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Interaction of GHK Tripeptide with Receptors Targeted in Some Cancer Studies: A Theoretical Approach with Molecular Docking

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ABSTRACT

Cancer, defined as the uncontrolled growth and proliferation of cells, is a serious disease seen in many people around the world. For this reason, a lot of work has been done and continues to be done by scientists for the diagnosis and treatment of cancer. It is known that various receptors are targeted in studies on cancers. In this study, ER, PR, EGFR and HER2 receptors, which are among the most frequently used target receptors, were selected. GHK is a tripeptide that has important benefits such as increasing cancer resistance and reversing cancer cells. In this study, the complex structures formed by the most commonly used target receptors (ER, PR, EGFR and HER2) and the GHK tripeptide were examined. These complex structures were obtained by molecular docking method that is a molecular modeling method used to predict how a receptor interacts with small molecules. As a result of the study, binding affinities, close interactions, and interaction types of GHK and receptors were determined, and interaction profiles with various drugs (such as tamoxifen, erlotinib and neratinib) in the literature were examined comparatively. In the light of the findings obtained in the studies, it was determined that the GHK tripeptide gave similar interaction profiles with the drugs used in cancer treatment.

Keywords:
Peptide, GHK, EGFR, Cancer, Docking

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INTRODUCTION

Normal cells grow and multiply for new cells needed in the body. When normal cells get old or damaged, they die and are replaced by new cells. With the disruption of this natural process, cancer cells are formed. Cancer is a disease that occurs when some cells in the body grow and multiply uncontrollably. These cells form tumors, which are lumps of tissue. These tumors may begin to spread throughout the body (1).

Breast cancer, which is one of the hormonal cancer types, is one of the most common cancer types in women. Estrogen and progesterone are hormones that are seen as potential risks in breast cancer (2, 3). Overexpression of estrogen and progesterone receptors are prominent distinguishing features in breast cancer cases. Therefore, studies have been made for breast cancer therapeutics to target these receptors (4-6). Estrogen receptor is one of the targets used not only in breast cancer, but also in treatment and prevention studies related to prostate, colon, and ovarian cancer (7, 8). Overexpression of progesterone is associated with overexpression of estrogen. PR overexpression, observed with estrogen overexpression, plays an important role in better diagnosis of PR+ breast cancer and higher response to hormonal therapy (2). Other important receptors in breast cancer are epidermal growth factor receptor (EGFR/HER1) and epidermal growth factor receptor 2 (EGFR2/HER2) (2, 9, 10). Overexpression of EGFR is also observed in breast cancer (11) and affects cell signaling and play a role in oncogenesis (12). In ER-, PR- and HER2- breast cancer (triple negative breast cancer) studies, EGFR level was found to be increased. Since treatment is limited in triple negative breast cancer, the use of EGFR antagonists is at the forefront of treatment strategies (13, 14). Additionally, more than half of non-small cell lung carcinomas express EGFR. Therefore, EGFR has an important place in lung cancer studies (15). Signaling pathways that occur in EGFR activation in colon cancer are also important (16). Another target used in anti-cancer studies is HER2 (12, 17). Both EGFR (HER1) and HER2 are used as target inhibitors in HER-2+ breast cancer. The limitations of single-targeting used in treatment studies are tried to be overcome with

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multi-targeted studies. These types of studies have shown that drug therapy is more effective (12, 18). Apart from breast cancer studies, HER2 is also prominent in other cancer types. In non-small cell lung cancer, HER2 is a biomarker of cancer proliferation (19). It has been reported in studies that HER2 gene mutations can be associated with the response to targeted agents in non-small cell lung cancer (20).

