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Molecular Docking Simulation of Cinchona Alkaloids Derivatives to Search New Active Anti-cancer Agent

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Abstract

One of the triggers of breast cancer is the estrogen hormone. Estrogen α-receptors play a role in breast development and growth. Estrogen receptors as molecular targets have been widely reported. Cinchona alkaloids have been known to function as anti-malarial, but recent studies have shown that cinchona alkaloids have other potential such as anti-cancer, anti-tumor, anti-microbial, anti-HBV, anti-inflammatory, anti-oxidant, anti-obesity. Cinchona alkaloids have been developed as anti-cancer agent. They have comparable reactivity and selectivity of cinchona alkaloids functional to anti-cancer agent. In this study, molecular modeling has been performed on forty-four cinchona alkaloid derivatives. These cinchona alkaloids derived compounds have been evaluated as anti-cancer agent. Herein, we observed molecular interactions between selectivity of cinchona alkaloids with estrogen α receptors (ER-α). The docking simulation showed 3 cinchona alkaloids derivatives (cinchonine 2-chlorobenzoate, cinchonidine benzoate and cinchonine 2-(4-hydroxyphenyl) acetate) have lower Gibbs free energy and and low kinetic inhibition compared to tamoxifen (as standard commercial) and there are 14 compounds that have relatively the same activity as tamoxifen from 44 alkaloid chincona derivatives.

Keywords: anti-cancer, cinchona alkaloids derivatives, cinchonidine, cinchonine, molecular docking, quinidine, quinine.

Simulasi Penambatan Molekul Senyawa Turunan Alkaloid Sinkona untuk Mencari Agen Aktif Anti-Kanker Baru

Abstrak

Salah satu pemicu kanker payudara adalah hormon estrogen. Reseptor estrogen α berperan dalam perkembangan dan pertumbuhan payudara. Reseptor estrogen sebagai target uji telah banyak dilaporkan. Alkaloid sinkona telah diketahui berfungsi sebagai anti-malaria, tetapi penelitian terbaru menunjukkan bahwa alkaloid sinkona memiliki potensi lain seperti anti-kanker, anti-tumor, anti-mikroba, anti-HBV, anti-inflamasi, anti-oksidan, anti-kegemukan. Alkaloid sinkona telah dikembangkan sebagai agen anti kanker. Mereka memiliki reaktivitas dan selektivitas alkaloid sinkona yang sebanding dengan agen anti-kanker. Dalam penelitian ini, pemodelan molekuler telah dilakukan pada empat puluh empat senyawa turunan alkaloid sinkona yang telah dievaluasi sebagai agen anti kanker. Kami mengamati interaksi molekuler antara selektivitas alkaloid sinkona dengan reseptor estrogen α (ER- α). Hasil simulasi *docking* menunjukkan ada 3 senyawa turunan alkaloid sinkona (sinkonin 2-klorobenzoat, sinkonidin benzoate dan sinkonin 2-(4-hidroksifenil) asetat) yang mempunyai energi bebas dan kinetika inhibisi yang lebih rendah dibandingkan dengan tamoksifen (sebagai standar komersial) dan ada 14 senyawa yang aktivitasnya relatif sama dengan tamoksifen dari 44 senyawa turunan alkaloid sinkona.

Kata Kunci: anti-kanker, derivat alkaloid sinkona, sinkonidin, sinkonin, penambatan molekul, kuinidin, kuinin.

