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Research Article

Momordica charantia (Bitter Melon) Fruit Bioactive Compounds and Potential Inhibitory Effects of Breast Cancer-Related Enzymes: *In silico* Approaches

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Abstract

Breast cancer rates are on the rise, particularly among women. Ongoing research is focused on finding effective treatments for this form of cancer. For centuries, plants have been harnessed for their therapeutic properties, with their chemical compounds shedding light on drug development for a wide range of ailments. This investigation aims to explore the potential of certain bioactive 17 compounds present in *Momordica charantia* (MC) fruit, known to inhibit the growth of breast cancer tumours. Specifically, the study delves into their interactions with critical enzymes—epidermal growth factor receptor (EFGR) and nudix-linked to moiety X-5 (NUDT5)—that are implicated in breast cancer development, utilizing *in silico* methods. For this purpose, firstly, iGemdock, DockThor and SwissDock were used for the first evaluation and it was observed that the binding affinities of bioactive compounds. In all three docking, compound **16** (Momordicoside L) has shown better results than standard molecules in EGFR and NUDT5. Therefore, docking was applied for compound **16** in HER2 and HER3, revealing a notably high binding affinity, especially for HER2. The results indicate that compound **16** is a potent inhibitor candidate for EGFR, HER2, HER3, and NUDT5, paving the way for further studies.

Keywords: *Momordica charantia*, Momordicoside L, Docking, Breast cancer, *In silico*

Introduction

The incidence of cancer is increasing day by day and cancer is a very difficult process with its economic, sociological and psychological effects. Especially in women, breast cancer is a type of cancer that develops in the breast tissue, usually in the milk-producing glands called lobules or the ducts that connect the lobules to the nipple. Risk factors for breast cancer include age, family history of breast cancer, genetic mutations (such as BRCA1 and BRCA2), early menstruation or late menopause, exposure to radiation, hormone replacement therapy, obesity, and alcohol consumption. Regular screenings and early detection can play a critical role in improving survival rates and managing breast cancer effectively. In addition, the intake of preventive and/or treatment nutrients is important in reducing the risk of breast cancer. In particular, plants may help to treatment of breast cancer in terms of both low side effects and reliability (Harbeck et al., 2019; Łukasiewicz et al., 2021).

Enzymes activities can change in cancer cells and especially the increase in the activity of some enzymes can ocur tumor development. Therefore, inhibition of cancer-related enzymes constitutes a preventive and tumor-decreasing treatment approach (Jin et al., 2022). Additionally, cancer is divided into different subgroups in different tissues. Treatment approaches and development according to tissue and sub-type of cancer rather than general treatment approaches can be a powerful perspective in terms of both patient health and time. In particular, although there are many subtypes of breast cancer, triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC), which are extremely aggressive types, account for 7-28% and 1-5% of total breast cancer statistics worldwide, respectively (Funakoshi et al., 2019; Swaminathan et al., 2023).

In particular, one of the biggest challenges in the treatment of TNBC and IBC is the absence of selective toxicity that causes a decrease in the therapeutic index and compromises the prognosis. Therefore, substandard doses are applied to prevent damage to normal healthy cells. TNBC has many different subtypes and different characteristics of these subtypes. TNBC subtypes are divided into six based on unique gene expression profiles and tumors into four different subtypes based on RNA and DNA profile analysis.

All subtypes and their molecular risks are presented in Table 1 and it is seen that the activity of EGFR increases especially in BL2, MSL and MES subtypes. Since these subtypes account for approximately 44% of TNBC, EGFR inhibitor therapy can be a powerful approach to the treatment (Nandini et al., 2021).

A hydrolases enzyme NUDT5 (also called NUDIX5) is involved in the metabolism of ADP-ribose and 8-oxo-guanine and has been identified as a key factor in ATP production in the nucleus of BRCA cells. Because of its role in ATP production for BRCA cells, NUDT5 has become an important enzyme in the treatment of breast cancer (Wright and Beato, 2021). Inhibition of these enzymes by the same molecules may allow it to be considered a multi-target drug.

Although many drugs have been developed, due to the possible side effects of drugs and the scarcity of multiple target drugs, patients use more drugs and are frequently exposed to side effects accordingly. Plants have been used

and continue to be used in the treatment of many diseases since ancient times. It is a great advantage that they have few side effects and can be easily grown and consumed. Until today, many drug-active substances have been isolated from plants and research is still continuing.

Table 1. The subtypes of the TNBC and their molecular risk factors

	Subtype	Abbrevatioons	Molecular Risk Features
Six TNBC subtypes	Basal-Like 1	BL1	Cell cycle ↑, ATR/BRCA genes ↑
	Basal-Like 2	BL2	Growt factor pathways (e.g. EGFR) ↑, Metabolic pathways ↑
	İmmunomodulatory	IM	Immune cell pathways ↑, cytokine pathways ↑
	Mesenchymal	M	Cellular motility pathways ↑, ECM-receptor interaction ↑, Differentiation pathways ↑
	Mesenchymal Stem-Like	MSL	Growt factor pathways (e.g. EGFR) ↑, Experssion of proliferating genes ↑,
	Luminal Androgen Receptor	LAR	Androgen receptors ↑
Four TNBC subtypes	Luminal AR	LAR	Androgen receptors ↑
	Mesenchymal Enrich	MES	Growt factor pathways (e.g. EGFR) ↑
	Basal-Like İmmunosuppressive	BLIS	Immuno suppressive molecules ↑
	Basal-Like İmmune Activated	BLIA	Immuno activated molecules ↑

Momordica charantia (MC), also known as bitter melon, is a tropical vine that belongs to the gourd family. It is cultivated for its edible fruit, which is commonly used in traditional medicine and various cuisines around the world. MC contains compounds such as momordicin and charantin that exhibit anti-cancer activities. Some research suggests that these compounds may help inhibit breast cancer cell growth, induce apoptosis (cell death), and suppress cancer progression (Muhammad et al., 2019; Feng et al., 2023; Psilopatis et al., 2023).

