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Exploring a Computational Method for Evaluating the Epinecidin-1 and Its Variants Binding Efficacy with Breast Cancer Receptor (HER-2)

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Abstract

Host defense peptides are short oligopeptides, which function as the first line of defense mechanism in the host organisms against various microbial infections. Despite their antimicrobial and anti-inflammatory activity, they also exhibit anticancer activity. Harnessing this property and using in silico tools, we iterated alanine, cysteine and lysine residues of a host defense peptide Epinecidin-1(Epi-1). Strong binding to the breast cancer receptor, HER-2 was set as the bait criteria and the affinities were compared to the FDA recommended commercial drugs such as Abemaciclib, Alpelisib, Everolimus, Exemestane and Paclitaxel. Doing so, we found that some of the iterated variants had obtained binding affinity values higher or equivalent to the commercial drugs. In the present study, out of the 60 created Epi-1 variants, 3 variants have strong affinity to the HER2 receptor. The variants alanine 9, cysteine 16 and lysine 1 have 10, 8 and 10 interactions respectively with the HER2 receptor. Moreover, alanine 9 has the closest binding distance (2.05872 Å) with the HER2 receptor among all other counterparts. The present finding is unequivocal evidence that these variants of Epi-1 peptides have a high binding affinity near to commercial chemotherapeutics. Thus, short peptides engineered for therapeutic application using the knowledge of computational biology is one of the best ways to find an alternative for anticancer chemotherapeutics. Designing molecules via this computational methodology will save time and resources for the new drug development in the pharmaceutical industry.

Keywords Epinecidin-1 · HER2 · Autodock vina · Protein docking · Breast cancer

Abbreviations

AD	Autodock
CAP	Cationic antimicrobial peptides
CD340	Cluster of differentiation 340
EGF	Epidermal growth factor
Epi-1	Epinecidin-1
ErbB	Erythroblastic oncogene
FDA	Food and drug administration
HER	Human epidermal growth factor receptor
HLP	Half-life of peptide
HDP	Host defence peptide
RMSD	Root mean square deviation

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Introduction

Cancer is one of the leading causes of death all over the globe with an estimated around 19.3 million cases and 10 million deaths in the year 2020 (Sung et al. 2021). Though the death rate due to breast cancer has been continuously stable for the last few decades, nevertheless it remains a foremost health dispute amongst developed and developing countries. Breast cancer is the first leading cause of women's cancer disease and recorded fifth mortality worldwide, and it is responsible for almost one-third of all cancer diagnoses (Ma and Jemal 2013). In 2020, around 2.3 million breast cancer incidences were diagnosed and 684,996 deaths were reported worldwide (GLOBOCAN 2020). Breast cancer is extremely heterogeneous and affects the function of normal mammary epithelial cells. In the development of an embryo and adult tissues, the epidermal growth factor receptor (ErbB2) or cluster of differentiation 340 (CD340) are the central mediators of cell proliferation and differentiation, and their inappropriate activation is linked to the development and severity of many cancers (Cho et al. 2003).

The human epidermal growth factor receptor, especially the HER2/ ErbB2 is overexpressed in 20–30% of breast tumors than any other 3 receptors like, HER1, (ErbB1), HER3 (ErbB3), and HER4 (ErbB4) (Mitri et al. 2012) that are associated with more malignant phenotype, high recurrence rate (Meric-Bernstam and Hung 2006), chemotherapy resistance and poor prognosis. The dimerization and activation of the receptor (which transmits downstream growth signals), occurs when ligands bind to these related receptors, while no ligand that specifically binds to HER2 has been discovered (Fu et al. 2014). ErbB2 receptor is an extracellular region with four domains arranged as a tandem repeat of a two-domain unit, a single membrane traversal and a cytoplasmic tyrosine kinase 6 tail end. When a ligand binds to the extracellular region, it causes receptor dimerization and cytoplasmic kinase activation, which further causes auto phosphorylation to start the downstream signaling events.

Several anticancer approaches are available towards inhibiting EGF-stimulated signal transduction. Monoclonal antibody-based drugs, especially trastuzumab, have effective binding efficacy to the eco domain of HER2. The clinical studies revealed that 70% of breast cancer patients are found to be resistant against currently available chemotherapeutic treatments (Kute et al. 2004). So there is an urgent need for potential and target specific anticancer therapy that can overcome the limitations and harmful effects of the current treatment options. Cationic antimicrobial peptides (CAP) are identified as innate defense molecules and characterized as future chemotherapeutic drugs either using alone or with antibiotics, which are under clinical trials, along with their antimicrobial potential, some CAP possesses membranolytic activity on mammalian cells (Sarkar et al. 2021). Due to the increasing anionicity on the tumor cell membranes than normal healthy cell membranes, there is a significant increase in selectivity and specificity of CAP towards the cancer cells (Riedl et al. 2011). Epinecidin (Epi-1) (21 aa), a cationic host defense peptide (HDP) obtained from *Epinephelus coioides,* is proved to have antimicrobial (Pan et al. 2007; Neshani et al. 2019), anticancer (Chen et al. 2009; Neshani et al. 2019), immunostimulatory (Neshani et al. 2019) and wound healing (Huang et al. 2017) activities. Due to poor stability and non-availability of effective binding studies, Epi-1 could not be used as an effective drug for human welfare.

Development of a comprehensive drug product takes approximately twelve years or more and would have its effect in increasing the expected cost of the marketed medicine substantially (Zenie 1994; Usta et al. 2015). This time-consuming and costly approach may result in a delay in medicine development or even failure. Thus predicting the effectiveness and failures; before the production and clinical-stage is an effective tool to lower down drug development cost and time (Lionberger 2008). The in silico method is one of the most preferable tools, protein-peptide docking using Autodock Vina, the best in silico process tool and the most cited (18,587 google scholar citations) docking software by the research community (Trott and Olson 2009). AutoDock Vina significantly improved the average accuracy of the binding mode of predictions while running two orders of magnitude faster with multithreading. AD Vina is an exciting development, in terms of efficiency but also due to it being an open-source tool among the most accurate classical binding affinity prediction tools (Ciemny et al. 2018).

Earlier reports are evidences that introducing alanine, cysteine and lysine in the host defense peptides or proteins results in enhancing antimicrobial, anticancer and wound healing activities in addition to their stability (Montigiani et al. 1996; Boutureira and Bernardes 2015; Cutrona et al. 2015), So far no related research reports are available for making epinecidin-1 variants for this line of research. This is the first in silico analysis with 60 variants of Epi-1 done by mutating at each position of amino acids with alanine, or cysteine or lysine in order to evaluate their structural stability and binding affinity towards the breast cancer receptor, HER2 signaling protein.

Methodology

Workplace Setup

In the present docking analysis, we used the Windows 10 operating system, and all the working place folders were in the administrative drive because all the docking processes are programming oriented. All the collected protein, ligand and programming files were placed in a single folder. For this study, a high-speed accessible RAM and an i5 Intel core processor computer was employed in collecting the data and processing the data using Autodock Vina (Trott and Olson 2009). The overall work plan of molecular docking of Epi-1 variants with HER2 is schematically shown in Fig. 1.

Protein Pre-Preparation

Human epidermal growth factor receptor 2 (HER2), gene-encoded ErbB2 receptor protein (PDB ID: 1N8Z) was retrieved from protein data bank (https://www.rcsb.org/structure/1N8Z), which consists of Chain A—Herceptin Fab (antibody) light chain (sequence length-214), Chain B—Herceptin Fab (antibody) heavy chain (sequence length-220), and Chain C—Receptor protein-tyrosine kinase (ErbB-2). In developing embryo and adult tissues, ErbB receptors are central mediators of cellular proliferation and their inadequate activation is linked to the growth and severity of many cancers (Tang et al. 1998).

