CHEMICAL STRUCTURE OPTIMIZATION OF LUPEOL AS ER-A AND HER2 INHIBITOR

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ABSTRACT

Objectives: Lupeol, a triterpenoid isolated from Kasturi (*Mangifera casturi*) fruit has been known for having several pharmacological activities, including anticancer properties. Lupeol showed antiproliferative activity toward many cancer cells line including breast cancer. Lupeol showed promising potency as both ER-α and HER2 inhibitors, although still lower than known ER-α and HER2 Inhibitors. Chemical structure optimization of lupeol was predicted could increase the affinity of lupeol derivatives against ER-α and HER2. This study aims to determine lupeol derivative with the highest affinity against ER-α and HER2.

Methods: All ligands were sketched and optimized using Gaussian 03W with Hartree–Fock method basis set 3-21G. Molecular docking was performed using Autodock 4.2.6 on several modified chemical structure of lupeol against active site of ER-α and HER2. The main parameter used was the free energy of binding and inhibition constants as affinity marker.

Results: The docking results show that lupeol derivative with an amine group (Lupeol-2) and ethyl group (Lupeol-4) at position C3 provide the highest affinity with the free energy of binding and dissociation constant −12.24 kcal/mol and 1.07 nM for ER-α also −9.63 kcal/mol and 86.94 nM for HER2, respectively. Interestingly, although lupeol derivatives showed higher affinity toward ER-α, their amino acid residues were closer to the interaction on HER2.

Conclusion: These results predict that lupeol have greater potential to be developed as a HER2 inhibitor. Further, derivate lupeol-4 should be potential to be developed as HER2-positive breast cancer therapy.

Keywords: Breast cancer, Docking, ER-α, HER2, Lupeol.

INTRODUCTION

The search for various pharmacological activities of the Kasturi, especially from the fruit, has shown a very promising development. Not only as an antioxidant [1], the kasturi fruit extract also exhibits immunomodulatory [2], anti-inflammatory [3], even antileukemia properties [4]. Various secondary metabolite compounds have been isolated from Kasturi fruit, including gallic acid, methyl gallate, 2,3-dihydroxybenzoic acid, dihydroxyquercetin, glucogallin, β-sitosterol, and lupeol [4-6].

Lupeol, a triterpenoid compound isolated from the fruit of Kasturi is known to have anti-inflammatory and anticancer activity. Several studies have demonstrated lupeol anticancer activity in some cancer cells, such as blood cancer [7], lung cancer, cervical cancer, skin cancer, and breast cancer [8]. Lupeol especially showed good anticancer activity in breast cancer for both ER-α-positive and HER2-positive breast cancer. Lupeol anticancer activity is predicted to be associated with antiproliferation and induced apoptotic effects of cancer cells [9].

Despite showing potential for therapy both types of breast cancer, lupeol anticancer potency is still lower than the currently available treatment options for each type of breast cancer [10]. Tamoxifen, a prodrug of 4-hydroxytamoxifen is currently one of the most common options for the treatment of ER-α-positive breast cancer [11], while various tyrosine-kinase inhibitors are also being developed as a HER2-positive breast cancer treatment, one of them is TAK-285 [12]. One of the ways to increase lupeol affinity with both ER-α and HER2 receptors is to optimize the lupeol chemical structure by modifying its pharmacophore [13]. Determination of the main pharmacophore with

the greatest influence on the affinity of a ligand on the receptor can be done by removing or modifying each pharmacophore of the ligand one by one [14]. The modified ligands were reexamined by the molecular docking method to see the affinity change of the modified ligand [15,16].

This study aims to find the best modification of chemical structure in pharmacophore from lupeol as ER-α and HER2 inhibitor which gives the highest affinity. The modified results were then re-tested and compared with known ER-α and HER2 inhibitors to determine the lupeol potential for development as a therapy for ER-α-positive and HER2-positive breast cancer.

