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Design and molecular docking of novel 5-*O*-Benzoylpinostrobin derivatives as anti-breast cancer

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ABSTRACT

Objectives: This study aims to design the 5-*O*-Benzoylpinostrobin derivative with the most potent anti-breast cancer activity along with the most dominant type of receptor for the compound. **Methods:** Molecular docking was performed using AutoDock 4.2.6 on four types of breast cancer receptors consisting of estrogen receptors α and β, progesterone receptors, and human epidermal growth factor receptor 2 (HER2), both in the form of binding to agonist and antagonist ligands. The parameters used were the free energy of binding (ΔG) and the dissociation constant (K_i) as an affinity marker and similarity of amino acid residues as interactions similarity indicator. **Results:** The benzoylpinostrobin derivative shows affinities for all receptors, but the highest was shown against HER2 receptors by 4-Nitro-5-*O*-benzoylpinostrobin. The ligand provided the most negative ΔG and the lowest K_i toward the antagonist form of HER2 with −12.79 kcal/mol and 0.42 nM, respectively. That affinity is 4 times higher than lapatinib, which is known as a potent HER2 inhibitor. Interestingly, the ligand has fewer Van der Waals interactions with amino acids than lapatinib, but the affinity shown is higher. **Conclusion:** Based on the study result, it can be considered that 4-Nitro-5-*O*-benzoylpinostrobin was the most potential modifications of pinostrobin as anti-breast cancer, especially for HER2-positive breast cancer.

Keywords: Benzoylpinostrobin, breast cancer, human epidermal growth factor receptor 2, molecular docking, pinostrobin

INTRODUCTION

Pinostrobin, a flavanone contained in *Boesenbergia pandurata*, is a marker compound for these plants. The pinostrobin content is the most abundant in the rhizome compared to other secondary metabolites, including other well-known active metabolites such as pinocembrin and panduratin A.[1] Pinostrobin is known to have various pharmacological activities and has been proven through laboratory tests, including anti-inflammatory,[2] antiproliferative,[3] antimicrobial,[4,5] anti-ulcer,[6] and anticancer.[7-10]

Among the various pharmacological potentials, the anticancer activity of pinostrobin is one of the most interesting and has been studied previously. Research by Sukardiman *et al*.[8] showed that pinostrobin isolated from the *B. pandurata* rhizome had cytotoxic activity against fibrosarcoma in mice induced by carcinogens, while the study of Junior[9] shows that pinostrobin has a very potent antiproliferative effect on breast cancer cells and leukemia. Other studies by Atun and Arianingrum[10] also showed that the cytotoxic activity of pinostrobin in several breast cancer cells was better than the *B. pandurata* chloroform extract, especially for T47D breast cancer cells. Although promising, the development of pinostrobin for the treatment of cancer, especially breast cancer, is still slow. Even though it has potential, the activity shown by pinostrobin is still lower than the breast anticancer drugs currently available on the market.

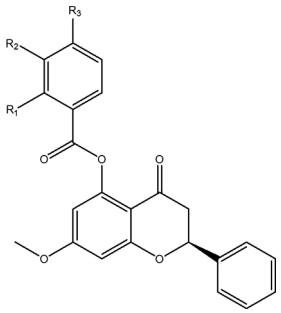
The method that can be done to increase the activity of a compound is to design and synthesize derivatives using certain functional groups to obtain derivative compounds with higher pharmacological activity.[11] The selection of modified groups and functional groups that will be added is a vital point in the design of derivatives of active metabolites of medicinal plants.[12] Determination of functional groups to be added is generally based on consideration of the pharmacological activities to be achieved, where the functional groups must be synergistic with the pharmacological activities of metabolites.[13] In the case of

pinostrobin, a functional group with potential cytotoxic activity can be added to one of the groups of pinostrobin.

The design and synthesis of pinostrobin derivatives to improve anticancer activity have been done before, for example, in the study of Poerwono *et al.*,[14] who reported a modification of the addition of the prenyl group to increase the cytotoxic activity of the pinostrobin derivative on SK-BR-3, MCF-7, PC-3, and Colo-320DM cancer cells. The exciting thing is that the previous structural modification was very rarely carried out on the only hydroxyl group of pinostrobin. One of them that is well known and is widely developed and can be added to that position is the benzoyl group. Some studies show that benzoyl derivatives from a compound can increase their cytotoxic activity compared to parent compounds.[15] Rationally, the addition of a benzoyl group to pinostrobin to form a 5-*O*-Benzoylpinostrobin derivative is predicted to increase its cytotoxic activity compared to the parent pinostrobin.

