Investigating new inhibitors with potential advantages for combating HER2-overexpressing head and neck squamous cancer cells; a practical and virtual screening study

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Abstract

Head and neck squamous cell carcinoma (HNSCC) are between most common cancer worldwide. Most HNSCC tumors are characterized by higher expression of human epidermal growth factor receptor 2 (HER2) that is related to resistance to chemotherapy and radiotherapy. Accordingly, HER2 has been proposed as a fair target for specific chemotherapy. Lapatinib as a potent inhibitor of the EGFR family were proposed for the treatment of HER2-positive HNSCC cases. In current study, at first step we used the crystal structure of HER1-lapatinib complex (PDB ID: 1XKK) to deeply investigate lapatinib interaction with HER1. Based on the HER1-lapatinib crystal structure and conserved structure of EGFR family, we made a confirmed coordination to survey HER2-lapatinib interactions. After investigation of lapatinib with HER1 and HER2 structures *via* docking approach, we evaluated lapatinib effect on HN5 cells as HER2-overxpressing HNSCC-originating cell line. At last, we used created 3D coordination to introduce other efficient and specific inhibitors for HER1 and HER2 based on virtual screening processing according on structural similarity to lapatinib. Also, Pharmacokinetic properties of indicated ligands were assessed by ADMET *in silico* modelling tool. Obtained results showed, in accordance with our obtained docking result while the presence of lapatinib could enhance the optimum effect of cisplatin on ablation of HN5 cells it could not empower the specific effect of cisplatin on HN5 cells when compared with normal HER2-expressing MCF-7 cells. Following, based on virtual screening process, we introduce agents with high and specific affinity for HER1 and HER2.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) with more than 600,000 new cases annually is supposed as the seventh most commonly form of cancer worldwide, accounting for approximately 278,000 deaths in 2020. It is predicted that incidence of head and neck cancer increase 30% annually by 2030 [1]. Surgery and radiotherapy are standard treatments for stage I and II of HNSCC, while various types of chemotherapy are added to the treatment protocols in the stages III and IV [2]. Platinum agents (e.g. cisplatin and carboplatin) alone or in combination with 5-fluorouracil, paclitaxel plus 5-fluorouracil, cetuximab plus platinum agents, and cetuximab in combination with 5-fluorouracil are the prevalent approved treatment protocols for HNSCC patients [3]. Cetuximab as a monoclonal antibody which inhibits epidermal growth factor receptor (EGFR), is a molecular targeting agent for the treatment of primary or recurrent/metastatic HNSCC. In approximately 90% of patients with HNSCC, overexpression of EGFRs has been reported. However, response rate to cetuximab as a single agent is lower than 13% in HNSCC cases and is not correlated with EGFR (HER1, ErbB1) expression levels in the primary tumors [4, 5].

The four closely related members of the EGFR family (HER1/ ErbB-1, HER2/ErbB-2, HER3/ErbB-3 and HER4/ErbB-4) which are expressed in many tissues have critical roles in the regulation of cell growth and proliferation and also in cell differentiation during development [6]. Each member of this family is a membrane-spanning receptor with an internal tyrosine kinase domain. Ligand binding to the external domain of EGFRs leads to receptor homo/hetero-dimerization, triggering of intracellular tyrosine kinase

domain autophosphorylation and initiation of signaling cascades [7]. Among the members of EGFR family, only dimerization of HER2 as an orphan receptor can occur both ligand-independently (in case of HER2 homodimerization) or ligand-dependently (in case of heterodimer formation with other members of EGFR family). Amplification of the signals from the other HER family members has been suggested as the main role of HER2 [8].

There is some evidence that HER2 amplification is an early event in cancers and correlates with increased propensity to metastasize, more aggressive disease, and poorer survival. Overexpression of HER2 has been reported in various cancers including breast (15–30%), colon, bladder (23–80%), gastric (10–30%), lung (20%), ovary (20–30%) and head and neck (1–35%) [9], suggesting that it may be a suitable target for antigen-directed chemo-immunotherapy [10].

Here, HN5 cell line was treated with a combination of two antineoplastic drugs, lapatinib (a tyrosine kinas inhibitor) [10] and cisplatin (a DNA alkylating agent) [7]. Following, based on the acquired results and available crystal structure of HER1-lapatinib complex, we also performed a virtual screening study for identification of new efficient and specific inhibitors for HER1 and HER2 for treatment of HNSCC cases with HER-overexpression.

2. Material and Methods

2.1. Materials

Cisplatin and lapatinib were bought from Millipore-Sigma (Darmstadt, Germany). The MTT assay kit was bought from Atocel (Hungary) and DMEM medium was obtained from Gibco (USA). The cell lines HN5 and MCF-7 were obtained from the Pasteur Institute of Iran (Tehran, Iran). All other chemicals and reagents were purchased from Merck (Darmstadt, Germany).

2.2. Investigating EGFR and HER1 interaction with lapatinib

We used 1xkk.pdb file to clarify lapatinib interaction with EGFR. Based on the performed analysis,

we also implemented an internal validation phase, where lapatinib was docked against the EGFR in 1xkk.pdb model. Based on highly conserved sequence and structural similarity among EGFRs family, we also used 1xkk.PDB file to present a 3D coordination model for HER2-lapatinib interaction with using 3pp0.PDB file for HER2 coordination. We used AutoDock-Tools 4.2 software for the determination of grids and converting of file formats (Morris, Huey et al. 2009). AutoDock-Vina (Trott and Olson 2010) was used for automated docking to find the lowest-energy poses of lapatinib against EGFRs.

For visualization of protein structures, depicting the protein-ligand interactions, and rendering of images, we used VMD (Humphrey, Dalke et al. 1996), Pymol (DeLano 2002), LIGPLOT (Wallace, Laskowski et al. 1995), and UCSF Chimera programs (Pettersen, Goddard et al. 2004).

2.3. Determination of HER2 expression level in the cultured cells

Two cell lines, one with HER2 overexpression (HN5) and another with normal level of HER2 expression (MCF-7) were cultured at 37°C, 5%CO_2 conditions in DMEM high glucose medium (Gibco, Manchester, UK) supplemented with FBS 10%. After reaching 70% confluency, the cells were washed twice with PBS, and lysed with RIPA buffer. The HER2 expression levels in the cultured cells were evaluated by immunoblotting technique after total protein separation on SDS-PAGE as described previously [11].

2.4. Treatment of cultured cells with cisplatin, cisplatin/lapatinib

The HN5 and MCF-7 cells were cultured in DMEM medium as described above. After reaching 70% confluency, the cells were washed twice with PBS, detached by trypsin 0.25%, and centrifuged at 1000 g for 5 minutes. By discarding the supernatant, the cell suspensions were prepared and seeded at approximately 1×10^4 cells $well^{-1}$ into 96-well cell culture plates (Nunc, Roskilde, Denmark). The cells were incubated overnight at 37 °C, 5% CO_2, in a humidified incubator. Then, the cells were exposed to: (1) various concentrations of cisplatin (0.75, 1.5, 3, 6, 10, and 12 $\mu g.ml^{-1}$), (2) a fixed concentration of cisplatin (3 $\mu g\ ml^{-1}$) in combination with various concentrations of lapatinib (0.015, 0.031, 0.062, 0.125, and 0.25 $\mu g\ ml^{-1}$). After four hours of treatment, the cells were washed with PBS and reincubated for 72 hours in the fresh medium under the same conditions but without addition of drugs. At the end of incubation, the cells were washed twice with PBS and viability was determined by MTT assay [12] using the microplate reader BioTek ELX800 (Winooski, United States).

