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In silico designing and optimization of anti-breast cancer antibody mimetic oligopeptide targeting HER-2 in women

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ABSTRACT

Overexpression of HER-2 is of frequent (20–30%) occurrence in breast cancer. Therapeutic targeting of HER-2 with humanized antibody derived oligopeptide may be a promising approach to the treatment of breast cancer. HER-2 gene is part of a family of genes that play critical roles in regulating transmembrane growth of breast cancer cells. Pertuzumab, a recombinant humanized monoclonal antibody (2C4), binds to extracellular domain II of the HER-2 receptor and inhibits its ability to dimerize with other HER receptors blocking the cell growth, signaling and apoptosis induction. The unique binding pocket on HER-2 for pertuzumab provides an important target domain for creation of new anticancer drugs. In the present work an efficient oligopeptide was designed by our computational method that interacts with pertuzumab binding sites of HER-2. In silico docking study demonstrated the best specific interaction of RASPADREV oligopeptide with the dimerization domain in the HER-2 molecule among various screened oligopeptides. ADMET and SAR properties prove the drug likeness of designed oligopeptide as having value 0.98.

1. Introduction

Breast cancer is the 2nd leading cause of deaths in women today and it is the most common cancer among women. The two strong risk factors for developing breast cancer include being female (only 1% of cases are diagnosed in males) and increasing age. 1 of 8 women is affected by breast cancer and one third of women die from breast cancer [1,2]. Infiltrating ductal carcinoma is the most common type of breast cancer, representing 78% of all malignancies. It in infiltrated through the wall of the duct then invades the fatty tissue of the breast and at this point it may be metastasize [2,3]. Studies show that approximately 25% of breast cancer patients have tumors that are human epidermal growth factor receptor 2 (HER-2) positive [4]. HER-2 positive tumors tend to grow and spread more quickly than tumors that are not HER-2 positive. HER-2 gene is part of a family of genes that promotes the growth of cancer cells. A fraction of breast cancers, as part of their development, undergo gene amplification [5,6]. Instead of having two gene copies of the HER-2 gene in a normal cell, there are multiple copies. As a result, there is far more expression of the HER-2 protein on the cell surface, resulting in aberrant cell growth. An estimated 20–25% of breast cancers make these extra copies of the HER-2 gene. A normal breast cell might have 20,000 HER-2 receptors; a breast cancer cell could have as many as 1.5 million. Approximately 40,000 women are diagnosed each year in the United States with HER-2 positive breast cancer [2].

HER proteins are transmembrane growth factor receptors that activate intracellular signaling pathways in response to extracellular signals [3]. The structure of extracellular region of HER-2 is divided into four domains, designated domain I (extracellular ligand-binding domain), domain II (transmembrane domain), domain III (intracellular catalytic tyrosine kinase domain) and domain IV (carboxyl terminal signaling tail). Domain I extends from 1 to 195 residues, domain II extends from 196 to 320 residues, domain III proceeds from 321 to 488 and domain IV starts from 489 and ends in 560 [2]. Upon ligand binding, the extracellular domain changes into its active conformation leading to receptor dimerization and transphosphorylation of their C-terminal tails. Dimeriza-

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tion brings the intracellular kinase domains of the two epidermal growth factor receptors (ErbBs) into proximity, allowing transphosphorylation of tyrosines on the C-terminal tail of one ErbB by the kinase domain of the other. These phosphorylated tyrosine residues activate multiple intracellular secondary messenger pathways leading to further downstream signaling [1,2]. This, in turn, leads to the stimulation of cell proliferation, invasion, and anti-apoptosis [7].

HER-2 over-expression is associated with increased tumor growth, increased rate of metastasis, and decreased overall survival rate for the patient [1,2]. Therapeutic approaches are being developed to block the effects of HER-2 over-expression in breast cancer. A monoclonal antibody (mAb) against HER-2, called trastuzumab, was approved for treatment of over expressed HER-2 protein. Another mAb pertuzumab mediates the antibody dependent cytotoxic effects as trastuzumab but in addition to cytotoxic effects, pertuzumab binding directly inhibits ErbB2 association with its partner receptors, blocking the signaling cascade at its source [2]. Alanine substitution studies on HER-2 indicated that histine, valine, serine, leucine and lysine were strongly related to the binding with pertuzumab [2,4] and are important for heterodimerization also. Pertuzumab light chain variable domain extends from 1 to 109 residues and heavy chain variable domain is 1–113 residues in length. Light chain constant domain begins from 110 and ends in 214 residues and heavy chain constant domain extends from 114 to 216 residues. Most of the residues of the pertuzumab that bind to HER-2 extracellular domain are of heavy chain [2]. The alanine-scan revealed that most of the side-chains that contribute to antigen binding are located in the heavy chain.