This study aimed to investigate the effects of unusual bioactive compounds found in MC fruit on enzymes that are important in breast cancer and to present a basic molecule or molecules in drug development by *in silico* method.

Materials and Methods

Selected bioactive compounds of Momordica charantia fruit and ligand preparation

17 bioactive compounds of MC fruit were listed in the Table 2 and these compunds were collected in Dr. Duke's Phytochemical and Ethnobotanical database (https://phytochem.nal.usda.gov) with exclude common compounds (Duke, 1992). The 3D structures of bioactive compounds and standards were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as .sdf format (Kim et al., 2015).

As a standard, different compounds were used for each enzyme (Table 3) and these compounds were 4-anilinoquinazoline and 7-[[5-(3,4-dichlorophenyl)-1,3, 4-oxadiazol-2-yl]methyl]-1,3-dimethyl-8-piperazin-1-yl-purine-2,6-dione (9CH). The structural optimization and energy minimization of all ligands and standards were performed using the Avogadro software with the help of UFF (Universal Force Field) before conducting molecular docking analysis under (Rappe et al., 1992). After that, the ligands were saved as pdb files for the molecular docking process.

Table 2. Selected bioactive compounds of *Momordica charantia* fruit.

No	PubChem CID	Phytochemicals
Compound 1	5202	5-Hydroxytryptamine
Compound 2	5281115	Alpha-eleostearic-acid
Compound 3	5281331	Alpha-spinasterol
Compound 4	3081416	Ascorbigen
Compound 5	12309060	Beta-sitosterol-D-glucoside
Compound 6	137199997	Charine
Compound 7	99474	Diosgenin
Compound 8	246983	Lanosterol
Compound 9	57518366	Momordicin
Compound 10	131751850	Momordicoside E
Compound 11	44445566	Momordicoside F1
Compound 12	44445567	Momordicoside F2
Compound 13	91895422	Momordicoside G
Compound 14	71717036	Momordicoside I
Compound 15	57330180	Momordicoside K
Compound 16	101743788	Momordicoside L
Compound 17	849	Pipecolic acid

Table 3. The list of standard compounds of EGFR and NUDT5.

Standard	PubChem CID	Target Enzyme
4-Anilinoquinazoline	324081	EGFR
9CH	132472992	NUDT5

Preparation of proteins

3D protein structure files of EGFR (PDB ID: 1M17; 2.60 Å, X ray diffraction) (Stamos et al., 2002) and NUDT5 (PDB ID: 5NWH; 2.60 Å, X ray diffraction) (Page et al., 2018) were obtained from RCSB Protein Data Bank (http://www.rcsb.org). The active side of the proteins were determined by using DeepSite server (https://playmolecule.com/deepsite/) and after the determination of the active sides of the proteins (Table 4) (Jiménez et al., 2017), selected ligands were docked the protein by using iGemdock software, DockThor and SwissDock online molecular docking server.

Table 4. The grip parameters of EGFR and NUDT5.

Protein	Center grid parameters			Grid box sizes
	x	y	z	Å^3
EGFR	25	2	53	20x20x20
NUDT5	9.4	-14.8	-19.2	20x20x20

Molecular docking studies

iGemdock is a free and easy to use for molecular docking applications and provides interactive interfaces to prepare both the binding site of the target enzyme and the screening compounds, and generates protein–compound interaction profiles of electrostatic (E), hydrogen-bonding (H) and Van der Waal's (VDW) interactions (Hsu et al., 2011). One of its important advantages is that it docks at the sites of the bound ligands. In this way, it is easier to understand the interactions of inhibitor-bound proteins with new inhibitors and to discover potential inhibitors. In the study, it used the "standard" protocol by setting a population size of 200, with 70 generations, and 2 solutions. DockThor (www.dockthor.lncc.br/v2/) is a molecular docking server that is freely available through the web server and utilises the same ligand and protein receptor files from the uses MMFFLigand and PdbThorBox in-house tools for its docking algorithm along with the MMFF94S53 force field (de Magalhães et al., 2014; Santos et al., 2020; Guedes et al., 2021a Guedes et al., 2021b). SwissDock is a specialized online platform designed for the docking of small molecules onto target proteins. Utilizing the EADock DSS engine, it integrates setup scripts to address common issues and facilitate the curation of both the target protein and ligand input files (Grosdidier et al., 2011a; Grosdidier et al., 2011b). The 3D and 2D interaction of ligand and protein from results monitored using PoseView (https://proteins.plus/) (Stierand and Rarey, 2010; Schöning-Stierand et al., 2020).

Furthermore, compounds with higher affinity than standards for multiple target proteins, including HER2 and HER3, which are members of the ErbB receptor tyrosine kinase family, have been subjected to docking studies. HER2 (PDB ID: 3PP0) (Aertgeerts et al., 2011) and HER3 (PDB ID: 3LMG) (Shi et al., 2010) downloaded from the Protein Data Bank sites, and for the identified ligands, the grid box dimensions will be set as X: 35.82 Å, Y: 44.10 Å, Z: -11.69 Å (HER2) and X: 11.93 Å, Y: -29.20 Å, Z: 45.83 Å (HER3), with a grid box size of 20x20x20 Å^3 for the docking process (Olivero-Acosta et al., 2017).