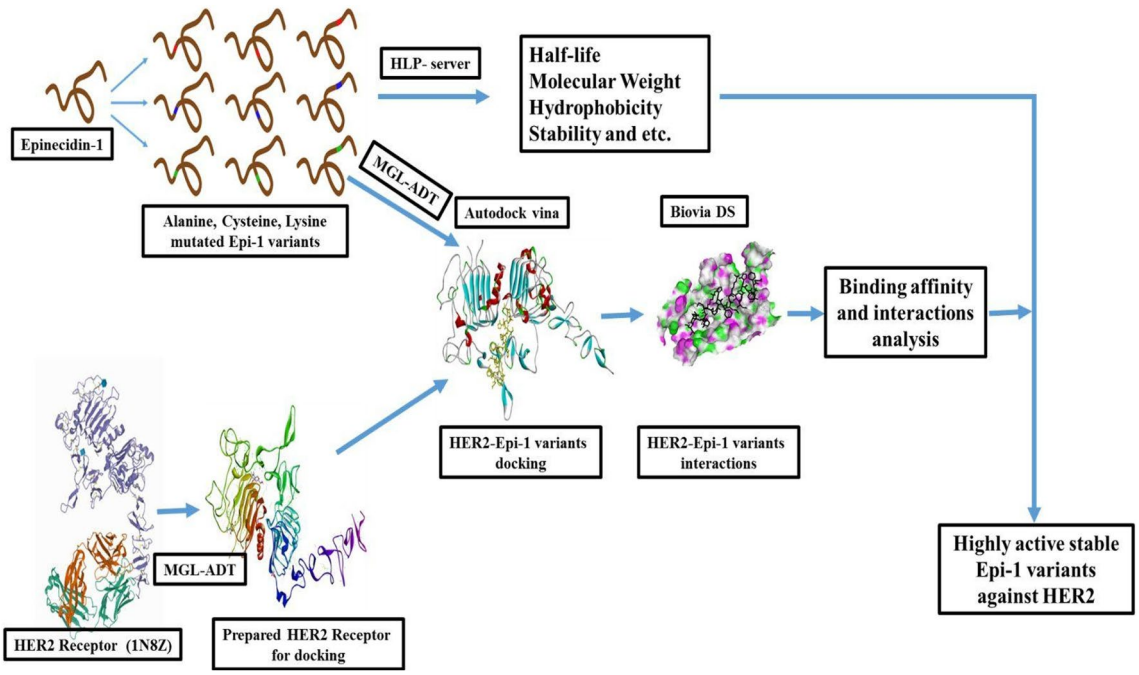


Fig. 1 Schematic view of overall work plan of molecular docking of Epinecidin-1 variants with HER2 receptor

Ligand Pre-Preparation

Epinecidin-1 is a 21 amino acid mature peptide, the sequence of Epi-1 (ID: 4201) was retrieved from the Database of Antimicrobial Activity and Structure of Peptide (DBAASP is accessible at http://www.biomedicine.org.ge/dbaasp/) (Pirtskhalava et al. 2021). In the epinecidin-1 sequence, each position of amino acid was replaced with Ala/Cys/Lys amino acids individually. Alanine, cysteine and lysine are retrieved and saved in protein data bank format (PDB format) with help of open Babel GUI tool version 3.1.1, which is used in converting FASTA format to PDB format (O'Boyle et al. 2011). In this way, 60 new Epi-1 variants were obtained with little structural differences, since at a time only one amino acid was modified.

Structural Property Analysis

All the new sixty Epi-1 variant's structural properties and half-life stability were analyzed with the HLP web-based server; HLP is a server that predicts peptide half-life in a intestine-like environment. It will predict/calculate the half-life of mutant peptides as well as their physicochemical properties (e.g. charge, polarity, hydrophobicity, and volume) (Sharma et al. 2014).

Protein and Ligand Preparation for Docking

Receptor protein crystal structure was accessed from Protein Data Bank (ID: 1N8Z) and was prepared using the Molecular Graphical Laboratory Autodock Tools (MGLADT) (Morris et al. 2009), Autodock Vina is a powerful, popular, reproducible and open resource tool for molecular docking studies. Removal of the bound complex molecules like, interrupting molecules, complex antibodies, non-essential water molecules and heteroatoms, adding hydrogens to the polar region, the addition of Kollman charges to the protein, which are template values for each amino acid that was derived from the corresponding electrostatic potential using quantum mechanics and the same above-mentioned tool was used to prepare the protein for the docking process also. The co-crystallized ligand was extracted from the active site to reveal the coordinate of the grid box, which is around the binding pocket. The prepared protein molecule is saved as a.pdbqt (pdb-protein data bank, q-charges, t-type of atoms) format into the particular folder. The modified pictorial diagram of prepared HER2 protein is shown in Fig. 2.

The wild-type Epi-1 and designed sixty Epi-1 variant peptide molecules were processed and addition of hydrogen atoms to polar regions of the peptides, with the addition of Kollman charges to each peptide molecule. All the new mutated peptide molecules had nearly 78–90 number of different torsion angles, but for the docking study, the first 10

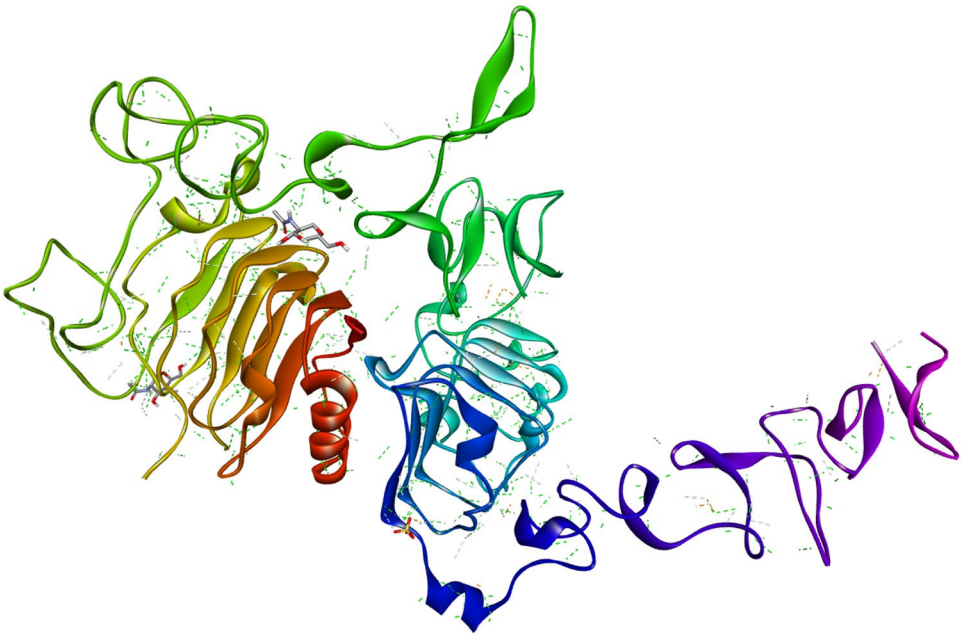


Fig. 2 Three-dimensional structure of the breast cancer cell receptor HER2 (Epidermal growth factor receptor (EGFR)) Protein (PDB ID: 1N8Z)

active torsion angles were only considered. The prepared active ligand molecules were saved as a.pdbqt format in the particular folder, which contains the prepared protein.

Receptor Gridbox Preparation

The Gridbox is the location of the particular area selected for the ligand to bind in the docking study, which helps to bind the ligands with the exact receptor site; the grid box is the margin of the receptor area of the protein molecule, without the grid generation process, ligand docking is not possible. A prepared protein structure with proper bond order and formal charges are necessary for receptor grid generation. To create a grid box, four tabs in the receptor grid generation were used. Receptor, location, limits, and rotatability are the four important elements (tabs) used for creating the grid box. The generated grid box had XYZ dimensional structure and size in angstrom (Å). The grid resolution was centered as 13.274, 88.167 and 129.816 along X, Y and Z axes respectively, for a grid size of $90 \times 80 \times 96$ Å to define the binding site respectively.

Molecular Docking Using Autodock Vina

Before starting the docking process, the folder should contain vina, vina_licence, vina_split, vina_windows perl programs, prepared protein and ligands in.pdbqt format, the configuration file in the text format, which contains the receptor protein name, receptor protein grid box coordinates, size of the grid box and ligand's name in the text file.

After the folder setup, the administrator command prompt was used to run the docking process. Here first we opened the docking folder in the command prompt, then used the perl programming to run the docking process by giving the input of configuration file and ligand names. The resulting interaction was compared with commercially available anticancer drugs, Abemaciclib, Alpelisib, Doxorubicin, Everolimus, Exemestane and Paclitaxel for similar active site determination using the same grid box dimension. All the above commercial anticancer drugs were retrieved from https://go.drugbank.com/ (Wishart et al. 2018) which is recommended by Food and Drug Administration (FDA) for breast cancer (NCI 2021).

Result and Discussion

Drug discovery is a very tedious and expensive process with an average of 10 to 12 years. It includes identification, characterization, validation and production at a small and large scale. Every process takes 1 or 2 years to complete even in highly sophisticated laboratories with well-trained scientists (Steinwandter et al. 2019). A group of computational, mathematical and biological scientists join together to work on the above problems with the available data to develop a new field in bioscience called bioinformatics. In this century with the help of bioinformatics and reverse engineering technology, the designing of a novel drug molecule could be easy within a short time without utilizing tons of chemicals polluting the environment, and scarifying hundreds of laboratory animals.

Peptide-receptor interactions are significant signaling phenomena between the cells and other external molecules. This specific interaction is effectively carried out via

hydrophobic, noncovalent and van der Waals interactions, which is of immense significance for signal transduction, immunoreaction and gene regulations. Molecular docking is an advanced scientific method to determine the protein–protein, protein-peptide and protein-nucleic acids interactions using computer tools. Though many online open software tools are available for researchers to do various docking studies, their specificity and reliability vary/not sufficient enough to meet the expectations, however, Auto dock Vina is one of the most promising and reliable software in this line.

