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In Silico Interaction Study of the Hydrazine Compound as an Antibody Drug Conjugate Linker Against Pertuzumab

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Abstract

Breast cancer can be initiated, one way or another, by overexpression of the HER-2 protein which can induce dimerization, resulting in metastasis in breast cancer cells. Pertuzumab is one of the methods used to treat metastatic breast cancer cells which has spread to other parts of the body. Hydrazine is a kind of hydro-nitrogen compound with the formula N2H4. The hydrazine compound acts as a strong reducing agent through the hydrogenation reaction. The aim of research is to study the characteristics of hydrazine as a linker with pertuzumab as an antibody to develop an antibody-drug-conjugate in silico with molecular docking. The docking method is used to know the conformation and free energy ties involved in the interaction between molecules (pertuzumab) and the ligands (hydrazine). In silico molecular docking was carried out with some number of stages such as ligand preparation, preparation of macromolecules, validation molecular docking method, docking hydrazine with pertuzumab protein and intermediate data analysis optimized hydrazine compound with which pertuzumab protein the lower mark energy bond so the stronger and stable the bond that occurs between hydrazine compound and pertuzumab protein. The result of docking is in the form of binding affinity from the results of adding pertuzumab protein with the hydrazine ligand which is -6.8 which shows conformation formed stable. Visualization results from complex molecule hydrazine compound against pertuzumab protein own identical confirmation on the part side active binding from pertuzumab protein macromolecules. The hydrazine compound can be linked to pertuzumab and shows a potential new drug development in the targeting of antibody-drug conjugate breast cancer.

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Introduction

Antibody-drug conjugate (ADC) is complex target agents composed of drugs scaffold dependent cytotoxicity antibodies. After bonding with surface antigens cells targeted by antibodies specifically, ADC is internalized by tumor cells and processed by the endo lysosomal system (Criscitiello et al, 2021).

Pertuzumab is a monoclonal antibody with HER2 as the target. Pertuzumab can interact with subdomain II of the HER2 extracellular domain playing an important role in the heterodimerization process. Pertuzumab binding with the HER2 receptor can cause inhibition of the heterodimerization process induced by heregulin (HRG), in addition HER2 receptor inhibitory process heterodimerization also occurs in other HER families including HER3 and HER4 including EGFR so that the growth process of tumor cells will be inhibited. In preclinical trials, pertuzumab this can hinder the growth of cancer cells in conditions when HER2 is not expressed in a way excessive matter this is caused because there is inhibition of heterodimerization receptors. Pertuzumab in a way synergistic combined with trastuzumab which can be done in vitro inhibit the growth of HERZ-overexpressing cells (Wong & Hurvitz, 2014). Pertuzumab in a synergistic way together with trastuzumab inhibits growth of breast cancer cells, BT474, causes reduced proliferation and increased apoptosis in cells. Effect of growth inhibition this is especially caused by a slowdown in the cycle cells (Pondé et al, 2016).

Component the main ADC consists of antibody, cytotoxic payload, and bridging chemistry. The ideal ADC drug remains stable in circulating blood, achieving therapeutic targets in a way accurate, and ultimately releasing the load of cytotoxicity around the target (eg cell cancer). Every element can influence the efficacy and safety end of ADC, and so general ADC development is necessary consider all components main this, including selection of target antigen, antibody, cytotoxic payload, bridging, as well as method conjugation (Fu et al, 2022).

Linkers are compound linking biochemistry antibodies with the load (criscitiello et al, 2021). The linker in the ADC bridges antibodies with cytotoxic drugs. This matter is one of the key factors associated with ADC stability and charge release profile, and because it is important for the therapeutic end of ADC. Hydrazine is sensitive link to acid (sensitive to pH). General hydrazine related ADC stable in circulating blood but hydrolyzed for release of cytotoxic load in lysosomes (pH 4.8) and endosomes (pH 5.5 – 6.2) after internalization into targeted cancer cells. However, hydrolysis of hydrazine bonds is not completely limited to lysosomes, and sometimes hydrolysis also occurs in plasma, so reduce targeting efficiency and off target effects. So far, hydrazine linker containing ADC is mainly used in violent hematology (Fu et al, 2022).