2. Materials and methods

The structure of the HER-2/pertuzumab complex (PDB entry 1S78) was used as the initial structure for oligopeptide design and 1–560 amino acid residues of 1S78A were only selected as they comprise the extracellular domain region of HER-2 [8]. The extracellular domain part was subjected to energy minimization by Gromos96. Energy minimized model was evaluated by Ramachandran's map using PROCHECK (a program to check the stereochemical quality of protein structures) [9] and environment profile using VERIFY-3D graph (structure evaluation server) [10]. The energy minimized model was subjugated for the identification of active site and the contact surface between HER-2 extracellular domain and pertuzumab was used as a target spot of our computational peptide design system. Docking of the HER-2 extracellular domain with pertuzumab was performed using AutoDock 4.2 to explore the binding site residues of the pertuzumab involved in interaction with the active residues of the HER-2 domain II. After docking of the pertuzumab antibody with target binding domain II, the residues interacting with the active site with high affinity were selected for the design of new sequential oligopeptide. Structure of proposed oligopeptide LPRAEDTVS was modeled using Mobyle server at RPRS portal that employs a *de novo* approach named PEP-FOLD to predict peptide structures from amino acid sequence [11–13]. PEP-FOLD is based on structural alphabet SA letters to describe the conformations of four consecutive residues [14,15], couples the predicted series of SA letters to a greedy algorithm [16–18] and a coarse-grained force field [12,18]. Binding energy of the designed oligopeptide was calculated by AutoDock. To enhance the binding affinity of the designed oligopeptide, amino acid positions within the oligopeptide were altered randomly and the structure of seven derived oligopeptides were modeled using Mobyle server. Molecular weight and solvation energy of oligopeptide structure were calculated by Protein Modeling tool and Solvation Energy protocol of Accelrys Discovery Studio 2.0 respectively. Simulations of XPROLIG forcefield typed best model of oligopeptide structures were performed using Standard Dynamics Cascade (SDC) protocol of Discovery Studio to obtain structure of lowest energy conformation. Docking of the energy minimized HER-2 extracellular domain with all the oligopeptides in their lowest energy conformation was carried out using Autodock 4.2 to identify the stability of model-oligopeptide complexes [19]. Screening of the new oligopeptides derived from the original one was done against HER-2 for the identification of best oligopeptide on the basis of highly populated cluster of docked poses with minimum docking energy. In order to predict the bioavailability of the newly proposed best oligopeptide, calculation of the ADMET properties and SAR studies were performed by Peptide Property Calculator server and Pharma Algorithms software respectively. Finally drug score of the best selected oligopeptide was predicted by PASS software.

3. Results

Table 1 depicts the percentile residue of the HER-2 extracellular domain in various regions of Ramachandran plot (Fig. 1). Energy minimized model was considered the best model (Fig. 2) as it has maximum number of residues in the core region. The different minimized energies for the model is listed in Table 2. After energy minimization the residue information of the new model was analyzed and is depicted in Table 3. The structure was further checked by VERIFY-3D graph. The compatibility score above zero in the VERIFY-3D graph confirmed the acceptable side-chain environments. The energy profile obtained from the VERIFY-3D program showed all residues of the protein are in satisfactory condition. It was observed that 87.68% of the residues had an averaged 3D–1D score >0.2.

Docking analysis indicated that VAL286, SER288, LEU295 and HIS296 of HER-2 were strongly involved in binding with pertuzumab (Table 4). 9mer oligopeptide LPRETVSAD was

Table 1
Ramachandran plot analysis.