Epinecidin-1 Variants

Host defense peptides (HDPs), the short peptides (< 100 amino acids) with amphipathic secondary structures are naturally produced by all living organisms against foreign pathogens. Most are alpha-helix followed by beta sheets and random coil secondary structures with a wide spectrum of bioactivities. The significant lacking of these peptides is their short half-life due to hydrophobic alpha-helix structure, protease degradation and secretion in a meager amount in the host organism and, which is highly influenced on the pathogenic infection. To overcome this issue, in recent days the HDPs are synthesized recombinantly in sophisticated laboratories for enhancing their therapeutic applications, which requires high environment polluting chemicals and expertise.

Epinecidin-1 is one of the several hundred HDPs identified from the orange-spotted grouper fish, *Epinephelus coioides* it exhibits diverse pharmacological properties including antimicrobial (Pan et al. 2007; Neshani et al. 2019), anticancer (Chen et al. 2009; Neshani et al. 2019) anti-inflammatory and wound healing properties (Huang et al. 2017). To enhance this peptide stability and bioactivity, we aim to create variants employing changing specific amino acids in Epi-1. Earlier studies are evidences that changing the amino acids like alanine (Montigiani et al. 1996), cysteine (Boutureira and Bernardes 2015) and lysine (Cutrona et al. 2015) in the HDPs helps to improve their bioactivity and stability. Moreover, earlier reports also revealed that altering biophysical properties could enhance stability, net charge, amphipathic nature, secondary structure, etc. (Reddy et al. 2004; Teixeira et al. 2012). The results showed that in all the 60 created variants, the structural confirmation was maintained as in the original and 3 variants showed enhanced binding activity when compared to its counterpart.

Structural Stability Analysis

The structural biophysical properties of Epi-1 and its 60 variants were analyzed with HLP web-based server for its stability, half-life, hydrophobicity, molecular weight, surface accessibility, charge, and optical rotation and their relative stability were calculated in the intestine-like microenvironment. The detailed results were tabulated (Tables 1, 2 and 3), these are evidence that the peptide's biophysical properties are highly altered in the variants. The highlights are, the half-life of the Epi-1 is 0.842 s, while the 21st alanine mutated Epi-1 variants exhibit a higher half-life (0.939 s), in the case of cysteine mutated Epi-1, except cysteine1, cysteine9, cysteine14 and cysteine19, all others have higher half-life compared to their counterparts. Similarly, the lysine mutated Epi-1 peptides, lysine1, lysine9, lysine14 and lysine19 have a lesser half-life when compared with wild type Epi-1 peptides.

While creating a variant, the molecular weight of the Epi-1 has increased in the alanine mutated Epi-1 peptides, at positions 1, 9, 14, and 19, while the remaining alanine variants show comparatively less molecular weight when compared with the wild type Epi-1. All the cysteine mutated Epi-1 show higher molecular weight than the wild type Epi-1. Among the lysine mutated Epi-1, only lysine mutated at 1, 4, 11 and 12 positions exhibit less molecular weight, while the rest show higher molecular weight.

Autodock Vina

The Autodock Vina results were generated as a text file. The results of docking as a value of Root Mean Square Deviation (RMSD) were stored in the particular folder in text file format, which contains the result of the first ten active torsion angles of the ligand docking with the receptor HER2. All the sixty ligands binding results were stored individually in the same folder.

Autodock Vina Split

The results were also stored as a complex of receptor HER2 and ligand complex, each result containing the ten complexes in a single.pdbqt format file. Autodock Vina split program split the ten individual results into a separate.pdbqt format file.

Validation of Result with PyMol

The HER2 protein and individual result molecules were opened in the PyMol 2.5 to validate the best binding torsion angle of the particular ligand peptide. Among the ten binding affinity values, the first value in the RMSD table showed maximum docking affinity with the receptor HER2 and the binding affinity values were expressed in kcal/mol.

Table 1 Structural properties of Epinecidin-1 and its alanine mutated variants peptide sequence (mutated alanine residue shown in red color and high half-life showing bold)

S. No.	Peptides Sequence	Mutation Position	Half-life(sec)	Stability	Hydrophobicity (KJ/mol)	Molecular weight	Surface Accessibility (Å)	Charge	Optical Rotation	Relative Stability
1.	GFIFHIIKGLFHAGKMIHGLV	Wild-type	0.842	Normal	-2.184	2336.24	36.381	3.5	-7.846	3.270
2.	AFIFHIIKGLFHAGKMIHGLV	1	0.215	Normal	-2.933	2350.26	36.538	3.5	-7.760	3.322
3.	GAIFHIIKGLFHAGKMIHGLV	2	0.841	Normal	-2.395	2260.14	36.490	3.5	-6.118	3.125
4.	GFAFHIIKGLFHAGKMIHGLV	3	0.752	Normal	-2.514	2294.15	36.619	3.5	-8.351	3.137
5.	GFIAHIIKGLFHAGKMIHGLV	4	0.841	Normal	-2.395	2260.14	36.490	3.5	-6.118	3.125
6.	GFIFAIIKGLFHAGKMIHGLV	5	0.712	Normal	-3.733	2270.17	35.290	3.0	-5.927	3.210
7.	GFIFHAIKGLFHAGKMIHGLV	6	0.752	Normal	-2.514	2294.15	36.619	3.5	-8.351	3.137
8.	GFIFHIAKGLFHAGKMIHGLV	7	0.752	Normal	-2.514	2294.15	36.619	3.5	-8.351	3.137
9.	GFIFHIIAGLFHAGKMIHGLV	8	0.608	Normal	-4.886	2279.14	32.80	2.5	-8.456	3.236
10.	GFIFHIIKALFHAGKMIHGLV	**9**	**0.215**	Normal	-2.933	2350.26	36.538	3.5	-7.760	3.322
11.	GFIFHIIKGAFHAGKMIHGLV	10	0.660	Normal	-2.576	2294.15	36.390	3.5	-7.237	3.130
12.	GFIFHIIKGLAHAGKMIHGLV	11	0.841	Normal	-2.395	2260.14	36.490	3.5	-6.118	3.125
13.	GFIFHIIKGLFAAGKMIHGLV	12	0.712	Normal	-3.733	2270.17	35.290	3.0	-5.927	3.210
14.	GFIFHIIKGLFHAAKMIHGLV	14	0.215	Normal	-2.933	2350.26	36.538	3.5	-7.760	3.322
15.	GFIFHIIKGLFHAGAMIHGLV	15	0.608	Normal	-4.886	2279.14	32.800	2.5	-8.456	3.236
16.	GFIFHIIKGLFHAGKAIHGLV	16	0.823	Normal	-2.457	2276.12	36.110	3.5	-7.284	3.169
17.	GFIFHIIKGLFHAGKMAHGLV	17	0.752	Normal	-2.514	2294.15	36.619	3.5	-8.351	3.137
18.	GFIFHIIKGLFHAGKMIAGLV	18	0.712	Normal	-3.733	2270.17	35.290	3.0	-5.927	3.210
19.	GFIFHIIKGLFHAGKMIHALV	19	0.215	Normal	-2.933	2350.26	36.538	3.5	-7.760	3.322
20.	GFIFHIIKGLFHAGKMIHGAV	20	0.660	Normal	-2.576	2294.15	36.390	3.5	-7.237	3.130
21.	GFIFHIIKGLFHAGKMIHGLA	21	0.939	Normal	-2.614	2308.18	36.576	3.5	-8.029	3.180

Virtual Screening of Docking Results with DS Visualization Studio

The prepared protein and extracted individual ligands from complex results were converted back into.pdb file format, a rigid format of the molecules to visualize with the Biovia Discovery studio visualizer. The binding affinity between the receptor HER2 and Epi-1 variants were visualized and the interaction between the amino acids was demonstrated. All the 60 Epi-1 variants had a good binding affinity with receptor HER2 (Fig. 3).

The ligands confirmations of the alanine, cysteine and lysine mutated Epi-1 variants showed potential anticancer effects which are expressed in Table 4 along with the binding affinities they have against the receptor HER2. Mutated Epi-1 variants, alanine 9th (Alanine 9) showed -10.8 kcal/mol as the highest binding affinity, followed by the cysteine 16th (Cysteine 16) − 9.7 kcal/mol and lysine 1st (Lysine 1) − 9.5 kcal/mol (Fig. 4).

Alanine mutated Epi-1 variants showed a good binding affinity with receptor HER2 protein. Among the 20 designed alanine mutated epi-1 variants, the 9th position (Alanine 9) mutated variant showed the highest binding affinity. In these results, the seven amino acids from receptor HER2 protein interacted with alanine 9, of those, eleven interactions are hydrophobic and one with a hydrogen bond. From the receptor HER2 protein HIS448, Ala15, PRO478, PRO356, LEU355 and HIS447 are the amino acids that interact with alanine 9. In this interaction C:HIS448:HD1−D:LEU10:O showed the closest interaction with 2.05872 Å. The detailed results are shown in Table 5.