Hydrazine is a hydro nitrogen compound that has the formula N_2H_4 molecules and can be found in solution form. Hydrazine is a strong reductor and widely used in field water treatment, in particular in boiler feed water systems (Boiler Feed Water) as an oxygen binder. The hydrazine compound has chemical characteristics as a strong reducing agent because hydrazine plays a role as donor group hydrogen can reduce bond double to become bond single through reaction hydrogenation. Hydrazine has superiority to others, considering its own low level of corrosion (Vachlepi, 2018).

The main problem in ADC development for cancer is adequate identification and validation of antigenic targets for component mAb. A number of factors need attention in antigen selection. First, to reduce target and resulting toxicity index therapeutic that can be accepted for ADC, the ideal target antigen owns a high level of expression in tumors and few or no there is expression in normal tissue, or at least expression limited to certain tissue types. Second, the target antigen must be on the surface cells to be able to be accessed by circulating mAb. Third, it must become an internalized antigen so, after bonding, the ADC is transported to in cells, where cytotoxic agents can give the effect. However, it has been reported that the ADC does internalized can show significant toxicity in a number of cases and that the ADC often causes strong observer effects (Beck et al, 2017).

The docking method is a method used to predict orientation between one molecule and another molecule when electrostatic interaction occurs between each other to form a stable bond. The principle of docking is a technique for placing ligands inside active continued receptors with molecule evaluation based on conformation structure and electrostatic properties. Docking simulation can be done to obtain more understanding good to mechanism work something compound chemistry or macromolecules such as protein or peptide, on a molecular scale so that it is possible for designing drug based structures (Syahutra et al, 2014).

Component main ADC used for developing important ADC in choosing cancer for bridge conjugation between antibodies and cytotoxic drugs. Study this aims to know does hydrazine have potential to hinder HER-2 receptor, in matter we take advantage of this molecular docking method to predict what is this hydrazine can be bonded with pertuzumab or no pertuzumab chosen because is antibody interacting monoclonals with subdomain II of the HER2 extracellular domain, which plays an important role in the heterodimerization process. pertuzumab can inhibit the heterodimerization process induced by heregulin (HRG) at HER2, HER3, HER4, and EGFR receptors, thereby hindering the growth of tumor cells. In addition, pertuzumab has also been proven to synergistically hinder the growth of breast cancer cells when

combined with trastuzumab. Apart from that, other research also shows that pertuzumab has potency to hinder excessive expression of the HER-2 protein and can be bound with HER-2, so it has potency as anti-breast cancer.

We chose the Hydrazine ligand because it is a common hydrazine related ADC stable in circulating blood but hydrolyzed for release of cytotoxic load in lysosomes and endosomes, besides that sometimes hydrolysis also occurs in plasma, so reduce targeting efficiency and off target effects. Then peruzumab this is used to treat metastatic breast cancer that is cancer cell breast the cancer has spread to other parts of the body.

Method

Material

3 dimensional structure The hydrazine compound is downloaded on the website https://pubchem.ncbi.nlm.nih.gov/. The structure of the HER-2 target protein pertuzumab (PDB ID: 3PP0) was downloaded on the PDB (protein data bank) website, namely http://www.rcsb.org/pdb/home/home.do.

Tools

A set of Lenovo laptops with Windows 10 specifications are equipped with the Pymol program and the Pyrex program.

Procedure

Procedure initial steps before carrying out the mooring process molecule that is setup structure macromolecules the receptors and ligands that will be used. Downloading macromolecules with use Pubchem and GDP. Then Her-2 pertuzumab (PDB ID: 4 LLU) was performed via GDP. The structure obtained this separated from remaining nonstandard residue there is in macromolecules for obtain structure molecule pure ready to stage furthermore with use device soft Discovery Studio Visualizer 2020. Then the ligands are prepared formerly with click open babel, select plus sign insect new item then select the ligand file in the form of a pdf file and select minimize selected, the energy already minimized to make it easier in the docking process. Then convert selected to autodock ligand. Ligand has been prepared with Open Babel which is a soft device for changing several file formats chemistry. Device This works as search conformers and 2D drawing, batch conversion and substructure and similarity search. How to drag the receptor and out ligand 1 in Discovery Studio visualization then look at the results in 2D and 3D shapes.

