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In silico predictive for modification of chalcone with pyrazole derivatives as a novel therapeutic compound for targeted breast cancer treatment

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ABSTRACT

One of the most common triggers of breast cancer is over-expression of estrogen receptor alpha (ERα). Long-term therapy of tamoxifen, an ERα antagonist, can reduce patient's quality of life because of its side effects. In the previous study, 2',4'-dihydroxy-6-methoxy-3,5-dimethylchalcone (ChalcEA) was isolated as an active compound from the *Eugenia aquea* leaves that is responsible for breast cancer treatment with positive ERα, however, the potency is lower than tamoxifen. The aim of this study is to find the best-modified chalcone that binds well with the ERα. Drug design approaches used in this study were Structure-Based (Autodock 4.1) and Ligand-Based (LiganScout 4.1). Prediction of absorption, distribution, and toxicity parameters was employed using preADMET and Toxtree. Interactions between tamoxifen and ERα were determined and the differences in the binding modes between tamoxifen and chalcones were observed. Modifina3 had pharmacophore fit score value of 76.42% and the molecular docking studies showed the lowest free energy binding (ΔG) of −11.07 kcal/mol while tamoxifen of −10.15 kcal/mol. Modifina3 had absorption and distribution properties with the percentage human intestinal absorption of 95.90%, $CaCO_2$ of 46.95%, and protein plasma binding of 93.55%. Toxicity prediction of Modifina3 was categorized in class III and risk assessment requires compound specific toxicity data. These results suggest that Modifina3 has the potency to be a novel therapeutic compound for potent ERα inhibitor targeted breast cancer.

INTRODUCTION

Base on International Agency for Research on cancer, with emphasis on geographical differences in 20 countries in the world, The GLOBOCAN 2018 estimates of cancer mortality and incidence (Bergman *et al*., 2000). The first prevalence of cancer in women is breast cancer, the second is colorectal and lung cancer (for incidence), followed by vice versa (for mortality), and the fourth, for mortality and incidence, is cervical cancer (Ferlay *et al*., 2010).

Breast tissues differentiation and development influence by ovarian hormones (Bernstein and Press, 1998). Breast is one of the estrogen-responsive target tissue induces of cancer development. Tamoxifen is one of the nonsteroidal compounds (Bentrem and Craig Jordan, 2002), which has been studied, whose effect is varied as agonists or antagonists based on the investigation of a gene or particular organ system (Rojas and Stuckey, 2016).

Estrogen has a play role in growth, development, and in the pathology of bones, breast, and uterus. Estrogen receptor is classified into two subtypes, ERα (Estrogen Receptor-α) and Erβ (Payne *et al*., 2008). The role of ERα is in cell proliferation (Fox *et al*., 2008), and ERα found in endometrial, mammary epithelial cells which are the origin cell for growth in most breast

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cancers, ovarian stromal cell, and hypothalamus (Levin, 2005). ERα plays an important and responsible role as the most common trigger of breast cancer (Narod, 2011). Various molecules have been an investigation to find out compounds that bind well with ERα, as a crucial receptor for breast cancer (Cragg *et al*., 1997). The most widely used as hormonal therapy of breast cancer is tamoxifen. The risk of recurrence and death of breast cancer are reduced by tamoxifen when given as adjuvant therapy. It also provides effective palliation for patients with metastatic breast cancer (Yang *et al*., 2013). Tamoxifen is a Selective Estrogen Receptor Modulator (SERM) that has antagonist activity to breast cancer but agonist activity to other receptors, especially in the uterus (Fisher *et al*., 2005). Tamoxifen is given to women who have stopped menstruation with ERα+ tumors. Tamoxifen plays a crucial role in breast cancer therapy. Tamoxifen can reduce breast cancer relapse significantly (Davies *et al*., 2013). Tamoxifen interacts with co-repressors, thus inhibiting expression of estrogen-dependent response genes (Chang, 2012). Besides the benefit of tamoxifen, there are adverse effects in the uterus (Fisher *et al*., 2005). Depending on the results of the study, endometrial cancer risk increased from 1.5 to 6.9 fold (Cohen, 2004). The risk of adverse effect in the uterus increases with accumulative usage and longer duration of tamoxifen therapy (Bergman *et al*., 2000). The risk of endometrial malignancy increases significantly with an increased body weight of postmenopausal females. Besides that, patients with ER+ show the intrinsic resistance to SERM not depend on ER increased (Fan *et al*., 2015). The mechanism of tamoxifen resistance occurs by loss of ERα expression, which leads to the removal of ligand for tamoxifen, change mechanism of co-activators or co-regulators, stimulate kinases and ER phosphorylation, change profile pharmacokinetic of metabolites active of tamoxifen, regulation of apoptosis, and antioxidant protein-mediated cell survival (Chang, 2012). Because of these cases, an alternative treatment was needed through the natural compound. Drug discovery from medicinal plants has played an important role in cancer treatment and, indeed, the newest therapy of herbal medicine practiced in fighting cancer (Prasad *et al*., 2006).

