Pharmacy Reports

https://doi.org/10.51511/pr.19

RESEARCH ARTICLE

Open Access

The potency of alpha-humulene as HER-2 inhibitor by molecular docking

I Made Harimbawa Putra, I Putu Ari Anggara Catur Pratama, Komang Dian Aditya Putra, G. A. Desya Pradnyaswari, Ni Putu Linda Laksmiani*

Department of Pharmacy, Faculty of Mathematics and Natural Science, Udayana University, Bukit Jimbaran, Badung, Bali 80361, Indonesia

*Corresponding author: Bukit Jimbaran Campus, Udayana University, Badung, Bali 80361, Indonesia. Email: laksmini@unud.ac.id

Abstract: HER-2 overexpression is present in approximately 20% of breast cancer. This research aims to study the interactions of α-humulene to HER-2 protein by using *in silico* molecular docking. The experiment was carried out by HER-2 protein preparation (PDB ID 3PP0), docking validation, α-humulene optimization, and α-humulene docking. The results showed that α-humulene had binding energy of -7.50 kcal/mol, Van der Waals binding energy of -7.48 kcal/mol, and electrostatic energy of -0.02 kcal/mol. α-Humulene is potential as anti-breast cancer towards HER-2 *in silico*.

Keywords: alpha-humulene, anti-breast cancer, HER-2, in silico, molecular docking

Introduction

Breast cancer is a malignant tumor in the mammary gland tissue originating from the ductal epithelium or its lobules [1]. About 20% of breast cancer cases are caused by overexpression of HER-2 [2]. The human epidermal growth factor (HER-2) plays a role in proliferation, migration, cell survival and growth [3]. HER-2 overexpression is associated with more aggressive disease, a higher recurrence rate, and shortened survival. In addition, HER-2+ breast cancer has a higher preference for metastasis to the brain [4].

Breast cancer is generally treated by chemotherapy, surgery, radiotherapy, or combination. However, this therapy has several side effects, such as hair loss and drug resistance [5]. Hair loss after chemotherapy is caused by an unspecific drug target [6]. Conventional chemotherapy kills both cancer cells and normal cells. Therefore, it is urgent to develop anticancer drugs that kill cancer cells specifically. One of the drug sources is natural ingredients.

α-Humulene is a monocyclic sesquiterpene that is commonly found in the *Zingiberaceae* family (Figure 1) [7]. Extracts from *Zingiberaceae* were tested on MCF-7 breast cancer and HT-29 colon cancer cell lines and produced the small IC_{50} value from the 11 plants of the *Zingiberaceae* group [8]. However, no study was found to elucidate α-humulene as anti-breast cancer *in silico*. Molecular docking is a computational simulation to predict the binding between a ligand and a protein, in which ligand is docked to the active site of the receptor [9]. This research aims to study α-humulene activity as anti-breast cancer by inhibiting HER-2 *in silico*.

Methods

Protein preparation

The HER-2 protein (PDB ID: 3PP0) was downloaded from http://www.rcsb.org/. This target protein was prepared using Chimera 1.11.1 by separating the protein sequence from 03Q native ligand.

Validation of docking

The docking method was validated using the Autodock Tools application (Autodock 4.2 and Autogrid) by redocking (2-{2-[4-({5-chloro-6-[3-(trifluoromethyl) phenoxy]pyridine-3-yl}amino)-5H-pyrrolo[3,2d] pyrimidin-5-yl]ethoxy}ethanol) (03Q) native ligand to the prepared HER-2 protein. The grid box was arranged by adjusting the coordinate size of the grid center (X = 16.387 Å, Y = 17.394 Å, Z = 26.218 Å) and grid size (X = 40 Å, Y = 40 Å, Z = 40 Å). The validation parameter of docking protocol was the value of root mean square deviation (RMSD), which is valid if the value of RMSD is less than ≤ 2.0 Å.

α-Humulene structure optimization

The downloaded 3D structure of α-humulene was optimized using HyperChem 8. The optimization

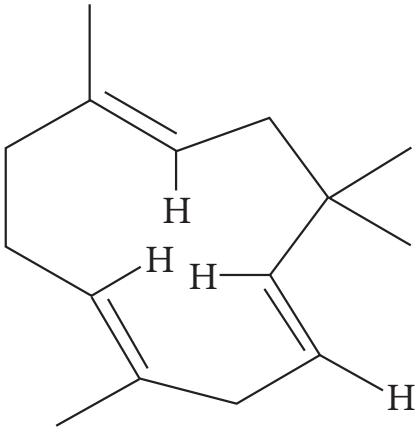

Figure 1. Two dimensional (2D) of α-humulene structure

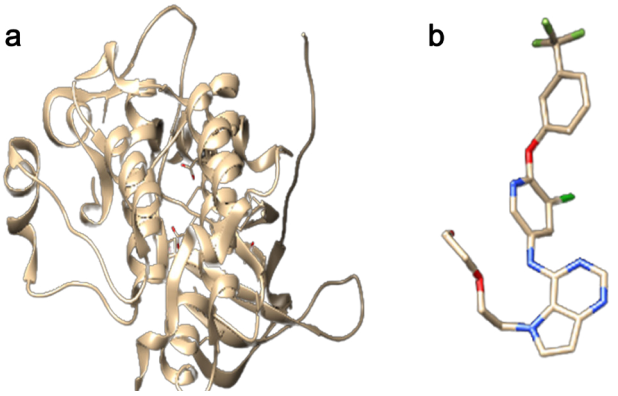

Figure 2. The results of protein preparation. (A) Structure of prepared HER-2 protein target (B) 03Q native ligand