GHK is a tripeptide with antioxidant, anti-inflammatory, wound healing, ulcer, anticancer and bone tissue healing properties (21, 22). GHK has many studies on the preservation and repair of tissues (23). In addition to these properties of GHK, it has also proven to be effective in modulating a number of genes. A study with GHK found that it reversed the pathological expression of the gene in metastasis-prone colon cancer and was effective in directing gene expression to healthy remodeling in COPD lungs (24, 25). In a study, it was reported that GHK reduce the viability of glioblastoma cells at higher concentrations while the viability of L929 cells stay the same as the control (21). In a study with GHK-Cu complex, gene effects on MCF-7 breast and PC3 prostate cancer cell lines were investigated (26). It is also known that the GHK-Cu complex provides regeneration of the lung and liver. In addition, studies on human cancer cells (SH-SY5Y, U937 and breast cancer cells) have shown that GHK activates programmed cell death and inhibits cell growth (23, 27, 28).

Molecular docking method is a molecular modeling method frequently used in drug development studies. In this method, it is aimed to predict the complex structure of the drug candidate molecule (ligand) and the selected macromolecule (receptor), and to determine the most stable structure of this complex structure and to find the most appropriate pose of the ligand. That is, the ligand-receptor complex is studied at the atomic level and the interaction profile of the ligand in the active site of the receptor is determined (29, 30). Afterwards, a theoretical background is obtained by comparing the interaction profile of the studied disease-related drug molecules in the active site of the receptor with the interaction profile of the investigated molecule (ligand).

In this study, 4 different receptors (ER, PR, EGFR and HER2), which are known as target receptors in cancer studies, were determined and their interactions with GHK tripeptide were investigated by molecular docking method for the first time. The interaction profiles of GHK tripeptide with these receptors were compared with the interaction profiles of cancer drug molecules such as tamoxifen, erlotinib and neratinib.

MATERIAL AND METHODS

With the molecular docking study, it was aimed to obtain an estimate of the complex structures of the GHK tripeptide with the receptors targeted in cancer studies. The binding mode of the GHK tripeptide with the binding sites of ER, PR, EGFR and HER2 were determined. Firstly, GHK and all receptors were prepared via AutoDock Tools 1.5.6. Estrogen receptor (PDB ID: 1A52) (31), Progesterone receptor (PDB ID: 2OVM) (32), epidermal growth factor receptor (PDB ID: 1M17) (33) and Receptor tyrosine-protein kinase erbB-2 (HER2) (PDB ID: 3RCD) (34) were downloaded from PDB DataBank (https://www.rcsb.org/). Receptors were prepared for molecular docking studies by removing water, ions, and other ligands and adding polar hydrogens. After ligand and receptors were prepared, grid boxes were adjusted as 30Åx30Åx30Å. All molecular docking studies were run using AutoDock Vina (35). After molecular docking studies were completed successfully, initial visualizations were performed with Pymol program (36), then interaction types and distances (Å) of ligand-receptor complexes were determined and visualized by Discovery Studio Visualizer 2019 (37).

RESULTS AND DISCUSSION

Estrogen Receptor (PDB ID: 1A52)

ER+ breast cancer constitutes the majority of breast cancers (38). ERα, which is abundant in cancerous tissues, is associated with cancer inhibition, but may also contribute to cancer progression. For these reasons, Estrogen receptor alpha (ERα) is an important receptor frequently used in theoretical studies on breast cancer (39). In this study, estrogen receptor and GHK tripeptide was docked and determined the binding energy of best docking pose as -6.7 kcal/mol by AutoDock Vina program. GHK in the active site of Erα, close interactions and interaction types of GHK-ERα complex were shown in Figure 1. Additionally, detailed interaction types and distances between GHK-ERα complex were tabulated in Table 1.