METHODS

Preparation of ligands

The ligand used was lupeol and derivatives as shown in Table 1. Modification of lupeol derivatives was performed on C3 (Lupeol-1 to Lupeol-10) and C20 atoms (Lupeol-11). The two-dimension structure of lupeol and derivatives is shown in Fig. 1.

Structures of lupeol and derivatives were sketched using GaussView 3.08 Software from Gaussian, Inc. All structures were geometry optimized by Hartree-Fock method basis set 3-21G with Gaussian 03 W software from Gaussian, Inc. Geometry optimization provided an ideal conformation of following compounds that approaching conformation of these compounds in nature [17]. Optimized structures format changed from.log to.pdb using Open Babel 2.4.1 software [18]. Docking program used in this study was Autodock 4.2.6 from The Scripps Research Institute. All ligands then are given the charge and set torque using software AutoDockTools 1.5.6 [19].

Preparation of receptors

The molecular structure of ER-α in complexes with 4-hydroxytamoxifen (protein data bank [PDB] ID 3ERT) and HER2 in complexes with TAK-285 (PDB ID 3PP0) was obtained from website of PDB http://www. rscb.org. The receptors were downloaded in.pdb format and then removed the unused portion, added the non-polar hydrogen group, given the charge, and set the grid box size and coordinate using software AutoDockTools 1.5.6 [19]. The used structure of ER-α is the active site which binds with 4-hydroxytamoxifen as a cocrystal ligand. 4-hydroxytamoxifen or afimoxifene is a potent ER-α inhibitor that can slowing cell proliferation rate of cancer cells in ER-α-positive breast cancer [20]. While the used structure of HER2 is a tyrosine-kinase domain which binds with TAK-285, a potent small molecule tyrosine kinase inhibitor of HER2 as a cocrystal ligand [21]. Tyrosine-kinase domain was chosen because the active site is known and already used for the development of HER2 inhibitors [22].

Validation of docking process

The method used for docking validation was redocking the cocrystal ligand into the active site of each receptor. The parameters observed in validation are root-mean-square deviation (RMSD) of each cocrystal ligands at the selected binding site. RMSD scores describe the average difference in position of the atoms of the redocking ligand with the crystallographic results. Docking programs are preferred to predict results from experimental poses with RMSD no more than 2 Å. Smaller RMSD indicates that position of redocking results ligand was closer to crystallography results ligand [23,24].

Molecular docking

Molecular docking is done using software AutoDock 4.2.6 from The Scripps Research Institute. Docking for all test ligand performed in same way as validation process with similar size and position of grid box [24]. The main parameter used in docking process was the free energy of binding (ΔG), dissociation constant (K_i), amino acid residues and number of hydrogen bonds [25]. Ligand affinity to the receptor in docking method is determined by ΔG and K_i scores. The more negative ΔG and lower K_i indicated higher ligand affinity toward active site of the used receptor [26]. Test ligand with the highest affinity was compared with validation result of cocrystal ligand to determine the potency of test ligand as each receptors inhibitor [17]. The amino acid residues of selected test ligand for each receptor then compared with amino acid residues of cocrystal ligand to assess the similarity of interaction between test and cocrystal ligand. The more similar amino acid residues are indicating a higher probability that the test ligand will have similar activity with the cocrystal ligand [27].

RESULTS AND DISCUSSION

Validation was performed on the active site of each receptor using cocrystal ligand as references for determining the size and coordinates of each grid box. The redocking results are shown in Fig. 2.

In Fig. 2 can be seen that the position of each redocking ligand almost overlapped with the crystallographic ligand position both on ER-α and HER2 receptors. The RMSD scores of each receptor also <2Å, indicate that both 3ERT and 3PP0 receptors are valid for use in the docking process [23]. Other parameters observed in the validation of receptors such as ΔG, K_i, amino acid residues, and a number of hydrogen bonds including the size and coordinate of the grid box are shown in Table 2.

Docking was performed using Autodock 4.2.6 at the active site of ER-α, and HER2 receptors with 100 genetic algorithms run to improve the accuracy of docking result [19]. For each test ligand, one poses with the most negative ΔG and the lowest K_i was selected as representatives of test ligand [17]. The docking results data of all test ligands to both receptors were compared each other as shown in Tables 3-8.

