed to analyze the activity of zerumbone as an anticancer agent targeting EGFR by molecular docking approach. Because it is considered that zerumbone may provide an alternative to erlotinib for cancer treatment. It is also aimed to evaluate the activity of zerumbone in comparison to curcumin which is a natural compound and has a potential with an anti-EGFR activity. So, the binding interactions of EGFR with zerumbone and curcumin were investigated by computer simulations such as molecular electrostatic potential analysis and molecular docking. Moreover, results were compared with the binding interactions of EGFR with erlotinib.

MATERIAL AND METHOD

The initial geometries of zerumbone and curcumin were drawn by Gauss View 5.0 [20], then they were optimized with DFT (Density Functional Theory) method in Gaussian 09 [21] (B3LYP [22,23] with the 6-31G(D, P) basis set).

For the purpose of understanding how electrostatic interactions affect binding of EGFR with zerumbone and curcumin, molecular electrostatic potential (MEP) and the highest occupied molecular orbital -lowest unoccupied molecular orbital (HOMO-LUMO) analyses were achieved at B3LYP/6-31G(D, P) level [24,25]. In the light of MEP analysis, the binding modes of EGFR for zerumbone and curcumin were searched by molecular docking. Molecular docking is powerful tool to examine receptor-ligand interactions. The best binding conformations between the receptor and ligand can be demonstrated by molecular docking analysis, which calculates the best binding energy using the following equation:

$$\Delta G = \Delta G_{vdW} + \Delta G_{Hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{desolv}$$

where the energy terms are represented as follows: van der Waals term ΔG_{vdW}, H-bonding term ΔG_{Hbond}, the electrostatic term ΔG_{elec}, torsional free energy term ΔG_{tor} when the ligand transits from the unbound to the bound state, and the desolvation term ΔG_{desolv}.

The inter-molecular interactions were calculated by selecting EGFR tyrosine kinase as receptor defined with Protein Data Bank (PDB) ID: 1M17 and zerumbone as ligand molecule (Figure 1). Later, curcumin having two tautomeric forms (keto and enol form) was chosen as ligand. In this stage, two forms were optimized at B3LYP/6-31G(D, P) level. Molecular docking analysis was performed with keto form having the lower energy obtained from optimization stage, as keto form possess has a lower energy of about 13 kcal/mol than enol form. Molecular docking studies were performed by AutoDockVina v.1.5.4 program [26]. First of all, in the arranging of the receptor and ligands MGL Tools v.1.5.4 have been used to convert into .pdbqt format [27]. Discover Studio Visualizer software was operated for visualization of interactions between the receptor and ligands [28]. Crystal data of the receptor was obtained from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank website [29]. In order to validate this molecular docking studies, cocrystal ligand erlotinib was redocked to EGFR (PDB ID: 1M17).