Rational drug discovery and development of new active agents or leads is utilizing *in silico* study. Ligand-based drug design and structure-based drug design (SBDD) were used as a modern method in drug discovery (Dror *et al*., 2004). One of the natural compound, which has potential as anti-breast cancer, is chalcone. Chalcone is one of the flavonoid groups secondary metabolite that is found in many plants (Prasad *et al*., 2006). In the previous study, 2',4'-dihydroxy-6-methoxy-3,5-dimethylchalcone was isolated from leaves of *Eugenia aquea* and was further investigated on to breast cancer therapy (Subarnas *et al*., 2015). The results showed that the chalcone isolates reduce cell proliferation against Michigan Cancer Foundation-7 human breast cell using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide bioassay in a dose-dependent manner with the half maximal inhibitory concentration (IC_{50}) of 74.5 µg/ml (250 µM) and induce apoptosis via the activation of poly (adenosine diphosphate-ribose) polymerase (Subarnas *et al*., 2015). However, this IC_{50} was categorized in moderate potential and this compound cannot compete with tamoxifen due to its lack of hydrophobic tail. So, we need an effort to improve 2',4'-dihydroxy-6-methoxy-3,5-dimethylchalcone potency and the aim of this study is to find the best-modified chalcone that binds well with ERα by replacement of the carbonyl group of that chalcone that binds well with the ERα.

MATERIALS AND METHODS

Molecular docking simulation

3ERT taken from protein data bank (PDB) used as a standard was complex ERα with 4-hydroxytamoxifen (4-OHT). The ligand and macromolecule structures were separated using Discovery Studio 4.0. The SBDD using molecular docking simulation methods has been carried out in the previous study (Muchtaridi *et al*., 2014; 2017). Using AutoDockTools 1.5.6, all the ligands and receptor were prepared for docking simulation and protonated. The solvation and default Kollman charge parameters were designate to the macromolecule atoms. Addition of Gasteiger charges to molecule as a ligand atom A grid box comprised of 40 × 40 × 40 points distance by 0.375 Å and was focused on the ER binding site (x = 30.282, y = −1.913, and z = 24.207). The bond strength of the atom in the ligand was calculated using an Autogrid (Morris *et al*., 2009). The Lamarckian genetic algorithm (LGA) specifications were 100 runs, elitism of 1, the mutation rate of 0.02, the population size of 100, and a crossover rate of 0.08 band 10,000,000 energy evaluations (Ikram *et al*., 2015). A root means square deviation was used for clustering the results of docked conformation, tolerance of 1.0 Å. The docking outcomes were imaged using Discovery Studio Visualizer 4.0.

Pharmacophore modeling

Ligand-based drug design using pharmacophore fit score calculated the qualified element of the corresponding site of the compound to the features of pharmacophore model and was used to interpret the value of the corresponding site from the pharmacophore model. A 3-D pharmacophore model using LiganScout 4.1 was derived from the X-ray derived structure of ERα that binds with 4-OHT (Wolber and Langer, 2005).

preADMET and Toxtree

In silico, pharmacokinetic properties and toxicities were predicted using preADMET and Toxtree software, which are available online (Lee *et al*., 2003). preADMET (v2.0) online at https://preadmet.bmdrc.kr/ is a web-based application for predicting absorption and distribution data using *in silico* method. The Toxtree v2.3.16 that we used was available online at https://sourceforge.net/projects/toxtree/files/toxtree/Toxtree-v.2.6.13/Toxtree-v2.6.13-setup.exe/download, used to predict the toxicity of compounds.