2.5. Screening based on targeted binding.

Previously described confirmed model of HER1 and HER2 were used for automated docking.

Of 140 identified chemical structures in the ligand search step considering 75% Tanimoto threshold in Pubchem data center based on chemical skeleton similarity to lapatinib to find the lowest-energy poses of the small molecule against indicated targets with using InstaDock-Tools software [13]

2.6. Pharmacokinetic and toxicity properties of top compounds

Physicochemical, pharmacokinetic, and toxicity properties of lapatinib, top three selected compounds with highest affinity for HER1 and HER2 were determined by SwissADME (Daina, Michielin et al. 2017) and PASS online web resource [14].

2.7. Statistical analysis

All the experiments were accomplished in triplicate. Data were analyzed using two-way ANOVA and all values were presented as mean ± SD. Values of $p<0.05$ were considered as statistically significant.

3. Results and discussion

3.1. Clarification of lapatinib interaction with HER1 and creation of a model to investigate HER2-lapatinib interaction.

The interaction of lapatinib with HER1 was investigated based on the available crystal structure 1xkk.pdb (figure 1). We also docked lapatinib against HER1 to perform a validation phase study for lapatinib interaction with HER1 based on the indicated PDB file (Figure 1). As indicated in Figure 1, lapatinib is docked fairly against HER1 as mainly overlayed to its coordination in 1xkk.pdb file with $\Delta G_{binding}$=10 kcal.mol^{-1}. Lapatinib generally categorized as poor soluble molecule (Table 1) and so extensively interact with HER1 molecule through many hydrophobic residues such Met766, Leu777, Cys775, Leu788, Thr790, Arg776, Lys745, Leu792, Leu844, Ala743, Gln791, Gly796, Asn842, Met793, Gly721, Arg841, Gly719, Leu718, Val726, and Met1002 and also via its Cl atom make two electrostatic interaction with Asp855 and Thr854 residues of HER1 molecule (Figure 1B).

As previously reported, members of EGFR family showed a high degree of similarity [15]. Based on the alignment study were performed on internal tyrosine kinase domain of HER1 (from 1xkk.pdb file) and similar domain of HER2 (from 3pp0.pdb file), internal tyrosine kinase domain of HER1 showed 78% identity and 87% similarity to internal tyrosine kinase domain of HER2 (Figure 2). In result of such similar primary structure (Figure 2), 3D coordination of internal tyrosine kinase domain of HER2 is so near to internal tyrosine kinase domain of HER1 and so we used 3D coordination of HER1 from 1xkk.pdb file for investigation of lapatinib interaction with HER2 (Figure 1). As indicated in figure 1, lapatinib was docked against HER2 as matched to internal kinase domain of HER1 with $\Delta G_{binding}$=9 kcal.mol^{-1} (Figure 1A) that acquired through hydrophobic interactions of lapatinib with Leu785, Lys753, Thr798, Leu852, Leu796, Val734, Asn850, Leu726, Asp863, Cys805, Asp808, Asp845, Ala730, Ser728, Pro885 and two H-bonds with Arg849 and Thr862 (Figure 1C). Predicted higher affinity of lapatinib for HER1 in compare to HER2 is in confirmed with previously evaluated IC_{50} values that reported by K. Aertgeerts et al. [16]. They reported 3±0.2 nm IC_{50} of lapatinib for HER1 and 13±1 nm IC_{50} of lapatinib for HER1 [16].

3.2. Treatment of HN5 cells with cisplatin

Cisplatin as a traditional clinical agent for chemotherapy of tumors, causes cell death by formation of DNA adducts and consequent blockage of replication and transcription. Cisplatin-based chemoradiotherapy is still considered as the first-line established standard protocol to treat patients with locally advanced HNSCC [17]. In current study, HN5 and MCF-7 cell lines were treated with various concentrations of cisplatin. Based on the results, cisplatin at concentrations 3, 6, 10, and 12 µg.ml^{-1} reduced the viability of MCF-7 cells to 91.79, 66.59, 83.37, and 84.35%, respectively. In a similar situation, cell viability of HN5 cells was reduced to 85.29, 88.64, 54.79, 34.07, 21.87, and 17.35% after treatment with cisplatin at concentrations 0.75, 1.5, 3, 6, 10, and 12 µg.ml^{-1} as respectively (Figure 3A). The antiproliferative effect of cisplatin on MCF-7 cells has already been studied and it is shown that MCF-7 cells compared to the other breast cancer cell lines are more resistant to cisplatin [18]. The higher resistance of MCF-7 cells to cisplatin is associated with the higher levels of the anti-apoptotic protein Bcl-2 in these cells. Here, as it can be seen, cisplatin induced a more potent toxic effect on HN5 cells than on MCF-7 cells. A dose-dependent reduction in HN5 cell viability was seen especially at cisplatin concentrations higher than 3 µg.ml^{-1}. Although 12 µg.ml^{-1} could seem a high dose for a chemotherapy

agent that may cause serious side but at this concentration of cisplatin, the percentage difference between viable MCF-7 and HN5 cells reached to 67% (Figure 3A).

3.3. Treatment of HN5 cells with a combination of cisplatin and lapatinib

High HER2 expression in HNSCC patients is associated with worse prognosis, high rates of recurrence, and decreased overall survival (OS) [19]. The 5-year OS and the 5-year disease-free survival (DFS) probabilities were meaningfully lower for HER2-positive HNSCC patients compared to HER2-negative ones [9]. HER2 expression level is also higher in the case of metastases of laryngeal tumors rather than corresponding primary tumors [20]. Co-expression of HER2 and EGFR has been reported in HNSCC tumors [21]. A study on the HNSCC tumors in The Cancer Proteome Atlas (TCPA) database revealed that the expression of HER2 and its activated (phosphorylated) form is correlated with EGFR expression. Wheeler and colleagues showed dysregulation of EGFR internalization/degradation and subsequent activation of HER2 and HER3 in cetuximab-resistant HNSCC cell lines [19]. It is possible to overcome resistance to cetuximab by using tyrosine kinase inhibitors (TKIs), including gefitinib and erlotinib or monoclonal antibody 2C4 against HER2 [22]. Together, these findings emphasize on the potential of HER2 as a molecular target for HNSCC therapy [5, 23].

Lapatinib targets tyrosine kinase domain of both EGFR and HER2 (Figure 1) and hence is under investigation in multimodal therapy of advanced HNSCC [24]. Before measurement of cell viability in the presence of lapatinib, HER2 expression level in the MCF-7 and HN5 cells were determined using immunoblotting technique. Based on the obtained results shown in Figure 4, level of HER2 expression in HN5 cells was more than 16 times higher than that of MCF-7 cells (Figure 4).

Previous reports also confirmed HER1 and HER2 co-expression in MCF-7 cell line by western blot [25] and flow cytometry approaches [26] and also higher expression of HER1 and HER2 in HN5 cell line in compare to MCF-7 cells [25, 26]. While it's a potent and confirmed crosstalk between members of EGFRs [25] and also they have high degree of homology and similarity [27], specific targeting of HER2 is still could seems as greatest potential for efficient cure of tumors with HER2 high-expression and therefore, MCF-7 cells with lower expression of HER1 and HER2 could be a suitable control cell.