Research methods

Ligand Preparation

The ligand was downloaded via the Pubchem website with the compound name hydrazine and with the code 9321 optimized using the pymol program and the pyrex program.

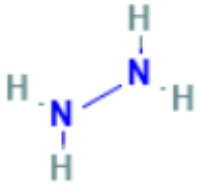


Figure 1. 2D ligand structure of hydazine with code 9321

Preparation of Macromolecules

Preparation Macromolecules with download on the Protein Data Bank (PDB) with protein code 4llu, then the protein is purified use application PyMol for separate native ligands on proteins.

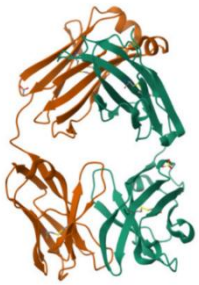


Figure 2. Macromolecular structure of Pertuzumab with code 4LLU

Validation of Molecular Docking Methods

Validation molecular docking method was carried out using pyrex docking results are saved with PDB format later done analysis beginning bond Ligand-Protein complex uses application PyMol and save complex with PDB format for analyzed bond between more protein ligands continue in the Discovery Studio (DS) application. Validation parameters method is Root Mean Square Deviation (RMSD). RMSD that can be obtained accepted is ≤ 3.0 Å.

Docking Hydrazine with Pertuzumab Protein

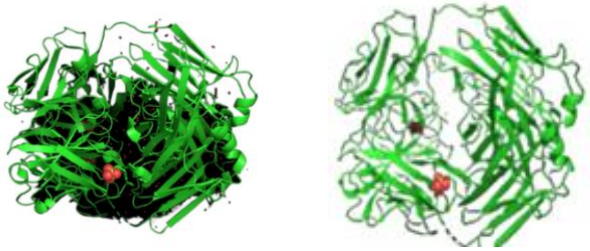
Hydrazine compound that has been optimized docking to the existing HER-2 protein the native ligand is removed using the application pymol with the same docking procedure like moment validation method. The analysis results show the bond conformation of compounds in proteins with bond energy values and hydrogen bonds.

Data analysis

The results of molecular docking are energy bonds and hydrogen ties formed. Bond energy is used to indicate the strength of the bond between a compound and a protein. The lower the bond energy value, the stronger and more stable the bond formed. The type of hydrogen bond formed is used to analyze the interaction mechanism formed.

Results and Discussion

Before The molecular docking process is carried out over previously conformation (forms molecules in space three dimensions due to rotation on the shaft bond single) then the molecules are cleaned and put together with ligands that have been optimized using the Pyrex program, then the molecules and ligands are put together to see the bonds using the Discovery Studio program.



(a) (b)

Figure 3. Preparation process macromolecules for clean protein with its native ligand use application PyMol on: (a) Pertuzumab protein before cleaning, (b) Pertuzumbab protein after cleaning

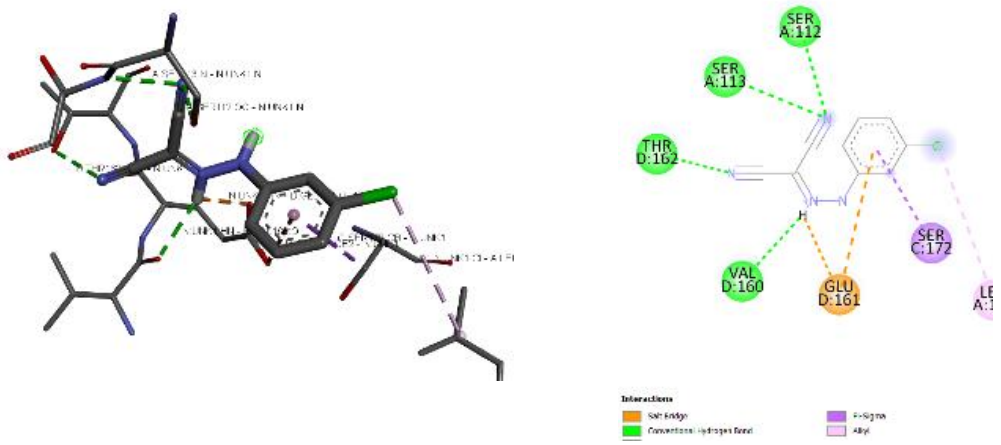
Based on the results of the protein ligands that we prepared before and after cleaning, these protein macromolecules were prepared first by removing water molecules and natural ligands. The aim of preparing protein macromolecules is to ensure that stable molecular interactions are formed at the active site of the macromolecules at the docking simulation stage.