Table 1. Validation parameters of HER-2 target protein and 03Q native ligand

Protein target	Ligand	Conformations	RMSD (Å)	Energy Vdw_Hb_ desolv (kcal/mol)	Energy elec (kcal/mol)	Binding energy (kcal/ mol)
HER-2	03Q native ligand	1*	0.62	-13.57	-0.01	-10.60
		2	0.71	-12.74	-0.02	-10.50
		3	0.72	-13.13	-0.09	-10.49
		4	1.18	-13.22	-0.04	-10.28
		5	0.69	-13.43	-0.04	-10.24
		6	2.59	-11.92	-0.03	-9.78
		7	2.76	-10.92	+0.03	-9.74
		8	3.53	-13.49	+0.01	-8.97
		9	2.61	-11.25	+0.02	-8.24
		10	2.51	-12.71	-0.01	-7.90

step was carried out using the AM1 (Austin Model 1) semi-empirical computational method and single-point calculations and geometry optimization.

Docking α-humulene to the HER-2 protein target

The optimized α-humulene was then docked to the prepared HER-2 target protein using the Autodock 4.2 program. The results gave α-humulene conformation with the lowest binding energy to the target protein. The interaction analysis showed the types of bindings such as hydrogen bonds, Van der Waals, hydrophobic, and electrostatics.

Results

Preparation of HER-2 protein target

The preparation of HER-2 target protein provided and the protein and 03Q native ligand (Figure 2).

Docking validation

The validation parameter of the docking protocol was the RMSD value. The obtained RMSD value was 0.62Å which is valid (< 2.0 Å). This validation also produced the binding energy between HER-2 target protein and 03Q native ligand. The binding energy of the selected conformation with the lowest RMSD value was -13.57 kcal/mol for Van der Waals, -0.01 kcal/mol for electrostatic, and -10.60 kcal/mol for free binding energy (Table 1). The visualization indicated the hydrogen bonding occurs between 03Q native ligand with MET 801 residue (Figure 3).

Optimization of α-humulene

The 3-dimensional structure (3D) of α-humulene was optimized using single-point calculations and geometry optimizations. The single point energy calculation of

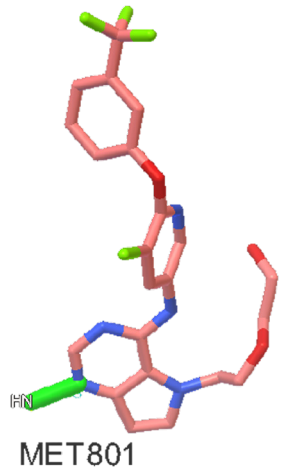

Figure 3. Visualization of 03Q native ligand with HER-2

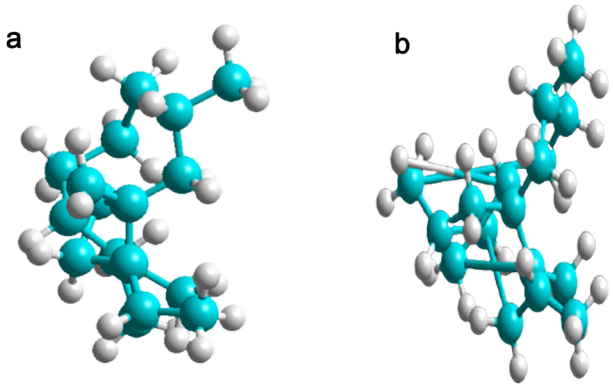

Figure 4. 3D structure α-humulene. (A) single point calculation result, and (B) geometry optimization result

Table 2. α-Humulene docking result on HER-2 protein

Protein target	Ligand	Conformations	Energy Vdw_Hb_ desolv (kcal/mol)	Energy elec (kcal/mol)	Binding energy (kcal/mol)
HER-2	α-humulene	1*	-7.48	-0.02	-7.50
		2	-6.39	-0.00	-6.39
		3	-6.39	+0.01	-6.38
		4	-7.31	-0.02	-7.33
		5	-6.39	+0.01	-6.38
		6	-6.39	+0.01	-6.38
		7	-6.39	-0.00	-6.39
		8	-6.39	+0.01	-6.38
		9	-6.39	-0.00	-6.39
		10	-6.39	-0.00	-6.39

α-humulene was -3800.11 kcal/mol, and the energy of geometric optimization was -4497.19 kcal/mol. The 3D structures of single-point and geometry optimization of α-humulene are displayed in Figure 4.