For the combination treatment of MCF-7 and HN5 cell lines, 3 $\mu g.ml^{-1}$ of cisplatin as optimized concentration plus 0.015, 0.031, 0.062, 0.125, and 0.25 $\mu g.ml^{-1}$ of lapatinib were applied. Based on the obtained results, viability of MCF-7 cells was decreased to 62.98, 62.59, 53.46, 53.56 and 44.70%, while these values were 44.83, 53.19, 39.56, 26.99 and 19.25% for HN5 cells in the respective combined doses (Figure 3B). Lapatinib did not induce a significant dose dependent cytotoxic effect on MCF-7 cells in concentrations <0.25 $\mu g.ml^{-1}$, while it remarkably reduced the HN5 cell viability in 0.062 $\mu g.ml^{-1}$ (Figure 3B). This effect may be the result of higher expression of HER1 and HER2 in HN5 cells as demonstrated before (Figure 4). Studies on the antiproliferative effect of cisplatin/lapatinib combination therapy have shown that lapatinib combined with cisplatin caused an additive growth inhibitory effect on HN5 cells and therefore suggested EGFR- or HER2-overexpressing in HNSCC malignancies [28]. In addition, the

combined effect of lapatinib and cisplatin on colony formation (CF) of epithelial cells of individual HNSCC was investigated in short term ex vivo assays, showing that lapatinib suppressed the CF of epithelial HNSCC cells and its efficacy was increased in combination with cisplatin. Still, simultaneously they warn about significant heterogeneity in the response of HNSCC to lapatinib as alone or when it combined with cisplatin [29].

As indicated above (Figure 1, 3, and 4), although lapatinib in lower doses in comparison to cisplatin could diminish viability of HN5 cells in comparison to HN5 cells that treated with cisplatin alone with negligible effect on MCF-7 cells but lapatinib could not induce such distances that seen between viability of MCF-7 and HN5 cells in ≥10 µg of cisplatin. Due to the higher expression of HER1 and HER2 in HN5 cells as originated from HNSCC, it seems compounds with higher affinity for HER1 [26] and especially HER2 (figure 4) could be assumed as a more potent and specific chemotherapeutic agent against HNSCC cases.

3.4. Virtual screening to discover new inhibitors for HER1 based on lapatinib chemical structure.

As indicated in the methods section, 140 compounds with structural similarity to lapatinib ligand and with recorded cure properties were chosen for docking against HER1 and HER2.

While $\Delta G_{binding}$=10.0 was calculated for lapatinib coordination in 1xkk.pdb file, the top-3 chemical structures with higher affinity for HER1 were including compound 127036333: [3-[4-[3-Chloro-4-[(3-fluorophenyl)methoxy]anilino]thieno[3,2-d]pyrimidin-6-yl]phenyl]-piperidin-1-ylmethanone, compound No.145974775: N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-(2-morpholin-4-ylpyrimidin-4-yl)quinazolin-4-amine, and compound No.145952005: N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-(4-morpholin-4-ylpyrimidin-2-yl)quinazolin-4-amine with -12.3, -12.3, and -12.2 kcal.mol^{-1} as $\Delta G_{binding}$ as respectively showed highest binding affinity for HER1 (Figure 5). Also, 7.41, 9.02, 9.02, and 8.95 as pKi were calculated for lapatinib, 127036333, 145974775, and 145952005 as respectively.

Compound 127036333 was previously introduced by Jennifer L Woodring and her colleagues as an agent that could use against parasites that cause trypanosomiasis, leishmaniasis, and malaria. Its most potent effect was its antiparasitic activity against Plasmodium falciparum D6 with EC50 1.17 µM [30]. As depicted in figure 6A, Compound 127036333 through Asp800, Leu1001, Gly796, Leu718, Met793, Leu792, Ala743, Leu844, Val726, Leu858, Asp855, Thr845, Lys745, Met766, Cys775, and Phe856 residues make hydrophobic interactions with HER1 and via Cys797 and Thr790 make two H-bond by the protein. Furthermore, pharmaceutical properties of 127036333 was predicted (Table 1) and it has a pharmaceutical attribute like lapatinib and could not induce other antagonist effect with Pa (probability "to be active")>0.7.

Compound 145974775 previously were introduced as inhibitor for trypanosome proliferation and shows EC50 0.069 µM against Plasmodium falciparum [31]. It also through two H-bonds via Met793 and Thr790 and also extended hydrophobic cavity that made by Met1002, Leu718, Ala743, Gly796, Ser720, Leu844, Leu792, Arg841, Thr854, Gln791, Phe856, Val726, Lys745, Asp855, Leu858, Cys775, and Met766 have

considerable affinity for HER1 (Figure 6B). It has higher water solubility in compare to lapatinib (Table 1) and also show Pa ≥0.729 as antagonist of Raf kinase C. compound 145952005 is also presented before with antiparasitic activity against plasmodium falciparum D6 with EC50 0.9 μM and could not induce other antagonist effect with Pa>0,7. As presented in figure 6C, this compound through H-bonds via Met793 and Thr790 and hydrophobic interactions through Leu718, Met1002, Gly796, Ala743, Arg841, Ser720, Val726, Leu792, Leu844Lys745, Gln791, Asp855, Thr854, Phe856, Leu858, Cys775, and Met766 has great affinity for HER1.

3.5. Specific and potent inhibitor discovery through a virtual screening process for HER2 based on lapatinib chemical structure

Between selected structures with structural similarity to lapatinib three compounds including compound No.162667484: N-[4-[3-chloro-4-[3-(trifluoromethyl)phenoxy]anilino]quinazolin-6-yl]-2-(dimethylamino)acetamide (Pubchem ID:162667484), compound No.162643878: N-[4-[3-chloro-4-[3-(trifluoromethyl)phenoxy]anilino]quinazolin-6-yl]-2-(4-methylpiperazin-1-yl)acetamide (Pubchem ID: 162643878), and compound No.127035117: N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-(5-methyl-6-morpholin-4-ylpyridin-3-yl)quinazolin-4-amine (Pubchem ID: 127035117) showed lowest $\Delta G_{binding}$ as -12.9 (pKi=9.46), -12.5 (pKi=9.17), -12.2 (pKi=8.95) kcal.mol^{-1} for HER2 as respectively (Figure 7). While lowest $\Delta G_{binding}$ for lapatinib against HER2 was predicted -9.0 with pKi=7.41, it seems introduced inhibitors have higher affinity and specificity for HER2 (Figure 7).

162667484 and 162643878 previously reported by T. A. Elwaie et al as potent and specific inhibitor for HER2. 162667484 induce its most intense effect with IC50=0.408 μM as antiproliferative activity against human AU565 cells assessed as reduction in cell viability incubated for 72 hrs by WST8 assay and 162643878 induce its most effective antiproliferative activity against human BT474 cells assessed as reduction in cell viability incubated for 72 hrs by WST8 assay with IC50=0.408 μM [32]. To our knowledge there is no data about any possible effect 162667484 and 162643878 on HN5 cell line. 2D interaction of 162667484 and 162643878 as docked to HER2 with lowest $\Delta G_{binding}$ were presented in figure 8A and 8B as respectively. Based on the predicted pharmaceutical properties and in compared to lapatinib, just 162643878 shows high GI absorption as considerable attribute and neither of 162667484 nor 162643878 do not show any Pa>0.7 for registered targets on the PASS online server.