This study aims to determine the derivative of 5-*O*-Benzoylpinostrobin with the highest potential anti-breast cancer and determine the type of breast cancer receptor that is most sensitive to the compound. Molecular modeling is carried out using variations in substituents in the benzoyl group of the 5-*O*-Benzoylpinostrobin derivative and the method used to predict activity is molecular docking. The molecular docking is an effective method predicted interactions and preferred orientation when bound of ligand to macromolecular target to create a stable and preferred complex.[16] Selection of substituents was carried out based on the Topliss model to obtain a rational approach considering lipophilic and electronic properties.[17] There are three main types of receptors that have a significant role in the occurrence of breast cancer consisting of estrogen (ER) and progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2). Overexpression of all three or one of these receptors is one of the leading causes of breast cancer.[18] In this study, all three receptors were used as test receptors.

Table 1: The 5-*O*-Benzoylpinostrobin derivatives test compound



Compounds name	Functional group		
	R_1	R_2	R_3
5-*O*-Benzoylpinostrobin	H	H	H
2-Chloro-5-*O*-benzoylpinostrobin	Cl	H	H
3-Chloro-5-*O*-benzoylpinostrobin	H	Cl	H
4-Chloro-5-*O*-benzoylpinostrobin	H	H	Cl
2,4-Dichloro-5-*O*-benzoylpinostrobin	Cl	H	Cl
3,4-Dichloro-5-*O*-benzoylpinostrobin	H	Cl	Cl
4-Bromo-5-*O*-benzoylpinostrobin	H	H	Br
4-Fluoro-5-*O*-benzoylpinostrobin	H	H	F
4-Nitro-5-*O*-benzoylpinostrobin	H	H	NO_2
4-Methyl-5-*O*-benzoylpinostrobin	H	H	CH_3
4-Methoxy-5-*O*-benzoylpinostrobin	H	H	OCH_3
4-Trifluoromethyl -5-*O*-benzoylpinostrobin	H	H	CF
4-*t*-Butyl-5-*O*-benzoylpinostrobin	H	H	$(CH_3)_3$

MATERIALS AND METHODS

Materials

The hardware used is the ASUS A46CB series Ultrabook with an Intel™ Core i5-3337U@1.8 GHz and Windows 7 Ultimate 64-bit SP-1 operating system. The software used is HyperChem 7.5 from Hypercube, Inc., Open Babel 2.4.1 from OpenBabel. org., AutoDockTools 1.5.6, and AutoDock 4.2.6 software from The Scripps Research Institute, Inc.[19] Information on three-dimensional structures of receptor proteins obtained from the website of Protein Data Banks (http://rcsb.org).

Ligands Preparation

The test ligands used consisted of pinostrobin and also a 5-*O*-Benzoylpinostrobin parent and substituted, as shown in Table 1. The two-dimensional structure was sketched using HyperChem 7.5 from Hypercube, Inc. with geometry optimization *ab initio* basis set 3–21. Optimization was done by Polak-Ribiere algorithm and RMS Gradient 0.1 kcal/mol. Optimization with large basis sets was carried out to obtain the ideal molecular conformation which approves conformation of these compounds in nature.[20] The optimized structure then changes the format from.hin to.pdb using Open Babel 2.4.1 software.[21] The use of Open Babel makes it very easy to change ligands from one format to another without losing their ideal conformation.[22] Docking software used was AutoDock 4.2.6 from The Scripps Research Institute. One of the advantages of AutoDock 4 is that it can provide predictive value for the dissociation constant (K_i), which can give predictions for the *in vitro* analysis process later. All ligands are then given the charge and set torque using AutoDockTools 1.5.6.[23]

Receptors Preparation

Receptors are downloaded in the format. pdb then the unused part including water molecules is removed, added non-polar hydrogen, given charged, and arranged size and coordinate grid using AutoDockTools 1.5.6.[19] The size and coordinates of the grid box are adjusted automatically with the ligand cocrystal position of each receptor by making the ligand position the center of the grid box.[24] Seven protein structures are used as receptors consisting of ERα which binds to agonist (PDB ID 1QKU) and antagonist (PDB ID 3ERT) ligands, ERβ with agonist (PDB ID 5TOA) and antagonist (PDB ID 1L2J) ligands, PR with agonist (PDB ID 3D90) and antagonist (PDB ID 2OVM) ligands, and also HER2 with antagonist ligand (PDB ID 3PP0). All of these receptors are known to have a very significant role in the treatment of breast cancer as a target of therapy and have been previously investigated as target

receptors for anti-breast cancer compounds. The receptor part used is an active site that has a cocrystal ligand, both in the form of natural ligand and known agonist or antagonist compounds. Especially for HER2 receptors as comparative ligands also used lapatinib, a HER2 inhibitor used in clinical therapy for HER2-positive breast cancer.