Cysteine mutated Epi-1 variant exhibited an efficient binding affinity with receptor HER2 protein. Among the 21 designed cysteine mutated epi-1 variants, the 16th position (Cysteine 16) mutated variant showed the highest binding affinity. Further, the results revealed that eight amino acids from receptor HER2 protein have interacted with Cysteine 16, and all eight interactions were hydrophobic. From the receptor HER2 protein ALA248, LEU224, VAL286, VAL292, ILE413, PHE236, HIS245 and PRO294 are the amino acids that interact with Cysteine 16. In this interaction, D:CYS16–C:VAL292 explored the most proximate interaction with 3.6535 Å (Table 6).

Similarly, Lysine mutated Epi-1 variant also displayed a strong binding affinity with receptor HER2 protein. The first position (Lysine 1) mutated variants revealed good binding affinity when compared with other 18 lysine mutated Epi-1 variants. In these results, among the ten amino acids from

Table 2 Structural properties of Epinecidin-1 and its cysteine mutated variants peptide sequence e (mutated cysteine shown in red color and high half-life showing bold)

S. No.	Peptides Sequence	Mutation Position	Half-life(sec)	Stability	Hydrophobicity (KJ/mol)	Molecular weight	Surface Accessibility(Å)	Charge	Optical Rotation	Relative Stability
1.	GFIFHIIKGLFHAGKMIHGLV	Wild type	0.842	Normal	-2.184	2336.24	36.381	3.5	-7.846	3.270
2.	**C**FIFHIIKGLFHAGKMIHGLV	1	0.631	Normal	-3.014	3071.3	35.952	3.5	-8.632	3.342
3.	G**C**IFHIIKGLFHAGKMIHGLV	2	1.349	High	-2.476	2942.3	35.905	3.5	-6.989	3.145
4.	GF**C**FHIIKGLFHAGKMIHGLV	3	1.297	High	-2.595	2962.8	36.033	3.5	-9.222	3.157
5.	GFI**C**HIIKGLFHAGKMIHGLV	4	1.349	High	-2.476	2942.3	35.905	3.5	-6.989	3.145
6.	GFIF**C**IIKGLFHAGKMIHGLV	5	1.278	High	-3.814	2978.7	34.705	3.0	-6.799	3.230
7.	GFIFH**C**IKGLFHAGKMIHGLV	6	1.297	High	-2.595	2962.8	36.033	3.5	-9.222	3.157
8.	GFIFHI**C**KGLFHAGKMIHGLV	7	1.297	High	-2.595	2962.8	36.033	3.5	-9.222	3.157
9.	GFIFHII**C**GLFHAGKMIHGLV	8	1.116	High	-4.967	2955.7	32.214	2.5	-9.327	3.257
10.	GFIFHIIK**C**LFHAGKMIHGLV	9	0.631	Normal	-3.014	3071.3	35.952	3.5	-8.632	3.342
11.	GFIFHIIKG**C**FHAGKMIHGLV	10	1.177	High	-2.657	2962.8	35.805	3.5	-8.108	3.151
12.	GFIFHIIKGL**C**HAGKMIHGLV	11	1.349	High	-2.476	2942.3	35.905	3.5	-6.989	3.145
13.	GFIFHIIKGLF**C**AGKMIHGLV	12	1.278	High	-3.814	2978.7	34.705	3.0	-6.799	3.230
14.	GFIFHIIKGLFH**C**GKMIHGLV	13	1.283	High	-2.895	3043.0	35.795	3.5	-8.718	3.290
15.	GFIFHIIKGLFHA**C**KMIHGLV	14	0.631	Normal	-3.014	3071.3	35.952	3.5	-8.632	3.342
16.	GFIFHIIKGLFHAG**C**MIHGLV	15	1.116	High	-4.967	2955.7	32.214	2.5	-9.327	3.257
17.	GFIFHIIKGLFHAGK**C**IHGLV	**16**	**1.331**	High	-2.538	2969.1	35.524	3.5	-8.156	3.190
18.	GFIFHIIKGLFHAGKM**C**HGLV	17	1.297	High	-2.595	2962.8	36.033	3.5	-9.222	3.157
19.	GFIFHIIKGLFHAGKMI**C**GLV	18	1.278	High	-3.814	2978.7	34.705	3.0	-6.799	3.230
20.	GFIFHIIKGLFHAGKMIH**C**LV	19	0.631	Normal	-3.014	3071.3	35.952	3.5	-8.632	3.342
21.	GFIFHIIKGLFHAGKMIHG**C**V	20	1.177	High	-2.657	2962.8	35.805	3.5	-8.108	3.151
22.	GFIFHIIKGLFHAGKMIHGL**C**	21	1.487	High	-2.695	2989.9	35.990	3.5	-8.900	3.201

the lysine interacted receptor HER2, nine interactions are hydrophobic and one is hydrogen-bonded. From the receptor HER2 protein HIS245, ALA248, LEU244, VAL292, VAL286, ILE413, PHE236, HIS245 and PRO294 are the amino acids that interacted with the lysine 1 mutated variant. C:HIS245:HD1–D:PHE2:O shows very close interaction by a hydrogen bond distance of 2.92144 Å between peptide and receptor (Table 7).

The commercial anticancer drugs namely, Abemaciclib, Alpelisib, Everolimus, Exemestane and Paclitaxel also showed a strong binding affinity with receptor HER2 protein and the RMSD values of binding affinity were − 7.5, − 8.2, − 11.1, − 8.8 and − 8.0 respectively (Table 8). The binding interactions were visualized by Discovery studio visualizer (Fig. 5).

The anticancer drug, abemaciclib has three hydrogen-bonded and two hydrophobic interactions with HER2 protein. Among these, C:CYS246:HN-:UNL1:F shows the closest binding affinity with 2.66842 Å distance between the conventional hydrogen bonds (Halogen (Fluorine) interaction). Alpelisib has five hydrogen bonding, five hydrophobic bonding, one halogen, one electrostatic and Pi-sulfur interactions with receptor HER2 protein, among those:UNL1:HN–C:ARG332:O is the closest binding affinity, which is 2.13391 Å distance between the conventional hydrogen bond interaction. Everolimus have four hydrogen bonding and three hydrophobic bonding interactions with receptor HER2, among those C:TYR387:HH–:UNK0:O is the closest binding affinity, which is 2.53039 Å distance between the conventional hydrogen bonding interaction. Exemestane has a C:SER441:HG−:UNL1:O single hydrogen bonding binding affinity with receptor HER2 protein, the binding distance is 2.60407 Å between the interactions. Finally, the Paclitaxel have three hydrogen bonding, one electrostatic and one hydrophobic interaction with receptor HER2 protein, among those C:LYS10:HZ2−:UNL1:O is the closest binding affinity, which is 2.32208 Å distance between the conventional hydrogen bond interaction. The detailed results are shown in Table 9. From these results, commercial anticancer drug everolimus showed a better binding affinity as well as higher RMSD value among the five drugs.

The mutated epi-1 variants showed a greater binding affinity compared to the commercial anticancer drugs abemaciclib, alpelisib, exemestane and paclitaxel. The average binding affinity value of alanine (− 9.065 kcal/mol), cysteine

Table 3 Structural properties of Epinecidin-1 and its lysine mutated variants peptide sequence e (mutated lysine shown in red color and high half-life showing bold)