Like the drugs (tamoxifen and exemestane), used to treat breast cancer, and estradiol, one of the three naturally produced estrogen hormones in the body, GHK also interacted with the ERα. GHK in the active site of ERα formed hydrogen bonds, pi-alkyl and van der Waals (vdW) interactions. GHK tripeptide formed 3 hydrogen bonds with Thr-347 residue of ERα (~2.71 Å, 2.71 Å and 3.03 Å). The tripeptide also formed carbon hydrogen bond with Gly-521

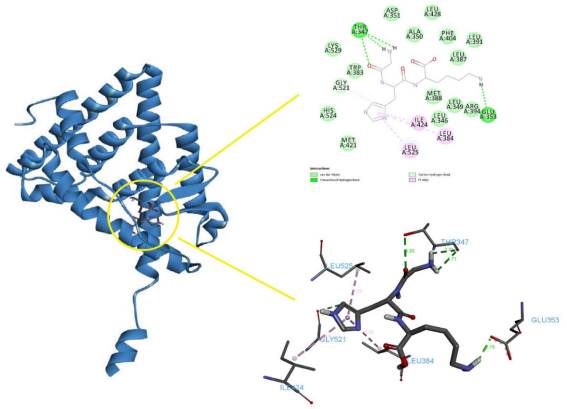

Figure 1. The close interactions of GHK tripeptide at ERα active site.

Table 1. The interaction types and distances (Å) of GHK- ERα complex.

Receptor ERα PDBID:1A52; with -6.7 kcal/mol Docking Score Energy		
Residue	**Interaction Type**	**Distance (Å)**
Thr-347	H-Bond	2.71 2.71 3.03
Glu-353	H-Bond	2.15
Gly-521	Carbon Hydrogen Bond	3.38
Leu-384	Pi-Alkyl	5.36
Ile-424	Pi-Alkyl	5.18
Leu-525	Pi-Alkyl	5.16
Leu-346, Leu-349, Ala-350, Asp-351, Trp-383, Leu-387, Met-388, Leu-391, Arg-394, Phe-404, Met-421, Leu-428, His-524, Lys-529	Van der Waals	

residue (~3.38 Å). Looking at other interactions, it was seen that GHK formed pi-alkyl interactions with Leu-384, Ile-424 and Leu-525 residues of ERα and vdW interactions with Leu-346, Leu-349, Ala-350, Asp-351, Trp-383, Leu-387, Met-388, Leu-391, Arg-394, Phe-404, Met-421, Leu-428, His-524 and Lys-529 residues of ERα. Mani et al. reported important residues forming the active site of ERα (9). According to this literature information, GHK tripeptide interacted with some important residues. These residues were Glu-353, Leu-525, Leu-384, His-524, Met-388, Leu-346, Arg-394, Ala-350, Phe-404 and Leu-387. In the study of Mani et al., the close interactions of two important molecules, estradiol and tamoxifen, with ERα residues were presented (9). When the interactions of GHK and these molecules (estradiol and tamoxifen) with the ERα were compared, it was determined that the close interaction residues between the molecules (estradiol and tamoxifen) and the ERα completely interacted with GHK tripeptide. In another docking study mentioned in the literature, exemestane, which is used in the treatment of breast cancer, was docked with the ERα and it was reported that the exemestane made hydrogen bonds with Glu-353, Arg-394 and His-524 residues of ER (40). In this study, GHK tripeptide interacted with these three residues via hydrogen bonding with Glu-353, vdW interactions with Arg-394 and His-524. As a result, when compared with the literature, it was determined that the GHK tripeptide interacts in the active region of the ER and even interacts with the same residues with the molecules used in breast cancer.

Progesterone receptor (PDB ID: 2OVM)

Progesterone receptors as well as ER are prognostic biomarkers in hormone-dependent breast cancers. Most breast cancers are ER+, PR+ or both positive (9, 41). In this study, progesterone receptor (PR) and GHK tripeptide was docked and determined the binding energy of best docking pose as -6.1 kcal/mol by AutoDock Vina program. GHK in the active site of PR, close interactions and interaction types of GHK-PR complex were shown in Figure 2. Additionally, detailed interaction types and distances between GHK-PR complex were tabulated in Table 2.