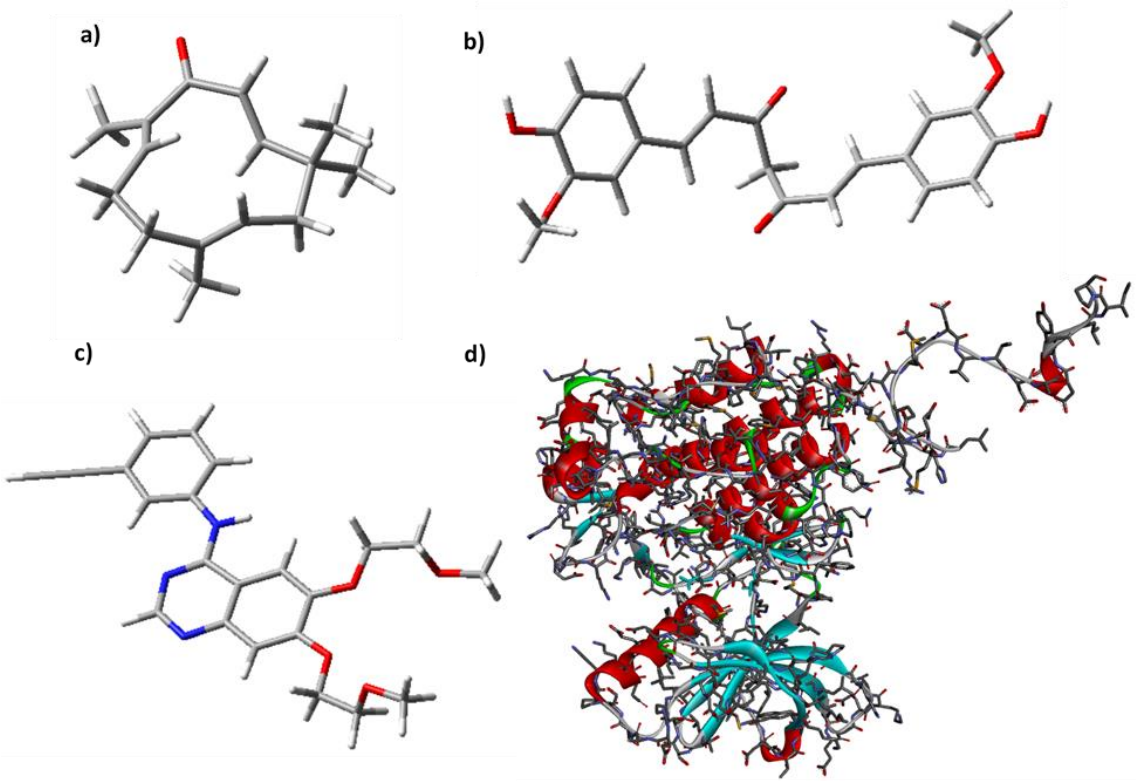

Figure 1. Optimized structures of zerumbone (a), curcumin (b) and (c) erlotinib at B3LYP/6-31G(D, P) level and the structure of 1M17* (d) (*obtained from the RCSB Protein Data Bank website [29]).

RESULT AND DISCUSSION

In the current study, the binding of zerumbone and curcumin to the tyrosine kinase domain in EGFR and inhibition of EGFR activity by molecular docking compared to that of erlotinib were discussed.

Electronic Properties of Zerumbone and Curcumin: Molecular Electrostatic Potential and HOMO-LUMO Analyses

MEP and HOMO-LUMO analyses was used to evaluate electronic properties of zerumbone and curcumin. MEP is a common tool to clarify the reactive behavior (electrophilicity, nucleophilicity), the recognition of biological processes and H-bonding interactions [25]. HOMO defines as the outer orbital containing electrons tends to give them as an electron donor, whereas LUMO can accept electrons and the LUMO energy is directly associated with electron affinity [24].

The oxygen atom of zerumbone has electron-rich site (corresponding to a red zone) with the highest negative potential which is in charge of the electrophilic attack, while the other parts of this molecule (green zone) have neutral areas, according to MEP diagram shown in Figure 2. While H atoms of phenolic hydroxyl in curcumin are responsible for nucleophilic attack which have the highest positive potential (blue zone), keto groups (C=O) of curcumin is in charge of electrophilic attack because of the highest negative potential.

The global reactivity parameters such as electronegativity (χ), chemical potential (μ), hardness (η), electrophilicity index (ω), and softness (σ) is computed by using Koopman's theorem [30]. While HOMO-LUMO distribution of zerumbone is the same trend, HOMO of curcumin is localized on the middle of the molecule and LUMO is distributed throughout the whole molecule. According to HOMO-LUMO analysis (Table 1), low electrophilicity index (ω) (low $|\mu|$ and high η values) of zerumbone means that its nucleophilic behavior is well. On the contrary, curcumin shows good electrophilic character with high $|\mu|$ and low η values.

Table 1. HOMO-LUMO energies (eV) and calculated global reactivity parameters of zerumbone, curcumin and erlotinib at B3LYP/6-31G(d,p) level.