HER-2 receptor	Core	Allowed	Generic	Disallowed
Extracellular domain (before energy minimization)	81.4	17.5	1.1	0.0
Extracellular domain (after energy minimization)	82.7	16.2	1.1	0.0

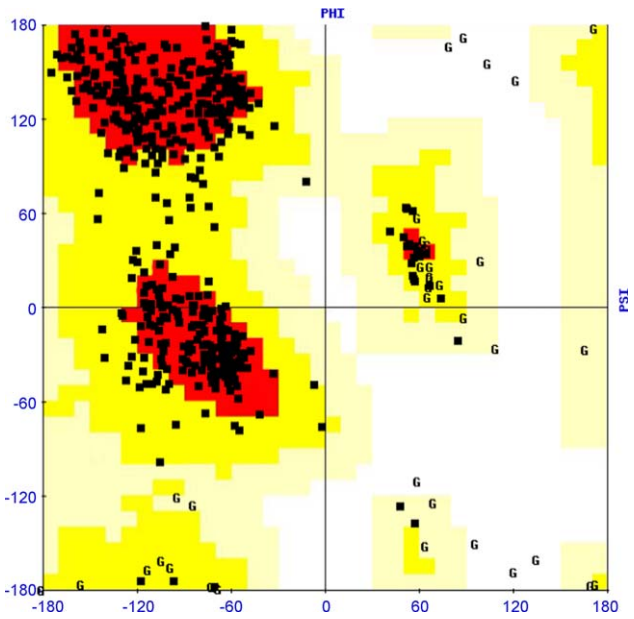

Fig. 1. Ramachandran plot of energy minimized model.

Table 2
Various minimized energies of the HER-2 extracellular protein model in kJ/mol.

Bonds	Angles	Torsions	Improper	Non-bonded	Electrostatic	Total
477.476	2305.286	4004.742	529.844	−18095.51	−17207.79	−27985.957

Fig. 2. 3D structure of HER-2 extracellular domain after energy minimization.

Table 3
Residue information of the model2 after energy minimization.

Residue information	Amino acids	Value
Residues in most favoured region [a,b,l]	388	82.7%
Residues in additional allowed Region [a,b,l,p]	76	16.2%
Residues in generously allowed region [~a,~b,~l,~p]	5	1.1%
Residues in disallowed region	0	0.0%
Number of non-glycine and non-proline residues	469	100%
Number of end-residues (excl. Gly and Pro)	4	–
Number of glycine residues (shown as triangles)	44	–
Number of proline residues	34	–

Table 4
Residues of pertuzumab interacting with HER-2 receptor.

Active residues of receptor (HER-2)	Residues of ligand (pertuzumab)
VAL286→	H: SER113, H: SER112
SER288→	H: LEU11
LEU295→	H: ALA84, H: GLU85, H: ASP86, H: ARG83
HIS296→	H: ALA84, H: ARG83, H: PRO14, H: VAL111

designed by Mobyle server taking into consideration all the interacting residues of the pertuzumab. Seven other 9mers were derived from LPRETVSAD by random altering of the desired location of amino acid residues within the oligopeptide. Proteins tend to come in their lowest energy state by means of protein folding in host immune system [19]. Table 5 lists the molecular weight, solubility, initial and final (lowest) conformational potential energies of modeled oligopeptides. Maximum 314.974987 kcal/mol decrement in energy of oligopeptide was calculated by simulations among 10 alternative conformations in oligopeptide LPRAQNRET (Fig. 3A) while minimum energy reduction (41.245776 kcal/mol) was found in oligopeptide LPRAQNTVS (Fig. 3F) by energy minimizations of all the eight oligopeptides (Fig. 3A–H).

Various energies of seven derived and one original oligopeptide are listed in Table 6. Comparative analysis of oligopeptides interpret that new oligopeptide (RASPADREV) as shown in Fig. 4A; possess high binding affinity with highly populated cluster of docked poses of reasonable energy than the originally designed oligopeptide LPRETVSAD. Active residues of HER-2 to which the RASPADREV oligopeptide binds is depicted in Fig. 4B. Four atoms of HER-2 extracellular domain were found to be involved in hydrogen bond formation with the peptide and the residues involved were CYS289(HN):ASP6(O), SER288(HG):-PRO4(O), LEU295(HN):ALA2(O), THR268(HG1):ALA5(O).