S. No.	Peptides Sequence	Mutation Position	Half-life(sec)	Stability	Hydrophobicity (KJ/mol)	Molecular weight	Surface Accessibility(Å)	Charge	Optical Rotation	Relative Stability
1.	GFIFHIIKGLFHAGKMIHGLV	wild type	0.842	Normal	-2.184	2336.24	36.381	3.5	-7.846	3.270
2.	**K**FIFHIIKGLFHAGKMIHGLV	1	0.419	Normal	-0.862	2407.36	40.119	4.5	-7.151	3.356
3.	G**K**IFHIIKGLFHAGKMIHGLV	2	1.041	High	-0.324	2317.24	40.071	4.5	-5.508	3.159
4.	GF**K**FHIIKGLFHAGKMIHGLV	3	0.958	Normal	-0.443	2351.25	40.200	4.5	-7.741	3.170
5.	GFI**K**HIIKGLFHAGKMIHGLV	4	1.041	High	-0.324	2317.24	40.071	4.5	-5.508	3.159
6.	GFIF**K**IIKGLFHAGKMIHGLV	5	0.930	Normal	-1.662	2327.27	38.871	4.0	-5.318	3.243
7.	GFIFH**K**IKGLFHAGKMIHGLV	6	0.958	Normal	-0.443	2351.25	40.200	4.5	-7.741	3.170
8.	GFIFHI**K**KGLFHAGKMIHGLV	7	0.958	Normal	-0.443	2351.25	40.200	4.5	-7.741	3.170
9.	GFIFHIIK**K**LFHAGKMIHGLV	9	0.419	Normal	-0.862	2407.36	40.119	4.5	-7.151	3.356
10.	GFIFHIIKG**K**FHAGKMIHGLV	10	0.881	Normal	-0.505	2351.25	39.971	4.5	-6.627	3.164
11.	GFIFHIIKGL**K**HAGKMIHGLV	11	1.041	High	-0.324	2317.24	40.071	4.5	-5.508	3.159
12.	GFIFHIIKGLF**K**AGKMIHGLV	12	0.930	Normal	-1.662	2327.27	38.871	4.0	-5.318	3.243
13.	GFIFHIIKGLFH**K**GKMIHGLV	13	0.988	Normal	-0.743	2393.34	39.962	4.5	-7.237	3.304
14.	GFIFHIIKGLFHA**K**KMIHGLV	14	0.419	Normal	-0.862	2407.36	40.119	4.5	-7.151	3.356
15.	GFIFHIIKGLFHAGK**K**IHGLV	16	1.014	High	-0.386	2333.22	39.690	4.5	-6.675	3.203
16.	GFIFHIIKGLFHAGKM**K**HGLV	17	0.958	Normal	-0.443	2351.25	40.200	4.5	-7.741	3.170
17.	GFIFHIIKGLFHAGKMI**K**GLV	18	0.930	Normal	-1.662	2327.27	38.871	4.0	-5.318	3.243
18.	GFIFHIIKGLFHAGKMIH**K**LV	19	0.419	Normal	-0.862	2407.36	40.119	4.5	-7.151	3.356
19.	GFIFHIIKGLFHAGKMIHG**K**V	20	0.881	Normal	-0.505	2351.25	39.971	4.5	-6.627	3.164
20.	GFIFHIIKGLFHAGKMIHGL**K**	21	**1.141**	High	-0.543	2365.28	40.157	4.5	-7.419	3.214

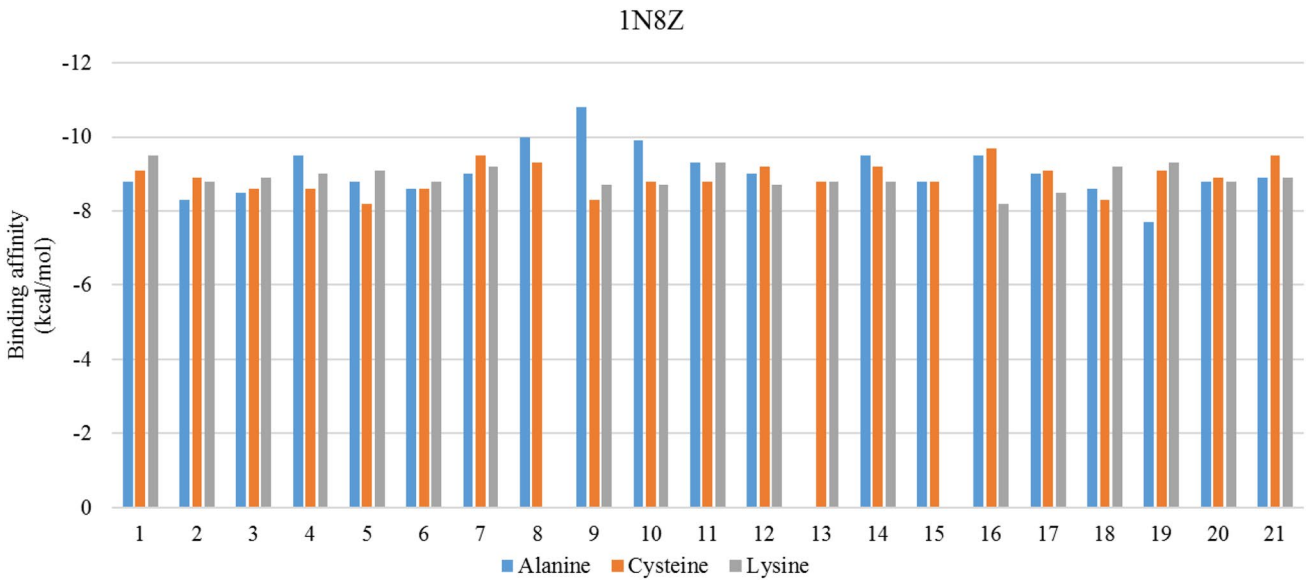


Fig. 3 Comparative analysis of the Alanine, Cysteine and Lysine Mutated Epinecidin-1 variants binding affinity with the breast cancer cell receptor HER2. **a** Alanine 9, **b** Cysteine 16, **c** Lysine 1

(− 8.92 kcal/mol) and lysine (− 8.95 kcal/mol) mutated epi-1 variants with HER2 protein is higher than the above mentioned commercial drugs. At the same time, alanine 9 (10 interactions), cysteine 16 (8 interactions) and lysine 1 (10 interactions) had more interactions than all other commercial drugs used in this study. Moreover, alanine 9 has the

Table 4 RMSD values of binding affinity between the breast cancer cell receptor HER2 with Epinecidin-1 alanine, cysteine and lysine mutated variants (The highest binding affinity of each amino acid modification results are shown in bold letters)

Position of amino acid change in the Epinecidine-1	Binding affinity (kcal/mole)	Position of amino acid change in the Epinecidine-1	Binding affinity (kcal/mole)	Position of amino acid change in the Epinecidine-1	Binding affinity (kcal/mole)
Epinecidin-1 (Wild type)	−8.0				
Alanine 1	−8.8	Cysteine 1	−9.1	**Lysine 1**	**−9.5**
Alanine 2	−8.3	Cysteine 2	−8.9	Lysine 2	−8.8
Alanine 3	−8.5	Cysteine 3	−8.6	Lysine 3	−8.9
Alanine 4	−9.5	Cysteine 4	−8.6	Lysine 4	−9.0
Alanine 5	−8.8	Cysteine 5	−8.2	Lysine 5	−9.1
Alanine 6	−8.6	Cysteine 6	−8.6	Lysine 6	−8.8
Alanine 7	−9.0	Cysteine 7	−9.5	Lysine 7	−9.2
Alanine 8	−10.0	Cysteine 8	−9.3	Lysine 9	-8.7
Alanine 9	**−10.8**	Cysteine 9	−8.3	Lysine 10	−8.7
Alanine 10	−9.9	Cysteine 10	−8.8	Lysine 11	−9.3
Alanine 11	−9.3	Cysteine 11	−8.8	Lysine 12	−8.7
Alanine 12	−9.0	Cysteine 12	−9.2	Lysine 13	−8.8
Alanine 14	−9.5	Cysteine 13	−8.8	Lysine 14	−8.8
Alanine 15	−8.8	Cysteine 14	−9.2	Lysine 16	−8.2
Alanine 16	−9.5	Cysteine 15	−8.8	Lysine 17	−8.5
Alanine 17	−9.0	**Cysteine 16**	**−9.7**	Lysine 18	−9.2
Alanine 18	−8.6	Cysteine 17	−9.1	Lysine 19	−9.3
Alanine 19	−7.7	Cysteine 18	−8.3	Lysine 20	−8.8
Alanine 20	−8.8	Cysteine 19	−9.1	Lysine21	−8.9
Alanine 21	−8.9	Cysteine 20	−8.9		
		Cysteine 21	−9.5		

closest binding distance (2.05872) with the HER2 protein among all other binding interactions.

