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In silico screening of inhibitors for HER2 from synthetic sources using swissdock software for the therapy of non-small cell lung cancer (NSCLC)

T Nivetha[1], Monisha M[2] , Gokul Paramasivam[3,] T Nivetha[1]

[1]Project Scholar, Department of Biomedical Engineering , Saveetha School of Engineering, Saveetha Institute of Medical And Technical Sciences, Saveetha University, Chennai, Tamilnadu. India. Pincode: 602105.

[2]Project Guide, Corresponding Author, Department of Biomedical Engineering , Saveetha School of Engineering, Saveetha Institute of Medical And Technical Sciences, Saveetha University, Chennai, Tamilnadu. India. Pincode: 602105.

[3]Project Co-Guide, Corresponding Author, Department of Biomedical Engineering , Saveetha School of Engineering, Saveetha Institute of Medical And Technical Sciences, Saveetha University, Chennai, Tamilnadu. India. Pincode: 602105.

ABSTRACT

Aim: Identifying suitable inhibitor molecules for Human epidermal growth factor receptor 2 (HER2) using SwizzDock by *in silico* screening of synthetic compounds that have exhibited anticancer properties. **Materials and Methods:** There were 51 samples analysed in the study. The 3D structures of 50 synthetic compounds and one reference compound were retrieved from NCBI – PubChem databases. The ligand molecule was prepared using the LigPrep wizard of the Schrodinger suite. SwissDock was used to predict the molecular interactions that may occur between the target protein and the small molecules. UCSF Chimera was used to predict the docking pose. Compounds with enhanced hits were analyzed and evaluated in PyMOL and Discovery Studio Visualizer respectively. **Results:** Synthetic compounds- Methotrexate, Aminopterin, Pemetrexed, Fulvestrant, Tamoxifen, Selinexor and Sobuzoxane were found to have enhanced binding affinity than the reference inhibitor Phenethyl isothiocyanate **(**PEITC) after molecular docking analysis. These compounds also had good amino acid interaction with HER2. **Conclusion:** The identified compounds had high binding affinity and good interaction with the amino acid residues of HER2. These inhibitors can be taken into consideration in future trials for the development of new anticancer drugs.

Keywords: HER2, Synthetic compounds, Inhibitors, Molecular docking, Anticancer drugs, Novel drug inhibitors, Computational biology.

INTRODUCTION

The research is about finding potent novel drug inhibitor molecules for HER2 from synthetic compounds that could be used for the therapy of non-small cell lung cancer (NSCLC) (Garrido-Castro and Felip 2013). In recent years it has also become a biomarker (Mitri, Constantine, and O'Regan 2012). The importance of the study is that the over-expression of HER2 has been shown to play an important role in the development and progression

of cancers. It is also found in other malignant tumors including NSCLC (13% to 55%) (Lohrisch 2001) (Lohrisch and Piccart 2001). Hence inhibiting it can stop the replication of cancer cells and lead to its death. The application of the study is that the identified compounds can be used to create new anticancer drugs that are better than the reference inhibitors used in current anticancer drugs (Garrido-Castro and Felip 2013) (Dong et al. 2019).

There have been around 25000 papers and 397 papers published in Google Scholar and ScienceDirect databases respectively related to the subject. Based on the literature survey, currently the most cited article was by Ménard *et.al.*, where they concluded that patients with HER2- positive or HER2-negative tumors benefit from CMF (cyclophosphamide, methotrexate, and fluorouracil) treatment (Ménard et al. 2001). This was overall the best paper related to the subject. Another article concluded that the epidermal growth factor receptor tyrosine kinase family and related downstream pathways play an important role in cancer development. It has also become a validated target in NSCLC (Spicer and Rudman 2010). Fukuda et al. in their study found that MM (mitomycin C and methotrexate) was effective and tolerable for Metastatic Breast Cancer patients even after aggressive treatment with ATCV (anthracycline, taxane, capecitabine, and vinorelbine) (Fukuda et al. 2015). Similarly in another study, Selinexor plus palbociclib was found to be highly effective in BT474 breast cancer cells. It showed synergistic inhibition of cell proliferation in HER2+ breast cancer cell line BT474 by down-regulation of CDK4/6 pathway (Chang et al. 2017). Previously our team has a rich experience in working on various research projects across multiple disciplines(Ezhilarasan et al. 2021; Balachandar et al. 2020; Muthukrishnan et al. 2020; Kavarthapu and Gurumoorthy 2021; Sarode et al. 2021; Hannah R et al. 2021; Sekar, Nallaswamy, and Lakshmanan 2020; Appavu et al. 2021; Menon et al. 2020; Gopalakrishnan et al. 2020; Arun Prakash et al. 2020)

The major setback in the existing research is the deficiency of small molecule inhibitors for HER2 for therapy of non-small cell lung cancer. HER2 is known to play an essential role in regulating the proliferation of epithelial cells. Since a disrupted HER2 can lead to the growth of tumours that may lead to cancers, it is necessary to regulate its activity. By increasing the number of inhibitors available for HER2, we can broaden the variety of anticancer drugs and can improve better treatment for cancer patients. The authors of this study are experienced in the field of computational biology. This study aims to identify inhibitor molecules for HER2 protein by *in silico* screening of compounds derived from alkaloids using SwizzDock software.