Compound	LUMO	HOMO	ΔE(eV)	IP(eV)	EA(eV)	χ (eV)	η(eV)	σ $(eV)^{-1}$	μ(eV)	ω(eV)
Zerumbone	-1.107	-6.194	5.087	6.194	1.107	3.650	2.544	0.197	-3.650	2.619
Curcumin	-1.942	-5.617	3.675	5.617	1.942	3.780	1.837	0.272	-3.780	3.887
Erlotinib	-1.137	-5.432	4.295	5.432	1.137	3.284	2.148	0.233	-3.284	2.512

ΔE (E_{LUMO}-E_{HOMO}): Band gap, IP **(-HOMO)**: Ionization potential, EA **(-LUMO)**: Electron affinity, χ **(*I*P+E*A*)/2**: Electronegativity, η **(IP−EA)/2**: Chemical hardness, σ **(1/2η)**: chemical softness, μ **(−(IP+EA)/2)**: Chemical potential, ω **(μ^2 /2η)**: Electrophilic index.

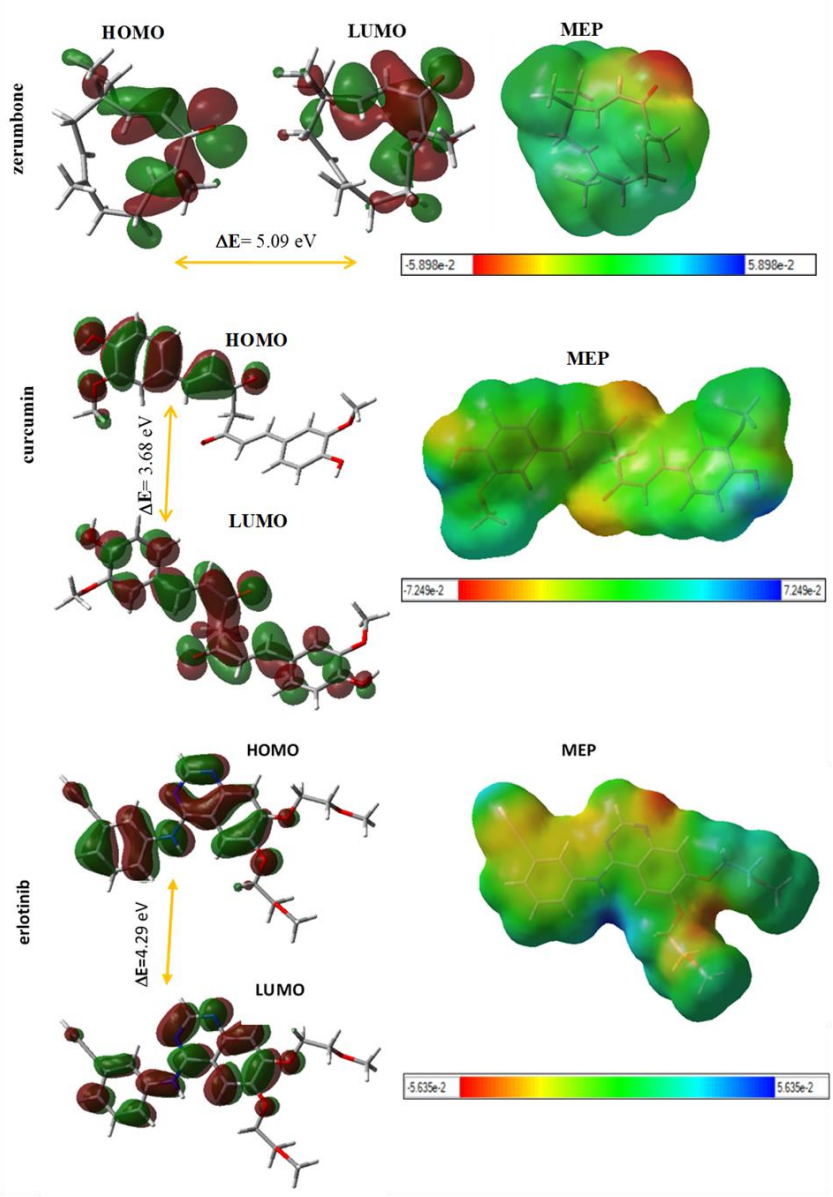

Figure 2. MEP and HOMO-LUMO drawing of zerumbone, curcumin and erlotinib.