RESULTS AND DISCUSSION

Modification of chalcone

Molecular docking of protein that binds with ligand is most widely used as Structure-Based Virtual Screening method. It is estimated affinity of the ligand and protein derived on its intermolecular interactions in the binding site. Chalcone derivatives are simple compounds, have ease of replacing hydrogen atom, easy and simple synthesis, with a numerous prospective effect as a new drug (Todorova, 2010). However, it is necessary to develop

chalcone as a new drug based on its chemical properties. The *in silico* study base on computer-aided drug design, especially to chalcone is needed for more valuable research (Gomes *et al*., 2017).

A number of compounds of pyrazolic chalcone derivatives were shown to have anticancer activity, based on 50% inhibitory concentration (IC_{50}) values (Hawash *et al*., 2017).

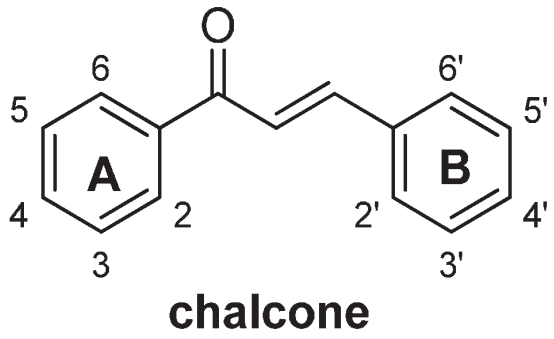
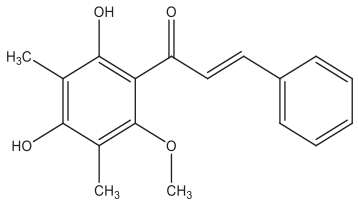
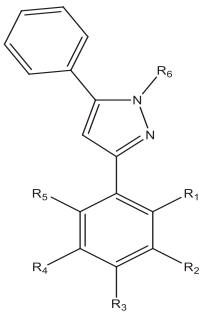


Figure 1. Basic structure of chalcone.

Table 1. Modification of chalcone.

ChalcEA	ChalcEA Derivatives (Modifina1-9)
	

Extension of the functional group to ring A and/or ring B (Fig. 1) can induce the activity of chalcone (Lahsasni *et al*., 2014).

In our study, in order to find the best-modified chalcone, we were replaced the carbonyl group of ChalcEA, to increase hydrophobicity, which is the important pharmacophore using hydrazine to produce pyrazole derivatives, to increase selectivity and activity against the ERα using rational drug design. The design of chalcone modifications is presented in Table 1.

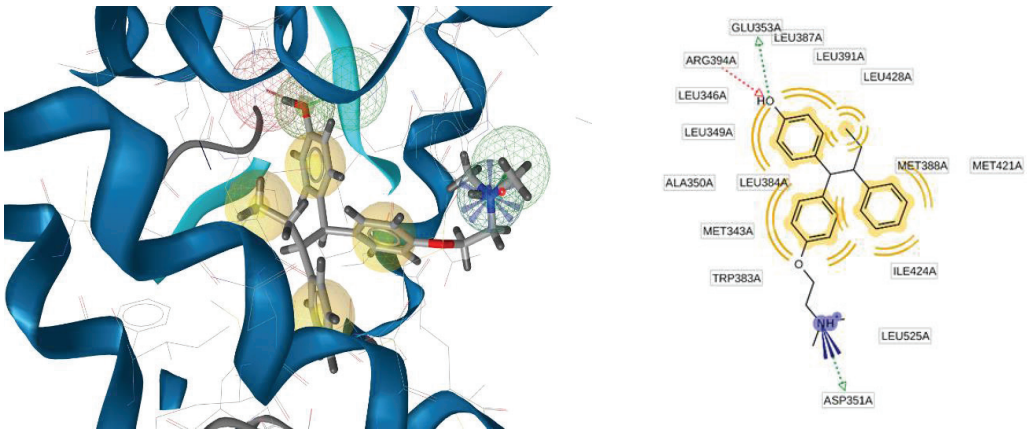
Pharmacophore modeling

The important feature that provides to biological activity is represented by pharmacophore (Wolber, 2008). Complexes of ligand-protein in PDB, the compound as a ligand separated from the protein and elucidated chemical properties of the ligand. Pharmacophore modeling representing the interaction of ligand and receptor which are generated from every one of these molecules and its surrounding them (Wolber and Langer, 2005). Figure 2a shows the pharmacophore of 4-OHT. The interaction between ERα (PDB code: 3ERT) with 4-OHT forms hydrophobic interactions predominantly with aromatic rings, hydrogen bond interactions, and positive ionizable interaction. The 2-D (Fig. 2b) pharmacophore modeling illustrates the interaction between a hydrophobic pocket with amino acids residue.