1. Introduction

Cancer is one of the leading causes of death because until now no effective treatment method has been found, especially for people with advanced cancer. Cancer occurs because of neoplasia, which is abnormal proliferation of cells in a tissue or organ, which causes neoplasms.[1] Cancer-causing neoplasms include teratoma, also include various types of cancer, including breast cancer. Of the various types of cancer, one of the most common cancers experienced by women is breast cancer. This cancer originates from breast supporting tissue.[2] It is estimated that in 2016 breast cancer reaches 29% of all cancer cases and around 14% causes death.[3] One risk factor for breast cancer is estrogen. Estrogen and estrogen receptors have an important role in the formation and development of breast cancer. Expression of estrogen α receptors (ER-α) which is larger than normal is one sign of the development of breast cancer cells. Estrogen performs its function by binding to estrogen receptors. This estrogen hormone often triggers the growth of cancer cells in the breast.[4] One example of cancer estrogen is the MCF-7 cancer cell, this cancer cell is one of the breast cancer cell models that is widely used in research.[5] Research of expression and function of ER-α especially from cancer specifically is very important to improve new strategies of prevention, diagnosis, and cancer therapy caused by estrogen. Drug design is starting with the determination of compounds that show important biological properties and ends with optimization steps, both the activity profile and the synthesis of chemical compounds. With advances in computational chemistry, researchers can use computers to optimize activity, geometry and reactivity before compounds are synthesized experimentally. This can avoid the synthesis of a compound that requires time and expensive costs, but the new compound does not have the activity as expected.[6]

Cinchona alkaloids are natural sources substances. They are natural products which can be isolated from cinchona tree bark. It can also be found in Andes mountain range, India, Java, Cameroon, and Vietnam and in some other Asian and African countries. Indonesia becomes the largest producer of cinchona throughout the world. The most abundant of these alkaloids is quinine, quinidine, cinchonine and cinchonidine, can comprise up to 16% by mass of the tree bark. The role of cinchona alkaloids in organic chemistry was firmly established with the discovery of their potential as resolving agents by Pasteur in 1853, which ushered in an era of racemate resolutions by the crystallization of diastereomeric salts.[7] Today, there are countless examples in which cinchona alkaloids are used as chiral resolving agents. Besides the classical resolution process, significant progress has also been made in the past two decades in the field of cinchona-based enantio separation of chiral, as well as in their use as enantioselective analytical tools. Literature study revealed that along with the antimalarial activity the cinchona alkaloids has other potentiality like anti-obesity, anti-cancer, anti-oxidant, anti-inflammatory, anti-microbial activity.[8-11] And also have been reported on cinchona alkaloids and modifications of compounds to obtain new biological activities.[12-16]

In this research study we would like to develop new potential activity of cinchona alkaloids derived compounds. Herein, we

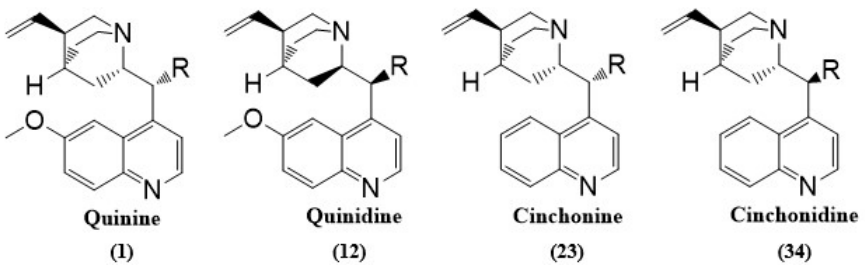

Figure 1. Structure of cinchona alkaloids : quinine(1), quinidine(12), cinchonine(23), cinchonidine(34)

designed and screened forty-four cinchona alkaloids derived compounds using molecular docking simulations and ligand-based virtual screening. Molecular docking is an important tool for designing and predicting of the new drug design discovery compounds. In this method predicted the active compound through experimental binding modes and affinity of a small molecule within the binding sites of the target receptor of interest.[17,18] We chose the ER-α protein as the target for virtual screening. The basis for crystallographic selection is 3ERT because 3ERT is the human crystallographic structure of the estrogen-alpha hormone and its resolution is good (the higher the resolution the better), and also external validation of the 3ERT crystallographic structure has been done.[19-25] It will be shown that docking simulation with the binding free energy function could be a valuable tool in screening assays. The docking simulation was used AutoDock 4.2 Program. The aim of the development and research of ER-α antagonists is needed because they are determined by current drug availability, disease burden and epidemiological trends (including the development of refractories/ disease resistance to available treatments), available drug properties in terms of efficacy, safety, cost and opportunity which is available through research and development activities in the academic and pharmaceutical industries, especially in the utilization of Indonesia's natural resources which have great potential for drug discovery, herein we utilize the potential of chincona alkaloids.