Docking α-humulene to HER-2 target protein

The docking process yielded ten bond conformations between α-humulene and HER-2 protein. The most stable conformation of α-humulene with the lowest binding energy had the Van der Waals and hydrophobic energy of -7.48 kcal/mol, electrostatic energy of -0.02 kcal/mol, and binding energy of -7.50 kcal/mol (Table 2). Visualization analysis showed that α-humulene did not form a hydrogen bonding with HER-2 protein target through MET 801 residue as 03Q native ligand.

Discussion

The docking protocol was valid, as shown by the RMSD of 0.62 Å (≤ 2.0 Å). The docking results showed that the binding energy of α-humulene to HER-2 protein was -7.50 kcal/mol, higher than 03Q native ligand with -10.60 kcal/mol. This finding implies that α-humulene is potential for HER-2 protein as indicated by the negative value of binding energy.

α-Humulene is one of the sesquiterpenes known for its anti-cancer activity. *In silico* study of eugenol sesquiterpenes against HER-2 protein obtained the binding energy value of -4.16 kcal/mol, higher than gefitinib with -7.05 kcal/mol as a control [10]. *In vitro* study indicated that α-humulene had a cytotoxic activity with an IC_{50} of 81.9 µg/mL against the MCF-7 breast cancer cell line [11].

Conclusion

Based on this study, α-humulene has the potential as an anti-breast cancer agent by *in silico* through the inhibition of the HER-2 protein.

Acknowledgment

None.

Funding

The authors thank to Direktorat Jenderal Pembelajaran dan Kemahasiswaan Kementerian Riset, Teknologi, dan Pendidikan Tinggi Republik Indonesia funded for this research in the Program Kreativitas Mahasiswa 2021 (grant number 015/E2/PPK/SPPK/PKM/2021).

Declaration of interest

The authors declare no competing interests.

Author contributions

IMHP conceptualized the study design; IMHP and IPAACP investigated the data; KDAP and GADP wrote the original draft; IMHP, KDAP and GADP reviewed and edited the final version; IMHP acquitted the funding; NPLL supervised all experiments. All authors read and approved the final manuscript.

Received: 25 September 2021
Accepted: 10 December 2021
Published online: 12 January 2022

References

1. Waks AG, Winer EP. Breast cancer treatment: A review. JAMA. 2019;321: 288-300. https://doi.org/10.1001/jama.2018.19323
2. Seol H, Lee HJ, Choi Y, Lee HE, Kim YJ, Kim JH, et al. Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance. Mod Pathol. 2012;25: 938-948. https://doi.org/10.1038/modpathol.2012.36
3. Franklin MC, Carey KD, Vajdos FF, Leahy DJ, de Vos AM, Sliwkowski MX. Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. Cancer Cell. 2004;5: 317-328. https://doi.org/10.1016/S1535-6108(04)00083-2
4. Mitri Z, Constantine T, O'Regan R. The HER2 receptor in breast cancer: pathophysiology, clinical use, and new advances in therapy. Chemother Res Pract. 2012;2012: 743193. https://doi.org/10.1155/2012/743193
5. Ogata R, Kishino E, Saitoh W, Koike Y, Kurebayashi J. Resistance to cyclin-dependent kinase (CDK) 4/6 inhibitors confers cross-resistance to other CDK inhibitors but not to chemotherapeutic agents in breast cancer cells. Breast Cancer. 2021;28: 206-215. https://doi.org/10.1007/s12282-020-01150-8
6. Chon SY, Champion RW, Geddes ER, Rashid RM. Chemotherapy-induced alopecia. J Am Acad Dermatol. 2012;67: e37-47. https://doi.org/10.1016/j.jaad.2011.02.026
7. Sharifi-Rad M, Varoni EM, Salehi B, Sharifi-Rad J, Matthews KR, Ayatollahi SA, et al. Plants of the genus zingiber as a source of bioactive phytochemicals: from tradition to pharmacy. Molecules. 2017;22. https://doi.org/10.3390/molecules22122145
8. Kirana C, Record IR, McIntosh GH, Jones GP. Screening for Antitumor Activity of 11 Species of Indonesian Zingiberaceae Using Human MCF-7 and HT-29 Cancer Cells. Pharm Biol. 2003;41: 271-276. https://doi.org/10.1076/phbi.41.4.271.15673
9. Meng X-Y, Zhang H-X, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des. 2011;7: 146-157. https://doi.org/10.2174/157340911795677602
10. Rohmah MK. Studi in silico kompleks ligand-reseptoreugenol daun basil (Ocimum basilicum L.) dengan reseptor HER-2 pada non-smallcell lung cancer (NSCLC) dengan kontrol gefitinib. JINTO. 2017;3: 71-78. https://doi.org/10.36733/medicamento.v3i2.894
11. Gautam N, Mantha AK, Mittal S. Essential oils and their constituents as anticancer agents: a mechanistic view. Biomed Res Int. 2014;2014: 154106. https://doi.org/10.1155/2014/154106