Peptide property calculator showed that RASPADREV oligopeptide (Fig. 5) having isoelectric point of 6.51 (Fig. 6A) will be neutral in nature. Overall interactive property calculation revealed the hydrophilic (Fig. 6B) character of this oligopeptide. It was explored by PHARMA algorithm that RASPADREV has good solubility and stability (susceptibility to acid hydrolysis in acidic environment of stomach) along with first-pass metabolism, P-gp efflux and Active transport but lower Passive absorption. In silico solubility of the oligopeptide in pure water was checked and was found to be highly soluble with log Sw: −3.07 and Sw: 0.847 mg/ml. Probability of oligopeptide solubility was analyzed under different concentrations and the results have shown that the oligopeptide might have a probability of 1 at >0.1 mg/ml, 1 at >1 mg/ml and 0.998 at >10 mg/ml. Quantitative solubility (log *S*, mmol/ml) was estimated at various physiologically important pH values and the log *S* values were: pH 1.7 (stomach): −0.11, pH 4.6 (duodenum): −3.31, pH 6.5 (jejunum and ileum): −3.06, pH 7.4 (blood): −3.07, pH 8.0 (colon): −3.07. Physicochemical property analysis was also carried out to check the various pharmacokinetic properties. It was predicted that the oligopeptide RASPADREV with molecular weight of 1003.12 has 22 hydrogen bond donors and 30 hydrogen bond acceptors with 33 rotatable bonds. To predict the ADMET properties, a precise calculation of pK_a is very important factor. We

Table 5
Analysis of oligopeptides.

Oligopeptide	Molecular weight (Da)	Solubility (kT)	Initial potential energy (kcal/mol)	Energy of lowest energy conformation
LPRAQNRET	1088.25	−470.6724	7.18581	−322.160797
LPRAETVSS	962.108	−314.7183	−95.56761	−271.513885
LPRAEDTVS	989.111	−411.785	−124.81933	−348.136414
PALRAQNRE	1058.22	−568.948	−62.74670	−320.056213
LPRETVSAD	989.111	−379.6511	−153.11972	−322.900085
LPRAQNTVS	989.157	−351.6615	−172.21272	−213.458496
RASPADREV	1003.12	−441.7675	−161.77857	−363.031494
PQAHRALVS	982.167	−393.3924	−20.01499	−192.487595