Validation of Docking Protocol

The docking process is preceded by a validation process, with the re-docking method using cocrystal ligands which have been extracted from receptors as test ligands and ligand cocrystal location as the active site.[25] The parameters observed in the

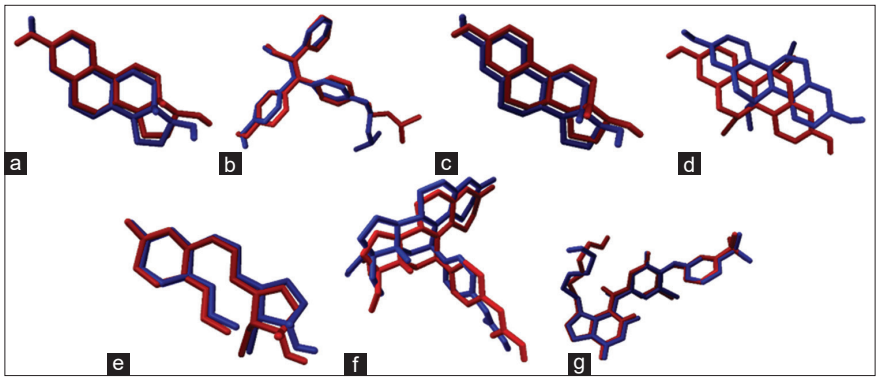


Figure 1: Overlays of redocking (blue) ligands with cocrystal ligands from crystallography (red) at receptors (a) 1QKU, (b) 3ERT, (c) 5TOA, (d) 1L2J, (e) 3D90, (f) 2OVM, and (g) 3PP0

Table 2: Results of the validation process

PDB ID	Cocrystal ligand	Grid Box Size (Å)	Grid Box Position	RMSD (Å)	ΔG (kcal/mol)	K_i (nM)	Amino acid residues	Number of hydrogen bonds
1QKU	Estradiol	60×60×60	X: 104.98 Y: 14.819 Z: 23.484	0.716	−10.62	16.35	353-Glu, 388-Met, 391-Leu, 404-Phe, 424-Ile, 521-Gly, 524-His, 525-Leu	3
3ERT	4-Hydroxytamoxifen	60×60×60	X: 30.01 Y: −1.913 Z: 24.207	0.993	−11.83	2.12	343-Met, 346-Leu, 347-Thr, 350-Ala, 351-Asp, 353-Glu, 383-Trp, 387-Leu, 394-Arg, 421-Met, 428-Leu, 521-Gly	2
5TOA	Estradiol	60×60×60	X: 19.789 Y: 43.343 Z: 15.491	0.727	−11.1	7.35	305-Glu, 336-Met, 340-Met, 343-Leu, 346-Arg, 356-Phe, 376-Ile, 380-Leu, 472-Gly, 475-His	3
1L2J	Tetrahydrochrysene	60×60×60	X: 31.926 Y: 82.682 Z: −11.054	1.431	−11.18	6.38	336-Met, 339-Leu, 340-Met, 343-Leu, 346-Arg, 376-Ile, 380-Ile, 472-Gly, 475-His	2
3D90	Levonorgestrel	60×60×60	X: −2.577 Y: −7.679 Z: 25.794	0.487	−10.75	13.21	715-Leu, 718-Leu, 721-Leu, 725-Gln, 759-Met, 766-Arg, 890-Tyr, 891-Cys	1
2OVM	Asoprisnil	60×60×60	X: −29.026 Y: 52.534 Z: 45.332	1.056	−12.99	0.3	718-Leu, 719-Asn, 722-Gly, 723-Glu, 725-Gln, 755-Trp, 756-Met, 759-Met, 760-Val, 766-Arg, 778-Phe, 797-Leu, 801-Met, 887-Leu, 890-Tyr, 891-Cys, 894-Thr	1
3PP0	SYR127063	60×60×60	X: 16.622 Y: 17.394 Z: 26.218	0.89	−10.39	24.07	734-Val, 751-Ala, 770-Glu, 774-Met, 785-Leu, 798-Thr, 799-Gln, 800-Leu, 801-Met, 849-Arg, 852-Leu, 862-Thr, 863-Asp	0

validation process are root-mean-square deviation (RMSD) of each ligand cocrystal at the selected binding site. The RMSD score illustrates the average difference in ligand atom position redocking with crystallographic results.[26] Docking software is preferred to predict results from experimental positions with RMSD no more than 2.0 Å. Smaller RMSD shows that the position of the redocking result is closer to the crystallographic ligand.[27]

Molecular Docking

The main objective of the molecular docking is to identify the energetically favorable binding modes of test ligands into the target receptor's binding site.[28] Docking for both test ligands performed in the same way as the validation process with similar size and position of grid box for each cocrystal ligands. To ensure that the test ligand binds to the ideal position for each ligand, the binding site orientation is carried out by the blind docking method, and the results of all test ligands show cavity with the highest affinity equal to the comparative ligand. For this reason, the same grid box size is used for the docking process with the validation process. Docking search parameter used are Lamarckian genetic algorithm with the number of genetic algorithm 100 runs, population size 150, the maximum number of energy evaluation is medium with 2,500,000, the maximum number of generations 27,000, with the default docking parameter used for run options. The primary parameter used in the docking process was the free energy of binding (ΔG), the dissociation constant (K_i), amino acid residues, and the number of hydrogen bonds.[29] ΔG and K_i scores determine ligand affinity to the receptor in the docking method. The more negative ΔG and lower K_i indicated higher ligand affinity toward the active site of the used receptor. All test ligand then compared with the validation result of cocrystal ligand to determine the potency of both test ligands as each receptors inhibitor.[30] The amino acid residues of both test ligands 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.[31] The two-dimensional visualization of ligand-receptor interactions was performed with Discovery Studio Visualizer v.19.1.0.18287 from BIOVIA.