MATERIALS AND METHODS

The study was done using softwares and online tools namely PyMol, SwissDock, UCSF Chimera and Discovery Studio Visualiser in Saveetha School Engineering. No ethical approvals were needed since no human samples were used. All screening and analysis were performed virtually. For power testing, the g power or the pre-test power was set at 80% (Jung et al. 2018). There were two groups considered in the study. 50 compounds derived from synthetic sources that had previously exhibited anticancer and antiproliferative activities were categorized as group 1. One reference inhibitor- Phenethyl isothiocyanate **(**PEITC) which had also inhibited cancerous cells was categorized as group 2. The total sample size for this study was 51.

The samples were prepared using public biological and chemical information providers. 3D structures of group 1 (synthetic compounds) were retrieved from NCBI – PubChem databases. These structures were then used for molecular docking analysis between the target protein and the sample ligands. Similarly, the 3D structures for group 2 (reference compound) – Phenethyl isothiocyanate **(**PEITC) was prepared using the PubChem database. The target protein is HER2. Structure of HER2 was obtained from the PDB ID - 3RCD. The protein was isolated from the ligand using PyMol software.

The testing setup used different softwares namely PyMol, UCSF Chimera and Discovery Studio Visualizer. The structures prepared are used for molecular docking analysis. The target and the ligands were screened using SwissDock that is used to predict the molecular interactions that may occur between a target protein and a small molecule. Data was collected based on the binding energy (kcal/mol) exhibited from each ligand. Later, the respective ΔG values (kcal/mol) were collected for all 50 synthetically derived compounds and one reference compound.

Compounds with enhanced hits were docked. The docked complexes were analyzed in PyMol software. UCSF Chimera was used to predict the docking pose. Ligands binding at the active sites were selected. Discovery

Studio Visualizer was used to evaluate the amino acid residue interactions between the protein and the ligand.

RESULTS

In Table 1, the results for screening of HER2 with inhibitors were tabulated. HER2 was docked with 50 synthetic compounds in SwissDock. The docking results showed that Methotrexate, Aminopterin, Pemetrexed, Fulvestrant, Tamoxifen, Selinexor and Sobuzoxane had better binding affinity, with ΔG corresponding to -8.85 kcal/mol, -9.68 kcal/mol, -9.46 kcal/mol, -9.17 kcal/mol, -8.73 kcal/mol, -8.63 and -8.98 kcal/mol respectively than the reference compound PEITC with ΔG= -6.88. Fig. 1 shows the interaction between HER2 and the synthetic compounds- (A) Methotrexate, (B) Aminopterin, (C) Pemetrexed, (D) Fulvestrant, (E) Tamoxifen, (F) Selinexor and (G) Sobuzoxane as a novel drug inhibitors. This was performed using Discovery studio visualizer. The structure of inhibitors are represented in red while the amino acid residues of HER2 are represented as grey.

Fig. 2 shows the interaction between HER2 and the reference inhibitor- PEITC. It was visualised using PyMol software. The structure of PEITC is represented in firebrick colour while the amino acid residues of HER2 are represented in cyan. The yellow structure is the HER2 protein. PEITC interacted with LYS753, LEU796, ALA751, SER783, PHE864 and ASP863 as novel drug inhibitors. The binding energy of PEITC was -6.88 kcal/mol. Fig. 3 shows the interaction between HER2 and Methotrexate. It was visualised using PyMol software. The structure of Methotrexate is represented in brown while the amino acid residues of HER2 are represented in cyan. The yellow structure is the HER2 protein. Methotrexate interacted with ALA751, VAL734, LEU800, PHE1004, ASP863 and THR862. The binding energy of Methotrexate was -8.85 kcal/mol.

Fig. 4 shows the interaction between HER2 and Aminopterin. It was visualised using PyMol software. The structure of Aminopterin is represented in lime green colour while the amino acid residues of HER2 are represented in blue. The yellow structure is the HER2 protein. Aminopterin interacted with ALA751, ASP808, CYS805, THR862, ASP863 and LEU976. The binding energy of Aminopterin was -9.68 kcal/mol. Fig. 5 shows the interaction between HER2 and Pemetrexed. It was visualised using PyMol software. The structure of Pemetrexed is represented in purple while the amino acid residues of HER2 are represented in pink. The yellow structure is the HER2 protein. Pemetrexed interacted with ALA751, THR798, ASP863, GLY804, CYS805 and ASP808. The binding energy of Pemetrexed was -9.46 kcal/mol.

Fig. 6 shows the interaction between HER2 and Fulvestrant. It was visualised using PyMol software. The structure of Fulvestrant is represented in magenta while the amino acid residues of HER2 are represented in blue. The yellow structure is the HER2 protein. Fulvestrant interacted with VAL734, PHE1004, GLY804, LEU852, ASP863 and LEU785. The binding energy of Fulvestrant was -9.17 kcal/mol. Fig. 7 shows the interaction between HER2 and Tamoxifen. It was visualised using PyMol software. The structure of Tamoxifen is represented in deep teal while the amino acid residues of HER2 are represented in red. The yellow structure is the HER2 protein. Tamoxifen interacted with LYS735, VAL734, ALA751, THR862, LEU852. The binding energy of Tamoxifen was -8.73 kcal/mol.