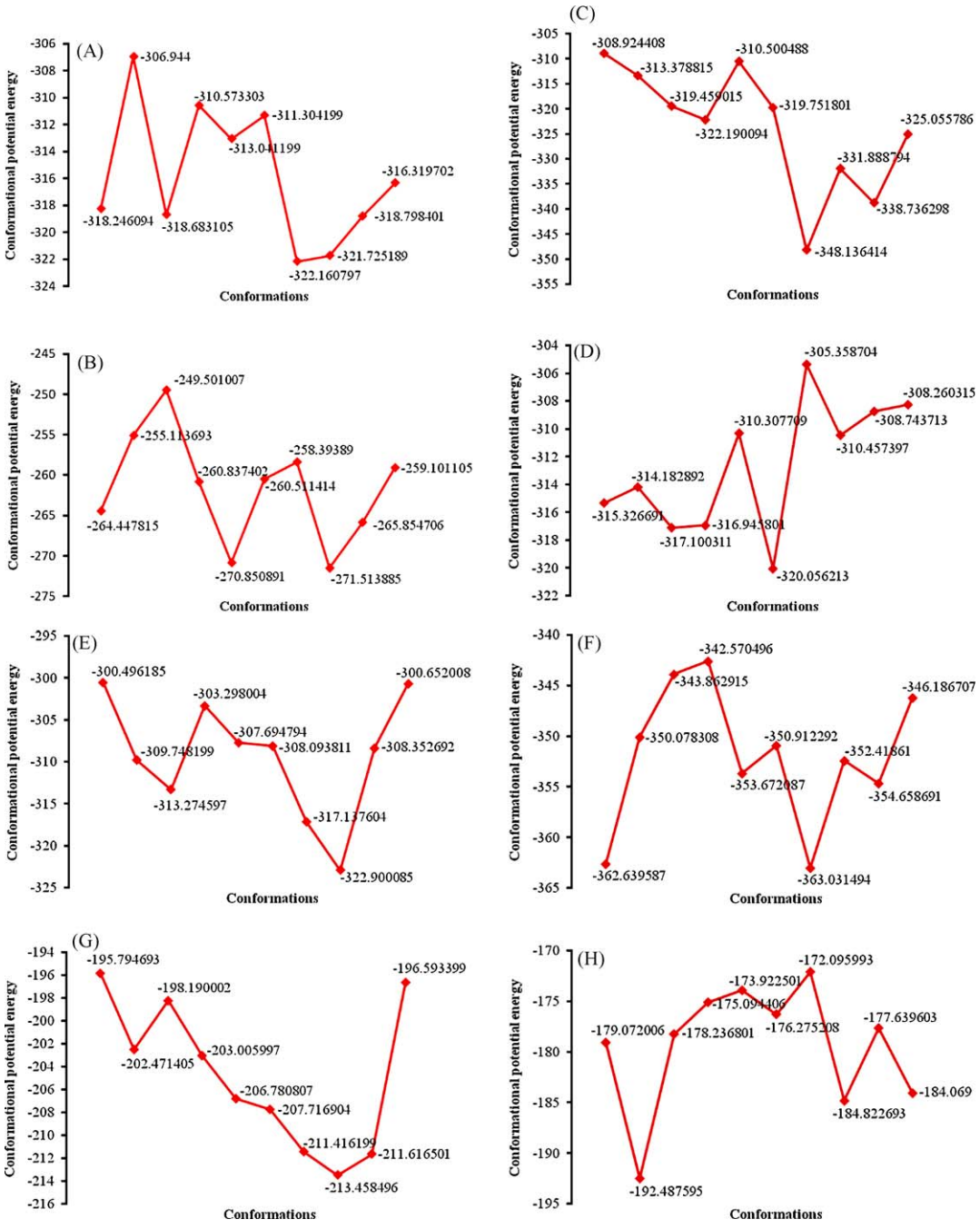

Fig. 3. (A–H) Conformational potential energies of alternative conformations of simulated oligopeptide structures.

Table 6
Various energies of oligopeptides calculated by AutoDock.

Oligopeptide	Binding energy	Ligand efficiency	Intermol energy	Vdw_hb_desolv energy	Electrostatic energy	Total Internal	Torsional energy	ClRMS
LPRAQNRET	−6.24e+027	−8.2105263 1579e+025	−6.24e+027	−6.24e+027	−3,810,000	−2.38	10.7	0.0
LPRAETVSS	0.39	0.01	−5.36	−4.44	−0.92	−3.86	9.6	0.0
LPRAEDTVS	−1.2e+022	−1.869565 21739e+020	−1.29e+022	5,010,000	−1.29e+022	−1.52	9.6	186.923
PALRAQNRE	−1.19e+013	−175,000,000 000	−1.19e+013	−1.19e+013	10,500,000	530,000	9.88	221.507
LPRETVSAD	−2.46e+036	−3.5652173 913e+034	−2.46e+036	−1.67e+017	−2.46e+036	77.62	9.6	189.534
LPRAQNTVS	−5.07e+037	−7.3478260 8696e035	−5.07e+037	3.98e+022	−5.07e+037	4.94	9.6	0.0
RASPADREV	−9.01e+012	−128,714,285 714	−9.01e+012	3,180,000	−9.01e+012	−6.36	9.6	0.0
PQAHRALVS	1.39	0.02	−4.72	−4.72	−4.25	−0.47	−2.39	8.51