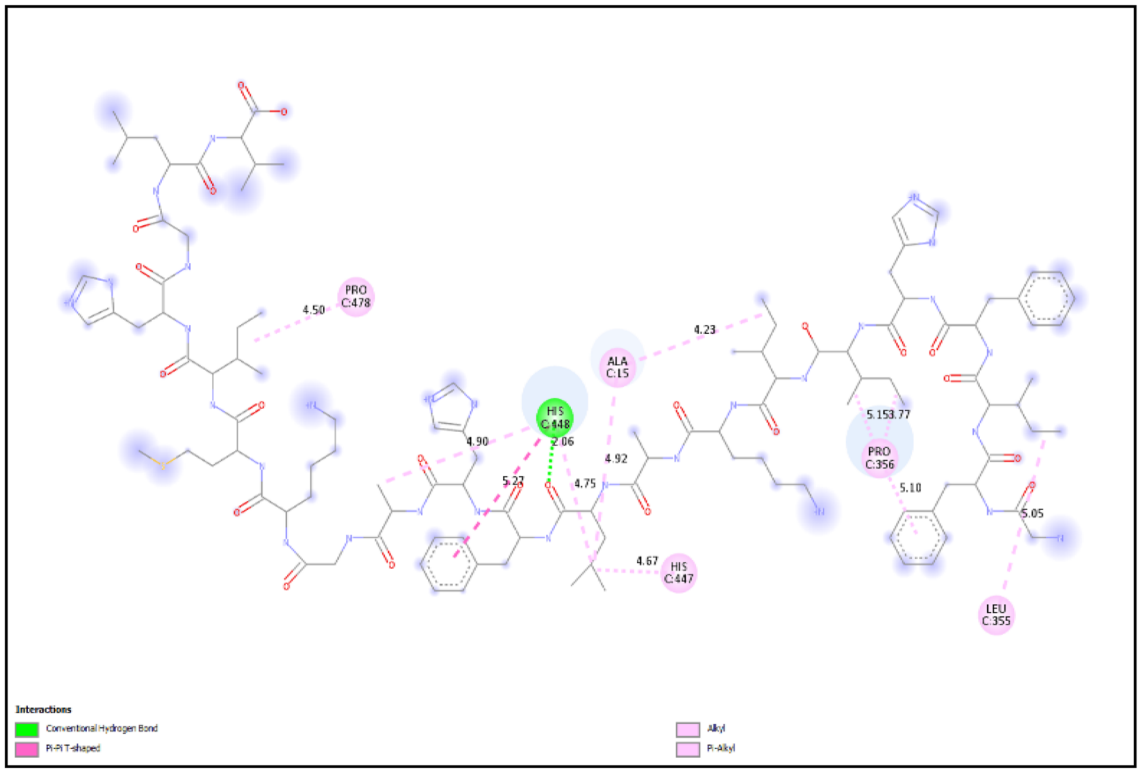
It is significant to point here that no specific or similar amino acid interaction between the commercial anticancer drugs with HER2 receptor was observed even though each antibiotic has the ability to control its further progenesis. Likewise, in the present study, we find that none of the Epi-1 variants shows similar amino acid interactions when binding with the HER2 receptor. Nevertheless, both drugs (commercial anticancer and Epi-1 variants) have interacted within the same binding pocket of the HER2 receptor. The present study illustrates the in silico analysis of the epinecidin-1 and its variants stability and interaction with breast cancer receptor HER2. The results are evidence that the variants of epi-1 have a strong affinity with breast cancer receptors when compared to its wild type counterparts and various recommended anticancer drugs. Especially the variants alanine 9 has significantly higher affinity (RMSD) value among the 60 created variants. However, when evaluating both stability and anticancer activity cysteine 16 showed the best results with the possibility of additional di-sulphide linkage. Further, this study clearly shows the possible binding sites of the peptide and receptors.

Statistical Analysis

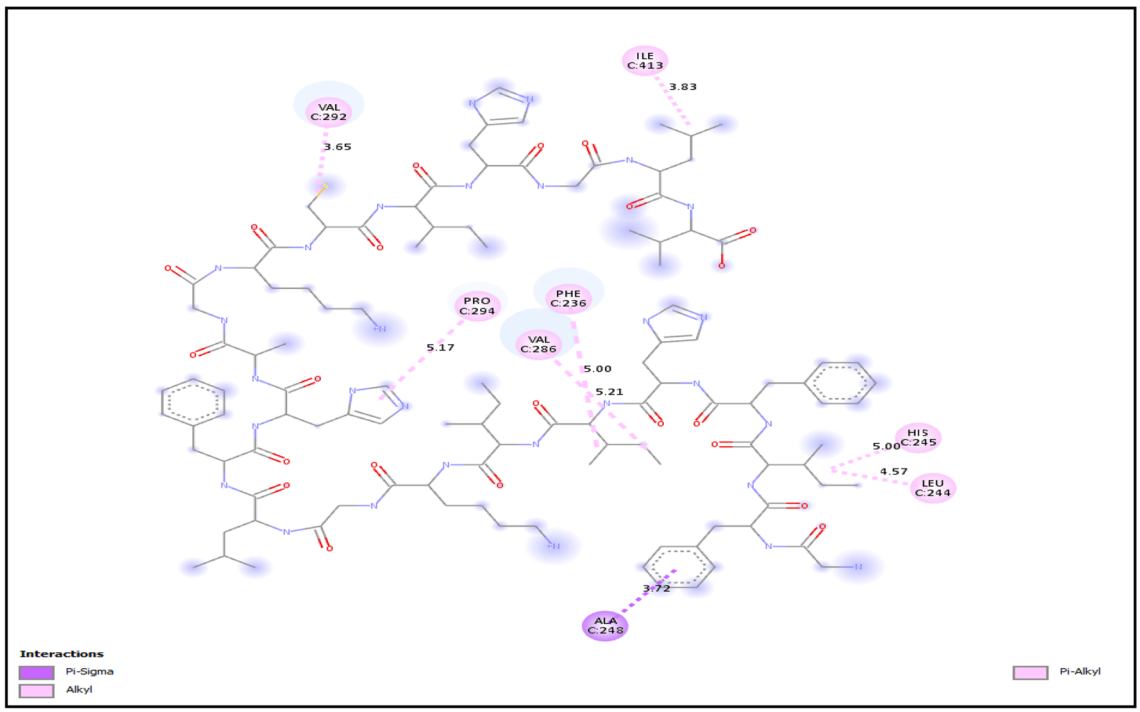
All data statistically assessed with ANOVA test, the Alanine, Cysteine and Lysine mutated peptides, positive control (Abemaciclib, Alpelisib, Doxorubicin, Everolimus, Exemestane and Paclitaxel) and control (Epinecidin-1) binding affinity results taken as a groups, a value of $P < 0.05$ was considered significant. Alanine, Cysteine and Lysine mutated peptide groups and positive control group have more significant with control (Epinecidin-1) group. The ANOVA results showing the $P < 0.0001$, since the test is more significant. (Fig. 6).

Conclusion

Computer-based interaction studies using reliable software are the most economically and technically sound method to predict the peptides and their variants bioactivities with various membrane receptors (including microbes and cancer). They prove to be the best method to obtain results in a short time span without using much sophisticated equipment. To confirm this analysis, specific variants in vitro and in vivo bioactivity assays are needed, which is the future goal of this study.

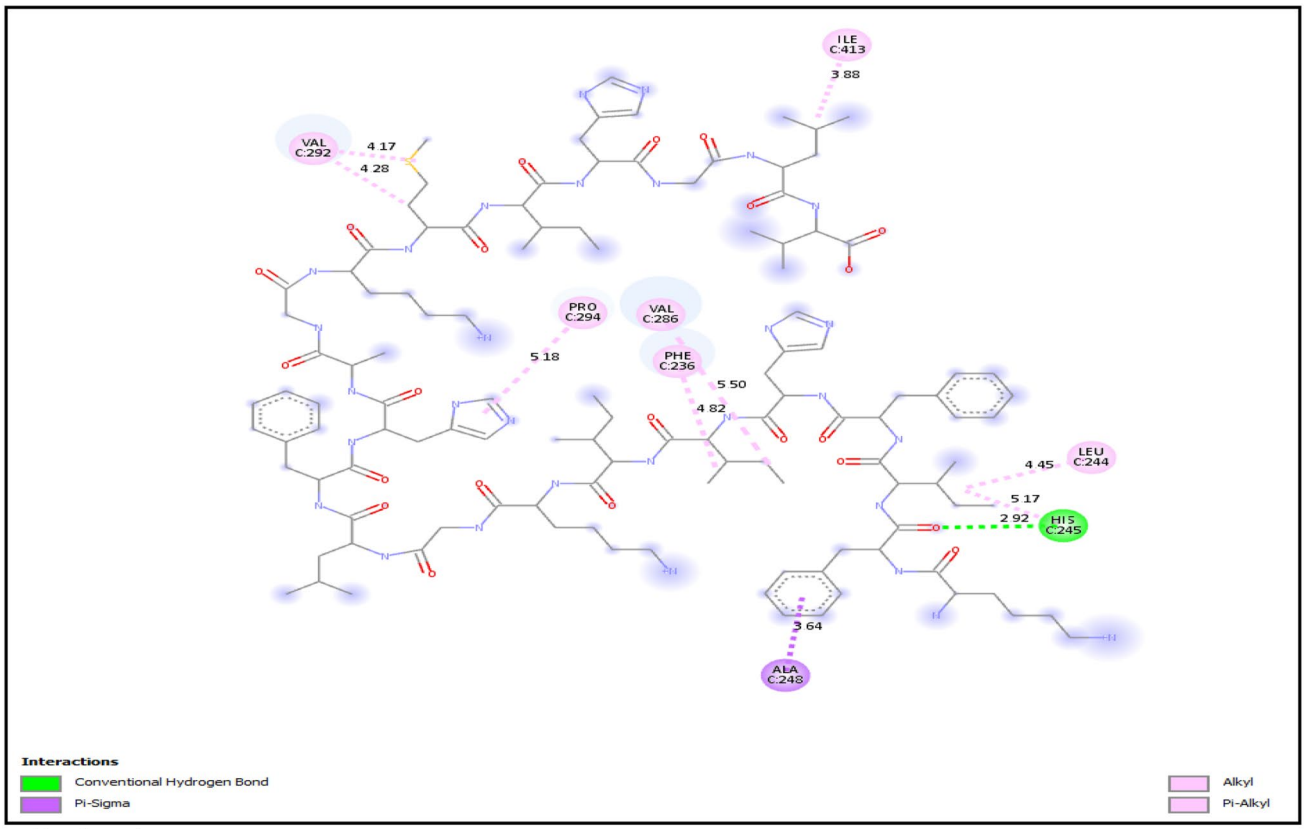


a. Alanine 9



b. Cysteine 16

Fig. 4 Binding interaction between the breast cancer cell receptor HER2 with Epinecidin-1 variant antimicrobial peptides. **a** with 9th alanine mutated variant, **b** with 16th position cysteine mutated variant, **c** with 1st position lysine mutated variants



c. Lysine 1

Fig. 4 (continued)