Ligands bound to the PDB structures of HER2 (CID: 33113, phosphoaminophosphonic acid-adenylate ester, AMP-PNP) and HER3 (CID: 16736274, 2-{2-[4-({5-Chloro-6-[3-(Trifluoromethyl)phenoxy]pyridin-3- Yl} amino)-5h-Pyrrolo[3,2-D]pyrimidin-5-Yl]ethoxyethanol, 03Q) were downloaded from the PubChem website for the purpose of redocking, and subsequently, energy minimizations were performed with the Avogadro program using the UFF method.

Protein flexibility-molecular dynamic (MD) simulation

After the protein-ligand interaction was determined of the target proteins, CABS-fex 2.0 server was used to evaluate the protein-ligand complex stability in this study for the multi target compounds and presented with RMSF (root mean square fuctuation) (http://biocomp.chem.uw.edu.pl/CABSflex2/index).

CABS-fex offers fast protein fexibility simulation and generates protein dynamic simulation at highly reduced system requirements. CABS-fex provides high resolution (10-ns) protein nearnative protein dynamics simulation and hence is very efective for evaluation of protein–ligand stability on real-time basis. Simulation in CABS-fex was set with default parameters, with 50 cycles (Jamroz et al., 2014; Kmiecik et al., 2016; Kurcinski et al., 2018).

Physicochemical properties of the compounds

The physicochemical properties of the compounds that are related to multiple target proteins were viewed on the ADMETLab 2.0 website (https://admetmesh.scbdd.com/) (Xiong et al., 2021). The macromolecular targets of the compounds, assumed as bioactive, were predicted using SwissTargetPrediction online tool (www.swisstargetprediction.ch/) (Daina et al., 2019).

Results

Molecular Docking

Heat graphs of molecular docking results for EGFR are presented in Figure 1. For iGemdock, compounds **10** (-130.96 kcal/mol), **5** (-110.44 kcal/mol), **12** (-97.67 kcal/mol), , **15** (-98.62 kcal/mol) and **16** (-107.24 kcal/mol), for DockThor, compounds **8** (-9.176 kcal/mol), **12** (-8.918 kcal/mol), **14** (-8.951 kcal/mol), **15** (-9.027 kcal/mol) and **16** (-9.036 kcal/mol) and for SwissDock, compounds **12** (-9.59 kcal/mol), **13** (-9.08 kcal/mol), **14** (-9.36 kcal/mol), **15** (-9.12 kcal/mol) and **16** (-9.04 kcal/mol) were determined that the the highest binding affinities. It was found that these compounds had higher affinities and better scores were obtained in each program

than the standard compound 4-anilinoquinazoline (-72.18, -7.326 and -7.30 kcal/mol, respectively). As a result of the overall evaluation of the results for EGFR, compounds **2**, **3**, **4**, **5**, **7**, **8**, **10**, **11**, **12**, **13**, **14**, **15** and **16** also gave better results than the standard in docking application.

NUDT5 results showed successful results in finding potential compounds. Compound **16** (-116.80, -8.040 and -10.17 kcal/mol, respectively) was found to have higher binding affinity than the standard molecule, 9CH (-109.86, -7.734 and -10.01 kcal/mol, respectively), in iGemdock, DockThor and Swissdock (Figure 2). In the general evaluation of the docking results, it is seen that the most potential target protein among the selected target proteins is EGFR and that compound **16** may be a multi-target molecule due to its better docking score in the standard for EGFR and NUDT5 in all three programs. Therefore, further analysis was carried out for compound **16**. The 3D and 2D protein-ligand interactions of iGemdock and DockThor results were examined in detail and compared (Figure 3 and 4). While iGemdock docks to a certain inhibitory region, docking is performed to the active center in DockThor. The amino acids with which compound **16** and standard molecules interact according to both target enzymes and programs are shown in Table 5.

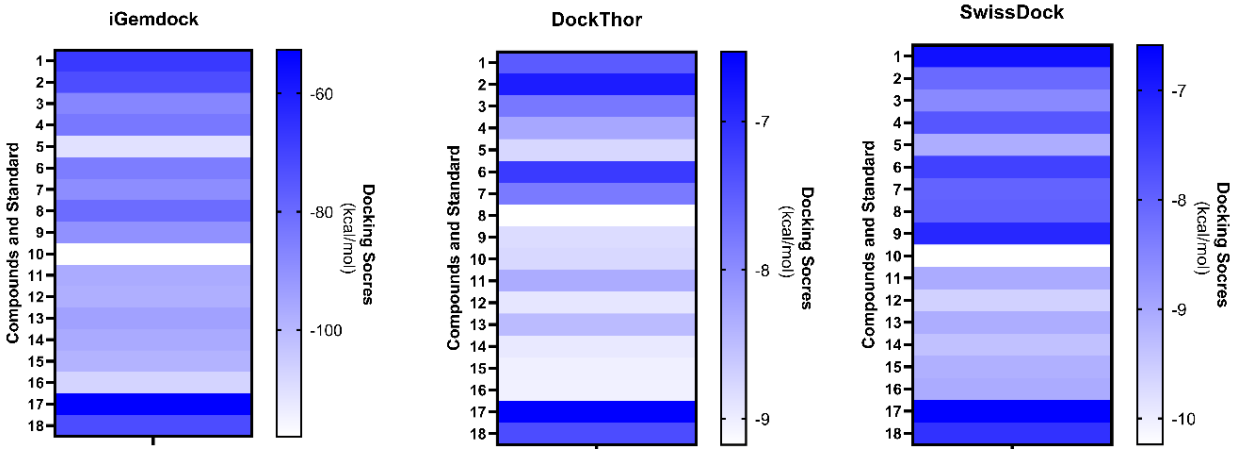

Fig. 1. The heat map of the docking results of the compounds for EGFR. Compound **18** means 4-anilinoquinazoline.