Fig. 8 shows the interaction between HER2 and Selinexor. It was visualised using PyMol software. The structure of Selinexor is represented in orange while the amino acid residues of HER2 are represented in cyan. The yellow structure is the HER2 protein. Selinexor interacted with LYS753, SER783, LEU726, ARG784, CYS805 and THR862. The binding energy of Selinexor was -8.63 kcal/mol. Fig. 9 shows the interaction between HER2 and Sobuzoxane. It was visualised using PyMol software. The structure of Sobuzoxane is represented in violet while the amino acid residues of HER2 are represented in red. The yellow structure is the HER2 protein. Sobuzoxane interacted with LYS753, PHE864, THR862, GLY804 and LEU785. The binding energy of Sobuzoxane was -8.98 kcal/mol.

DISCUSSION

The target protein HER2 was docked with 50 synthetic compounds and potent inhibitors for HER2 were found (Methotrexate, Aminopterin, Pemetrexed, Fulvestrant, Tamoxifen, Selinexor and Sobuzoxane) based on binding affinity and amino acid residue interactions. Methotrexate, Aminopterin, Pemetrexed, Fulvestrant, Tamoxifen, Selinexor and Sobuzoxane were found to have an enhanced binding affinity than the reference inhibitor PEITC after molecular docking analysis. Similar findings were reported by several other studies (Spicer and Rudman 2010). All the identified compounds were shown to exhibit anticancer properties (Fukuda

et al. 2015) (Chang et al. 2017). The seven compounds were shown to have enhanced binding affinity than the reference compound - PEITC. The binding energy of PEITC was found to be -6.88 kcal/mol while for the identified compounded it was as follows- Methotrexate = -8.85 kcal/mol, Aminopterin = -9.68 kcal/mol, Pemetrexed = -9.46 kcal/mol, Fulvestrant = -9.17 kcal/mol, Tamoxifen = -8.73 kcal/mol, Selinexor = -8.63 kcal/mol, Sobuzoxane = -8.98 kcal/mol. Interactions of the compounds were analyzed using Discovery Studio visualizer. It was found that the AA residues of HER2– LYS753, LEU796, ALA751, THR798, SER783, PHE864 and ASP863 interacted with the reference inhibitor PEITC. Methotrexate, Aminopterin, Pemetrexed, Fulvestrant, Tamoxifen, Selinexor and Sobuzoxane could be potential HER2 inhibitors for the therapy of non-small cell lung cancer (NSCLC). Similar findings have proved that the above mentioned natural compounds were good inhibitors of HER2 (Fukuda et al. 2015) (Chang et al. 2017). No contradicting statements were found since all literature agreed that the compounds exhibited anticancer activities.

The major limitation of this study is that it requires clinical trials to effectively determine its effect on HER2 for the therapy of non-small cell lung cancer (NSCLC). A ligand binding in virtual docking might not bind in similar fashion during experimentally validation. Some of the other factors affecting the research include capital, lack of targets and biomarkers for cancers, failing clinical trials for potential new anticancer drugs, insufficient knowledge about mechanisms involved and validity of highly efficient animal models.

These compounds not only had high binding affinity but also had good interaction with the amino acid residues of HER2. The identified novel drug inhibitor hits can be taken into consideration for future trials *in vitro* and *in vivo* for the development of anticancer drugs.

CONCLUSION

By using *in-silico* analysis, potent novel drug inhibitor molecules have been identified for HER2 protein for the therapy of non-small cell lung cancer (NSCLC) by screening of compounds derived from synthetic compounds using SwizzDock. Among the seven findings, Aminopterin had the best binding affinity with HER2.

DECLARATIONS

Conflict of interests

The authors declare no conflict of interest in this manuscript.

Authors contributions

Author TN was involved in data collection, data analysis and manuscript writing. Author MM was involved in Conceptualization. Author GP was involved in data validation and critical review of the manuscript.

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Tables and Figures:

Table 1: List of 50 synthetic compounds: Screening of HER2 inhibitors derived from natural sources - HER2 was docked with 50 Synthetic compounds in SwissDock. The docking results showed that Methotrexate, Aminopterin, Pemetrexed, Fulvestrant, Tamoxifen, Selinexor and Sobuzoxane had better binding affinity, with ΔG corresponding to -8.85 kcal/mol, -9.68kcal/mol, -9.46 kcal/mol, -9.17 kcal/mol, -8.73 kcal/mol, -8.63 and -8.98 kcal/mol respectively than the reference compound PEITC with ΔG= -6.88 kcal/mol