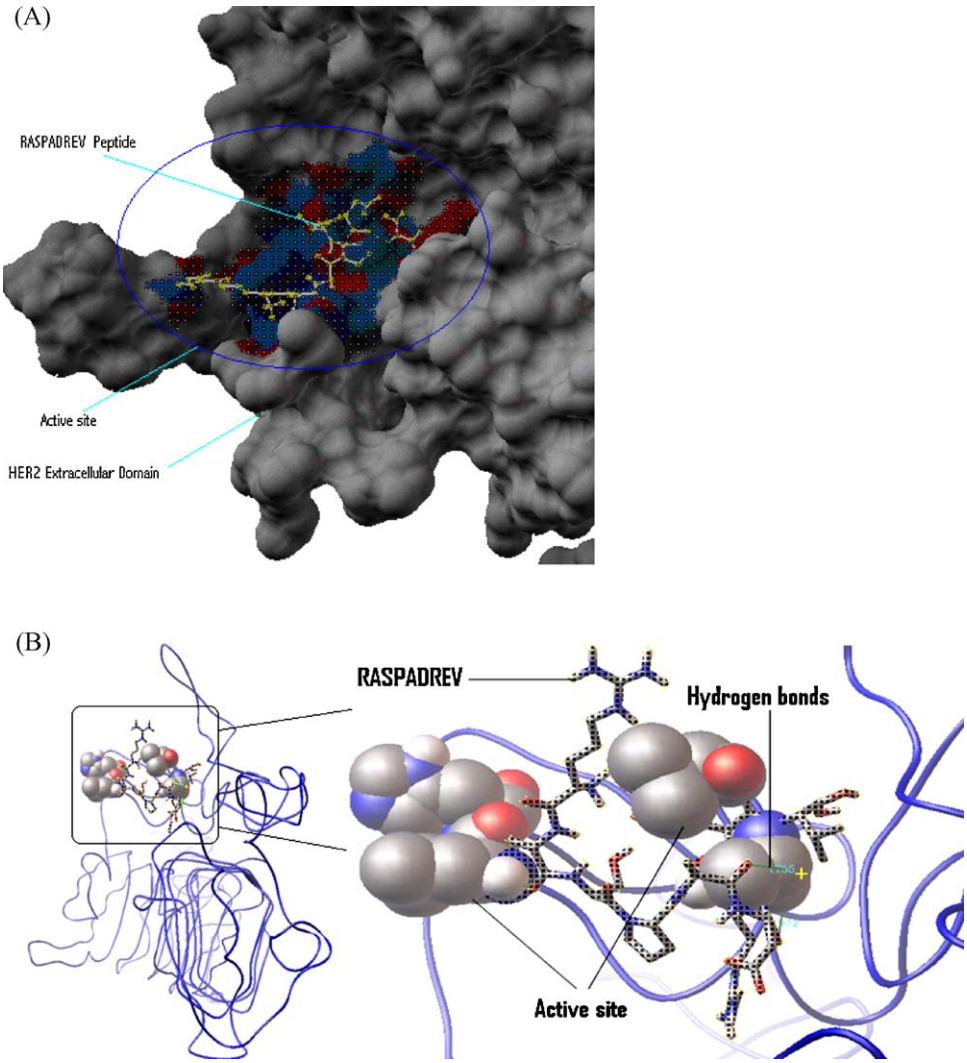

Fig. 4. (A and B) Oligopeptide RASPADREV binding with HER-2 extracellular domain.

examined ionization values of the oligopeptide and the strongest pK_a value of 4.90 ± 0.50 and 13.70 ± 0.50 were observed for acid and base respectively with 5 ionizable groups. Calculation of logarithm of the apparent octanol–water partition coefficient (log D) of the oligopeptide at various pH values were analyzed and were: pH 1.7 (stomach): −14.32, pH 4.6 (duodenum): −12.01, pH 6.5 (jejunum and ileum): −11.54, pH 7.4 (blood): −11.53, pH 8.0 (colon): −11.53. In silico probabilities of the various health effects of the oligopeptide were predicted and the observed values of 0.98, 0.99, 0.53, 0.97, 1.00, and 0.18 were identified for blood, cardiovascular system, gastrointestinal track, kidney, liver and lungs respectively. PASS software was used for evaluation of general biological potential of the designed oligopeptide and drug likeness value of 0.98 was predicted.

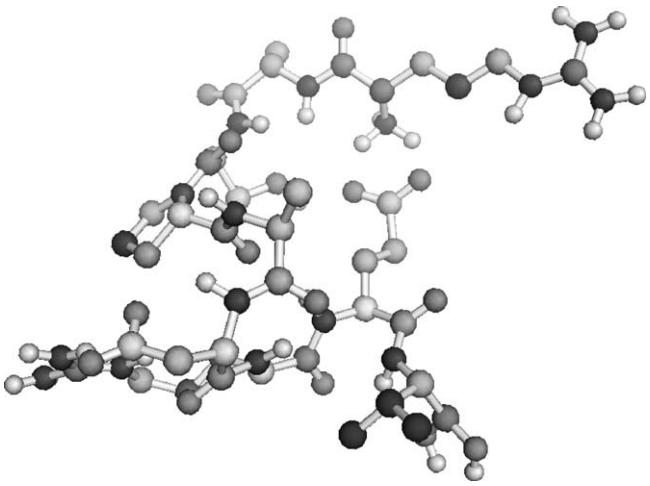

Fig. 5. 3D Structure view of the best oligopeptide (RASPADREV).