2. Methods

2.1. Instruments

The experiments were performed on a computer withIntel Core i5 -4460 processor (3.20 GHz) and the windows 7 Ultimate as the operating system. Software used in this work included the application molecular docking using Autodock 4.2.

2.2. Materials

The 2D and 3D structures of cinchona alkaloids compounds were drawn by Marvin Sketch and ChemDraw 12.0. The receptors used are alpha estrogen receptors taken from PDB (Protein Data Bank) with PDB ID : 3ERT.

2.3. Procedure

The receptors used are alpha estrogen receptors taken from PDB (Protein Data Bank) with PDB ID : 3ERT, and external validation has been carried out.Macromolecules were optimized by using software Autodock 4.2.3D structure of macromolecules added hydrogen atoms was then repaired of the charge by adding the partial charge Gasteiger and given force field using Autodock. Macromolecule structure and ligand to be docked firstly were optimized then after that each macromolecules and ligand were made their PDBQT file, also grid file parameter file (GPF). The calculated grid maps were dimension 40x40x40 centralized with the spacing of 0.375 Å with x, y, z (30.282, -1.913, 24.207). GPF would inform Autogrid which potential receptors needed to be calculated and the type of map types that must be counted and their location. While the DPF would inform Autodock which folder to be used, which ligand to be moved, including the center and the torsion of the ligand, what docking algorithm to be used, and the number of docking should be done. The results of molecular docking were visualized by using Autodock and Chimera. Docking outcome parameters were analyzed. Receptor-ligand binding energy obtained from the docking was sorted by their lowest energy. And through Chimera, it was observed that amino acid residue is the closest to the ligand.[26]

3. Results

Molecular modeling studies of the asymmetriccinchonaalkaloidswereconducted to determine the specific interaction between the cinchona alkaloids derivatives with ER-α protein as macromolecule targets. Herein, molecular modeling has been carried out on forty-four cinchona alkaloids derivatives. Modifications of quinine, quinidine, chinconine, chinconidine were carried out in 10 modifications. We performed the design of cinchona alkaloids derivatives compounds

Table 1. Design of cinchona alkaloids derivatives with R-substitution

No	Compound	MW	R-Substitution
1	Quinine	324.18	-OH
2	Quinine 3,3-dimethylbutanoate	422.26	$-OOCCH_2C(CH_3)_3$
3	Quinine 2-chlorobenzoate	462.17	$-OOCC_6H_4Cl$
4	Quinine 4-hydroxybenzoate	444.20	$-OOCC_6H_4OH$
5	Quinine 2-(4-hydroxyphenyl)acetate	458.22	$-OOCCH_2C_6H_4OH$
6	Quinine 2-hydroxy-3-methoxybenzoate	474.22	$-OOC\ C_6H_3OHOCH_3$
7	Quinine 2-nitrobenzoate	473.20	$-OOCC_6H_2(OH)_3$
8	Quinine nonanoate	464.30	$-OOC(CH_2)_7CH_3$
9	Quinine acetate	366.19	$-OOCCH_3$
10	Quinine benzoate	418.23	$-OOCC_6H_5$
11	Quinine propionate	380.21	$-OOCCH_2CH_3$
12	Quinidine	324.18	-OH
13	Quinidine 3,3-dimethylbutanoate	422.26	$-OOCCH_2C(CH_3)_3$
14	Quinidine 2-chlorobenzoate	462.17	$-OOCC_6H_4Cl$
15	Quinidine 4-hydroxybenzoate	444.20	$-OOCC_6H_4OH$
16	Quinidine 2-(4-hydroxyphenyl)acetate	458.22	$-OOCCH_2C_6H_4OH$
17	Quinidine 2-hydroxy-3-methoxybenzoate	474.22	$-OOC\ C_6H_3OHOCH_3$
18	Quinidine 2-nitrobenzoate	473.20	$-OOCC_6H_2(OH)_3$
19	Quinidine nonanoate	464.30	$-OOC(CH_2)_7CH_3$
20	Quinidine acetate	366.19	$-OOCCH_3$
21	Quinidine benzoate	418.23	$-OOCC_6H_5$
22	Quinidine propionate	380.21	$-OOCCH_2CH_3$
23	Cinchonine	294.17	-OH
24	Cinchonine 3,3-dimethylbutanoate	398.18	$-OOCCH_2C(CH_3)_3$
25	Cinchonine 2-chlorobenzoate	432.16	$-OOCC_6H_4Cl$
26	Cinchonine 4-hydroxybenzoate	414.19	$-OOCC_6H_4OH$
27	Cinchonine 2-(4-hydroxyphenyl)acetate	428.21	$-OOCCH_2C_6H_4OH$
28	Cinchonine 2-hydroxy-3-methoxybenzoate	444.20	$-OOC\ C_6H_3OHOCH_3$
29	Cinchonine 2-nitrobenzoate	443.18	$-OOCC_6H_2(OH)_3$