Pharmacophore fit scores indicate that chemical properties of the ligands are suitable to the feature of the 4-OHT structure-based pharmacophore model. Table 2 showed that Modifina1 and Modifina3 were the best two pharmacophores fit score, which means the chemical properties of that ligands are most suitable with the features of the 4-OHT pharmacophore model. However, Modifina5 and Modifina7 do not have pharmacophore fit score, which means that chemical features of those compounds are not aligned to the feature of the 4-OHT pharmacophore modeling.

Molecular docking results

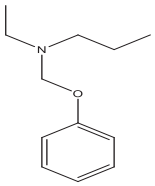
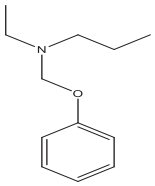
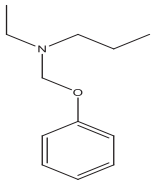
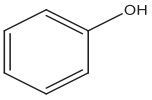
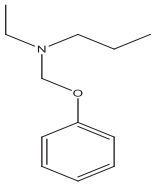
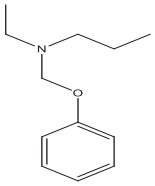
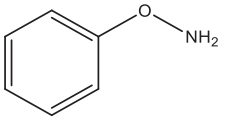
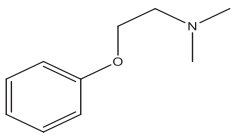
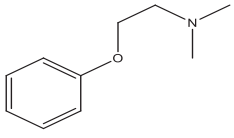
SBDD is a method that depends on possessing the knowledge of the 3-D structure of the receptor as a biological target



(a) (b)

Figure 2. (a) The 3-D pharmacophore modeling using LiganScout 4.1 based of 4-OHT that complexed with ERα f (PDBid: 3ERT). Yellow spheres, blue star, green and red arrows were illustrating of hydrophobic, positive ionizable, hydrogen bond donor, and acceptor interaction, respectively. (b) The 2-D pharmacophore modeling illustrates the interaction between hydrophobic pockets with the binding site residues.

Table 2. Pharmacophore fit score result.

No.	Molecule Name	R1	R2	R3	R4	R5	R6	Pharmacophore Fit Score (%)
1	Modifina1	OCH_3		OH		OH		76.44
2	Modifina2	OCH_3		OH	CH_3	OH		67.40
3	Modifina3	OCH_3	CH3	OH		OH		76.42
4	Modifina4	OCH_3		OH		OH		66.49
5	Modifina5							-
6	Modifina6	OCH_3		OH		OH		65.58
7	Modifina7							-
8	Modifina8		OH		OH			67.47
9	Modifina9				OH			66.87

(Kalyaanamoorthy and Chen, 2011). Structural determination of biological macromolecules by using X-Ray crystallographic is currently the most favored method (Smyth and Martin, 2000). Crystallographic ERα structure a that binds with 4-OHT from PDB code: 3ERT was preferred for molecular docking simulation of chalcone and its derivatives since they have suitable criteria for research resolution (1.9 Å). Molecular docking is commonly used for prediction of biology molecules complexes in molecular design. The fast results of free energy binding in Autodock come from combining a force field of empirical free energy with an LGA (Morris *et al*., 2009).

Validation of molecular docking is done first, before performing molecular docking simulation of ligand that will be tested, conducted by separating 4-OHT from ERα in PDB and docking it into active site to verify that the method running well as the bioactive conformation antagonist of 4-OHT. The best-docked antagonist bioactive ligand conformation is shown in Figure 3. Chalcone is a secondary metabolite and its derivatives have several anticancer activities. In addition, other advantages of chalcone are ic hardly interact with DNA and less mutagenic (Xu *et al*., 2015). Both synthesis and natural products chalcones have been proven in the various studies for important pathway or molecular targets in cancers. Chalcone has the advantages of being inexpensive, easily available, and less toxic. Moreover, chalcones are not difficult to synthesize, which makes them an attractive drug scaffold (Jandial *et al*., 2014). Chalcone was discovered in recent time as a potential and specific inhibitor. However, chalcone has cytotoxic activity. Replacement at positions 3, 4, and 5 of chalcone induced cytotoxic activity (Rangel *et al*., 2013). Modifying the carbonyl group of the chalcone with pyrazole group was proved to induce better cytotoxicity against many cancer cell line (Hawash *et al*., 2017). The aim of this study was to find the best-modified chalcone that binds well with the ERα and to focus on the modification at the position of the carbonyl group by pyrazole derivate to increase the hydrophobicity.