J. L. Woodring et al. at 2015 presented compound 127035117 as an anti-parasitic agent with the most brilliant effect as with previously was introduced as antiparasitic activity against Plasmodium falciparum D6 with EC50=0.26 μM [30]. As depicted in figure 8C, 127035117 through two H-bonds with Thr798 and Ser783 and an through extended hydrophobic network that formed by Asp808, Cys805, Gly804, Ser725, Leu762, Arg849, Leu852, Asp863, Leu796, Val734, Ala751, Lys753, The862, Phe864, Leu785, Met774 has higher affinity in compare to lapatinib for HER2. 127035117 showed predicted pharmaceutical properties similar lapatinib and do not show any Pa>0.7 for registered targets on PASS online server.

4. Conclusion

Newer and innovative protocols that do not rely only on a single agent's traditional cytotoxicity profile are required to provide a more specific, efficient, and improved form of cancer therapy. The combination of two or more therapeutic treatments to specifically target cancer-inducing or cell-sustaining pathways may be the cornerstone of cancer therapy [23]. Chemotherapy can be poisonous to patients with multiple side effects, and can also strongly reduce their immune system by affecting bone marrow cells and increasing susceptibility to other diseases [33]. Although combination therapy also can be toxic, the toxicity is commonly remarkably less because different pathways will be targeted, and lower therapeutic dosage of each individual drug is required. Further, monotherapy treatment is more susceptible to drug resistance because constant treatment with a single compound induces malignant cells to recruit alternative salvage pathways [34]. However, combination therapy that includes agents that target cancer stem cells (CSCs) would therefore reduce drug resistance and decrease the likelihood of relapse. Thus, using a combination of compounds that target different pathways could yield significant anti-cancer results [35]. All in all, it would be reasonable to suggest that more adequately well-designed clinical research studies that test the combination of a repurposed therapeutic agents and other cytotoxic agents should be performed to achieve greater efficacy expediently [23]. Based on the above points and for gaining a valid comparison between combination therapy and formulated and targeted therapy of HNSCC, we examined the effect of cisplatin as traditional and confirmed medicine for several types of HNSCC on HN5 cell line. Simultaneously we used MCF-7 cell line as malignant cells with normal HER2 expression as control cell line to evaluate potency and specificity of cisplatin and its combination therapy. As mentioned above and showed in Figure 2A, although 12 $\mu g.ml^{-1}$ commonly assume as a high concentration for a chemotherapy agent that may cause various inevitable side-effects, cisplatin in its highest concentration, 12 $\mu g.ml^{-1}$, significantly decreased the percent of HN5 cell viability to 17.35%, almost 67% lower than that of MCF-7 cells in the similar concentration (Figure 2A). In other side, the presence of lapatinib in nanogram range could deeply intensify the toxic effect of cisplatin in a dose-dependent manner (Figure 3B), but in the optimized condition (3 $\mu g.ml^{-1}$ of cisplatin+ 0.125 $\mu g.ml^{-1}$ of lapatinib) difference of percentage of cell viability between treated MCF-7 and HN5 cells just reached to 26.57%. Thus, we conclude that lapatinib could intensify the toxic effect of the optimized concentration of cisplatin on HN5 and MCF-7 cell lines, but could not be extended to 31.94% of difference of cell viability that was induced by 3 $\mu g.ml^{-1}$ of cisplatin alone and so using of more potent and specific inhibitor against HER1 and HER2 that over-express in many types of cancerous cells, like HNSCC, make these receptors suitable object for targeting [36]. To achieve this target, we also proceed a virtual screening based on the available crystal structure of HER1-lapatinib and homology modelling of HER2, and presented some new compounds with higher affinity for HER1 and HER2 and acceptable pharmaceutical properties that could be assessed for more effective and specific treatment of HNSCC cases with high expression of EGFR family without induction of undesirable drug interactions, enforcing side effects and simultaneously improving bioavailability by preventing the rapid degradation of drugs.

Abbreviations

MTT: 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide; TCPA: The Cancer Proteome Atlas; TCGA: The Cancer Genome Atlas; CSCs: Cancer Stem Cells; CF: Colony Formation; DFS: Disease-Free Survival; HNSCC: Head and Neck Squamous Cell Carcinoma; HER: Human Epidermal Growth Factor Receptor; OS: Overall Survival.

Declarations

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Author Contribution

In this research, Majid Zeinali, Aminollah Pourshohod, Ebrahim Barzegari, Akbar Akbari, Forouzan Absalan, Amir Mehranfar, and Mostafa Jamalan have been in charge of research, data collection, and analysis, and article writing.

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Table

Table 1 is available in the Supplementary Files section.