by modifying the secondary alcohol group on C-9. There are 10 carboxylic derivatives made ester with cinchona alkaloids. So total amount of cinchona alkaloids derivatives compounds as many as forty-four. We observed the molecular interactions between the selectivity of cinchona alkaloids and estrogen receptors α (ER-α). We chose the ER-α protein as a target for virtual screening because the estrogen hormone often triggers the growth of cancer cells in the breast. It is also because it is one of the breast cancer cell models that is widely used in research. The design of asymmetric cinchona alkaloid derivatives with R-substitution are shown in Table 1.

The molecular modeling results on 44 chincona alkaloids derivative compounds are shown in Table 2. In this table it shows the of free energy (ΔG), inhibition constanta (Ki) and hydrogen bonds that occur when molecular docking simulation.

Binding Interaction of chincona alkaloids derivative compounds are shown in Figure 2. In these figure show interaction between chincona alkaloids derivative compounds with ER-α residue amino acids in the active site of ER-α.

Table 2. Result of Docking Scores of molecular docking the cinchona alkaloids derivatives

No	Compound	Result of Docking		
		ΔG (kcal/mol)	Ki (nM)	H-bond
1	Quinine	-11.74	2.47	1
2	Quinine 3,3-dimethylbutanoate	-10.94	9.51	1
3	Quinine 2-chlorobenzoate	-11.46	3.97	1
4	Quinine 4-hydroxybenzoate	-11.26	5.59	1
5	Quinine 2-(4-hydroxyphenyl)acetate	-10.74	13.4	1
6	Quinine 2-hydroxy-3-methoxybenzoate	-8.54	553.72	0
7	Quinine 2-nitrobenzoate	-11.06	7.83	0
8	Quinine nonanoate	-10.16	35.78	0
9	Quinine acetate	-9.34	142.99	0
10	Quinine benzoate	-10.49	20.32	1
11	Quinine propionate	-9.9	55.45	0
12	Quinidine	-6.87	9.27	
13	Quinidine 3,3-dimethylbutanoate	-9.44	120.67	0
14	Quinidine 2-chlorobenzoate	-10.48	20.83	0
15	Quinidine 4-hydroxybenzoate	-10.08	40.82	2
16	Quinidine 2-(4-hydroxyphenyl)acetate	-10.97	9.04	1
17	Quinidine 2-hydroxy-3-methoxybenzoate	-9.36	138.79	0
18	Quinidine 2-nitrobenzoate	-10.06	42.58	0
19	Quinidine nonanoate	-9.37	135.41	0
20	Quinidine acetate	-9.01	250.38	0
21	Quinidine benzoate	-10.66	15.22	0
22	Quinidine propionate	-9.34	141.32	0
23	Cinchonine	-6.92	8.51	
24	Cinchonine 3,3-dimethylbutanoate	-10.32	27.11	0
25	Cinchonine 2-chlorobenzoate	-12.08	1.4	0
26	Cinchonine 4-hydroxybenzoate	-11.11	7.13	1
27	Cinchonine 2-(4-hydroxyphenyl)acetate	-11.94	1.78	2
28	Cinchonine 2-hydroxy-3-methoxybenzoate	-11.06	7.87	0
29	Cinchonine 2-nitrobenzoate	-10.85	11.09	0
30	Cinchonine nonanoate	-10.19	33.76	0
31	Cinchonine acetate	-9.33	145.54	0
32	Cinchonine benzoate	-11.10	7.28	0
33	Cinchonine propionate	-9.67	81.07	0
34	Cinchonidine	-6.93	8.36	
35	Cinchonidine 3,3-dimethylbutanoate	-10.42	23.01	0
36	Cinchonidine 2-chlorobenzoate	-11.61	3.07	1
37	Cinchonidine 4-hydroxybenzoate	-11.25	5.64	0
38	Cinchonidine 2-(4-hydroxyphenyl)acetate	-11.40	4.43	1
39	Cinchonidine 2-hydroxy-3-methoxybenzoate	-11.09	7.4	0
40	Cinchonidine 2-nitrobenzoate	-11.29	5.26	1
41	Cinchonidine nonanoate	-10.16	35.97	0
42	Cinchonidine acetate	-9.4	129.54	0
43	Cinchonidine benzoate	-11.75	2.43	1
44	Cinchonidine propionate	-9.56	97.97	0
45	Tamoxifen	-11.74	2.47	0