Chalcone modification based on ERα interaction with 4-OHT (Fig. 2a), molecular bond acceptors, and less than five hydrogen bond donor base on Lipinski's Rule of Five. Table 3 below shows molecular docking results using Autodock 4.2. The dimethylamino ethoxy group of 4-OHT elongated than the

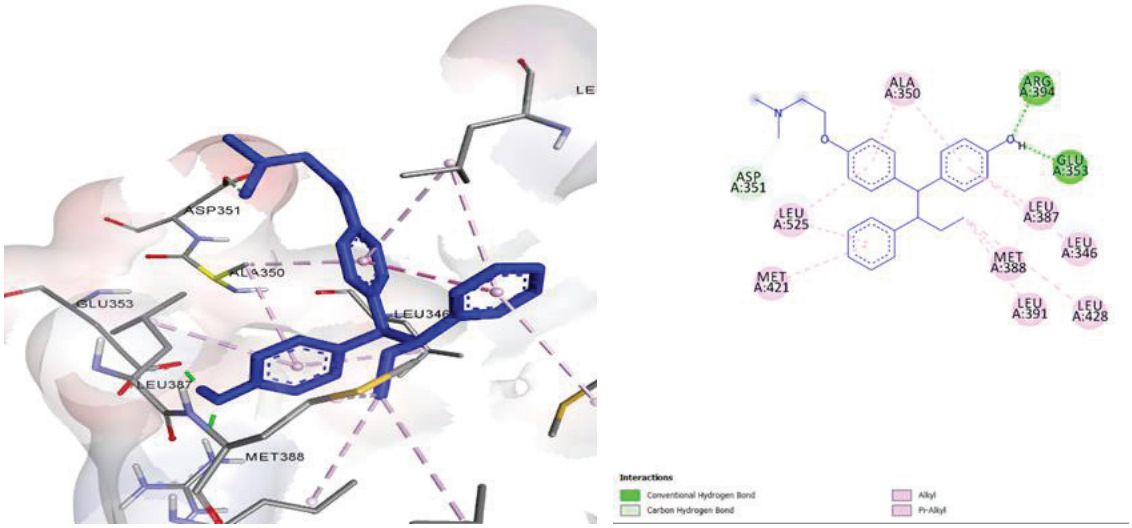


Figure 3. Result of molecular docking method validation. The best conformation of docking pose of 4_OHT with ERα (3 ERT) using Autodock 4.2.

Table 3. Molecular docking result.

Molecule name	Free energy binding (ΔG) kcal/mol	Amino acids residues
Tamoxifen	−10.15	Arg394, Glu353, Leu387, Ala350, Leu346, Met343, Met421, Leu525, Thr347, Asp351
ChalcEA	−8.91	Leu525, Met421, Leu346, Glu353, Leu391, Met388, Leu387, Ala350
Modifina1	−10.51	Glu353, Arg394, Leu346, Leu387, Met522, Met421, Ala350, Met421, Met522, Leu525, Met343
Modifina2	−9.71	Arg394, Leu387, Leu391, Leu525, Ala350, Trp383, Leu354, Leu349, Leu384, Leu346, Met421
Modifina3	−11.07	Arg394, Leu387, Leu346, Glu353, Leu525, Leu536, Trp383, Leu354, Ala350, Leu384, Leu349, Leu391
Modifina4	−10.11	Glu419, Glu353, Arg394, Leu387, Leu346, Met343, Leu391, Ala350, Met421
Modifina6	−9.89	Leu346, Arg394, Glu353, Met421, Leu525, Met343, Leu391, Ala350, Leu387
Modifina8	−9.6	Met343, Leu387, Met421, Leu391, Leu384, Ala350, Leu525
Modifina9	−9.28	Leu346, Asp351, Leu525, Phe404, Leu391, Leu349, Ala350, Met343, Met421