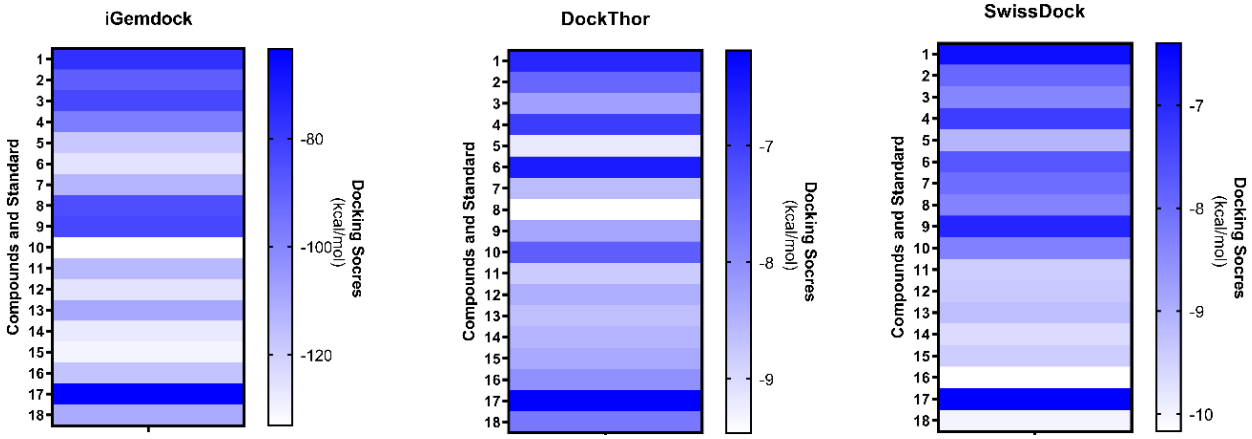

Fig. 2. The heat map of the docking results of the compounds for NUDT5. Compound **18** means 9CH.

Table 5. The interactions of the residues of compound **16** and standards for all docking.

	EGFR		**NUDT5**	
	DockThor	**iGemdock**	**DockThor**	**iGemdock**
Compound **16**	LEU23 LYS50 ASP142 ASP160	PHE28 VAL31 LYS50 PRO99 PHE100 CYS102 ASP160	PHE81A ALA83A GLY84A LEU85A MET119A	ALA83A GLY84A ASP179A ARG181A TYR23B THR32B TRP33B
Std	LYS50 LEU149 ASP160	LYS50 THR95 THR159	ARG38A LEU85A ARG71A GLU130A TRP33B	ALA83A GLY84A ASP179A ARG181A TYR23B THR32B TRP33B

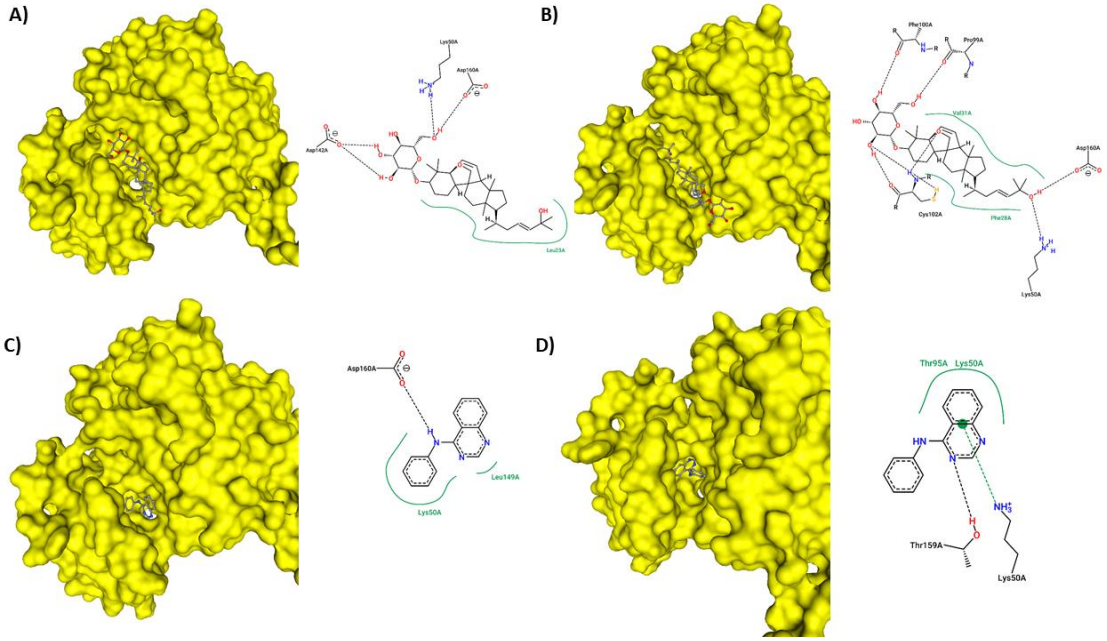

Fig. 3**.** The 3D and 2D EGFR-ligand interactions of DockThor and iGemdock. A) EGFR-compound **16** obtained from DockThor, B) EGFR-standard obtained from DockThor, C) EGFR-compound **16** obtained from iGemdock, D) EGFR-standard obtained from DockThor.