S.No.	Compound name	Estimated ΔG (kcal/mol)	FullFitness (kcal/mol)
1.	Methotrexate	-8.85	-2100.20
2.	Aminopterin	-9.68	-2107.57
3.	Thioguanine	-6.83	-2187.97
4.	Bedaquiline	-6.93	-1995.31
5.	Fludarabine	-7.58	-2021.16
6.	Gemcitabine	-7.02	-2067.62
7.	Pemetrexed	-9.46	-2162.36
8.	Aranose	-7.17	-2074.49
9.	Belinostat	-7.60	-2073.18
10.	Azacitidine	-7.00	-2162.36
11.	Bendamustine	-7.88	-2081.28
12.	Bexarotene	-7.56	-2024.17
13.	Bicalutamide	-8.09	-2038.53

14.	Chlorambucil	-7.33	-2069.21
15.	Enzalutamide	-7.74	-2031.39
16.	Fulvestrant	-9.17	-2038.62
17.	Tamoxifen	-8.73	-2016.41
18.	Flutamide	-6.81	-2056.60
19.	Megestrol acetate	-6.70	-2026.57
20.	Plerixafor	-7.34	-2033.55
21.	Nilutamide	-7.30	-2049.31
22.	Prednisone	-6.98	-2001.62
23.	Selinexor	-8.63	-1970.64
24.	Aminoglutethimide	-7.30	-2090.01
25.	Amsacrine	-7.51	-2044.44
26.	Bisantrene hydrochloride	-8.47	-2042.75
27.	Carboplatin	-6.48	-2095.97
28.	Fotemustine	-7.99	-2096.23
29.	Lonidamine	-7.47	-2036.54
30.	Nedaplatin	-6.06	-2063.78
31.	Pomalidomide	-7.05	-2104.97
32.	Porfimer sodium	-7.64	-2116.27
33.	Ranimustine	-7.51	-2052.16
34.	Sobuzoxane	-8.98	-2121.22
35.	Busulfan	-7.12	-2117.16
36.	Lomustine	-6.80	-2122.00
37.	Chlorotrianisene	-7.79	-2012.75
38.	Cyclophosphamide	-6.98	-2180.73

39.	Dacarbazine	-7.06	-2124.13
40.	Altretamine	-6.87	-2260.81
41.	Hydroxyurea	-7.41	-2104.64
42.	Ifosfamide	-6.95	-2184.27
43.	Mechlorethamine	-6.24	-2058.14
44.	Melphalan	-7.49	-2022.40
45.	Mitotane	-6.53	-2035.56
46.	Pipobroman	-7.28	-2089.47
47.	Procarbazine	-6.80	-2046.36
48.	Razoxane	-7.21	-2001.21
49.	Thiotepa	-7.13	-1397.55
50.	Triethylenemelamine	-7.95	-1500.09
51.	Phenethyl isothiocyanate (Reference)	-6.88	-2065.72