Molecular Docking Studies

Researches on finding new therapeutic agents in the treatment of cancer, which aims to inhibit excessive cell proliferation immediately and hinder the metastatic process, has always been a crucial issue. Cellular proteins or receptors have suggested as targets for anticancer drugs because of their roles in cancer cell survival and disease progression. Receptor tyrosine kinases (RTKs) regulate essential cellular processes such as cell proliferation, division and differentiation. EGFR is a member of the family of growth factor RTKs. Upon EGFR activation, several signaling transduction cascades like PI3K/Akt, Raf/MEK/Erk, and STAT pathways are then activated and lead to cell survival [31,32]. EGFR has been associated with a large number of epithelial tumors such as breast, lung, brain, prostate, and liver cancer [6]. It has become an important target in cancer therapy [33]. Due to overexpression of EGFR in NSCLC, targeting EGFR signaling is a well-established strategy for treatment of NSCLC.

Many small molecules have been designed and synthesized for EGFR inhibition such as gefitinib, erlotinib, and lapatinib (the first-generation EGFR inhibitors) [34]. Erlotinib inhibits the EGFR kinase through binding to the ATP binding site and a H-bond is formed with the NH backbone of Met769 at the hinge region of EGFR [35].

Recently, researches on new molecules that inhibit this receptor are turning to natural substances. Zerumbone and curcumin as natural compounds appear to be related to EGFR inhibition as demonstrated in previous studies [33,36–38].

The molecular docking studies play a critical role in new drug discovery, determining the exact binding target and the molecular events that may happen between the drug and the target. This method has been successfully used in many studies [6,8,39]. Docking studies of zerumbone and curcumin were conducted on EGFR kinase to specify if it interacts with EGFR in resembling case to erlotinib.

The binding energy of zerumbone is -7.6 kcal/mol (Table 2) and constructs one strong inter-molecular H-bond between NH in residue MET769 of the EGFR and =O of the zerumbone molecule. This N–H···O type H-bond between the zerumbone and the EGFR is reasonably strong with the value of 1.80 Å (Table 3). Additionally, the carbon H-bond occurs between CH atom in residue LEU768 and =O atom of the zerumbone. Moreover, there are three alkyl interactions between electron group of alkyl group (ALA719, VAL702 and LEU820) and carbon atom on the opposite side of oxygen as shown in Figure 3. The other interactions are of the van der Waals type (shown light green). These results pointed out that both strong H-bond and other interactions played an important role in the binding of zerumbone with EGFR.

Table 2. The binding affinity and RMSD values of best poses of **zerumbone, curcumin** and **erlotinib** docked into the receptor **1M17.**

Best Mode	Affinity (kcal/mol)	Distance from best mode (Å)	
		RMSD l.b.	RMSD u.b.
zerumbone	-7.6	0.000	0.000
curcumin	-8.0	0.000	0.000
erlotinib	-7.3	0.000	0.000

Curcumin containing the functional groups like a 3,5- dione group, methoxyl and hydroxyl groups interact with EGFR through these groups. Curcumin has two H-bonds. One of them is between C=O of curcumin and the backbone NH of MET769, the other is between the phenolic OH group and COO^- oxygen of ASP831 (Figure 4). H-bond between C=O and the backbone NH of MET769 is stronger than the bond between the phenolic OH group and COO^- oxygen of ASP831, their values are 1.87 Å and 2.84 Å, respectively. In addition, the carbon hydrogen bond exists between COO^- oxygen in GLU738 and methyl carbon in methoxy group of ligand. The interaction of π-electron cloud over an aromatic group and electron cloud of alkyl groups (ALA719, VAL702 and LYS721) represents to π-alkyl interactions.

Moreover, π–π stacking is between phenyl ring of PHE699 and phenyl ring bonded methoxy of ligand (Table 3). The binding energy of curcumin is -8.0 kcal/mol (Table 2).

Table 3. Interactions, their species and distances between **zerumbone, curcumin** and **erlotinib** and the **1M17**.