All test ligands in both ER-α and HER2 receptors show negative ΔG scores, indicating that the interaction between ER-α and HER2 receptors with all test ligands will occur spontaneously [24]. In ER-α

Table 1: Lupeol derivatives including modified functional groups

Compounds	R_1	R_2
Lupeol (L–0)	-OH	-CH$_2$
Lupeol derivative 1 (L–1)	-CH$_3$	-CH$_2$
Lupeol derivative 2 (L–2)	-NH$_2$	-CH$_2$
Lupeol derivative 3 (L–3)	-OCH$_3$	-CH$_2$
Lupeol derivative 4 (L–4)	-CH$_2$CH$_3$	-CH$_2$
Lupeol derivative 5 (L–5)	-NHCH$_3$	-CH$_2$
Lupeol derivative 6 (L–6)	-CH(CH$_3$)$_2$	-CH$_2$
Lupeol derivative 7 (L–7)	-N(CH$_3$)$_2$	-CH$_2$
Lupeol derivative 8 (L–8)	-C(CH$_3$)$_3$	-CH$_2$
Lupeol derivative 9 (L–9)	-CCH$_2$CH$_3$	-CH$_2$
Lupeol derivative 10 (L–10)	-COCH$_3$	-CH$_2$
Lupeol derivative 11 (L–11)	-OH	-COCH$_3$

Table 2: Validation results of NA receptors PDB ID 2HU4 with cocrystal ligand oseltamivir

Receptor	3ERT	3PP0
RMSD (Å)	1.155	0.731
ΔG (kcal/mol)	−11.87	−10.42
K_i (μM)	0,00201	0.02297
Amino acid residues	343-Met	726-Leu
	346-Leu	728-Ser
	347-Thr	729-Gly
	350-Ala	734-Val
	351-Asp	751-Ala
	353-Glu	753-Lys
	383-Trp	774-Met
	384-Leu	785-Leu
	387-Leu	796-Leu
	394-Arg	798-Thr
	428-Leu	799-Gln
	521-Gly	800-Leu
		801-Met
		852-Leu
		863-Asp
		864-Phe
Number of hydrogen bonds	2	0
Grid box coordinate	X= −30.01	X= −16.622
	Y= −1.913	Y=17.394
	Z=24.207	Z= −26.218
Grid Box size (Å)	60×60×60	40×40×40

PDB: Protein data bank, RMSD: Root-mean-square deviation

Table 3: Docking results of lupeol derivatives at ER-α receptor (1)

Ligand	L-0	L-1	L-2	L-3
ΔG (kcal/mol)	−10.03	−10.27	−12.24	−9.95
K_i (μM)	0.0441	0.0298	0.00107	0.05085
Amino acid residues	347-Thr	-	347-Thr	-
	351-Asp	-	351-Asp	-
	380-Glu	-	380-Glu	-
	383-Trp	383-Trp	383-Trp	383-Trp
	-	-	-	-
	522-Met	522-Met	522-Met	522-Met
	-	523-Glu	-	523-Glu
	525-Leu	525-Leu	525-Leu	525-Leu
	526-Tyr	526-Tyr	526-Tyr	526-Tyr
	536-Leu	-	536-Leu	-
	-	-	-	-
Number of hydrogen bonds	1	0	0	0

receptor, lupeol-2 show the most negative ΔG and the smallest K_i. In other words, lupeol-2 has the highest affinity to ER-α receptor. While

Table 4: Docking results of lupeol derivatives at ER-α receptor (2)

Ligand	L-4	L-5	L-6	L-7
ΔG (kcal/mol)	−10.23	−11.81	−10.53	−11.05
K_i (μM)	0.0316	0.00222	0.01913	0.00797
Amino acid residues	-	347-Thr	-	-
	-	351-Asp	-	351-Asp
	-	380-Glu	-	-
	383-Trp	383-Trp	383-Trp	383-Trp
	519-Asn	-	519-Asn	-
	522-Met	522-Met	522-Met	522-Met
	523-Glu	-	523-Glu	-
	525-Leu	525-Leu	525-Leu	525-Leu
	526-Tyr	526-Tyr	526-Tyr	526-Tyr
	-	536-Leu	-	536-Leu
	-	-	-	539-Leu
Number of hydrogen bonds	0	1	0	0