Table 5 Binding interaction between the receptor HER2 and Epinecidin-1 alanine 9 mutated variant

Name	Distance Å	Category	Types	From	From chemistry	To	To chemistry
C:HIS448:HD1 -D:LEU10:O	2.05872	Hydrogen Bond	Conventional Hydrogen Bond	C:HIS448:HD1	H-Donor	A:LEU10:O	H-Acceptor
C:HIS448—D:PHE11	5.2681	Hydrophobic	Pi-Pi T-shaped	C:HIS448	Pi-Orbitals	D:PHE11	Pi-Orbitals
C:PRO478—D:ILE17	4.50255	Hydrophobic	Alkyl	C:PRO478	Alkyl	D:ILE17	Alkyl
D:ILE6—C:PRO356	5.15130	Hydrophobic	Alkyl	D:ILE6	Alkyl	C:PRO356	Alkyl
D:ILE3—C:LEU355	5.05286	Hydrophobic	Alkyl	D:ILE3	Alkyl	C:LEU355	Alkyl
D:ILE6—C:PRO356	3.76543	Hydrophobic	Alkyl	D:ILE6	Alkyl	C:PRO356	Alkyl
C:HIS447—D:LEU10	4.67024	Hydrophobic	Pi-Alkyl	C:HIS447	Pi-Orbitals	D:LEU10	Alkyl
C:HIS448—D:LEU10	4.75253	Hydrophobic	Pi-Alkyl	C:HIS448	Pi-Orbitals	D:LEU10	Alkyl
C:HIS448—D:ALA13	4.89915	Hydrophobic	Pi-Alkyl	C:HIS448	Pi-Orbitals	D:ALA13	Alkyl
D:PHE2—C:PRO356	5.10159	Hydrophobic	Pi-Alkyl	D:PHE2	Pi-Orbitals	C:PRO356	Alkyl

*C- HER2 receptor, *D-Epinecidin-1 alanine 9 mutated variant

Table 6 Binding interaction between the receptor HER2 and Epinecidin-1 cysteine 16 mutated variants

Name	Distance Å	Category	Types	From	From chemistry	To	To chemistry
C:ALA248:CB—D:PHE2	3.71924	Hydrophobic	Pi-Sigma	C:ALA248:CB	C-H	D:PHE2	Pi-Orbitals
C:LEU244—D:ILE3	4.56578	Hydrophobic	Alkyl	C:LEU244	Alkyl	D:ILE3	Alkyl
D:ILE6—C:VAL286	5.21434	Hydrophobic	Alkyl	D:ILE6	Alkyl	C:VAL286	Alkyl
D:CYS16—C:VAL292	3.65350	Hydrophobic	Alkyl	D:CYS16	Alkyl	C:VAL292	Alkyl
D:LEU20—C:ILE413	3.83062	Hydrophobic	Alkyl	D:LEU20	Alkyl	C:ILE413	Alkyl
C:PHE236—D:ILE6	5.00361	Hydrophobic	Pi-Alkyl	C:PHE236	Pi-Orbitals	D:ILE6	Alkyl
C:HIS245—D:ILE3	5.00358	Hydrophobic	Pi-Alkyl	C:HIS245	Pi-Orbitals	D:ILE3	Alkyl
D:HIS12—C:PRO294	5.17489	Hydrophobic	Pi-Alkyl	D:HIS12	Pi-Orbitals	C:PRO294	Alkyl

*C- HER2 receptor, *D-Epinecidin-1 cysteine16 mutated variante

Table 7 Binding interaction between the receptor HER2 and Epinecidin-1 lysine 1 mutated variants

Name	Distance Å	Category	Types	From	From chemistry	To	To chemistry
C:HIS245:HD1—D:PHE2:O	2.92144	Hydrogen Bond	Conventional Hydrogen Bond	C:HIS245:HD1	H-Donor	D:PHE2:O	H-Acceptor
C:ALA248:CB—D:PHE2	3.64068	Hydrophobic	Pi-Sigma	C:ALA248:CB	C-H	D:PHE2	Pi-Orbitals
C:LEU244—D:ILE3	4.44847	Hydrophobic	Alkyl	C:LEU244	Alkyl	D:ILE3	Alkyl
C:VAL292—D:MET16	4.17237	Hydrophobic	Alkyl	C:VAL292	Alkyl	D:MET16	Alkyl
D:MET16—C:VAL292	4.2757	Hydrophobic	Alkyl	D:MET16	Alkyl	C:VAL292	Alkyl
D:ILE6—C:VAL286	5.49549	Hydrophobic	Alkyl	D:ILE6	Alkyl	C:VAL286	Alkyl
D:LEU20—C:ILE413	3.87553	Hydrophobic	Alkyl	D:LEU20	Alkyl	C:ILE413	Alkyl
C:PHE236—D:ILE6	4.81846	Hydrophobic	Pi-Alkyl	C:PHE236	Pi-Orbitals	D:ILE6	Alkyl
C:HIS245—D:ILE3	5.16911	Hydrophobic	Pi-Alkyl	C:HIS245	Pi-Orbitals	D:ILE3	Alkyl
D:HIS12—C:PRO294	5.18207	Hydrophobic	Pi-Alkyl	D:HIS12	Pi-Orbitals	C:PRO294	Alkyl

*C- HER2 receptor, *D-Epinecidin-1 lysine 1 mutated variant

Table 8 RMSD values of binding affinity between the breast cancer cell receptor HER2 with FDA recommended anticancer drugs

Commercial anticancer drug	Binding affinity (kcal/mole)
Abemaciclib	−7.5
Alpelisib	−8.2
Everolimus	**−11.1**
Exemestane	−8.8
Paclitaxel	−8.0