Residue	**Ligand group**	**Distance (Å)**	**Interaction**
zerumbone			
NH atom in MET769	=O atom	1.80	Conventional hydrogen bond
CH atom in LEU768	=O atom	2.77	Carbon hydrogen bond
alkyl in ALA719	carbon	5.56	Alkyl
alkyl in VAL702	carbon	5.87	Alkyl
alkyl in LEU820	carbon	6.69	Alkyl
curcumin			
NH atom in MET769	=O atom	1.87	Conventional hydrogen bond
COO^- oxygen in ASP831	phenolic OH	2.84	Conventional hydrogen bond
COO^- oxygen in GLU738	methyl carbon in methoxy group	3.37	Carbon hydrogen bond
Methyl carbon in VAL702	pi in phenyl ring linked methoxy	4.66	Pi-Sigma
Alkyl in ALA719	pi in phenyl ring linked methoxy	4.71	Pi-Alkyl
Alkyl in VAL702	pi in phenyl ring linked methoxy	6.00	Pi-Alkyl
Alkyl in LYS721	pi in phenyl ring linked methoxy	6.07	Pi-Alkyl
COO^- oxygen in ASP831	pi in phenyl ring linked methoxy	7.43	Pi-Anion
pi in phenyl ring in PHE699	pi in phenyl ring linked methoxy	7.78	Pi-Pi Stacked
erlotinib			
NH atom in MET769	– O atom	1.91	Conventional hydrogen bond
NH atom in LYS721	N atom	2.11	Conventional hydrogen bond
pi in phenyl ring in PHE699	pi in phenyl ring	4.47	Pi-Pi Stacked
Alkyl in VAL702	pi in quinazoline ring	4.83	Pi-Alkyl
Alkyl in LYS721	pi in quinazoline ring	4.86	Pi-Alkyl
Alkyl in VAL702	pi in quinazoline ring	5.16	Pi-Alkyl
COO^- oxygen in ASP831	pi in quinazoline ring	6.13	Pi-Anion
COO- oxygen in ASP831	pi in ethynylbenzene	6.28	Pi-Anion
alkyl in LEU820	pi in quinazoline ring	7.05	Pi-Alkyl

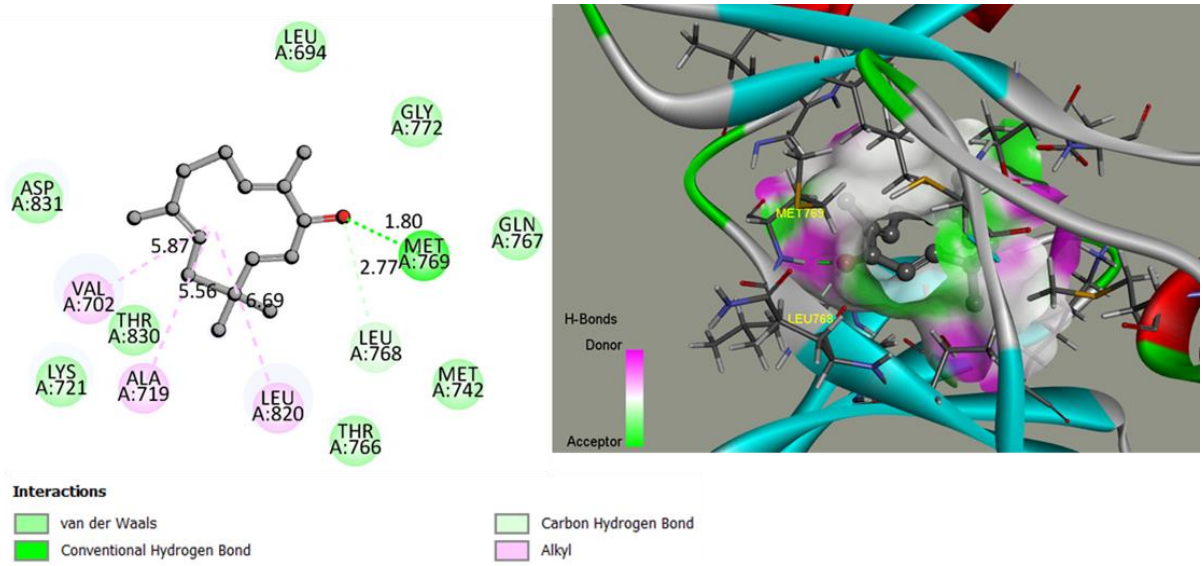

Figure 3. The interactions of **zerumbone** docked into the macromolecule **1M17** (left) and amino acid residue involved in hydrogen bond (green dotted line) (right).