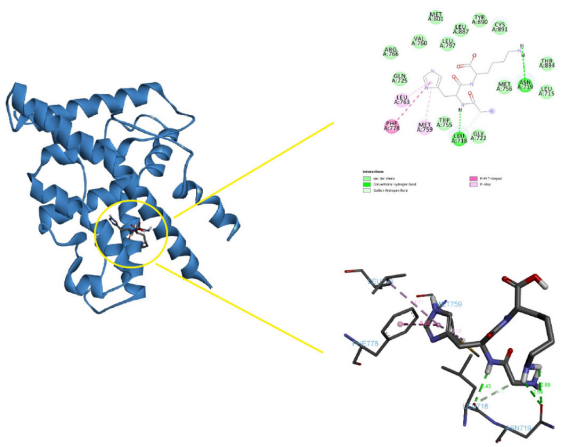

Figure 2. The close interactions of GHK tripeptide at PR active site.

As a result of docking of GHK with the progesterone receptor, it was determined that the GHK tripeptide formed hydrogen bonds, pi-alkyl, pi-pi t-shaped and vdW interactions at the determined active site of progesterone. Asoprisnil, a progesterone receptor modulator, (42) and tamoxifen, a drug used in the treatment of breast cancer, (9) interacted with the residues such as Leu-718, Asn-719, Gly-722, Gln-725, Met-756, Met-759, Val-760, Phe-778, Leu-887, Leu-797, Met-801, Tyr-890, Cys-891, Thr-894 in the progesterone active site, commonly. Hydrogen bonds with common residues Leu-718 (2.43 Å and 3.68 Å (carbon hydrogen bond)) and Asn-719 (2.59 Å and 2.69 Å), pi-alkyl interactions with Met-759 (5.15 Å), pi-pi T-shaped interactions with Phe-778 (5.22 Å), and vdW interactions with Gly-722, Gln-725, Met-756, Val-760, Leu-797, Met-801, Leu-887, Tyr-890, Cys-891,

Thr-894 were found in GHK tripeptide. Additionally, GHK interacted with Leu-763 residue of PR via pi-alkyl interaction (4.71 Å) and Trp-755, Arg-766 residues of PR via vdW interaction. In the literature, it was reported that tamoxifen and asoprisnil formed close interaction with Leu-763 (9) and Trp-755;Arg-766 (42), respectively. When the binding energies were compared, it was determined that the tripeptide had a strong binding energy as tamoxifen (-6.1 kcal/mol (9)), although not as strong as asoprisnil (-12.99 kcal/mol (42)).

Table 2. The interaction types and distances (Å) of GHK-PR complex.

Receptor PR PDBID: 2OVM ; with -6.1 kcal/mol Docking Score Energy		
Residue	**Interaction Type**	**Distance (Å)**
Leu-718	H-Bond	2.43
	Carbon Hydrogen Bond	3.68
Asn-719	H-Bond	2.59 2.69
Met-759	Pi-Alkyl	5.15
Leu-763	Pi-Alkyl	4.71
Phe-778	Pi-Pi T-Shaped	5.22
Leu-715, Gly-722, Gln-725, Trp-755, Met-756, Val-760, Arg-766, Leu-797, Met-801, Leu-887, Tyr-890, Cys-891, Thr-894	Van der Waals	

Epidermal Growth Factor Receptor (PDB ID: 1M17)

With the emergence of resistance cases in the treatment of breast cancer, the search for new drugs has become a necessity. Since new drugs are expected to have high anticancer activity and minimal side effects, studies focused on growth factor receptor (GFR) targeting. In studies, it was aimed to develop new drug types with the prediction of epidermal growth factor receptor (EGFR/HER1) signal pathway inhibition. Because overexpression of EGFR can cause uncontrolled cell growth (12, 43). In this study, EGFR and GHK tripeptide was docked and determined the binding energy of best docking pose as -6.2 kcal/mol by AutoDock Vina program. GHK in the active site of EGFR, close interactions and interaction types of GHK-EGFR complex were shown in Figure 3. Additionally, detailed interaction types and distances between GHK-EGFR complex were tabulated in Table 3.