Table 5: Docking results of lupeol derivatives at ER-α receptor (3)

Ligand	L-8	L-9	L-10	L-11
ΔG (kcal/mol)	−10.81	−10.43	−10.24	−10.57
K_i (μM)	0.01184	0.02258	0.03111	0.01789
Amino acid residues	-	-	-	351-Asp
	-	-	-	380-Glu
	383-Trp	383-Trp	383-Trp	383-Trp
	-	519-Asn	519-Asn	-
	522-Met	522-Met	522-Met	522-Met
	-	523-Glu	523-Glu	-
	525-Leu	525-Leu	525-Leu	525-Leu
	526-Tyr	526-Tyr	526-Tyr	526-Tyr
	-	-	-	536-Leu
Number of hydrogen bonds	0	0	0	2

Table 6: Docking results of lupeol derivatives at the HER2 receptor (1)

Ligand	L-0	L-1	L-2	L-3
ΔG (kcal/mol)	−9.25	−9.11	−6.86	−8.32
K_i (μM)	0.16548	0.20972	9.36	0.78037
Amino acid residues	726-Leu	726-Leu	726-Leu	726-Leu
	728-Ser	728-Ser	728-Ser	728-Ser
	-	-	-	-
	734-Val	734-Val	734-Val	734-Val
	751-Ala	751-Ala	751-Ala	751-Ala
	753-Lys	753-Lys	753-Lys	753-Lys
	783-Ser	783-Ser	783-Ser	783-Ser
	785-Leu	785-Leu	785-Leu	785-Leu
	796-Leu	796-Leu	796-Leu	796-Leu
	-	-	-	-
	798-Thr	798-Thr	798-Thr	798-Thr
	-	-	-	-
	-	-	-	-
	-	805-Cys	805-Cys	805-Cys
	849-Arg	849-Arg	849-Arg	849-Arg
	850-Asp	850-Asp	850-Asp	850-Asp
	852-Leu	852-Leu	852-Leu	852-Leu
	862-Thr	862-Thr	862-Thr	862-Thr
	863-Asp	863-Asp	863-Asp	-
	-	-	-	-
Number of hydrogen bonds	0	0	0	0

Table 7: Docking results of lupeol derivatives at the HER2 receptor (2)

Ligand	L-4	L-5	L-6	L-7
ΔG (kcal/mol)	−9.63	−7.42	−8.23	−5.92
K_i (μM)	0.08694	3.65	0.92474	46.1
Amino acid residues	726-Leu	726-Leu	726-Leu	726-Leu
	-	-	-	-
	734-Val	734-Val	734-Val	734-Val
	751-Ala	751-Ala	751-Ala	751-Ala
	753-Lys	753-Lys	753-Lys	753-Lys
	783-Ser	783-Ser	783-Ser	783-Ser
	785-Leu	785-Leu	785-Leu	785-Leu
	796-Leu	796-Leu	796-Leu	796-Leu
	-	-	-	-
	798-Thr	798-Thr	798-Thr	798-Thr
	-	-	800-Leu	800-Leu
	-	-	801-Met	801-Met
	804-Gly	804-Gly	804-Gly	804-Gly
	805-Cys	805-Cys	805-Cys	805-Cys
	849-Arg	849-Arg	-	-
	-	-	-	-
	852-Leu	852-Leu	852-Leu	852-Leu
	862-Thr	862-Thr	862-Thr	862-Thr
	863-Asp	863-Asp	863-Asp	863-Asp
	-	-	864-Phe	864-Phe
Number of hydrogen bonds	0	0	0	0
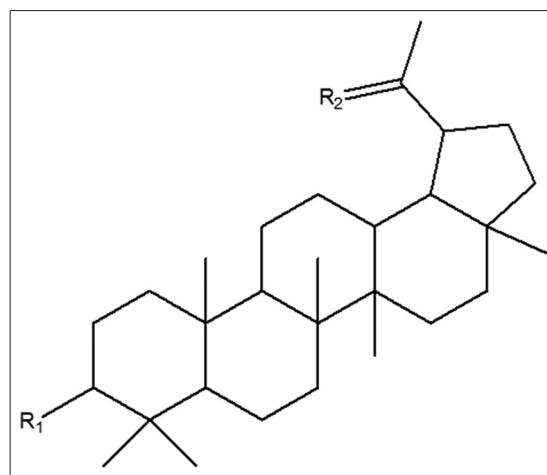
Fig. 1: Structure of lupeol [6]