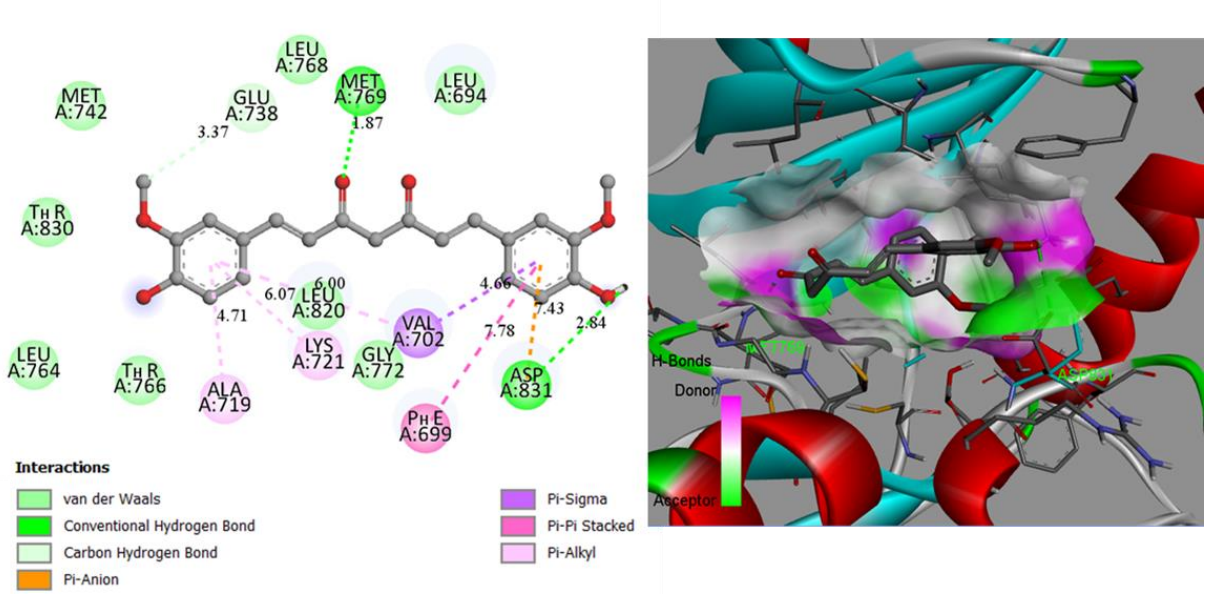

Figure 4. The interactions of **curcumin** docked into the macromolecule **1M17** (left) and amino acid residue involved in hydrogen bond (green dotted lines) (right).

The functional groups like ethynyl-phenyl and methoxy-ethoxy linked quinazoline in erlotinib interact with EGFR, in which two of the interactions are conventional hydrogen bonds. The others are π-alkyl, π–π stacking and π–anion interactions given in Table 3 and Figure 5.

Our results demonstrated that the binding energies of zerumbone and curcumin are very close to each other. Also their binding energies compared to that of erlotinib -7.3 kcal/mol in this study (-7.54 kcal/mol [40]), there is only 0.3 and 0.7 kcal/mol difference between them, respectively. While the zerumbone shows stronger H-bonding (1.80 Å), curcumin has two type H-bond, which the stronger one (1.87 Å) is the same as the interaction in zerumbone and the other one is weaker (2.84 Å). Both ligands interact with EGFR similar to erlotinib which has H-bond on MET769. H-bonds on MET769 of two

ligands are stronger than that of erlotinib (1.91 Å in this study) (2.70 Å [40]). Based on these values, it can be mentioned that zerumbone, curcumin and erlotinib bind strongly to the receptor.