carbonyl group of ChalcEA. This unlikeness makes it possible for higher computer free energy of binding (ΔG) of 4-OHT lower than ChalcEA (Muchtaridi *et al*., 2017). All of the modified chalcones have a free energy of binding (ΔG) lower than ChalcEA, except Modifina8 and 9, formed hydrogen bonds with Arg394 similar to 4-OHT. Modifina3 was the best compound with lowest free energy binding, so it has the highest affinity to bind properly with the ERα and interacted with Arg394, Leu387, Leu346, Glu353, Leu525, Leu536, Trp383, Leu354, Ala350, Leu384, Leu349, and Leu391. However, Modifina3 has more Leusin, which is hydrophobic amino acids residue, and amino acids residue polar Met421, Met343, and Asp351 interacted with 4-OHT which made the free energy binding (ΔG) of Modifina3 lower than 4-OHT which cause Modifina3 has the highest affinity to ERα. The hydrogen bonds formation with Arg394 and Glu353 is essential to ERα.

Interpretation of the *in silico* results

Modifina1 and Modifina3 show the best two pharmacophore fit scores (Fig. 4) of derivatives and Modifina3 has lowest ΔG free energy of binding (−11.07 kcal.mol^{-1}) than Modifina1 (−10.51 kcal.mol^{-1}) and 4-OHT (−10.15 kcal.mol^{-1}), respectively.

Figure 3 shows that the modification of carbonyl groups on Modifina1 blocking complete interaction with all three hydrophobic features of 4-OHT (Fig. 4a). On the other hand, the meta position of methyl groups on the ring of Modifina3 enables better alignment with the center of the hydrophobic features, thus resulting in a better pharmacophore fit score (Fig. 4b)

Modifina3 formed 10 hydrophobic interactions with Leu387, Leu346, Leu525, Leu536, Leu354, Ala350, Leu384, Leu349, Tryptopan383, and Leu391, and two hydrogen bonds with Leu387 and Arg394 (Fig. 5). The interaction of hydrogen bonds with Glu353 and Arg394 is necessary for binding to ER (Wang *et al*., 2010), which is in suitable with the results of previous docking studies requiring chalcone (Vasanthi, Reuben and Usha, 2016). Hydrophobic interactions were the most important site of binding of ChalcEA derivatives with ERα. Role of aromatics ring in binding interaction was shown in Fig. 4.

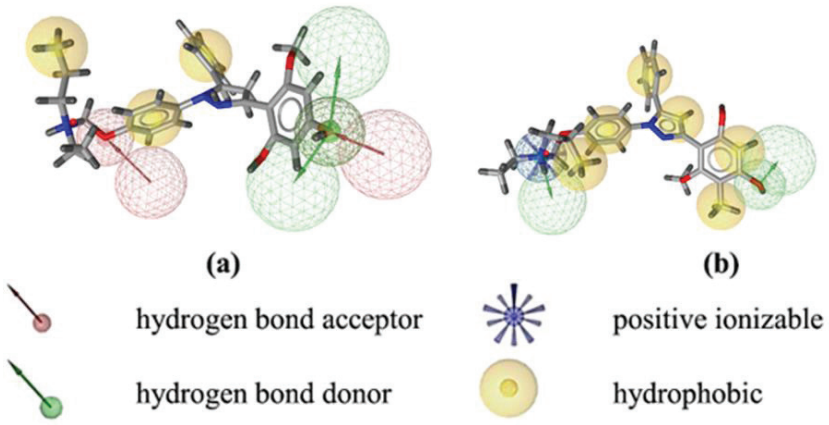


Figure 4. Fit of the (a) Modifina1 and (b) Modifina3 were derived from 4-OHT pharmacophore models with ERα (3ERT) by LigandScout 4.1 Advanced. Virtual screening was conducted leaving at least two features out.