It was determined that the interactions between GHK tripeptide and EGFR consisted of hydrogen bonding, pi interactions and van der Waals interactions. A closer look at the close interactions revealed that GHK forms 3 hydrogen bonds with EGFR through Asp-831. In a docking study of Erlotinib, a drug used in the treatment of lung and pancreatic cancer, and EGFR in the literature, close interactions with Asp-831 were observed (43). The GHK tripeptide made pi-alkyl interactions with Ala-719 and Lys-721 residues of EGFR. Erlotinib drug also made hydrophobic interactions (alkyl/pi-alkyl) interactions with these two residues (12). In other words, it was observed that GHK and Erlotinib have the same interaction types with the residues interacted with. GHK made pi-sigma interaction with Phe-699 and pi-donor hydrogen bond with Thr-766. Erlotinib had van der Waals interactions with these two residues (12). GHK also made pi-sulfur interactions with Cys-751 and Met-742. According to this study, Erlotinib's Val-702 with pi-sigma interaction, Leu-764 with alkyl interaction and Met-769 with hydrogen bonds were determined to have vdW interactions with GHK. GHK had similar vdW interactions with erlotinib and it was determined that the common residues that erlotinib and GHK interacted were Glu-738, Thr-830, Leu-820, Leu-768 and Gly-772. GHK also had vdW interactions with Cys-773, Arg-817, Asn-818 and Leu-694 residues.

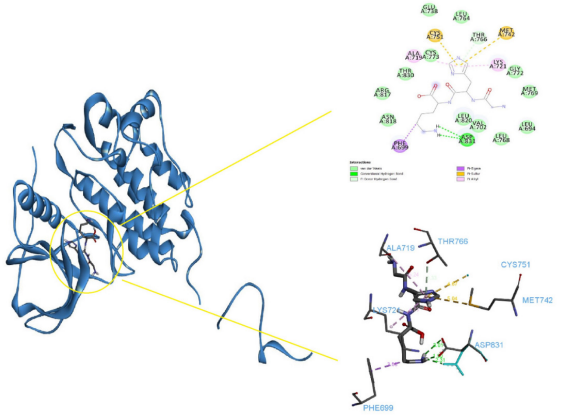

Figure 3. The close interactions of GHK tripeptide at EGFR active site.

Table 3. The interaction types and distances (Å) of GHK-EGFR complex.

Receptor EGFR PDBID: 1M17; with -6.2 kcal/mol Docking Score Energy		
Residue	**Interaction Type**	**Distance (Å)**
Asp-831	H-Bond	1.90 2.31 3.01
Phe-699	Pi-Sigma	3.53
Ala-719	Pi-Alkyl	5.24
Lys-721	Pi-Alkyl	4.73
Met-742	Pi-Sulfur	5.04
Cys-751	Pi-Sulfur	4.67
Thr-766	Pi-Donor Hydrogen Bond	3.33
Leu-694, Val-702, Glu-738, Leu-764, Leu-768, Met-769, Gly-772, Cys-773, Arg-817, Asn-818, Leu-820, Thr-830	Van der Waals	

Receptor tyrosine-protein kinase erbB-2 (PDB ID: 3RCD)

Another important receptor that stands out in studies on breast cancer is HER-2. About 15-20% of breast cancer types are HER-2 positive (18). HER protein family is involved in cell proliferation, differentiation, and migration (12). Overexpression of the HER protein is associated with breast cancer. A linear relationship between the growth of pathological tumor diameter and HER- expression has also been reported in literature studies (44-46). In this study, HER-2 and GHK tripeptide was docked and determined the binding energy of best docking pose as -7.1 kcal/mol by AutoDock Vina program. GHK in the active site of HER-2, close interactions and interaction types of GHK-HER-2 complex were shown in Figure 4. Additionally, detailed interaction types and distances between GHK-HER-2 complex were tabulated in Table 4.