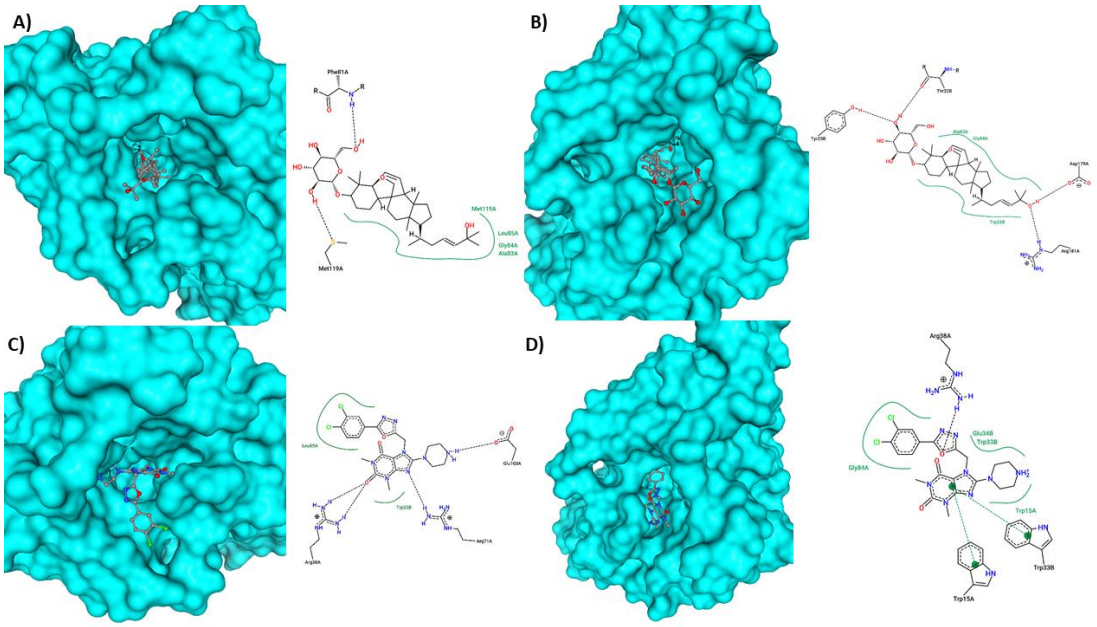

Fig. 4**.** The 3D and 2D NUDT5-ligand interactions of DockThor and iGemdock. A) NUDT5-compound **16** obtained from DockThor, B) NUDT5-standard obtained from DockThor, C) NUDT5-compound **16** obtained from iGemdock, D) NUDT5-standard obtained from DockThor.

Protein flexibility-molecular dynamic (MD) simulation

The RMSF graphs were drawn to understand the consistency of 2D protein-ligand interactions obtained from different programs, and a detailed evaluation of the amino acid residues involved in the interactions of each molecule was carried out. A molecular dynamic analysis was conducted for compound **16** with EGFR and NUDT5 active sites to provide a detailed analysis of interactions. The RMSF graphs for compound **16** and standard molecules with respect to EGFR and NUDT5 are presented in Figure 5.

The flexibility of a protein or a specific region of interest can be evaluated through RMSF values obtained from molecular dynamic simulations. Regions with significant flexibility within a protein can be highlighted by RMSF profiles. This information becomes crucial in molecular docking studies, as it indicates the adaptability of a flexible binding site to ligands with various conformations. The relationship between RMSF and molecular docking is symbiotic, as information about flexibility and dynamics obtained from RMSF analysis enhances the reliability of molecular docking studies. The integration of these techniques allows for a more comprehensive understanding of structure-function relationships within biological systems.

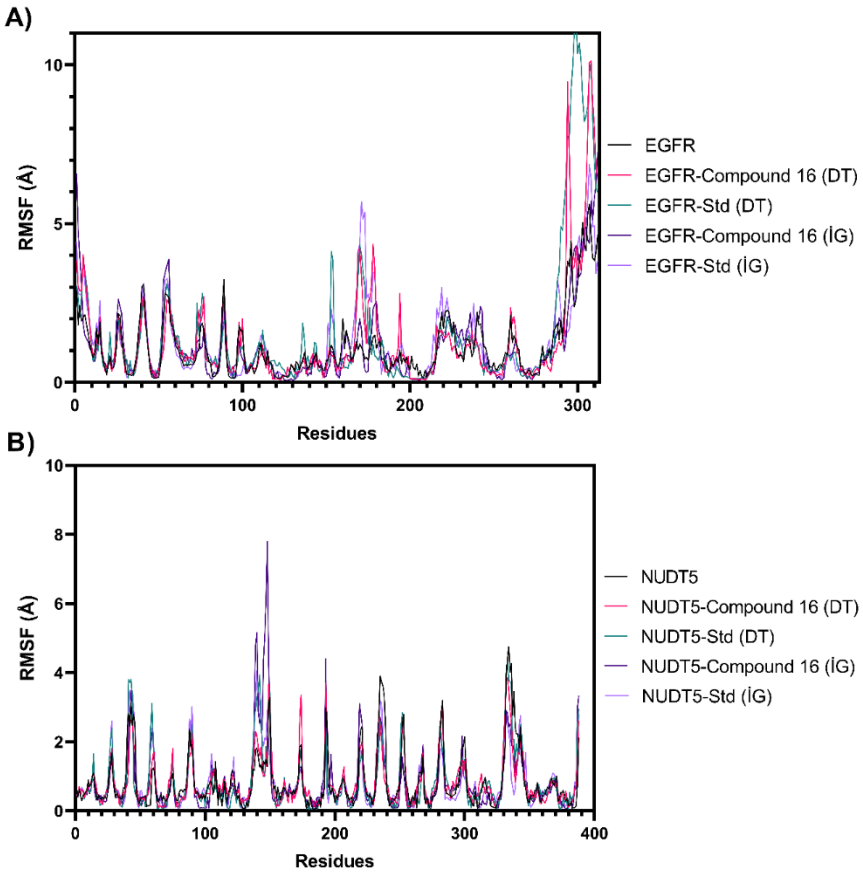

Fig. 5. A) The RMSF plots of the EGFR-ligands, B) the RMSF plots of the NUDT5-ligands. DT: obtained from Dockthor, IG: obtained from iGemdock.