Figures

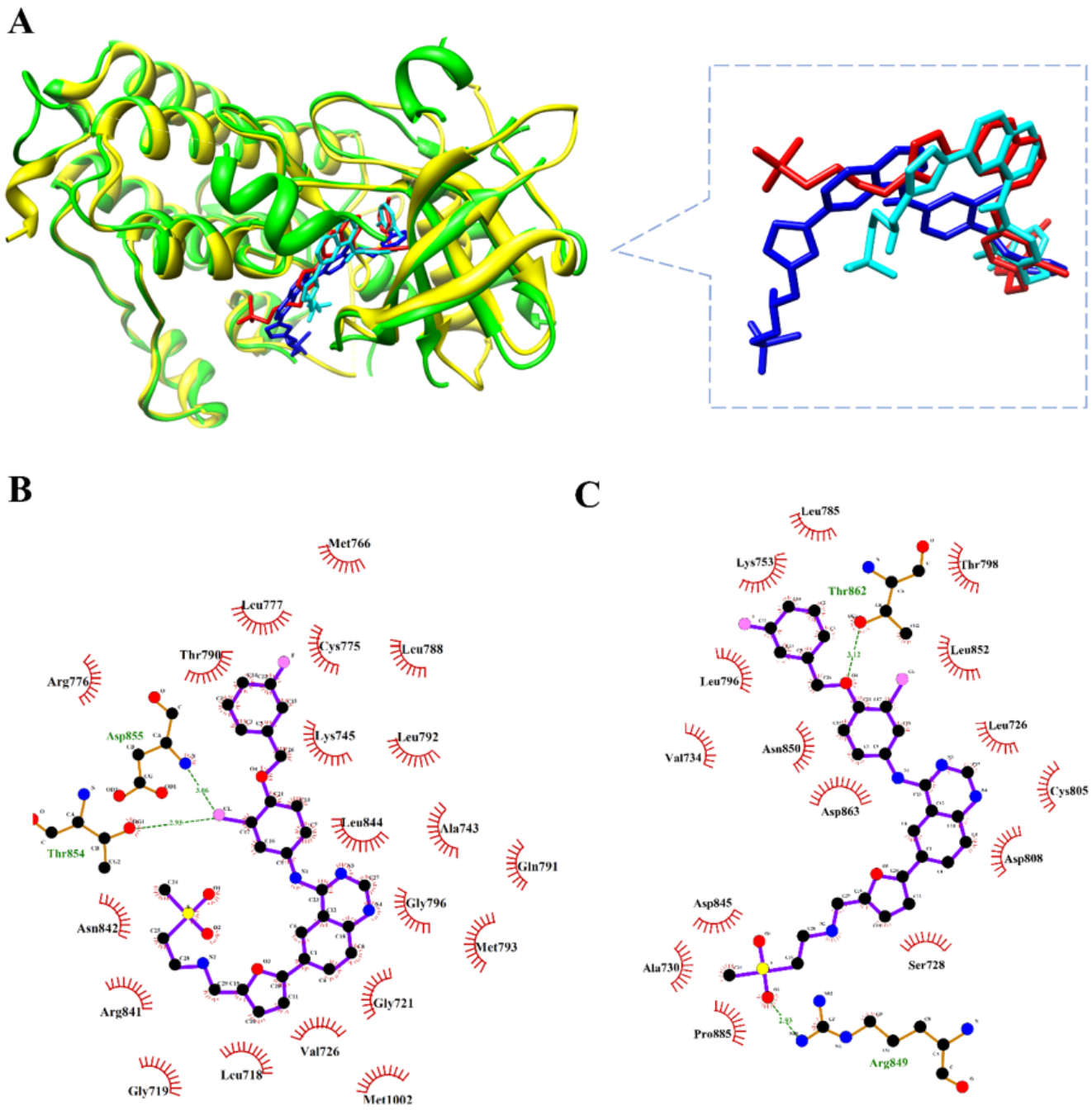


Figure 1

(A) Coordination of lapatinib in 1xkk.pdb file (Red), coordination of lapatinib (Cyan) as docked to HER1 (Green) with $\Delta G_{binding}$=-10.0 kcal.mol^{-1} and lapatinib (Blue) as docked to HER2 structure from 3pp0.pdb (Yellow) with $\Delta G_{binding}$=-9 kcal.mol^{-1}. 2D depiction of lapatinib as docked to HER1 with $\Delta G_{binding}$=-10 kcal.mol^{-1} **(B)** and 2D depiction of lapatinib-HER2 with $\Delta G_{binding}$=9 kcal.mol^{-1} **(C)**.

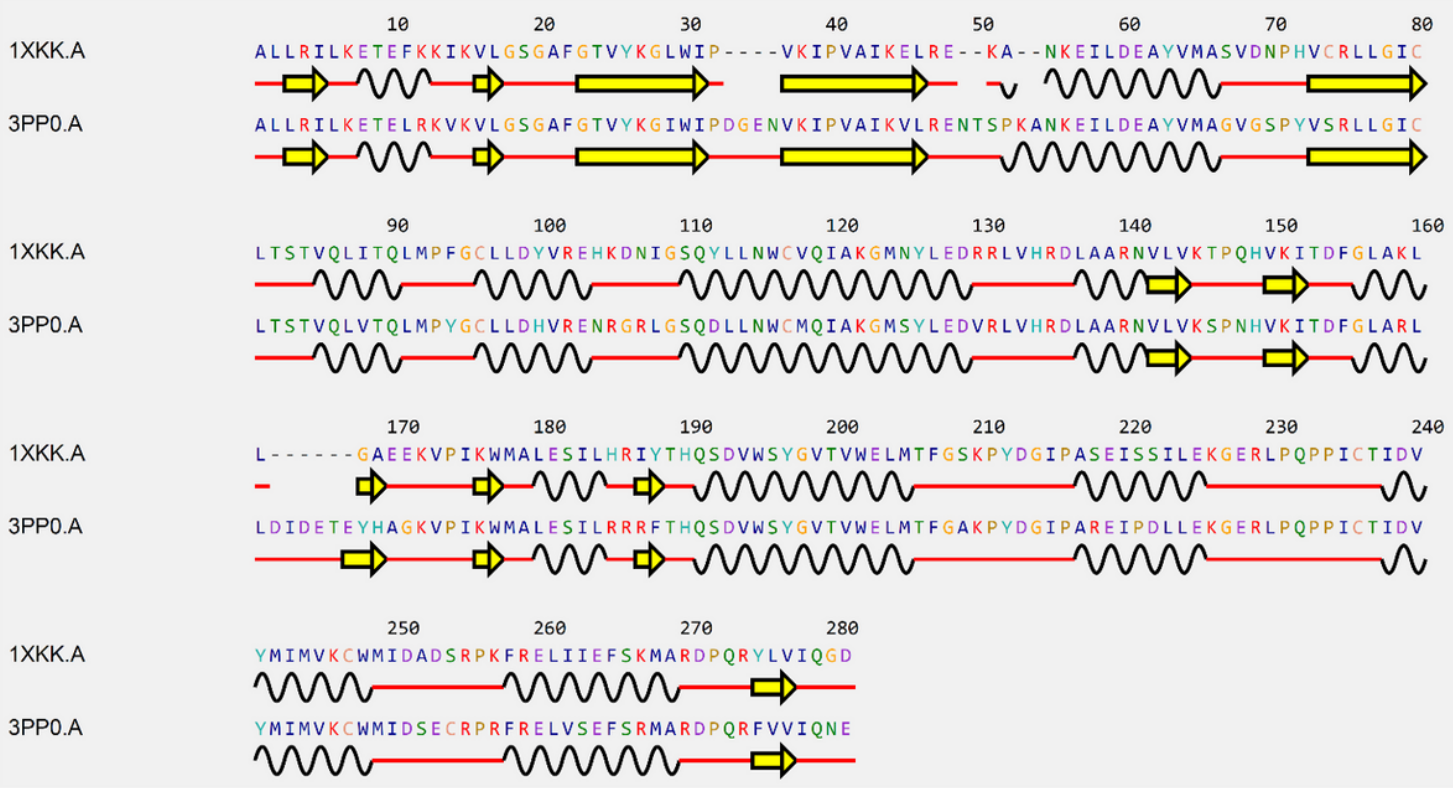


Figure 2

Sequence and 2D depiction of secondary structure of internal tyrosine kinase domain of HER1 (from 1xkk.pdb file) and HER2 (from 3pp0.pdb file).