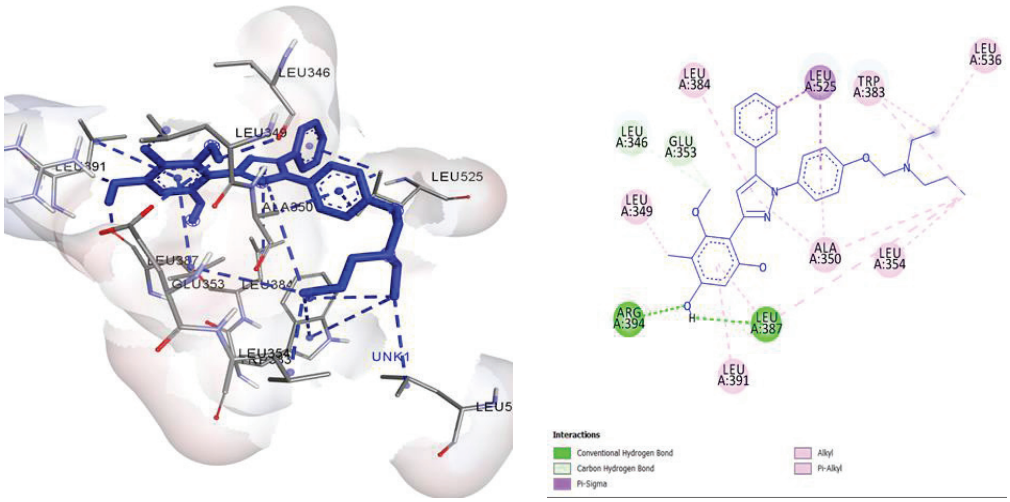


Figure 5. Molecular docking result of Modifina3. There is hydrogen bond with Arg394, Glu353, and Leu387 amino acids residues.

The cyclic compound of Modifina3 formed CH-pi hydrophobic binding with Ala350, Met421, and Leu525 (Fig. 6). In the previous study, the substituent of ring A and B of chalcone plays a role in the activity (Wang *et al*., 2010).

In silico prediction of absorption, distribution, and toxicity

In the latest decades, *in silico* absorption, distribution, metabolism, excretion/toxicity (ADME/T) modeling as a computation method for rational drug design with various models has been used by pharmaceutical scientists. The high analysis results and the efficient cost of the method made the simultaneous investigations of the compounds, including pharmacokinetic profile, safety, and activity. The compounds that used as a drug must have good ADME properties. The complex mechanism of *in vivo* process of a drug made the ADME prediction method more simple using major component or as several single processes (Thomas *et al*., 2008).

Determination of permeation across a monolayer of the human adenocarcinoma cell line, Caco-2 is a popular surrogate for ligands permeation across the human intestinal epithelium. Human intestinal absorption (HIA%) of compounds was predicted very important for identifying a potential drug candidate. Among HIA of all the modified chalcones, more than 90% represented well-absorbed compounds (70%–100%) in the intestines. The parameter of $CaCO_2$ cell permeability capability shows all the compounds have a medium permeability (20%–70%) (Thomas *et al*., 2008).

Protein plasma binding (PPB) is a significantly pharmacokinetic property of compounds in drug discovery and design. Effective and efficient *in silico* method for a pharmacokinetic profile of compounds. PPB is exactly related to drug distribution, metabolism, and clearance, which influence the efficacy and potency of drugs (Sun *et al*., 2018). A degree of PPB of a compound influences on the drug disposition, its action, and efficacy. PPB of all the compounds in Table 4 was more than 90%, which means all compounds will be well distributed in the body, chemical's strongly bound. However, transport across cell membranes or diffusion, and bind with a pharmacological target (receptor) only requires the unbound drug. If there is reversible

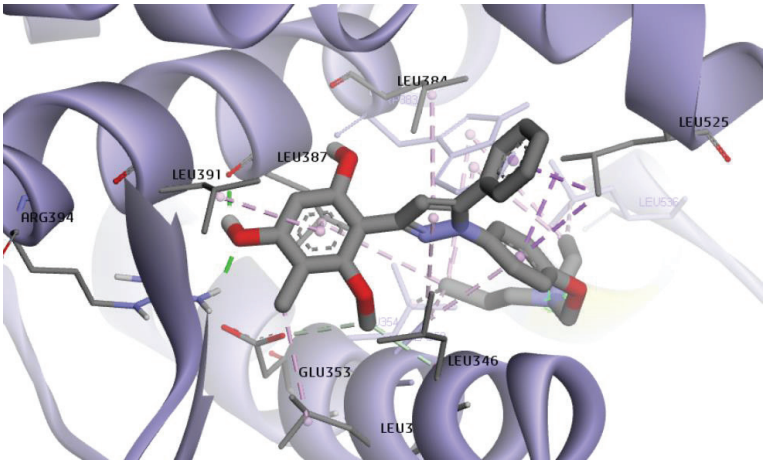


Figure 6. Interaction of Modifina3 within the binding site of ERα. Ion-ion interaction, hydrogen bond, and pi-alkyl interactions are represented in purple and green colored dashed lines, respectively.