RESULTS AND DISCUSSION

Validation is carried out at the active site of each receptor using the cocrystal ligand as a reference to determine the size and coordinates of each box.[32] Redocking results from this study were provided RMSD score in the range between 0.487 Å and 1.431 Å, indicated that each receptor used was valid for docking purposes.[33] Visualization of ligand overlays resulting from redocking with cocrystal ligands from crystallographic results is presented in Figure 1. Overall, the redocking process shows results that can be used for the docking process. Other parameters observed in the validation process are ΔG, K_i, amino acid residues, and the number of hydrogen bonds, including size and grid coordinates, as shown in Table 2.

Test ligands were sketched then performed geometry optimization using the Hartree-Fock method with basis set 3–21G. This method was *ab initio* approximation with a high confidence rate for *in silico* analysis.[34] Docking was performed using AutoDock 4.2.6 at each binding site with 100 genetic algorithms runs to improve the accuracy of docking results. The center of grid box is determined by the blind docking method using a large grid box (60 Å × 60 Å × 60 Å) to ensure that the binding site used is the one that has the highest suitability for each receptor.[29] For each test ligand, one poses with the most negative ΔG, and lowest K_i was selected as representatives of test ligand.[34] The ΔG and K_i values obtained from docking results of all test ligands to each binding sites were compared with each other, as shown in Table 3, while the comparison of amino acid residues and the number of hydrogen bonds from ligands with the highest affinity with references ligands are presented in Table 4.

The highest affinity prediction is shown by 4-Nitro-5-*O*-benzoylpinostrobin to the HER2 receptor, with an affinity predicted to be higher than lapatinib. The main difference in the position of the ligand from docking results mainly is the 4-Nitrobenzoyl group, which interacts in a different position from the position of the entire functional group of lapatinib. To facilitate observation, Discovery Studio Visualizer v.19.1.0.18287 is used to obtain a two-dimensional ligand-receptor interaction display. Visualization of the docking results for 4-Nitro-5-*O*-benzoylpinostrobin compared to lapatinib as well as pinostrobin, 5-*O*-Benzoylpinostrobin, and SYR127063 is presented in Figure 2.

The docking results show some interesting points, but before concluding on ligands with the highest affinity for each receptor, certain things must be considered. First, both ER and PR are hormonal receptors so that they can interact with both agonist and antagonist ligands at the same binding site. In other words, to assess the affinity of a ligand for ER and PR through a docking method, it is necessary to compare the affinity with the receptor that binds to the agonist and antagonist ligands.[35] Ideally, because ER/PR-positive breast cancer results from ER and PR overexpression, the test ligand must have a higher affinity as an antagonist than agonist ligands. The comparison of K_i value between agonist and antagonist ligand, as listed in Table 3, was compared to making the assessment. The comparison value higher than 1 indicates that the ligand tends to be an antagonist than an agonist ligand. The higher comparative value indicates higher affinity as the antagonist ligand.

At the ERα receptor, this requirement is only fulfilled in the compounds 3,4-Dichloro-5-*O*-benzoylpinostrobin, 3-Chloro-5-*O*-benzoylpinostrobin, 4-Bromo-5-*O*-benzoylpinostrobin, and 4-Chloro-5-*O*-benzoylpinostrobin. Comparison of the agonist: Antagonist affinity with the highest value indicated by 3,4-Dichloro-5-*O*-benzoylpinostrobin, with a comparison value of 41.01/19.39 = 2.12. These values indicate that 3,4-Dichloro-5-*O*-benzoylpinostrobin has a two-fold higher tendency as an antagonist than as an agonist ligand. However, the K_i antagonist value of 3,4-Dichloro-5-*O*-benzoylpinostrobin is higher than the cocrystal ligand, 4-Hydroxytamoxifen, with a comparison value of 19.38/2.12 = 9.14. In other words, 4-Hydroxytamoxifen is still 9 times more potent as ERα antagonist than 3,4-Dichloro-5-*O*-benzoylpinostrobin. The 4-Hydroxytamoxifen itself is a drug of choice for the treatment of ER-positive breast cancer mainly due to ERα overexpression.