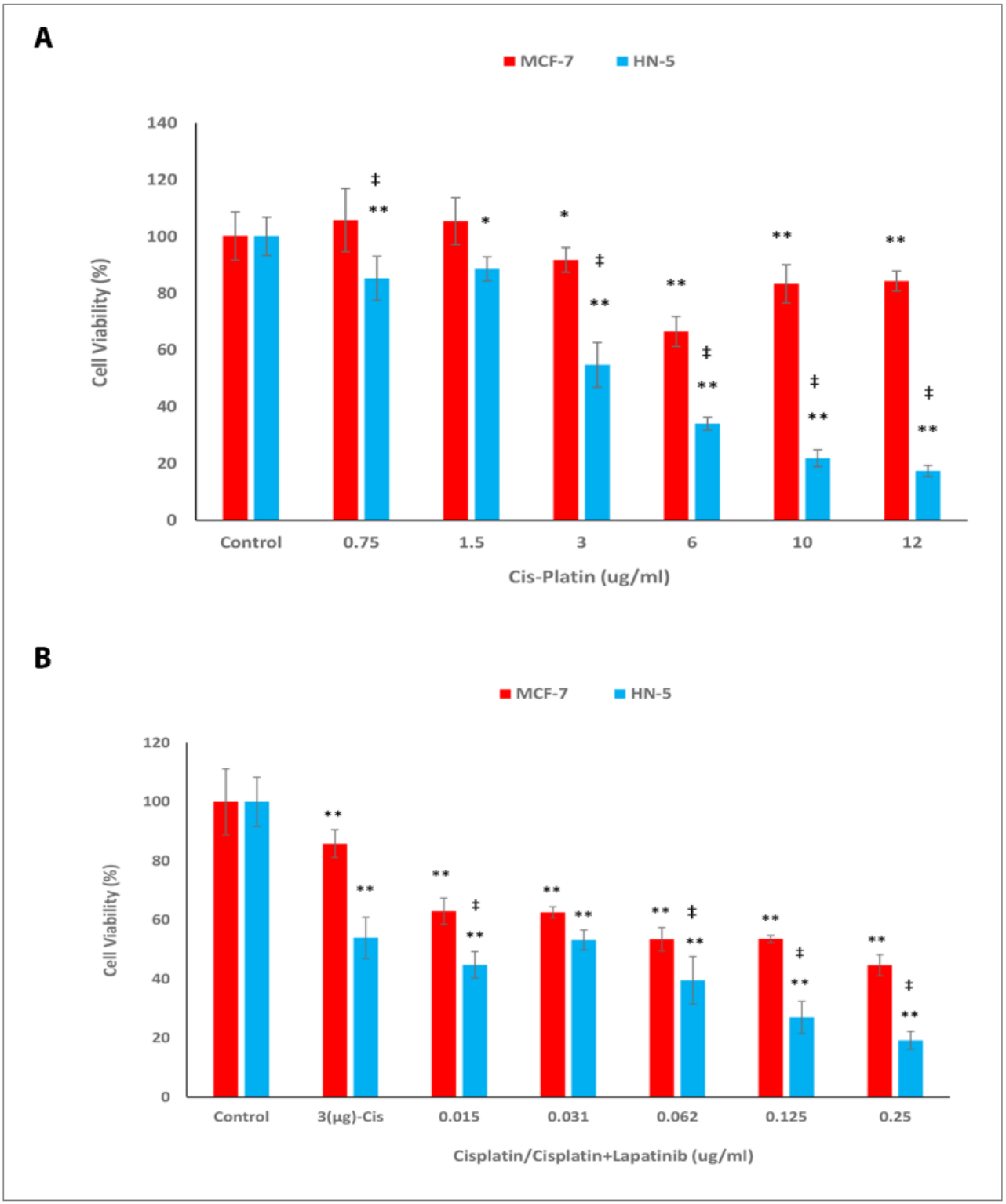


Figure 3

Cytotoxicity of cisplatin and cisplatin+lapatinib combination therapy. Cell lines MCF-7 and HN5 were treated with different concentrations of cisplatin **(A),** and 3 µg.ml^{-1} of cisplatin plus various concentrations of lapatinib **(B)** for 4 hours, and then the cell viability was evaluated by MTT assay after an incubation time of 72 h (*p<0.05 and **p<0.01, in comparison to the untreated control group, and ‡p< 0.01, in comparison to MCF-7 cell line).

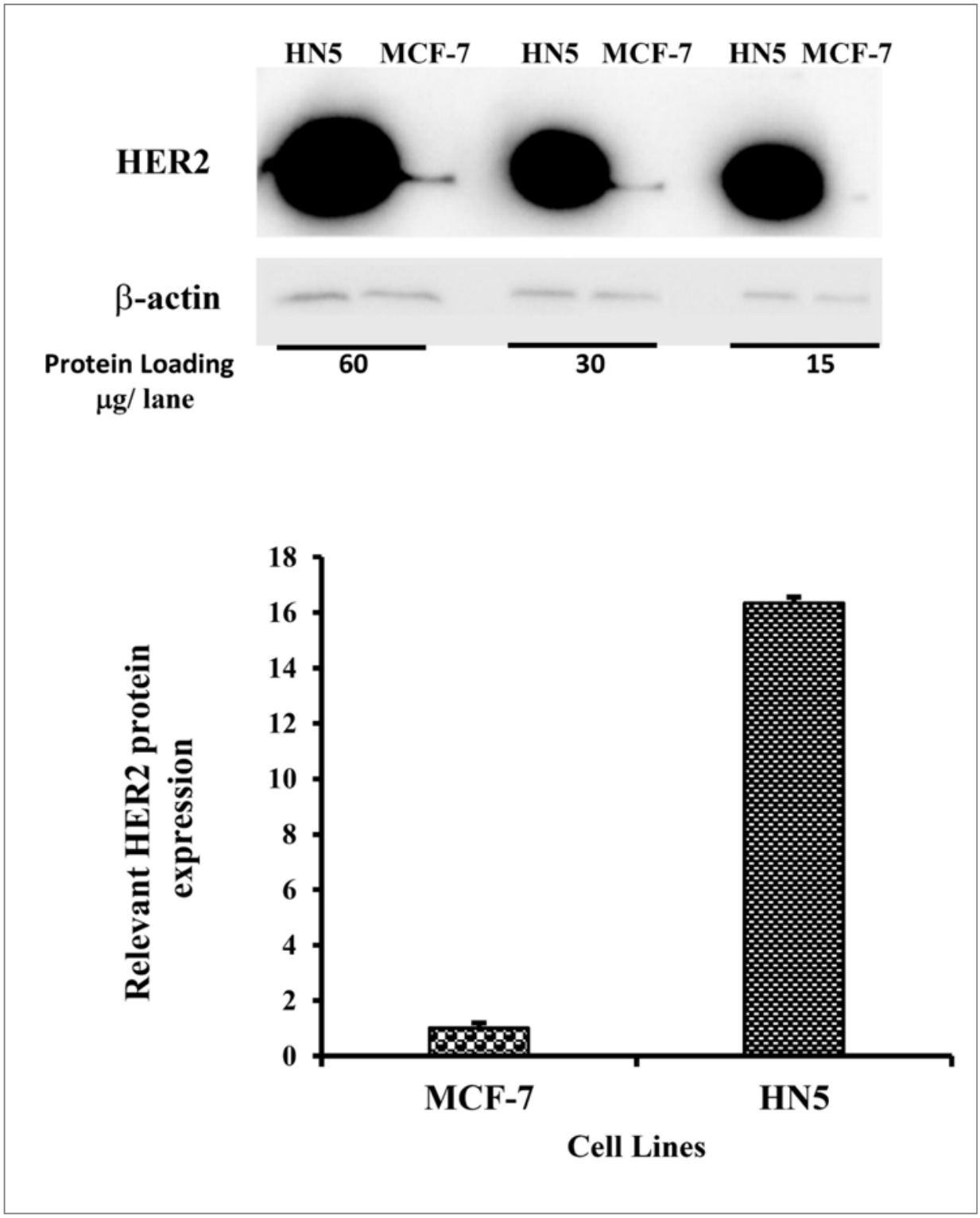
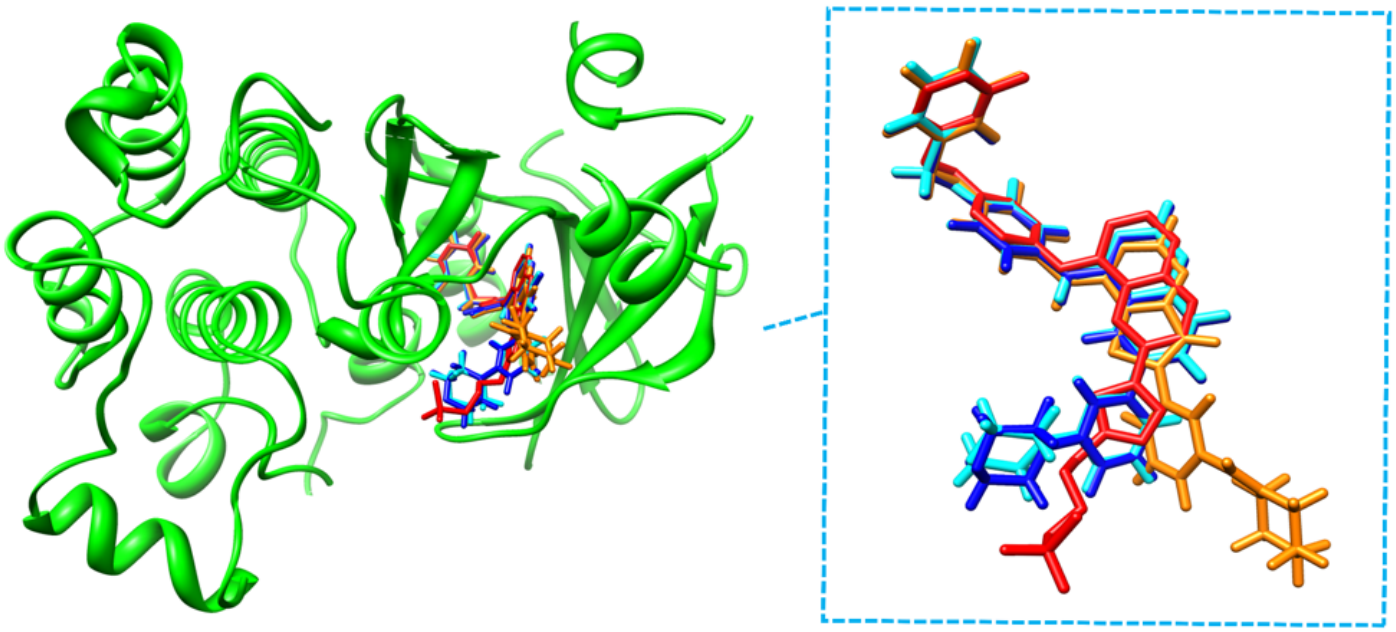