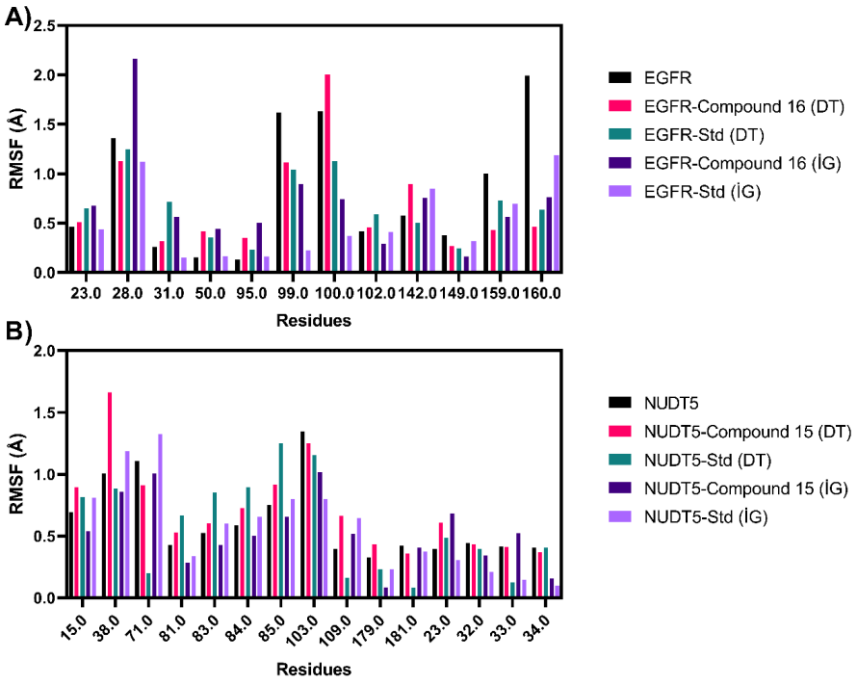

Fig. 6. The detailed RMSF anaylses of the active sides of target proteins. A) EGFR, B) NUDT5. DT: obtained from Dockthor, IG: obtained from iGemdock

For EGFR, common results were observed for LYS50 and ASP160 in both docking results. The values detected for compound **16** at LYS50 were 0.416 and 0.440 Å, and for the standard molecule 4-anilinoquine, they were 0.353 and 0.165 Å, respectively. These results suggest that compound **16** is more unstable specifically at LYS50. For

ASP160, the values for compound **16** were 0.465 and 0.760 Å, while for 4-anilinoquine, they were 0.635 and 1.184 Å, indicating that compound **16** forms a more stable structure at ASP160. In addition to these differences, average RMSF values for amino acid residues active in the active center for all molecules were calculated, resulting in the following: EGFR: 0.831 Å, EGFR-Compound **16** (DT): 0.696 Å, EGFR-Std (DT): 0.672 Å, EGFR-Compound **16** (IG): 0.709 Å, EGFR-Std (IG): 0.507 Å. These results suggest that compound **16** is consistent with docking results (Figure 6A).

In the study conducted for NUDT5, no common residues were observed between iGemdock and DockThor results. Therefore, each result was evaluated separately. In iGemdock results, TRP33B and GLY84A residues were found to interact commonly between compound 15 and 9CH. The RMSF values for each ligand were 0.407, 0.522 Å, and 0.146, 0.593 Å, respectively. Compound **16** appears to be more stable, especially for GLY84A. DockThor results indicate that only LEU85A residue is common between compound **16** and 9CH, with RMSF values of 0.473 and 0.434 Å, suggesting similar stability for both ligands. Average RMSF values for amino acid residues active in the active center for all molecules were as follows: NUDT5: 0.616 Å, NUDT5-Compound **16** (DT): 0.718 Å, NUDT5-Std (DT): 0.574 Å, NUDT5-Compound **16** (IG): 0.534 Å, NUDT5-Std (IG): 0.569 Å. These results indicate that compound **16** is consistent with docking results, especially for iGemdock (Figure 6B).

Molecular docking studies of HER2 and HER3

EGFR (HER1), is one of the members of the ERbB family, and there are three more proteins belonging to this protein family; HER2, HER3, and HER4. Particularly, HER2 and HER3 are key enzymes in cancer treatment. Therefore, in terms of designing multi-target molecules, the binding affinities of compound **16** to these two proteins have been examined, and it has been found that the binding affinities are high for both target proteins. For HER2, the binding affinities of compound **16** were observed to be -122.15, -10.911, and -7.95 kcal/mol, while for AMP-PNP, they were -108.72, -7.359, and -9.96 kcal/mol. The results for HER3 were close to the standard molecule, and for compound 15, they were -126.89, -8.458, and -9.30 kcal/mol, while for the standard, they were -123.39, -8.637, and -9.45 kcal/mol (Figure 7).

The results for HER2 and HER3 did not yield similar outcomes in all three docking processes, and conclusive judgments could not be made based on the docking results for compound **16**. However, the existing binding affinity for compound **16** suggests that it could serve as a fundamental structure in the development of new inhibitors for multi-target development.

Physicochemical properties of the compound 16

Physicochemical properties provide predictions about whether a molecule has drug potential or not and are the features that form drug similarity rules. Therefore, their evaluation constitutes an approach. The most important drug similarity rules are Lipinski, Pfizer, GSK and Golden Triangle rules. Among these rules, compound **16** was found to comply only with the Pfizer rule (logP > 3 and TPSA < 75) and not the other rules (Figure 8). Its physicochemical properties can be changed and made more suitable by adding another functional group or groups.