In Table 1. Represents results of addition of Pertuzumab Protein with neutral ligand of the Hydrazine ligand.

Table 1. Binding Affinity on hydrazyne

Ligand	Binding affinity	Mode	RMSD lower bound	RMSD upper bound
Hydrazyne-3_clean_445154_uff_E=172.22	-6.8	0	0.0	0.0
Hydrazyne-3_clean_445154_uff_E=172.22	-6.8	1	50,463	51,519
Hydrazyne -3_clean_445154_uff_E=172.22	-6.8	2	0.14	2,018
Hydrazyne -3_clean_445154_uff_E=172.22	-6.7	3	50,463	51,486
Hydrazyne -3_clean_445154_uff_E=172.22	-6.7	4	1,744	7,045

Table 1 shows that small binding affinity value namely -6.8 (increasingly negative) indicates that conformation formed stable or does not need great energy to do binding or interaction. While large binding affinity value shows not enough it's stable complex formed. For binding ligands and receptors with low binding affinity values, they have the potential to bind with high targets or receptors (Pujiastuti, 2017).



(a) (b)

Figure 4. 3D and 2D display results of ligand-protein fusion (a) 3D view of ligand-protein, (b) 2D view of ligand-protein

Observation further carried on to visualization of complex molecule hydrazine compounds against pertuzumab protein macromolecules. As shown by Figure 4. can be observed that second molecule of the test compound has identical conformations in parts side active binding from pertuzumab protein macromolecules. Then, if identified based on interactions molecularly formed, the hydrazine compound has interactions that are formed between hydrazine compound with pertuzumab protein consisting of 7 interactions that include 4 hydrogen bonds (with SER112, SER113, THR162, and VAL160), 2 hydrophobic interactions (with SER172 and LEU170), and 1 electrostatic interaction (with GLU161).

From the 2D view of the docking ligand that we observed, we can see GLU code, where For Glu That glutamine later If it reacts with HCl there will produce a chloride salt, in bond on That marked it with color orange, the description is salt bridge, which is an electrostatic bond. Because if amino acids react with HCl and NaOH the result is salt. Then there is a bond between N group with compiler The amino acid code is SER or serine, VAL or valine, and THR or threonine Where in the reaction between the ligand and the protein the bond forms hydrogen proves it's a strong bond. Then, as for building blocks of protein, amino acids with code SER or serine and LEU or Leucina are known bonded with branch benzene Cl and the protein forms bond hydrophobic, bonding marked hydrophobic the same bond colored purple and pink say pi-sigma and alkyl. In understanding hydrophobic alone is characteristic physique of something molecule (so-called hydrophobic molecule) which seems to be rejected from water mass. (Actually no there is strength

repulsion involved, this caused because no exists power pull). And in the 3D results of the union between ligand and protein, hydrogen bonds, hydrophobic bonds and electrostatic bonds are obtained.

From the explanation above, yes hydrogen and hydrophobic bonds can infer bonds between proteins and ligands important and indicative strong bonds between proteins and ligands, their importance knows predicted bond this for drug development applications, especially Antibodies Drug Conjugate binding between linker and antibody. So docking is great important and necessary exists evidence in vitro and in vivo for continuing in silico study. In 3D unification results between the ligand and the tagged protein with the existence of a non-bond with hydrogen and hydrophobic bond information.

Conclusion

Based on results have been obtained between hydrazine ligands and macro molecule petruzumab get a number of bonds among them hydrogen bond and hydrophobic bonds where marked by ties with SER and alkyl codes, which indicates that antibody drug conjugates can be made to use Hydrazine as a linker for paired with pertuzumab, and necessary exists continued study in vitro and in vivo.

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