Figure 4

HER2 protein expression levels in HN5 and MCF-5 cell lines. After harvesting the cells with RIPA buffer and protein assay, different amounts of cell lysates (60, 30, and 15 ug/lane) were loaded in a 10% SDS-page gel and HER2 was detected using the specific antibody against HER2. As indicated, the protein expression of HER2 is meaningfully over-expressed in HN5 cells compared to MCF-7 cell line.



Pubchem ID	Chemical Name	$\Delta G_{binding\ (kcal/mol)}$	Molecular Structure	Color
208908	Lapatinib in 1XKK.pdb file	10.0		Red
127036333	[3-[4-[3-Chloro-4-[(3-fluorophenyl)methoxy]anilino]thieno[3,2-d]pyrimidin-6-yl]phenyl]-piperidin-1-ylmethanone	-12.3		Orange
145974775	N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-(2-morpholin-4-ylpyrimidin-4-yl)quinazolin-4-amine	-12.3		Cyan
145952005	N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-(4-morpholin-4-ylpyrimidin-2-yl)quinazolin-4-amine	-12.2		Blue

Figure 5

Coordination of lapatinib (Red) as docked to HER1 (Green) with $\Delta G_{binding}$=-10.0 kcal.mol^{-1} and compound 127036333 (Orange), 145974775 (Cyan), 145974775 (Blue) as docked to HER1 with $\Delta G_{binding}$ -12.3, -12.3, and -12.2 kcal.mol^{-1} as respectively. Pubchem ID, chemical name, and chemical structure also mentioned.

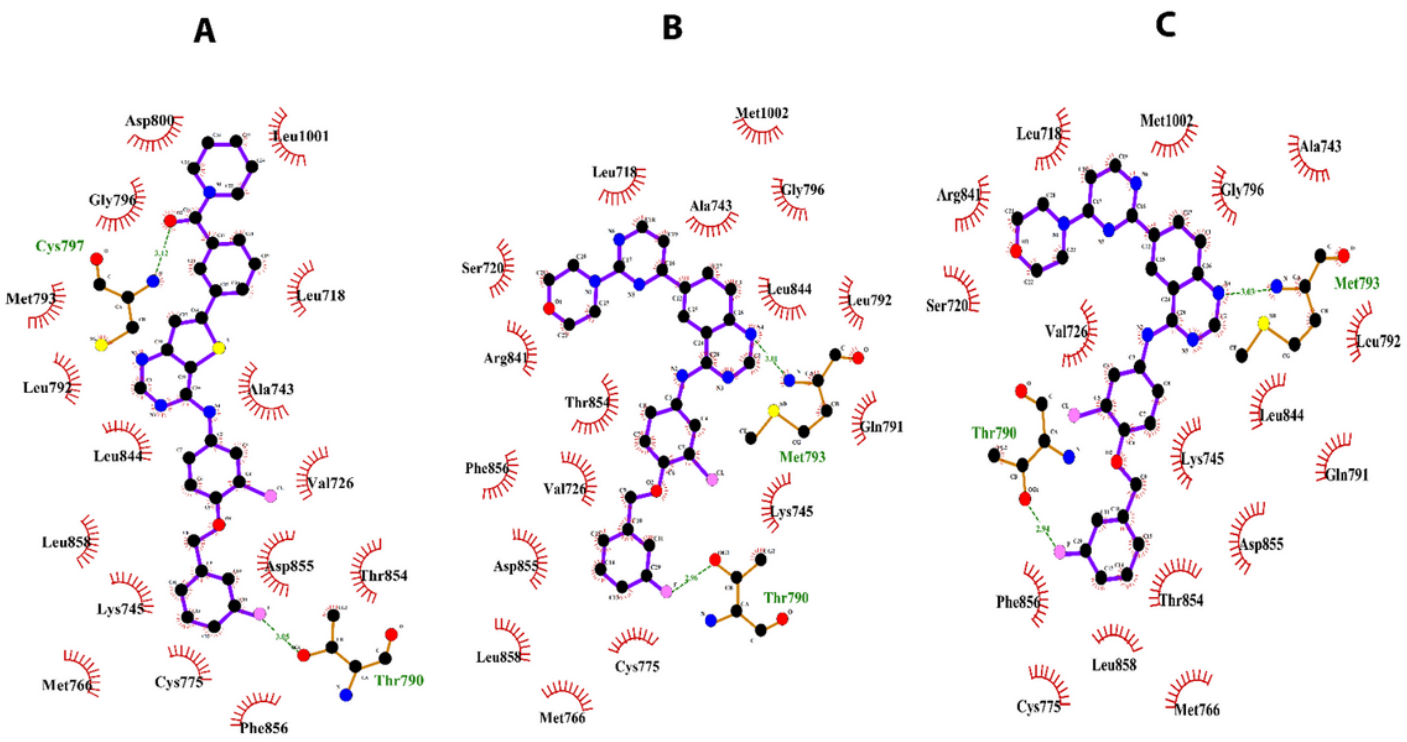
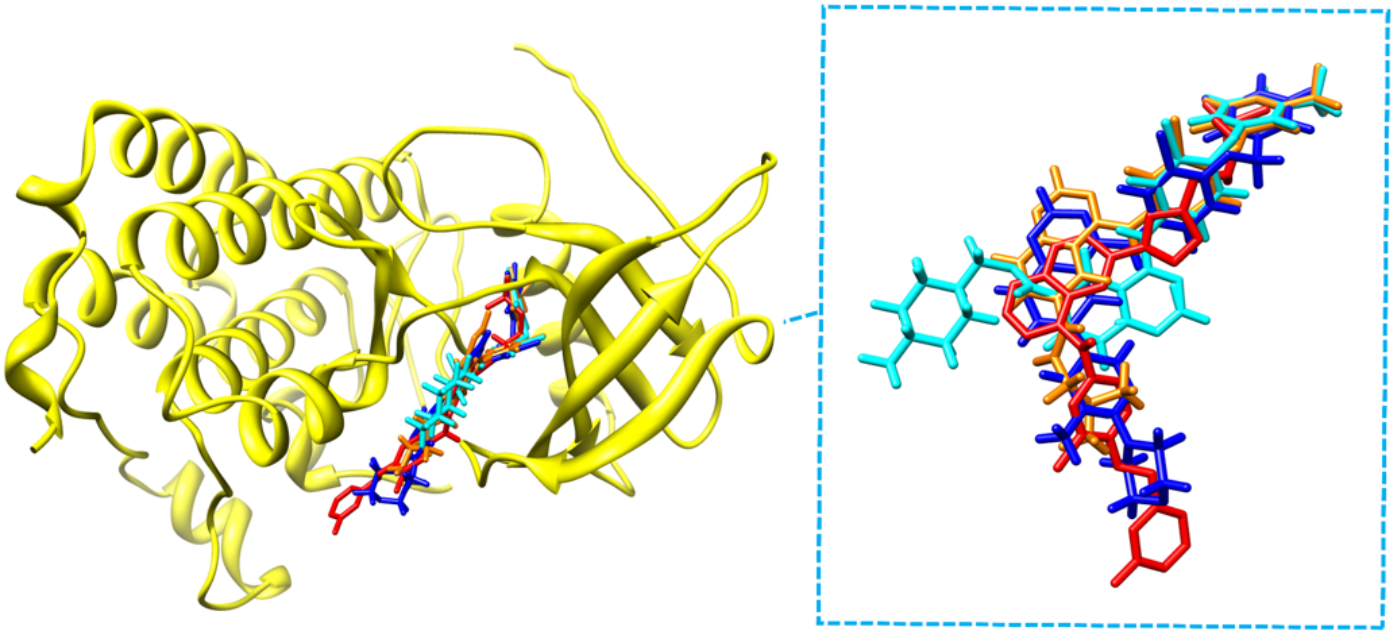