in the HER2 receptor, the most negative ΔG and the smallest K_i are indicated by lupeol-4.

The comparison of amino acid residues and the number of hydrogen bonds between the redocking results of the cocrystal ligand of each receptor with lupeol and the highest affinity derivative were performed to analyze the similarities and differences types of interactions between each ligand [25]. The comparison for the ER-α receptor as shown in Table 9 shows that 4-hydroxytamoxifen with lupeol-0 and lupeol-2 have considerable differences in amino acid residues. . Interestingly, the affinity indicated by 4-hydroxytamoxifen is still higher than lupeol-0, but lower than lupeol-2. The K_i score of lupeol-2 itself almost half from the K_i score of 4-hydroxytamoxifen, indicating the lupeol-2 affinity that is almost two-fold than 4-hydroxytamoxifen. These results indicate that modification of amino groups at the position of C number 3 can increase the affinity of lupeol against ER-α receptor.

Whether lupeol-0 and lupeol-2 have similar activity with 4-hydroxytamoxifen as an ER-α inhibitor or not is still unknown. However, amino acid residues shown by lupeol-0 and lupeol-2 have

very little in common with amino acid residues of 4-hydroxytamoxifen (3 out of 12). These results indicated that although lupeol-2 has a higher affinity than 4-hydroxytamoxifen toward ER-α, it is likely that lupeol-2 does not exhibit ER-α inhibitory activity as possessed by 4-hydroxytamoxifen. Further observation of the docking results shown in Fig. 3 shows the considerable position difference between 4-hydroxytamoxifen with lupeol-0 and lupeol-2.

The comparison for HER2 receptor as shown in Table 10 shows that amino acid residues between TAK-285 with lupeol-0 and lupeol-4 are not much different. In contrast to ER-α receptor, both lupeol-0 and lupeol-4 have lower affinity than TAK-285. However, the affinity of lupeol-4 is still higher than lupeol-0. The K_i score of lupeol-4 itself is almost half of the K_i score lupeol-0, but almost 4 times greater than the K_i score of TAK-285. These results indicate that the modification of ethyl
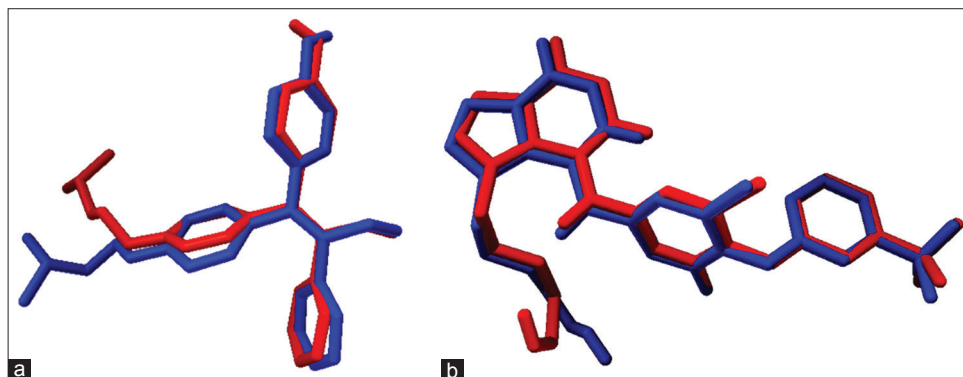