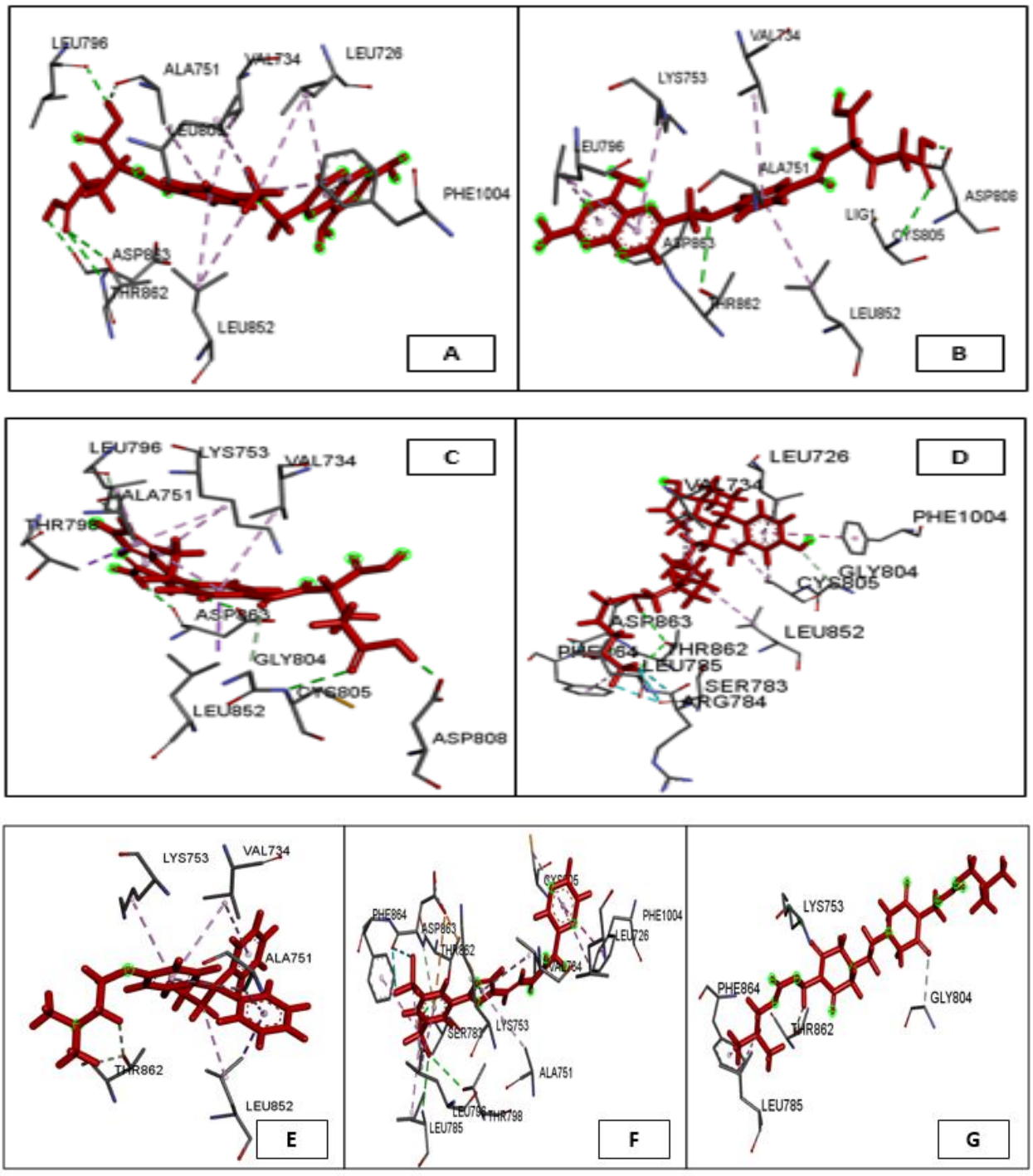

Fig. 1. Interaction of HER2 with synthetic compounds using Discovery studio visualizer - Interaction of HER2 with (A) Methotrexate, (B) Aminopterin, (C) Pemetrexed, (D) Fulvestrant, (E) Tamoxifen, (F) Selinexor and (G) Sobuzoxane. The structure of inhibitors are represented in red while the amino acid residues of HER2 are represented as grey.

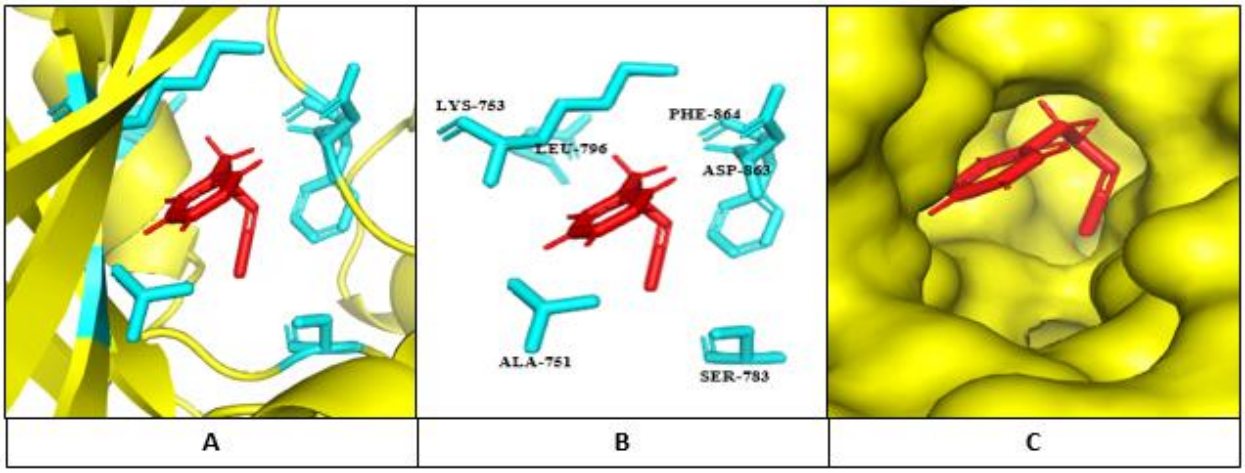

Fig. 2. (A) PEITC with cartoon HER2, (B) PEITC and amino acid residue interactions, (C) PEITC inside surface developed HER2. The structure of PEITC is represented in red while the amino acid residues of HER2 are represented in cyan. The yellow structure is the HER2 protein. PEITC interacted with LYS753, LEU796, ALA751, SER783, PHE864 and ASP863. The binding energy of PEITC was -6.88 kcal/mol.