4. Discussion

Unique binding pockets on HER-2 for pertuzumab have been resolved and provide the important target domains for creation of new anticancer drugs. It can also be a dominant technique for the identification of new lead compounds in drug discovery for physical screening of large libraries of oligopeptides against HER-2. In the present study we retrieved the extracellular domain of HER-2 from HER-2/pertuzumab complex (PDB entry 1S78). Extracellular domain region of ErbB2 (1S78A) containing first 560 amino acid residues comprising of four domains including domain 2 [2], was selected and was subjected to energy minimization by Gromos96. The structural difference by energy minimization was analyzed by Procheck and it was observed that there is an increment in the percentage of residues in the core region (Table 1). The final structure was further checked by VERIFY-3D graph and the results

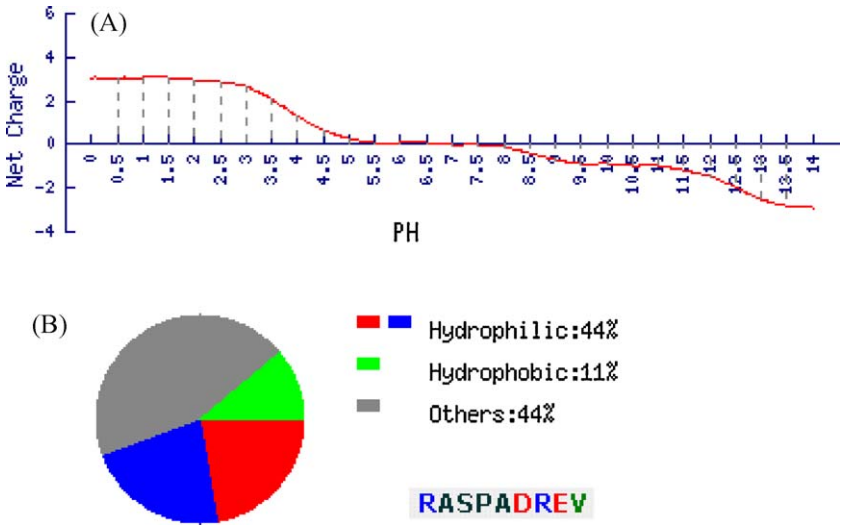

Fig. 6. (A) Analysis of isoelectric point of oligopeptide RASPADREV. (B) Analysis of interactive properties of oligopeptide RASPADREV.

showed 87.68% of the residues had an averaged 3D–1D score >0.2 which was acceptable. The model was later subjected to Docking studies. It was found that the structure having minimum binding energy takes less time to bind and binds more efficiently as compared to the structure having more binding energy. Hence the oligopeptide having the minimum binding energy towards HER-2 binding residues among the oligopeptides screened was selected as the best antagonistic oligopeptide against HER-2. Among the various screened oligopeptides, RASPADREV (Fig. 4) showed high specific binding activity to the unique surface pocket in the HER-2 molecule involving mainly four VAL286, SER288, LEU295, HIS296 amino acid residues, which is in the interaction domain of pertuzumab. Various properties of the oligopeptide like oral bioavailability (human), log Sw (solubility in pure water), log *S* (pH dependent aqueous solubility), distribution, physicochemical properties, log *P* (octanol–water partition coefficient), pK_a and ion fraction values, log *D* (pH dependent distribution coefficient), probabilities of health effects were predicted. The oligopeptide was also predicted to show good solubility and stability. Finally we examined the overall drug score by PASS software and result showed the drug likeness score of 0.98 which was acceptable.

5. Conclusion

Structure based design of oligopeptides have important implications in development of novel cost effective drugs. Owing to the limitations in experimental methods for determining binary interactions and structure determination of protein complexes, there is need for computational models to fill this increasing gap. Some percentage of breast cancer is caused by ErbB receptor dimerization. Out of the four ErbB receptors, HER-2 is preferred partner for dimerization. So, by blocking the dimerization site of HER-2 by antagonistic oligopeptide, intracellular signaling by the receptors can be prevented. We identified that the virtual oligopeptide RASPADREV possess high binding affinity towards the HER-2. The oligopeptide predicted to dock efficiently into the active site of human HER-2 has shown acceptable drug-likeness score with high solubility and stability. However, in vivo studies are desired to know the real efficacy of the oligopeptide and for use of this oligopeptide as an anti-breast cancer drug in future.

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