Table 4. ADME prediction result.

Compound	Absorption		Distribution
	HIA (%)	$CaCO_2$ cell (nm/second)	PPB (%)
Modifina1	95.77	40.57	99.47
Modifina2	95.77	45.34	90.97
Modifina3	95.90	46.95	93.55
Modifina4	92.75	12.09	100
Modifina6	93.29	13.16	100
Modifina7	100	44.82	96.14
Modifina8	95.47	42.01	100
Modifina9	95.47	47.42	97.74
Modifina10	96.57	44.42	100
Modifina11	97.88	21.53	100
ChalcEA	93.24	20.13	91.52
Tamoxifen	97.17	47.76	100

Table 5. Toxtree result.

Parameter	Tamoxifen	ChalcEA	Modifina3
Cramer rules	**High class 3**	**High class 3**	**High class 3**
Kroess thresholds of toxicological concern	Negligible risk	Negligible risk	Negligible risk
Decision tree	Risk assessment requires compound specific toxicity data	Risk assessment requires compound specific toxicity data	Risk assessment requires compound specific toxicity data
Benigni and Bosa	Negative for genotoxic and nongenotoxic carcinogenicity	Negative for genotoxic and nongenotoxic carcinogenicity	Negative for genotoxic and nongenotoxic carcinogenicity

protein plasma-drug binding, there will be a chemical balance between protein plasma-drug binding with the unbound drug. The bound part of the drug will become a reservoir and then release as unbound drug to maintain equilibrium.

In silico toxicity prediction

Prediction toxicity using a computational method is needed in the early step of drug development. Toxicity is the concentration level of the compounds which disturbs an organism or its substructure (Wang *et al*., 2015).

In the drug design and development, genotoxicity is the critical point in the *in vitro* toxicity assay. Information Technology development and growing experimental data made *in silico* screening and toxicity prediction interesting (Wang *et al*., 2015).

In silico toxicity risk was performed to check the genotoxic and carcinogenic effect of the compound. In Table 5 shown that the Modifina3 has nongenotoxic and non-carcinogenicity properties.

In order to improve the patient's quality of life through reduced side effect of the chemotherapeutic agents for breast cancer, Modifina3 is possibly potent for that activity. The molecular docking results showed that most of the residue which interacted with Modifina3 and 4-OHT were Arg394, Glu353, Leu387, Ala350, Leu346, and Leu525 almost similar. Agonistic effect of 4-OHT can be eliminated by Modifina3. In the previous study, molecular dynamic simulations on ERα showed that between Helix-11 and Helix-12 was very adjustable which made support the different conformations (for examples: apo-, agonist-, and antagonist-form) (Musfiroh *et al*., 2013). A hydrophobic cavity of the ligand binding domain (LBD) of ERα consists of residues from helices 3, 6, 7, 8, 11, and 12 (Morris *et al*., 2009). Based on the previous study, residues 536-544 in Helix-12 of the ER is the important thing responsible for the activity of agonist or antagonist of a ligand. For example, an antagonist such as 4-OHT is accommodated by helix-12 of the ERα LBD occludes the co-activator recognition channel resulting in antagonist activity.

As shown in Fig. 4, Modifina3 lacks hydrogen bond with His524. Interestingly, the loss of hydrogen bonding with His524 when 4-OHT is bound while agonist activity existing when the hydrogen bonding with His524 and the ERα agonist estradiol (Muchtaridi *et al*., 2014; 2017; Musfiroh *et al*., 2013). It is predicted that the Modifina3 has no agonist effect on ERα due to the loss of the hydrogen bonding with His524 when Modifina3 is bound.

CONCLUSION

Modifina3 has the potential to be a novel therapeutic compound for targeted breast cancer treatment due to its highest affinity, with high-class category toxicity but still can be used as a compound for drugs. Based on the further biological investigation, Modifina3 represents rational computationally designed compound prioritized.

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