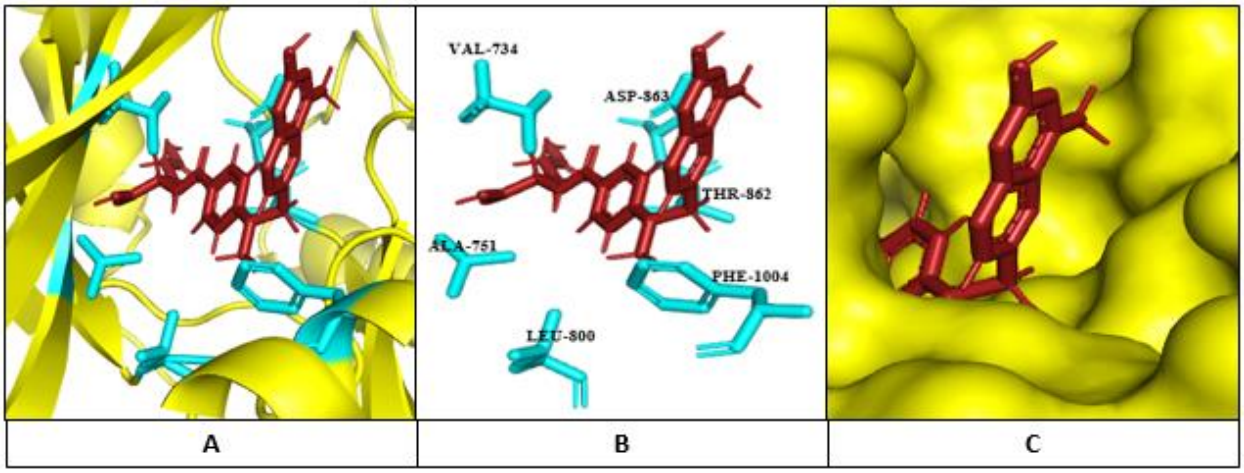

Fig. 3. (A) Methotrexate with cartoon HER2, (B) Methotrexate and amino acid residue interactions, (C) Methotrexate inside surface developed HER2. The structure of Methotrexate is represented in firebrick colour while the amino acid residues of HER2 are represented in cyan. The yellow structure is the HER2 protein. Methotrexate interacted with ALA751, VAL734, LEU800, PHE1004, ASP863 and THR862. The binding energy of Methotrexate was -8.85 kcal/mol.

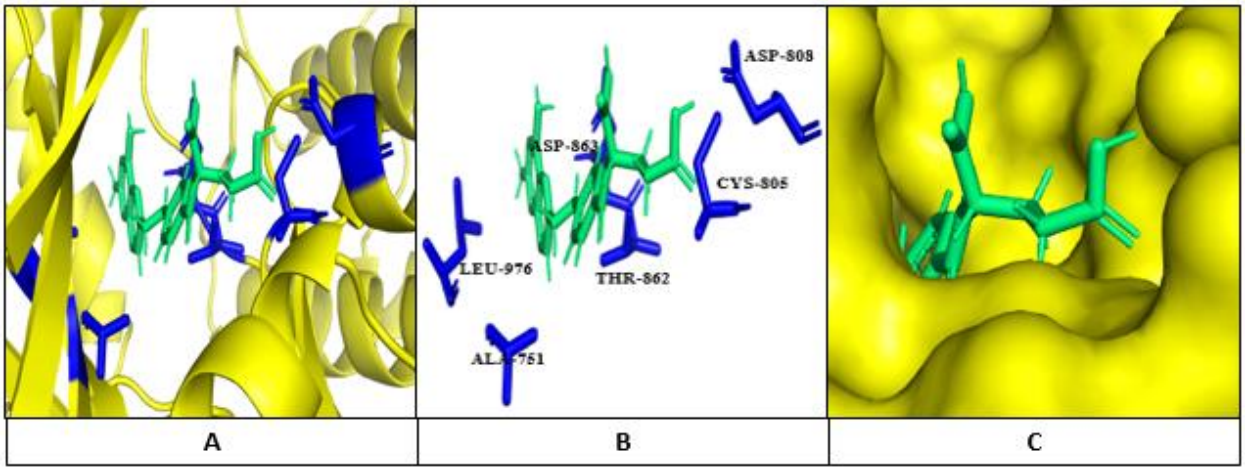

Fig. 4. (A) Aminopterin with cartoon HER2, (B) Aminopterin and amino acid residue interactions, (C) Aminopterin inside surface developed HER2. The structure of Methotrexate is represented in lime green colour while the amino acid residues of HER2 are represented in blue. The yellow structure is the HER2 protein. Aminopterin interacted with ALA751, ASP808, CYS805, THR862, ASP863 and LEU976. The binding energy of Aminopterin was -9.68 kcal/mol.