Fig. 2: Overlay of redocking (red) and crystallography (blue) ligand position; including 4-hydroxytamoxifen with root-mean-square deviation (RMSD): 1.155Å (a) and TAK-285 with RMSD: 0.731Å (b)
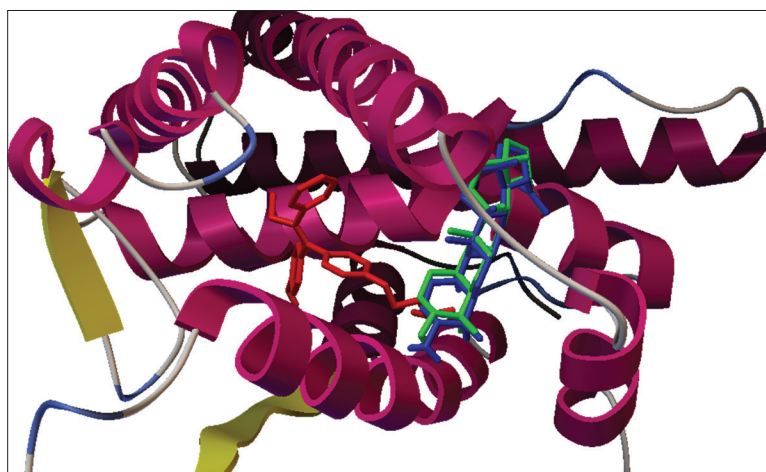

Fig. 3: Comparison of redocking result from 4-hydroxytamoxifen position (red) with docking result from lupeol-0 (blue) and lupeol-2 (green)
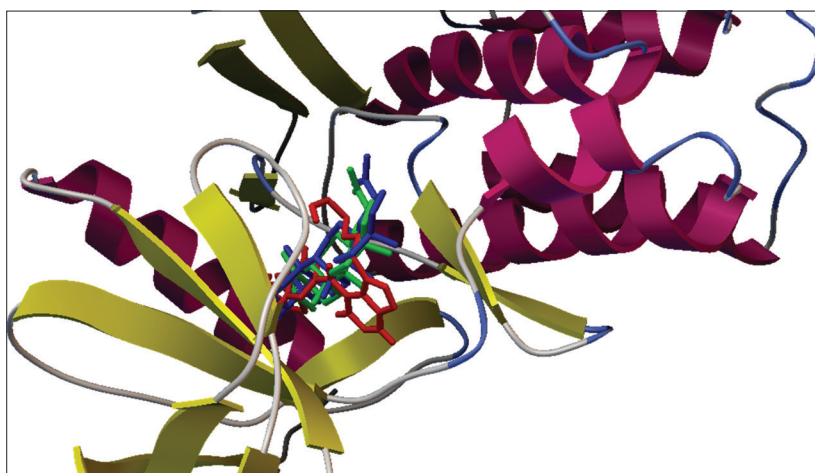

Fig. 4: Comparison of redocking result from TAK-285 position (red) with docking result from lupeol-0 (blue) and lupeol-4 (green)

Table 8: Docking results of lupeol derivatives at the HER2 receptor (3)

Ligand	L-8	L-9	L-10	L-11
ΔG (kcal/mol)	−1.18	−8.19	−9.33	−8.22
K_i (μM)	137080	0.99935	0.14539	0.94931
Amino acid residues	726-Leu	726-Leu	726-Leu	726-Leu
	728-Ser	-	-	-
	729-Gly	-	-	-
	734-Val	734-Val	734-Val	734-Val
	751-Ala	751-Ala	751-Ala	751-Ala
	753-Lys	753-Lys	753-Lys	753-Lys
	783-Ser	783-Ser	783-Ser	783-Ser
	785-Leu	785-Leu	785-Leu	-
	796-Leu	796-Leu	796-Leu	796-Leu
	797-Val	797-Val	-	-
	798-Thr	798-Thr	798-Thr	798-Thr
	-	-	-	-
	-	-	-	-
	-	-	804-Gly	-
	-	805-Cys	805-Cys	805-Cys
	-	849-Arg	849-Arg	849-Arg
	-	-	-	-
	852-Leu	852-Leu	852-Leu	852-Leu
	862-Thr	862-Thr	862-Thr	862-Thr
	863-Asp	863-Asp	863-Asp	-
	864-Phe	-	-	-
Number of hydrogen bonds	0	0	0	0