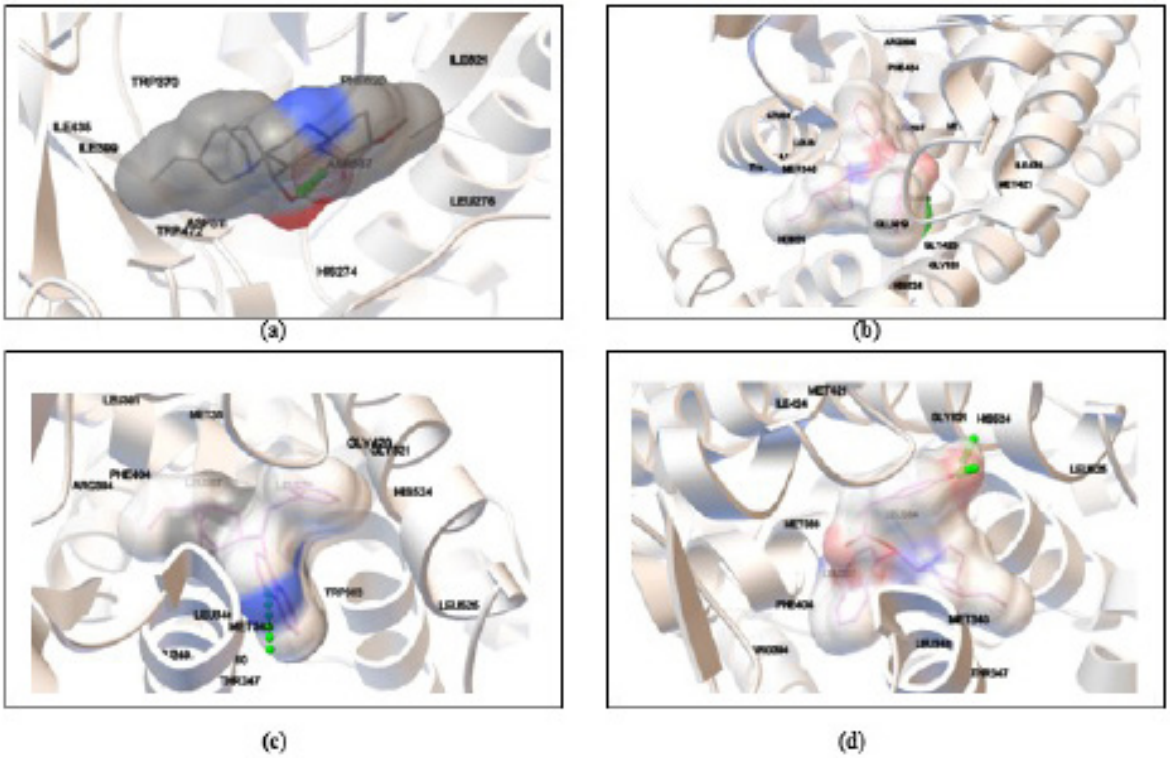

Figure 2. Binding mode of quinine(a), cinchonine 2-chlorobenzoate (b), cinchonidine benzoate(c), cinchonine 2-(4-hydroxyphenyl)acetate (d) in the active site of ER-α