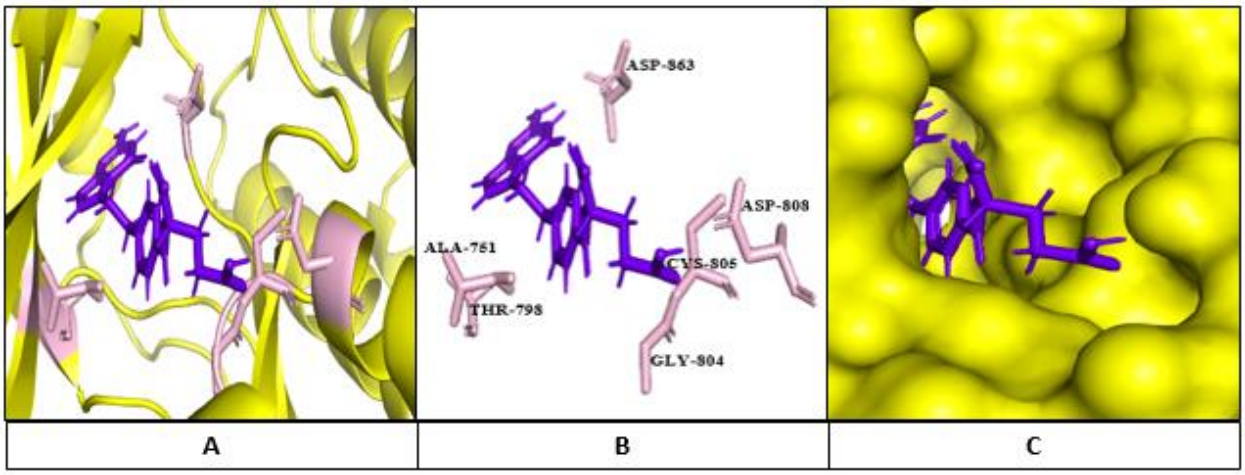

Fig. 5. (A) Pemetrexed with cartoon HER2, (B) Pemetrexed and amino acid residue interactions, (C) Pemetrexed inside surface developed HER2. The structure of Pemetrexed is represented in purple while the amino acid residues of HER2 are represented in pink. The yellow structure is the HER2 protein. Pemetrexed interacted with ALA751, THR798, ASP863, GLY804, CYS805 and ASP808. The binding energy of Pemetrexed was -9.46 kcal/mol.

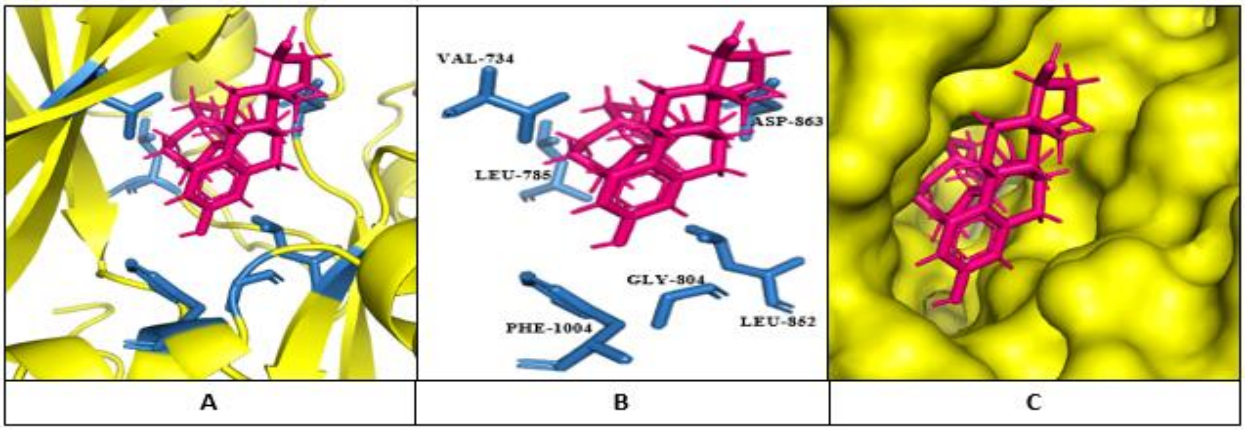

Fig. 6. (A) Fulvestrant with cartoon HER2, (B) Fulvestrant and amino acid residue interactions, (C) Fulvestrant inside surface developed HER2. The structure of Fulvestrant is represented in pink while the amino acid residues of HER2 are represented in blue. The yellow structure is the HER2 protein. Fulvestrant interacted with VAL734, PHE1004, GLY804, LEU852, ASP863 and LEU785. The binding energy of Fulvestrant was -9.17 kcal/mol.

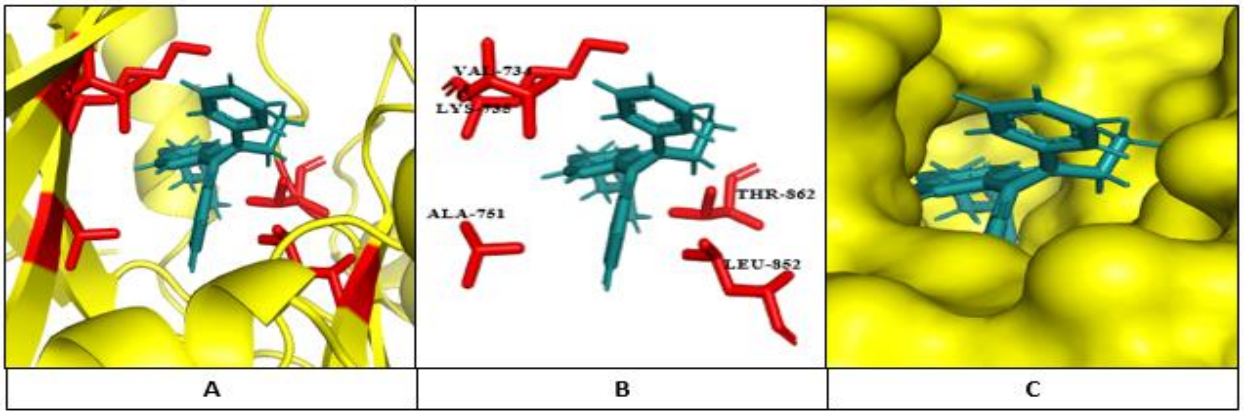

Fig. 7. (A) Tamoxifen with cartoon HER2, (B) Tamoxifen and amino acid residue interactions, (C) Tamoxifen inside surface developed HER2. The structure of Tamoxifen is represented in deep teal colour while the amino acid residues of HER2 are represented in blue. The yellow structure is the HER2 protein. Tamoxifen interacted with LYS735, VAL734, ALA751, THR862, LEU852. The binding energy of Tamoxifen was -8.73 kcal/mol.