Table 10: Comparison of redocking results of TAK-285 with docking result of lupeol-0 and lupeol-2 toward HER2

Ligand	TAK-285	L-0	L-4
ΔG (kcal/mol)	−10.42	−9.25	−9.63
K_i (μM)	0.02297	0.16548	0.08694
Amino acid residues	726-Leu	726-Leu	726-Leu
	728-Ser	728-Ser	-
	729-Gly	-	-
	734-Val	734-Val	734-Val
	751-Ala	751-Ala	751-Ala
	753-Lys	753-Lys	753-Lys
	774-Met	-	-
	-	783-Ser	783-Ser
	785-Leu	785-Leu	785-Leu
	796-Leu	796-Leu	796-Leu
	798-Thr	798-Thr	798-Thr
	799-Gln	-	-
	800-Leu	-	-
	801-Met	-	-
	-	-	804-Gly
	-	-	805-Cys
	-	849-Arg	849-Arg
	-	850-Asn	-
	852-Leu	852-Leu	852-Leu
	-	862-Thr	862-Thr
	863-Asp	863-Asp	863-Asp
	864-Phe	-	-
Number of hydrogen bonds	0	0	0

Table 9: Comparison of redocking results of 4-hydroxytamoxifen with docking result of lupeol-0 and lupeol-2 toward ER-α

Ligand	4-hydroxytamoxifen	L-0	L-2
ΔG (kcal/mol)	−11.87	−10.03	−12.24
K_i (μM)	0.00201	0.0441	0.00107
Amino acid residues	343-Met	-	-
	346-Leu	-	-
	347-Thr	347-Thr	347-Thr
	350-Ala	-	-
	351-Asp	351-Asp	351-Asp
	353-Glu	-	-
	-	380-Glu	380-Glu
	383-Trp	383-Trp	383-Trp
	384-Leu	-	-
	387-Leu	-	-
	394-Arg	-	-
	428-Leu	-	-
	521-Gly	-	-
	-	522-Met	522-Met
	-	525-Leu	525-Leu
	-	526-Tyr	526-Tyr
	-	536-Leu	536-Leu
Number of hydrogen bonds	2	1	0

CONCLUSION

The presents study was successfully found the optimal optimization of lupeol chemical structure as the ER-α and HER2 inhibitors, even yielding interesting results where the activity of lupeol derivatives is more likely as HER2 than ER-α inhibitor. Although the resulting affinity is still lower than comparative ligands, the potential indicated by lupeol derivatives, especially lupeol-4 which modified by the addition of ethyl group at the position of C number 3 as HER2 inhibitor is still promising. Further modification of atom C number 3 with other more lipophilic groups has the potential to increase the affinity of lupeol derivatives even further. Thus, the study clearly shows the promising potential for lupeol derivatives to be developed as a HER2 inhibitor in HER2-positive breast cancer therapy.

CONFLICT OF INTEREST

All authors are declared, there is no conflict of interest.

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groups at the position of C number 3 can multiply the affinity of lupeol toward HER2 receptor.

In contrast to ER-α receptor, the similarity of amino acid residues toward HER2 receptor represents a much greater number, even more than half of amino acid residues in TAK-285 (9 out of 16) also present in both lupeol-0 and lupeol-4. Regardless of the lower affinity, there is a great possibility that lupeol-0 especially lupeol-4 have similar HER2 inhibitory activity with TAK-285. Further observation of the docking results shown in Fig. 4 shows the similarity of binding positions between TAK-285 with lupeol-0 and lupeol-4.

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