The results for compound **16** have been obtained from the Swisstarget prediction application, which assesses the potential targets of small molecules on macromolecules using datasets. While phosphatase is identified as the primary target with 33%, kinases are second with 20%. In particular, tyrosine kinase is found to be a potential target in the sub-results (Figure 9).

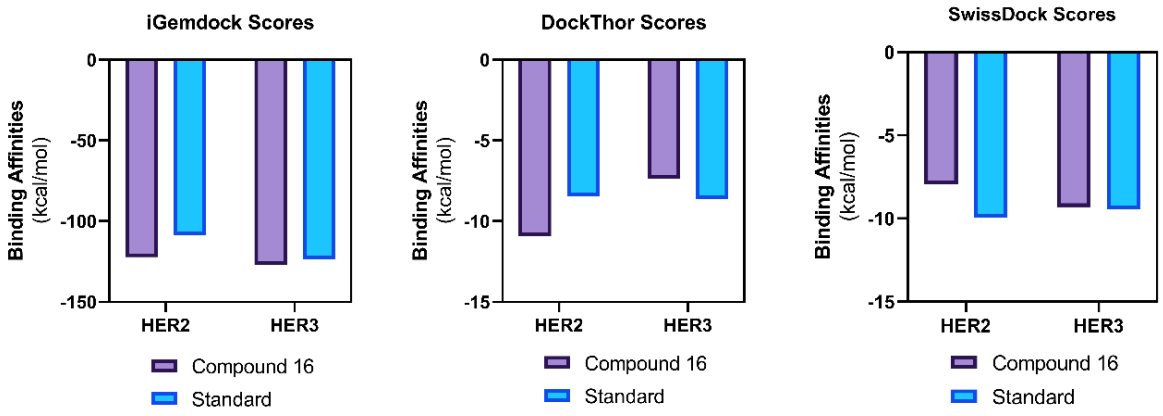

Fig. 7. The molecular docking scores for compound **16** and standard for each application.

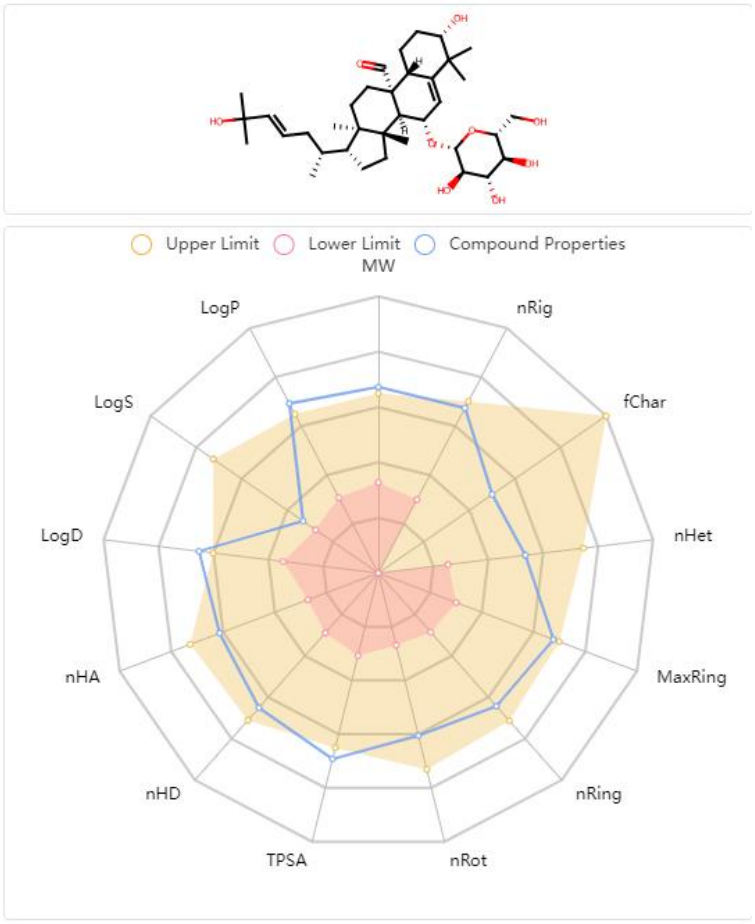

Fig. 8. The physicochemical properties of the compound **16**. MW: molecular weight, nRig: number of rigid bonds, fChar: formal charge, nHet: numbers of heteroatoms, MaxRing: numbers of atoms in the biggest ring, nRing: number of rings, nRot: number of rototable bonds, TPSA: topological polar surface area, nHD: number of hydrogen atoms donor, nHA: number of hydrogen atoms acceptors, LogD: logP at physiological pH 7.4, LogS: log of the aqueous solubility, LogP: log of the octanol/water partition coefficient.

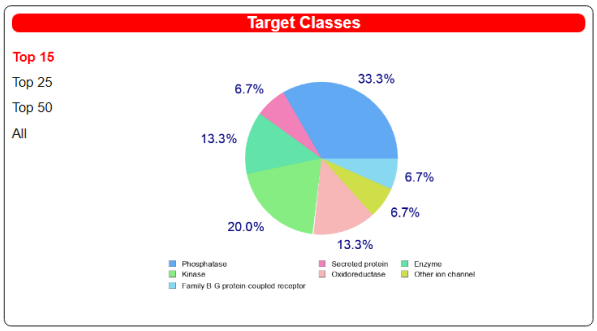

Fig. 9. The prediction of the target macromolecules of compound **16**.