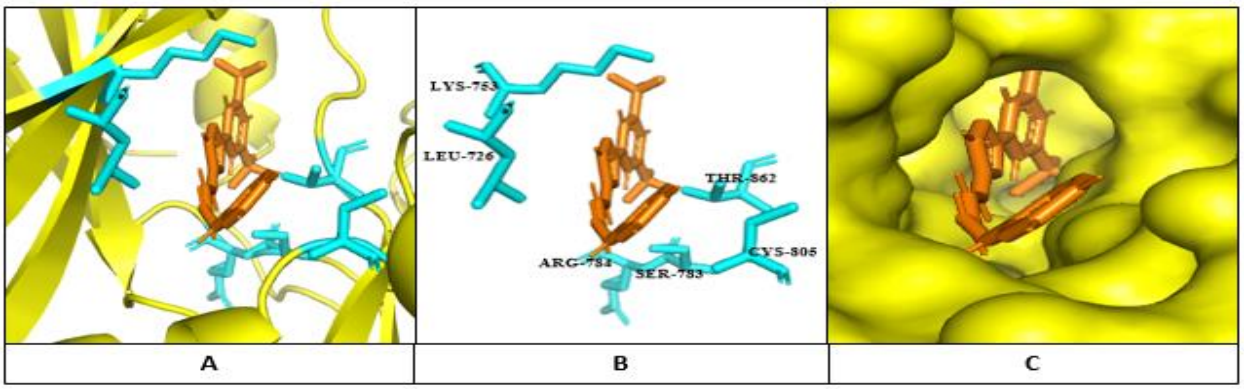

Fig. 8. (A) Selinexor with cartoon HER2, (B) Selinexor and amino acid residue interactions, (C) Selinexor inside surface developed HER2. The structure of Selinexor is represented in orange while the amino acid residues of HER2 are represented in cyan. The yellow structure is the HER2 protein. Selinexor interacted with LYS753, SER783, LEU726, ARG784, CYS805 and THR862. The binding energy of Selinexor was -8.63 kcal/mol.

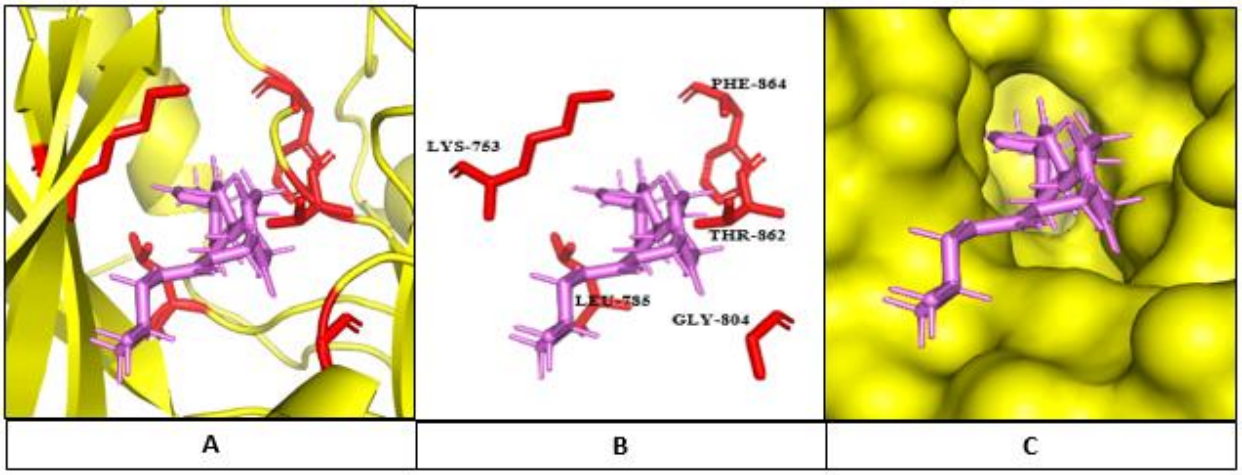

Fig. 9. (A) Sobuzoxane with cartoon HER2, (B) Sobuzoxane and amino acid residue interactions, (C) Sobuzoxane inside surface developed HER2. The structure of Sobuzoxane is represented in violet while the amino acid residues of HER2 are represented in red. The yellow structure is the HER2 protein. Sobuzoxane interacted with LYS753, PHE864, THR862, GLY804 and LEU785. The binding energy of Sobuzoxane was -8.98 kcal/mol.