Table 3: Comparison of ΔG and K_i values from docking results

Receptors	Ligand	ΔG (kcal/mol)	K_i (nM)
1QKU	2-Chloro-5-*O*-benzoylpinostrobin	−11.34	4.9
	3-Chloro-5-*O*-benzoylpinostrobin	−11.2	6.16
	4-Nitro-5-*O*-benzoylpinostrobin	−10.66	15.33
	5-*O*-Benzoylpinostrobin	−10.65	15.53
	Co-crystal (Estradiol)	−10.62	16.35
	2,4-Dichloro-5-*O*-benzoylpinostrobin	−10.62	16.55
	4-Methoxy-5-*O*-benzoylpinostrobin	−10.44	22.08
	4-Fluoro-5-*O*-benzoylpinostrobin	−10.36	25.26
	4-Methyl-5-*O*-benzoylpinostrobin	−10.1	39.26
	3,4-Dichloro-5-*O*-benzoylpinostrobin	−10.08	41.01
	4-Bromo-5-*O*-benzoylpinostrobin	−10.03	44.67
	4-Chloro-5-*O*-benzoylpinostrobin	−9.98	48.68
	4-Trifluoromethyl-5-*O*-benzoylpinostrobin	−9.89	56.21
	4-*t*-Butyl-5-*O*-benzoylpinostrobin	−9.59	93.96
	Pinostrobin	−7.97	1450
3ERT	Co-crystal (4-hydroxytamoxifen)	−11.83	2.12
	3,4-Dichloro-5-*O*-benzoylpinostrobin	−10.52	19.38
	3-Chloro-5-*O*-benzoylpinostrobin	−10.24	31.25
	4-Methoxy-5-*O*-benzoylpinostrobin	−10.21	32.72
	2,4-Dichloro-5-*O*-benzoylpinostrobin	−10.16	35.89
	4-Bromo-5-*O*-benzoylpinostrobin	−10.14	37.01
	4-Chloro-5-*O*-benzoylpinostrobin	−10.08	40.65
	4-Methyl-5-*O*-benzoylpinostrobin	−10.07	41.23
	2-Chloro-5-*O*-benzoylpinostrobin	−10.01	46.21
	4-*t*-Butyl-5-*O*-benzoylpinostrobin	−9.91	54.66
	4-Nitro-5-*O*-benzoylpinostrobin	−9.75	71.66
	4-Trifluoromethyl-5-*O*-benzoylpinostrobin	−9.48	112.79
	5-*O*-Benzoylpinostrobin	−9.47	114.24
	4-Fluoro-5-*O*-benzoylpinostrobin	−9.45	117.51
	Pinostrobin	−7.21	5150
5TOA	Co-crystal (Estradiol)	−11.1	7.35
	3-Chloro-5-*O*-benzoylpinostrobin	−9.27	160.65
	2,4-Dichloro-5-*O*-benzoylpinostrobin	−9.25	166.34
	2-Chloro-5-*O*-benzoylpinostrobin	−9.92	179.04
	5-*O*-Benzoylpinostrobin	−9.09	217.65
	4-Methoxy-5-*O*-benzoylpinostrobin	−8.97	264.58
	4-Methyl-5-*O*-benzoylpinostrobin	−8.71	411.57
	4-Fluoro-5-*O*-benzoylpinostrobin	−8.71	412.52
	4-Trifluoromethyl-5-*O*-benzoylpinostrobin	−8.6	497.47
	4-Bromo-5-*O*-benzoylpinostrobin	−8.38	716.93
	4-Chloro-5-*O*-benzoylpinostrobin	−8.29	831.23
	4-Nitro-5-*O*-benzoylpinostrobin	−8.14	1080
	Pinostrobin	−7.85	1770
	3,4-Dichloro-5-*O*-benzoylpinostrobin	−7.69	2300
	4-*t*-Butyl-5-*O*-benzoylpinostrobin	−7.26	4760

(Contd...)