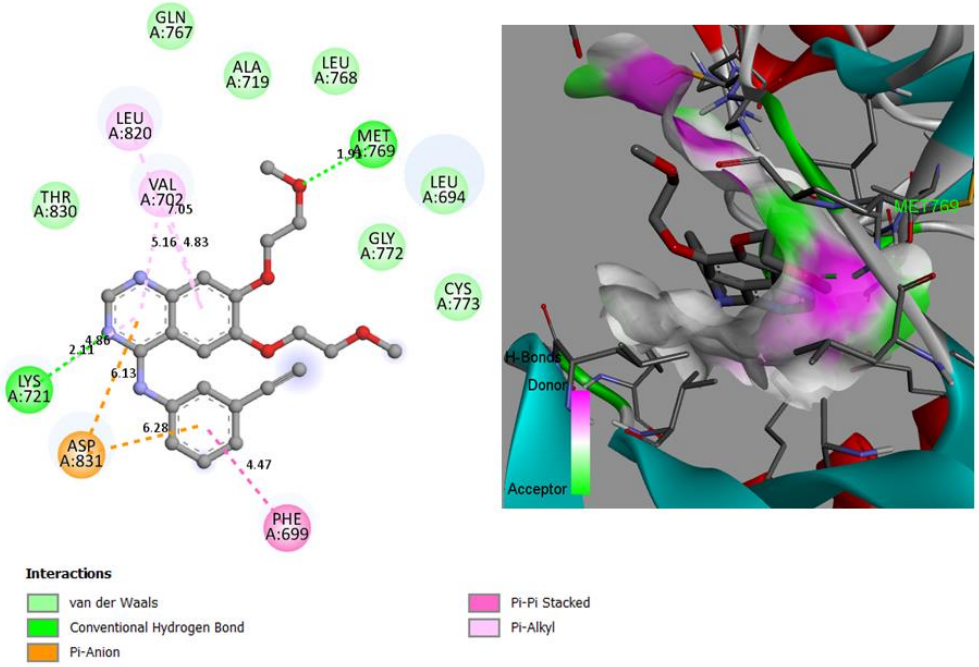

Figure 5. The interactions of **erlotinib** docked into the macromolecule **1M17** (left) and amino acid residue involved in hydrogen bond (green dotted lines) (right).

Band gap energy (**ΔE)** order is curcumin (3.68 eV) < erlotinib (4.29 eV) < zerumbone (5.09 eV) according to this study. The larger the band gap energy means the harder and more stable and less reactive the molecule. According to **ΔE** order, zerumbone is the most stable in binding to EGFR as a consequence of the highest energy gap. In this case, erlotinib containing the functional groups like methoxy-ethoxy bonded quinazoline is more reactive than zerumbone with the lower band gap, which is due to possess the large number of groups to interact with.

In conclusion, small molecules targeting EGFR have become prominent in cancer treatment, especially NSCLC. Natural products as anti-cancer compounds can be counted among the most important small molecule inhibitors. This study revealed that zerumbone has the potential as tyrosine kinase inhibitor that target EGFR due to the strong H-bond interactions and more stability. The oxygen atom of carboxyl in zerumbone being responsible for the electrophilic attack makes H-bond with NH atom in MET769, as did in curcumin. The oxygen of phenolic OH in curcumin resonates with the phenyl ring and becomes more positive (responsible for nucleophilic attack) and it makes H-bond with COO- oxygen of ASP831. Both ligands have same type H-bonds between the C=O group and the backbone NH of MET769, which are stronger than that of erlotinib. Based on this in silico results using docking studies, we can say that zerumbone may be effective in lung cancer treatment like erlotinib. However, *in vitro* and *in vivo* studies are necessary to understand the molecular mechanism of anti-cancer effect of zerumbone and also investigate its use in cancer treatment by targeting EGFR and its signaling components.

AUTHOR CONTRIBUTIONS

Concept: D.Y., A.K.; Design: D.Y., B.B., A.K.; Control: D.Y., A.K.; Sources: D.Y., B.B., A.K.; Materials: - ; Data Collection and/or Processing: A.K.; Analysis and/or İnterpretation: D.Y., B.B., A.K.;

Literature Review: D.Y., B.B., A.K.; Manuscript Writing: D.Y., B.B., A.K.; Critical Review: D.Y., B.B., A.K., 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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