Discussion and Conclusion

Breast cancer is a type of cancer that occurs due to many factors and has a high incidence, especially in women. In addition to its physiological effects, it also has many psychological effects. For this reason, studies on breast cancer are still ongoing and remain popular. Changes in enzyme activities may vary according to different types of cancer. Therefore, the enzyme-cancer relationship is being investigated and it is important to find natural or synthetic compounds that stop or prevent tumor development. The fact that a molecule can affect more than one related cancer enzyme can provide convenience in terms of treatment or prevention (Hong and Xu, 2022; Smolarz et al., 2022).

The effects of MC on breast cancer have been among the subjects that have been studied extensively in recent years (Sur and Ray, 2020). Extract of MC fruit has been suggested to inhibit the growth of breast cancer and modulate the signal transduction pathways for this inhibition. It is observed that bitter melon extract (BME) treatment has a significant effect on the uptake of 99mTc-PAC on MCF-7 cells which is a known estrogen receptor-positive breast carcinoma cell line (Ray et al., 2010). In addition, MC water extract (0.5% and 30%) applied with drinkin water prevented breast tumor development in SHN virgin fees (Nagasawa et al., 2002; Muhammad et al., 2017; Shim et al., 2018).

These effects on breast cancer cause MC to be considered a herbal treatment tool. Like many plants, MC contains different bioactive molecules in different parts. Determining the activities of these molecules can make them potential drugs or drug precursors to spesific enzymes. Interactions between growth factors and cell surface receptors regulate proliferation, survival, differentiation, and metabolism. Oncogenesis is

characterized by a loss of control over these critical biological processes. EGFR was one of the first members of the growth factor/receptor tyrosine kinase (RTK) family to be identified (Stamos et al., 2002). The findings supported epidermal growth factor receptor (EGFR) overexpression in a large number of triple-negative breast cancers (TNBC) patients and showed that EGFR inhibition may be advantageous in these patients (Tong et al., 2018). Therefore, EGFR inhibition is an alternative in the treatment of breast cancer. NUDT5 (also called NUDIX5) has been identified as a key factor in ATP production in the nucleus of BRCA cells. Because of its role in ATP production, NUDT5 has become an important enzyme in the treatment of breast cancer. Inhibition of these enzymes by the same molecules may allow it to be considered a multi-target drug.

MC has the highest nutritive values among cucurbits and contains ucurbitane-type triterpenoids, cucurbitane-type triterpene glycosides, phenolic acids, flavonoids, essential oils, fatty acids, amino acids, lectins, sterols and saponin (goyasaponins I, II and III) depends on part of the plant. Due to its rich content, many different biological activities have been examined and it is known to be effective in many different cancer types such as lung, prostate, breast, and liver. Triterpenoids and triterpene glycosides are found in MC fruit as well as they are known to have an inhibitory effect on EGFR. It was determined that pristimerin is a novel EGFR2-downregulated compound that is able to decrease fatty acid synthase and modulate the Akt, MAPK, and mTOR signaling pathways to influence metastasis and apoptosis of breast cancer cells (Lee et al., 2013). 20(S)-protopanaxatriol isolated from Panax ginseng was found to inhibit the activation of EGFR signaling pathways in lung cancer (Li et al., 2014). 20(R)-ginsenoside isolated from the same source inhibited the proliferation of lung cancer cells in Rg3 via EGFR/PI3K/AKT pathway (Dai et al., 2018; Wang et al., 2019). As a result of the in silico EGFR study in our study, when the results of both molecular docking results were compared, it was shown that compounds **3**, **4**, **5**, **7**, **8**, **11**, **12**, **13**, **14**, **15** and **16** had more binding affinity than the standard compound. It can be concluded that these compounds found in MC fruit can be used in EGFR inhibition.

It is known that the increase in the amount of ATP supports the development of breast cancer cells. Therefore, reducing the amount of ATP, especially in cancer cells, creates an approach to prevent tumor development. NUDTs, one of the hydrolase class enzymes, have been investigated for their relation with breast cancer, especially in recent years, and it has been reported that the activities of NUDT1, 2, 5, and 16 enzymes have increased in breast cancer tumors (Pickuop et al., 2019). In recent years, NUDT5 has become more remarkable due to its function for hormonal gene regulation of PARP1's poly-ADP-Ribose (PAR) synthesis in breast cancer cells. Hence, the discovery of compounds showing an inhibitory effect on NUDT5 continues (Page et al., 2018; Tong et al., 2018).

HER2 is one of the best-defined therapeutic targets in breast cancer, and overexpression of HER2 protein in breast cancer cells tends to make aggressive tumor cells grow and divide more rapidly (Schlam and Swain, 2021). HER3 heterodimerizes with receptor tyrosine kinases (RTK) to activate oncogenic signaling through the PI3K/AKT pathway. Increased expression of HER3 is associated with malignancies in various cancers, including ovarian, breast, prostate, stomach, bladder, lung, melanoma, colorectal, and squamous cell carcinoma. Co-expression of HER2 and HER3 is common in breast cancer cell lines (Mishra et al., 2018). Determining the binding affinities of molecules with a particular interest in EGFR, especially in the development of multi-targeted inhibitors, to HER2 and HER3 targets can enhance the molecule's drug candidacy.

Compound **16** (momordicoside L) is found in MC (Zhang et al., 2010) and known to show antidiabetic, anti-inflammatory, and antibacterial properties and an effective compound (Li et al., 2020). *In silico* analyses performed in our study, the results obtained from iGemdock, DockThor and SwissDock, it was determined that the binding affinities of compound **16** on EGFR and NUDT5 were higher than the standards and it also has high binding affinity for HER2 and HER3. These data show that compound **16** is a potent inhibitor of these enzymes and can be a breast cancer drug potentially.

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