4. Discussion

In this paper, we observed molecular interactions of estrogen-α when it was inhibited by cinchona alkaloids derivatives. The model of ER-α was made on the crystalline structure of alpha estrogen receptors taken from PDB (Protein Data Bank) with PDB ID : 3ERT, and external validation has been carried out. Validation method herein is pose selection whereby docking programs are used to re-dock into the target's active site a compound with a known conformation and orientation, typically from a co-crystal structure. Programs that are able to return poses below a preselected Root Mean Square Deviation (RMSD) value from the known conformation (usually 1.5 or 2 Å depending on ligand size) are considered to have performed successfully. Pose selection is then followed by scoring and ranking to study which of the available scorin.

Result of analysis of virtual screening with docking of simulation indicated by the value molecular docking Gibbs free energy (ΔG) was shown in Table 2. Parameters measured were Gibbs free energy (ΔG in kcal/mol) and constanta inhibition (Ki in μM) which were resulted from receptor-ligand interactions. Those parameters from virtual screening docking simulation that could predict the inhibition activity of ER-α are: low Gibbs free energy and low kinetic inhibition value.The Gibbs free energy (ΔG) resulted from the docking simulation showed that all cinchona alkaloids derivatives have lower Gibbs free energy and and low kinetic inhibition compared to tamoxifen. Cinchonine 2-chlorobenzoate, cinchonidine benzoate and cinchonine 2-(4-hydroxyphenyl)acetate which have the lowest Gibbs free energy compared to all the tested compounds. To obtain their binding modes in the active site were examined using AutoDock 4.2 program. AutoDock also calculated binding mode of all cinchona alkaloids derivatives in the active site of ER-α. It is known that the carbonyl oxygen of the cinchona alkaloids derivatives receives and donates a hydrogen bond from the side chain of Asp587, Arg571, Phe620, Ile435, Ile399, Asp398, Ile621, Asp511, Trp472, His274, Leu276, and to that of Trp370. These residue are believed to play a critical point in the catalytic mechanism.[23] The carbonyl group moiety looks to be an

effective chemical group for binding in the active site of ER-α and in that way for inhibiting its catalytic activity.[24]

Figure 2 showed the lowest-energy AutoDock conformation of the compound of quinine(a) cinchonine 2-chlorobenzoate (b), cinchonidine benzoate(c), cinchonine 2-(4-hydroxyphenyl)acetate (d) in the active site of ER-α. Compound of quinine, the alcohol oxygen on C-9 of the inhibitor is hydrogen bonded to the catalytic residue of Asp587, confirming the importance of the hydroxyl moiety for binding in the active site.

5. Conclusion

The virtual screening with docking of simulation in this studies were conducted on forty-four cinchona alkaloids derivatives against ER-α. The molecular docking analyses resulted in the ligand interactions with the binding site. The binding interaction of the ligands to the ER-α showed that conserved amino acid residues in ER-α protein played an important role in maintaining a functional conformation. The interactions mechanism of the enzyme and ligands in this study were useful to understand the prospective mechanism of the ER-α and ligands interactions. Two parameters from virtual screening docking simulation that could predict the inhibition activity of ER-α are: low Gibbs free energy and low kinetic inhibition value.The Gibbs free energy (ΔG) resulted from the docking simulation showed 3 cinchona alkaloids derivatives have lower Gibbs free energy and and low kinetic inhibition compared to tamoxifen (as standard commercial) and there are 14 compounds that have relatively the same activity as tamoxifen. Cinchonine 2-chlorobenzoate, cinchonidine benzoate and cinchonine 2-(4-hydroxyphenyl) acetate which have the lowest Gibbs free energy compared to all the tested compounds.

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