Table 3: *(Continued)*

Receptors	Ligand	ΔG (kcal/mol)	K_i (nM)
1L2J	4-Nitro-5-*O*-benzoylpinostrobin	−12.23	1.09
	4-*t*-Butyl-5-*O*-benzoylpinostrobin	−11.3	5.18
	Co-crystal (Tetrahydrochrysene)	−11.18	6.38
	2-Chloro-5-*O*-benzoylpinostrobin	−10.94	9.54
	4-Methyl-5-*O*-benzoylpinostrobin	−10.82	11.81
	3-Chloro-5-*O*-benzoylpinostrobin	−10.77	12.74
	3,4-Dichloro-5-*O*-benzoylpinostrobin	−10.75	13.17
	4-Chloro-5-*O*-benzoylpinostrobin	−10.62	16.46
	4-Bromo-5-*O*-benzoylpinostrobin	−10.4	23.97
	2,4-Dichloro-5-*O*-benzoylpinostrobin	−10.33	26.67
	5-*O*-Benzoylpinostrobin	−10.32	27.19
	4-Fluoro-5-*O*-benzoylpinostrobin	−10.2	33.37
	4-Trifluoromethyl-5-*O*-benzoylpinostrobin	−10.2	33.48
	4-Methoxy-5-*O*-benzoylpinostrobin	−10.17	35.18
	Pinostrobin	−8.37	734.51
3D90	2,4-Dichloro-5-*O*-benzoylpinostrobin	−11.43	4.19
	3,4-Dichloro-5-*O*-benzoylpinostrobin	−11.3	5.2
	4-Nitro-5-*O*-benzoylpinostrobin	−11.28	5.37
	4-Bromo-5-*O*-benzoylpinostrobin	−11.08	7.59
	4-Methyl-5-*O*-benzoylpinostrobin	−11	8.6
	4-Chloro-5-*O*-benzoylpinostrobin	−11	8.61
	4-Methoxy-5-*O*-benzoylpinostrobin	−10.94	9.63
	3-Chloro-5-*O*-benzoylpinostrobin	−10.85	11.16
	2-Chloro-5-*O*-benzoylpinostrobin	−10.82	11.68
	4-Trifluoromethyl-5-*O*-benzoylpinostrobin	−10.75	13.17
	Co-crystal (Levonorgestrel)	−10.75	13.21
	4-*t*-Butyl-5-*O*-benzoylpinostrobin	−10.58	17.42
	4-Fluoro-5-*O*-benzoylpinostrobin	−10.49	20.44
	5-*O*-Benzoylpinostrobin	−10.44	22.4
	Pinostrobin	−7.82	1840
2OVM	Co-crystal (Asoprisnil)	−12.99	0.3
	4-Nitro-5-*O*-benzoylpinostrobin	−12.97	0.31
	4-*t*-Butyl-5-*O*-benzoylpinostrobin	−11.07	7.65
	3,4-Dichloro-5-*O*-benzoylpinostrobin	−10.37	24.95
	3-Chloro-5-*O*-benzoylpinostrobin	−10.07	41.67
	4-Methoxy-5-*O*-benzoylpinostrobin	−10.05	42.91
	2-Chloro-5-*O*-benzoylpinostrobin	−9.92	53.47
	4-Bromo-5-*O*-benzoylpinostrobin	−9.88	56.79
	2,4-Dichloro-5-*O*-benzoylpinostrobin	−9.88	57.18
	4-Chloro-5-*O*-benzoylpinostrobin	−9.85	60.66
	4-Trifluoromethyl-5-*O*-benzoylpinostrobin	−9.73	73.39
	5-*O*-Benzoylpinostrobin	−9.69	78.43
	4-Methyl-5-*O*-benzoylpinostrobin	−9.65	84.31
	4-Fluoro-5-*O*-benzoylpinostrobin	−9.37	134.39
	Pinostrobin	−7.76	2060

(Contd...)

Table 3: *(Continued)*

Receptors	Ligand	ΔG (kcal/mol)	K_i (nM)
3PP0	4-Nitro-5-*O*-benzoylpinostrobin	−12.79	0.42
	4-*t*-Butyl-5-*O*-benzoylpinostrobin	−12.38	0.85
	Lapatinib	−11.97	1.69
	3,4-Dichloro-5-*O*-benzoylpinostrobin	−11.08	7.62
	2,4-Dichloro-5-*O*-benzoylpinostrobin	−10.87	10.72
	4-Bromo-5-*O*-benzoylpinostrobin	−10.77	12.85
	4-Trifluoromethyl-5-*O*-benzoylpinostrobin	−10.47	21.06
	3-Chloro-5-*O*-benzoylpinostrobin	−10.47	21.07
	4-Methoxy-5-*O*-benzoylpinostrobin	−10.43	22.76
	4-Chloro-5-*O*-benzoylpinostrobin	−10.41	23.23
	2-Chloro-5-*O*-benzoylpinostrobin	−10.41	23.42
	Co-crystal (SYR127063)	−10.39	24.07
	4-Methyl-5-*O*-benzoylpinostrobin	−10.36	25.36
	4-Fluoro-5-*O*-benzoylpinostrobin	−9.93	52.95
	5-*O*-Benzoylpinostrobin	−9.92	53.85
	Pinostrobin	−8.44	651.87

Table 4: Comparison of amino acid residues and the number of hydrogen bonds

Receptor	Amino acid residues			Number of hydrogen bonds	
	Comparative ligand	Ligand with the highest affinity	Similarity (%)	Comparative ligand	Ligand with the highest affinity
1QKU	353-Glu	-	75	3	1
	388-Met	388-Met			
	391-Leu	391-Leu			
	404-Phe	404-Phe			
	424-Ile	-			
	521-Gly	521-Gly			
	524-His	524-His			
	525-Leu	525-Leu			
3ERT	343-Met	-	66.67	2	0
	346-Leu	346-Leu			
	347-Thr	347-Thr			
	350-Ala	350-Ala			
	351-Asp	-			
	353-Glu	353-Glu			
	383-Trp	-			
	387-Leu	387-Leu			
	394-Arg	394-Arg			
	421-Met	421-Met			
	428-Leu	-			
	521-Gly	521-Gly			
5TOA	305-Glu	305-Glu	70	3	1
	336-Met	336-Met			
	340-Met	-			
	343-Leu	343-Leu			
	346-Arg	346-Arg			

(Contd...)