Figure 6

2D depiction of compound 127036333 (A), 145974775 (B), and 145974775 (C) as docked to HER1 with $\Delta G_{binding}$ -12.3, -12.3, and -12.2 kcal.mol^{-1} as respectively.



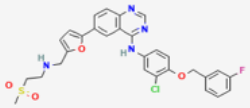
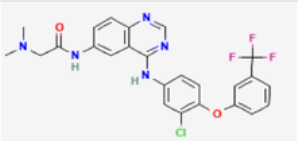
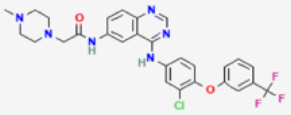
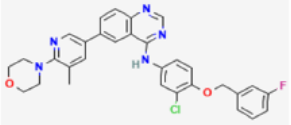
Pubchem ID	Chemical Name	$\Delta G_{binding\ (kcal/mol)}$	Molecular Structure	Color
208908	Lapatinib	-9.0		Red
162667484	N-[4-[3-chloro-4-[3-(trifluoromethyl)phenoxy]anilino]quinazolin-6-yl]-2-(dimethylamino)acetamide	-12.9		Orange
162643878	N-[4-[3-chloro-4-[3-(trifluoromethyl)phenoxy]anilino]quinazolin-6-yl]-2-(4-methylpiperazin-1-yl)acetamide	-12.5		Cyan
127035117	N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-(5-methyl-6-morpholin-4-ylpyridin-3-yl)quinazolin-4-amine	-12.2		Blue

Figure 7

Coordination of lapatinib (Red) as docked to HER2 (Yellow) with $\Delta G_{binding}$=-9.0 kcal.mol^{-1} and compound 162667484 (Orange), 162643878 (Cyan), 162643878 (Blue) as docked to HER2 with $\Delta G_{binding}$ -12.9, -12.5, and -12.2 kcal.mol^{-1} as respectively. Pubchem ID, chemical name, and chemical structure also mentioned.

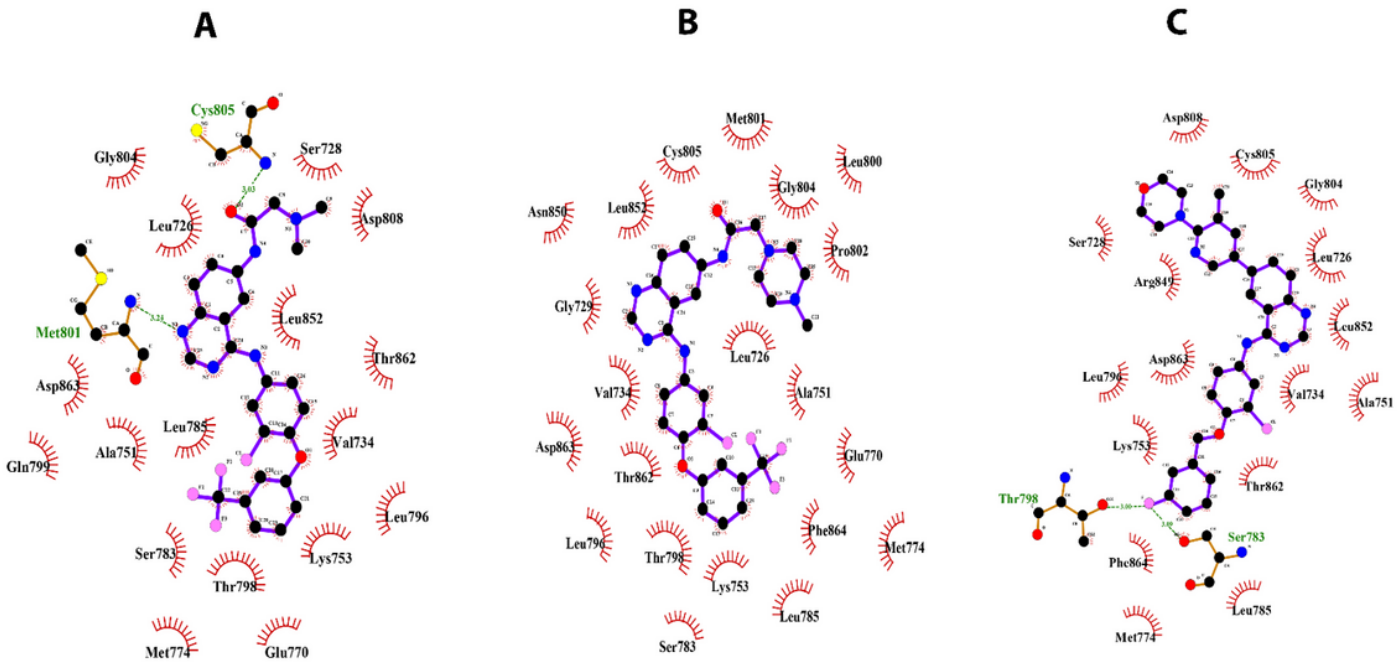


Figure 8

2D depiction of compound 162667484 (A), 162643878 (B), and 162643878 (C) as docked to HER2 with $\Delta G_{binding}$ -12.9, -12.5, and -12.2 kcal.mol^{-1} as respectively.

Supplementary Files

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- Table.docx