Bold indicates the best docking affinity among chosen drugs

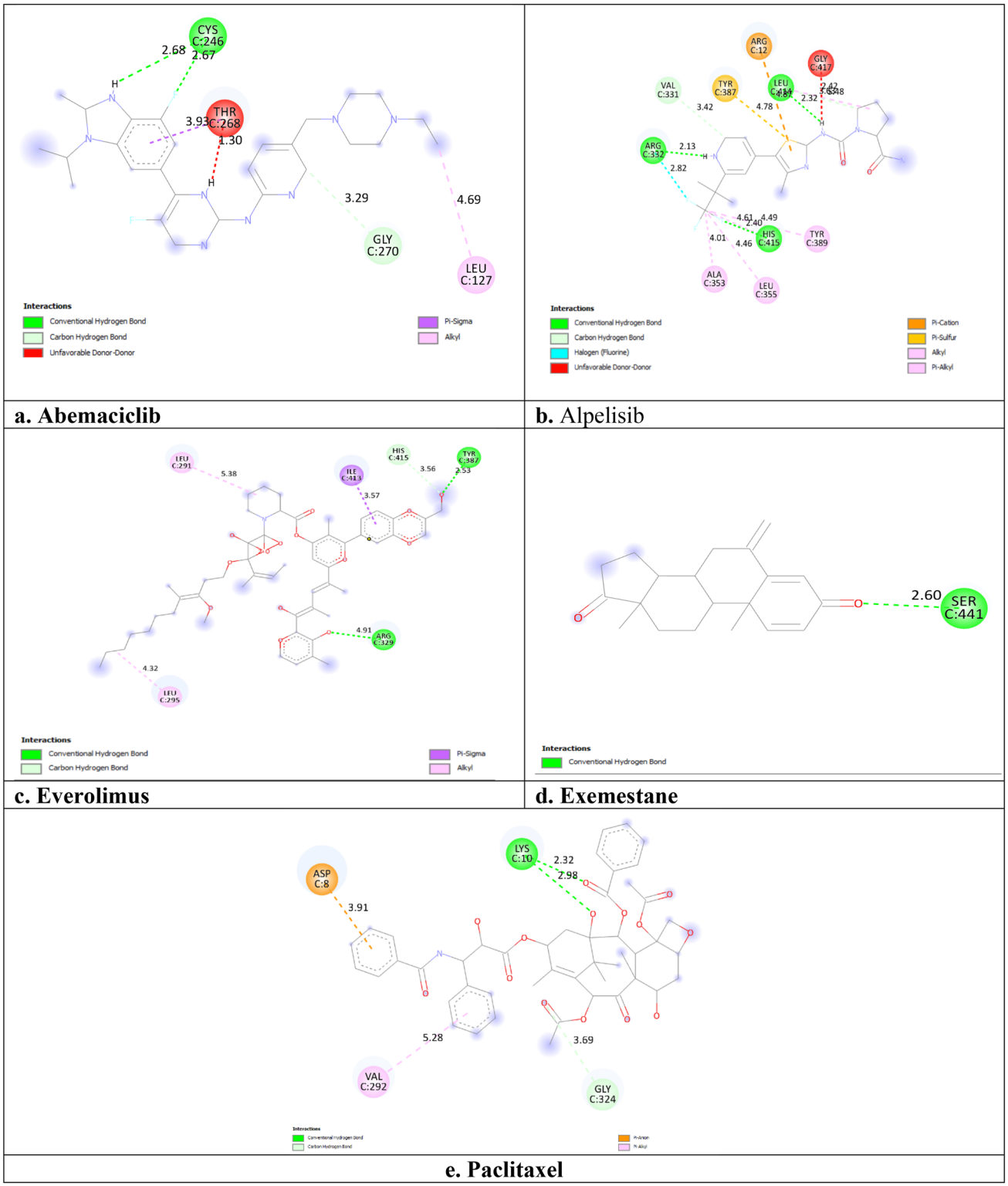


Fig. 5 Binding affinity between the breast cancer cell receptor HER2 protein and FDA recommended commercial anticancer drugs. **a** Abemaciclib, **b** Alpelisib, **c** Everolimus, **d** Exemestane and **e** Paclitaxel

Table 9 Binding interaction between the breast cancer cell receptor HER2 with FDA recommended anticancer drugs

Name	Distance Å	Category	Types	From	From chemistry	To	To chemistry
Abemaciclib							
C:CYS246:HN -:UNL1:F	2.66842	Hydrogen Bond;Halogen	Conventional Hydrogen Bond;Halogen (Fluorine)	C:CYS246:HN	H-Donor; Halogen Acceptor	:UNL1:F	H-Acceptor;Halogen
:UNL1:HN—C:CYS246:O	2.68459	Hydrogen Bond	Conventional Hydrogen Bond	:UNL1:HN	H-Donor	C:CYS246:O	H-Acceptor
:UNL1:C—C:GLY270:O	3.2916	Hydrogen Bond	Carbon Hydrogen Bond	:UNL1:C	H-Donor	C:GLY270:O	H-Acceptor
C:THR268:CG2 -:UNL1	3.92815	Hydrophobic	Pi-Sigma	C:THR268:CG2	C-H	:UNL1	Pi-Orbitals
:UNL1:C—C:LEU127	4.69413	Hydrophobic	Alkyl	:UNL1:C	Alkyl	C:LEU127	Alkyl
Alpelisib							
C:HIS415:HE2 -:UNL1:F	2.39636	Hydrogen Bond;Halogen	Conventional Hydrogen Bond;Halogen (Fluorine)	C:HIS415:HE2	H-Donor; Halogen Acceptor	:UNL1:F	H-Acceptor;Halogen
:UNL1:HN—C:ARG332:O	2.13391	Hydrogen Bond	Conventional Hydrogen Bond	:UNL1:HN	H-Donor	C:ARG332:O	H-Acceptor
:UNL1:HN—C:LEU414:O	2.31612	Hydrogen Bond	Conventional Hydrogen Bond	:UNL1:HN	H-Donor	C:LEU414:O	H-Acceptor
:UNL1:C—C:VAL331:O	3.4219	Hydrogen Bond	Carbon Hydrogen Bond	:UNL1:C	H-Donor	C:VAL331:O	H-Acceptor
:UNL1:C—C:LEU414:O	3.6343	Hydrogen Bond	Carbon Hydrogen Bond	:UNL1:C	H-Donor	C:LEU414:O	H-Acceptor
C:ARG332:O -:UNL1:F	2.82077	Halogen	Halogen (Fluorine)	C:ARG332:O	Halogen Acceptor	:UNL1:F	Halogen
C:ARG12:NH1 -:UNL1	4.86641	Electrostatic	Pi-Cation	C:ARG12:NH1	Positive	:UNL1	Pi-Orbitals
:UNL1:S—C:TYR387	4.77661	Other	Pi-Sulfur	:UNL1:S	Sulfur	C:TYR387	Pi-Orbitals
C:ALA353 -:UNL1:C	4.01164	Hydrophobic	Alkyl	C:ALA353	Alkyl	:UNL1:C	Alkyl
:UNL1:C—C:LEU355	4.45608	Hydrophobic	Alkyl	:UNL1:C	Alkyl	C:LEU355	Alkyl
:UNL1—C:LEU414	5.48307	Hydrophobic	Alkyl	:UNL1	Alkyl	C:LEU414	Alkyl
C:TYR389 -:UNL1:C	4.48648	Hydrophobic	Pi-Alkyl	C:TYR389	Pi-Orbitals	:UNL1:C	Alkyl
C:HIS415 -:UNL1:C	4.61273	Hydrophobic	Pi-Alkyl	C:HIS415	Pi-Orbitals	:UNL1:C	Alkyl
Everolimus							
C:ARG329:HH11 -:UNK0:O	2.78644	Hydrogen Bond	Conventional Hydrogen Bond	C:ARG329:HH11	H-Donor	:UNK0:O	H-Acceptor
C:ARG329:HH12 -:UNK0:O	2.71599	Hydrogen Bond	Conventional Hydrogen Bond	C:ARG329:HH12	H-Donor	:UNK0:O	H-Acceptor
C:TYR387:HH -:UNK0:O	2.53039	Hydrogen Bond	Conventional Hydrogen Bond	C:TYR387:HH	H-Donor	:UNK0:O	H-Acceptor

Table 9 (continued)

Name	Distance Å	Category	Types	From	From chemistry	To	To chemistry
C:HIS415:CD2 -:UNK0:O	3.55913	Hydrogen Bond	Carbon Hydrogen Bond	C:HIS415:CD2	H-Donor	:UNK0:O	H-Acceptor
C:ILE413:CG2 -:UNK0	3.57286	Hydrophobic	Pi-Sigma	C:ILE413:CG2	C-H	:UNK0	Pi-Orbitals
C:LEU291 -:UNK0	5.3845	Hydrophobic	Alkyl	C:LEU291	Alkyl	:UNK0	Alkyl
C:LEU295 -:UNK0	4.31547	Hydrophobic	Alkyl	C:LEU295	Alkyl	:UNK0	Alkyl
Exemestane							
C:SER441:HG -:UNL1:O	2.60407	Hydrogen Bond	Conventional Hydrogen Bond	C:SER441:HG	H-Donor	:UNL1:O	H-Acceptor
Paclitaxel							
C:LYS10:HZ2 -:UNL1:O	2.32208	Hydrogen Bond	Conventional Hydrogen Bond	C:LYS10:HZ2	H-Donor	:UNL1:O	H-Acceptor
C:LYS10:HZ1 -:UNL1:O	2.98318	Hydrogen Bond	Conventional Hydrogen Bond	C:LYS10:HZ1	H-Donor	:UNL1:O	H-Acceptor
C:GLY324:CA -:UNL1:O	3.69145	Hydrogen Bond	Carbon Hydrogen Bond	C:GLY324:CA	H-Donor	:UNL1:O	H-Acceptor
C:ASP8:OD2 -:UNL1	3.91174	Electrostatic	Pi-Anion	C:ASP8:OD2	Negative	:UNL1	Pi-Orbitals
:UNL1—C:VAL292	5.28115	Hydrophobic	Pi-Alkyl	:UNL1	Pi-Orbitals	C:VAL292	Alkyl

*C- HER2 receptor, *UNL-FDA approved breast cancer drugs

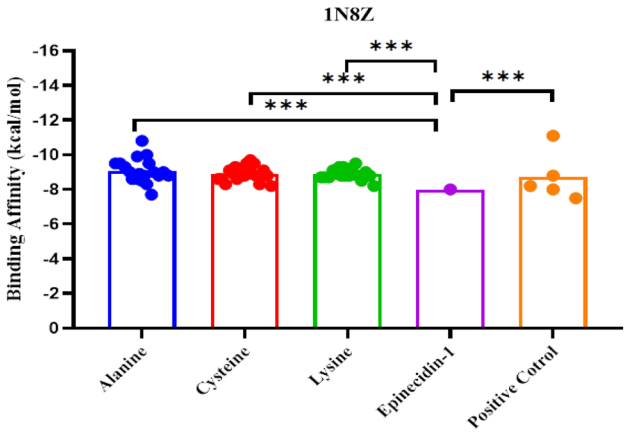


Fig. 6 Statistically analyzed ANOVA results for the Alanine, Cysteine and Lysine mutated peptides binding affinity compared with Epinecidin-1 and positive control (Abemaciclib, Alpelisib, Doxorubicin, Everolimus, Exemestane and Paclitaxel) groups. (***P < 0.0001)

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Declarations

Conflict of interest The author declares that they have no conflict of interest.

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