Table 4: *(Continued)*

Receptor	Amino acid residues			Number of hydrogen bonds	
	Comparative ligand	Ligand with the highest affinity	Similarity (%)	Comparative ligand	Ligand with the highest affinity
	356-Phe	356-Phe			
	376-Ile	376-Ile			
	380-Leu	-			
	472-Gly	-			
	475-His	475-His			
1L2J	336-Met	336-Met	88.89	2	1
	339-Leu	339-Leu			
	340-Met	340-Met			
	343-Leu	343-Leu			
	346-Arg	346-Arg			
	376-Ile	376-Ile			
	380-Leu	380-Leu			
	472-Gly	-			
	475-His	475-His			
3D90	715-Leu	715-Leu	62.5	1	0
	718-Leu	-			
	721-Leu	721-Leu			
	725-Gln	725-Gln			
	759-Met	759-Met			
	766-Arg	766-Arg			
	890-Tyr	-			
	891-Cys	-			
2OVM	718-Leu	-	64.71	1	1
	719-Asn	719-Asn			
	722-Gly	722-Gly			
	723-Glu	723-Glu			
	725-Gln	-			
	755-Trp	755-Trp			
	756-Met	756-Met			
	759-Met	759-Met			
	760-Val	760-Val			
	766-Arg	766-Arg			
	778-Phe	778-Phe			
	797-Leu	-			
	801-Met	801-Met			
	887-Leu	-			
	890-Tyr	-			
	891-Cys	891-Cys			
	894-Thr	-			
3PP0 (SYR127063 as comparative ligand)	798-Thr	734-Val	38.46	0	0
	799-Gln	-			
	800-Leu	-			
	801-Met	-			
	849-Arg	-			
	852-Leu	798-Thr			

(Contd...)

Table 4: *(Continued)*

Receptor	Amino acid residues			Number of hydrogen bonds	
	Comparative ligand	Ligand with the highest affinity	Similarity (%)	Comparative ligand	Ligand with the highest affinity
	862-Thr	-			
	863-Asp	-			
		-			
		849-Arg			
		852-Leu			
		-			
		863-Asp			
3PP0 (Lapatinib as comparative ligand)	726-Leu	726-Leu	52.38	0	0
	728-Ser	728-Ser			
	729-Glu	729-Glu			
	734-Val	734-Val			
	751-Ala	-			
	753-Lys	753-Lys			
	774-Met	-			
	785-Leu	-			
	796-Leu	796-Leu			
	798-Thr	798-Thr			
	799-Gln	-			
	800-Leu	-			
	801-Met	-			
	804-Gly	-			
	805-Cys	-			
	849-Arg	849-Arg			
	850-Asn	850-Asn			
	852-Leu	852-Leu			
	862-Thr	-			
	863-Asp	863-Asp			
	864-Phe	-			

However, resistance to 4-Hydroxytamoxifen is currently a problem in its use in therapy.[36]

Exciting results are shown in the ERβ receptor, where all test ligands have a higher affinity as the antagonist than agonist ligands. Comparison of the agonist: Antagonist affinity with the highest value indicated by 4-Nitro-5-*O*-benzoylpinostrobin, with a comparison value of 1080/1.09 = 990.83. These values indicate that 4-Nitro-5-*O*-benzoylpinostrobin has almost a 1000 times higher tendency as an antagonist than as an agonist ligand. Compared to the cocrystal ligand, in this case, tetrahydrochrysene, the K_i antagonist value of 4-Nitro-5-*O*-benzoylpinostrobin has a comparison value of 6.38/1.09 = 5.85, which means that 4-Nitro-5-*O*-benzoylpinostrobin is almost 6 times fold more potent than tetrahydrochrysene as an ERβ antagonist. These results indicate that 5-*O*-Benzoylpinostrobin derivatives tend to have a higher affinity for ERβ than ERα. This result is fascinating because the most promising ER-ligands for clinical use are those eliciting an ERβ-selective activation.[37] The 4-Nitro-5-*O*-benzoylpinostrobin itself meets particular requirements; hence, it is very promising to be used in the treatment of ER-positive breast cancer.