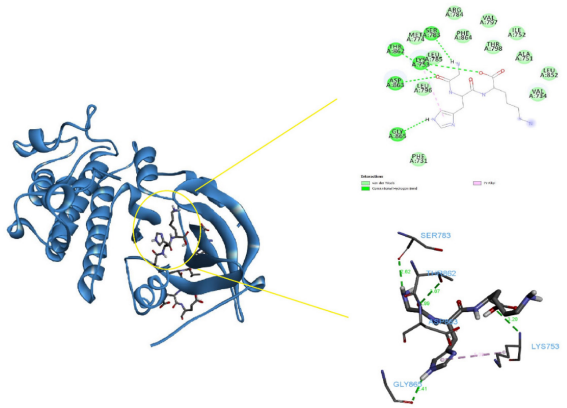

Figure 4. The close interactions of GHK tripeptide at HER2 active site.

GHK made hydrogen bond, pi-alkyl and vdW interactions with HER-2. GHK made a hydrogen bond with Gly-865 (2.41 Å). There was a vdW interaction between the reference drug Neratinib and Gly-865 (12). While the reference drugs Neratinib and TAK-285 made Carbon H-bond and vdW interactions with Asp-863, respectively, GHK made a hydrogen bond with this residue (2.99 Å). GHK, which has hydrogen bonding and pi-alkyl interactions with Lys-753, had similar interactions with drugs in the literature (neratinib (alkyl/pi-alkyl), TAK-285 (H-bond)). While GHK made a hydrogen bond with Thr-862 (3.07 Å), reference drugs made vdW interactions with this residue. With Ser-783, with which neratinib interacts with vdW, the GHK tripeptide formed a hydrogen bond (2.62 Å). When looking at the residues that GHK interacts with vdW, it was determined with the help of literature studies that all of them, except Arg-784, have various interactions with Neratinib and/or TAK-285 (12).

Table 4. The interaction types and distances (Å) of GHK-HER2 complex.

Receptor HER2 PDBID: 3RCD; with -7.1kcal/mol Docking Score Energy		
Residue	**Interaction Type**	**Distance (Å)**
Lys-753	H-Bond	3.20
	Pi-Alkyl	5.08
Ser-783	H-Bond	2.62
Thr-862	H-Bond	3.07
Asp-863	H-Bond	2.99
Gly-865	H-Bond	2.41
Phe-731, Val-734, Ala-751, Ile-752, Met-774, Arg-784, Leu-785, Leu-796, Val-797, Thr-798, Leu-852, Phe-864	Van der Waals	

CONCLUSION

In this study, the interactions of GHK tripeptide with 4 different receptors (ER, PR, EGFR, HER2) selected as targets in cancer studies were theoretically investigated for the first time. The interaction profile of GHK tripeptide with these four receptors is presented in comparison with the interaction profiles of various anticancer drugs. As a result of the investigations, it was determined that the GHK tripeptide has similar interaction profiles with tamoxifen, exemestane and neratinib used in the treatment of breast cancer. GHK had similar interactions with erlotinib, which is used in the treatment of lung and pancreatic cancer. GHK also had similar interaction profiles with asoprisnil, a progesterone receptor modulator, and TAK-285, a novel dual erbB protein kinase inhibitor that specifically targets the human epidermal growth factor receptor (EGFR) and HER2. The result of these theoretical studies has been a pioneering study as a glimmer of hope for investigating the anticancer activity of GHK with experimental methods and examining it in more detail in peptide studies.

CONFLICT OF INTEREST

The authors stated that did not have conflict of interests.

AUTHOR CONTRIBUTION

B. Bicak and S. Kecel Gunduz carried out the analyses and analyzed the data. The authors co-wrote the manuscript. All authors contributed to the finalization of the manuscript.

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