While at PR receptors, this requirement is only fulfilled on 4-Nitro-5-*O*-benzoylpinostrobin and 4-*t*-Butyl-5-*O*-benzoylpinostrobin. Comparison of the agonist: Antagonist affinity with the highest value indicated by 4-Nitro-5-*O*-benzoylpinostrobin, with a comparison value of 5.37/0.31 = 17.32. These values indicate that 4-Nitro-5-*O*-benzoylpinostrobin has a seventeen-fold higher tendency as an antagonist than agonist ligand. This value is almost similar to the K_i antagonist value of the cocrystal ligand, in this case, asoprisnil with a comparison value of 0.3/0.31 = 0.97, indicating that 4-Nitro-5-*O*-benzoylpinostrobin is almost as potent as asoprisnil as a PR antagonist. These results complement the previous analysis, which also placed 4-Nitro-5-*O*-benzoylpinostrobin as a 5-*O*-Benzoylpinostrobin derivative with the highest potential as an ER antagonist, especially ERβ. Overexpression in ER and PR itself often occurs together. Therefore, ligands that have antagonist activity on both are very potential to be developed in the treatment of ER/PR-positive breast cancer.[38]

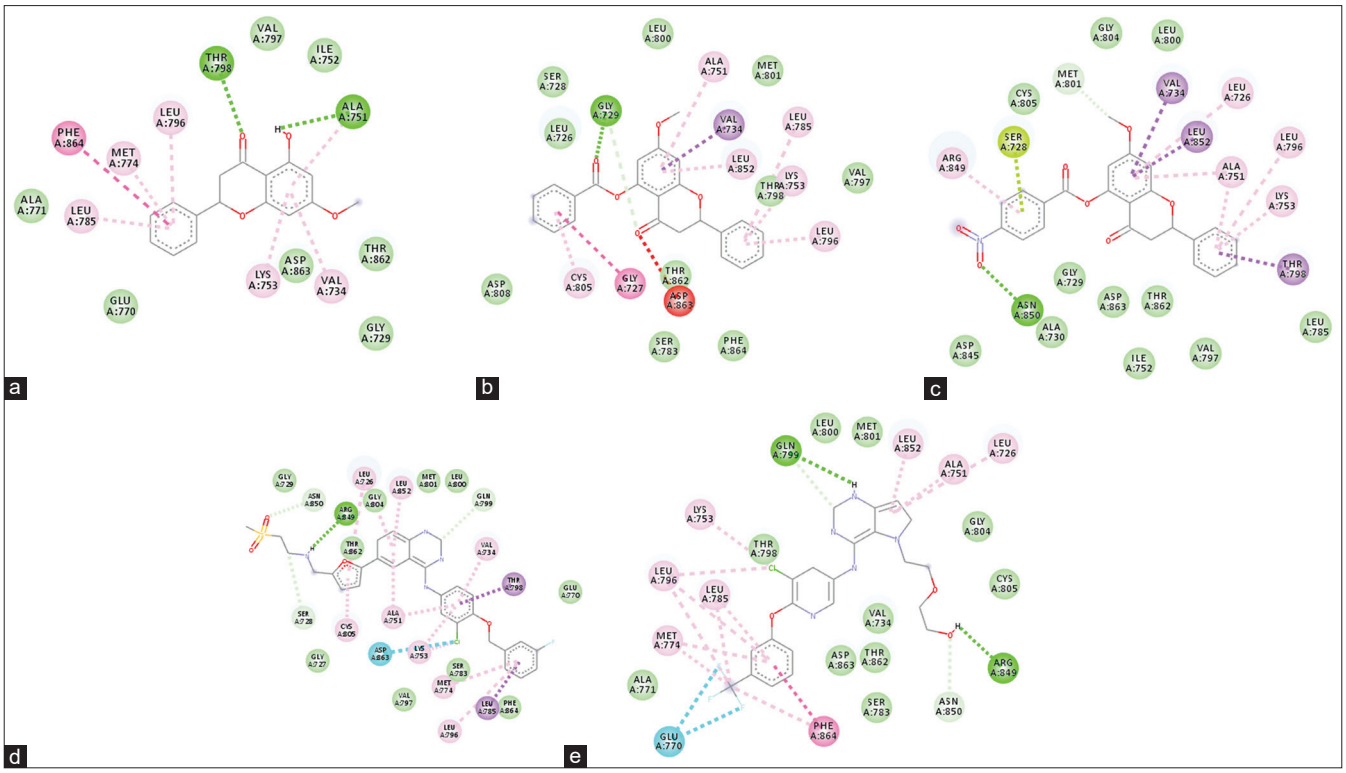


Figure 2: Two-dimensional comparison of docking results from pinostrobin (a), 5-*O*-Benzoylpinostrobin (b), 4-Nitro-5-*O*-benzoylpinostrobin (c), lapatinib (d), and cocrystal ligand SYR127063 (e) at human epidermal growth factor receptor 2 binding site

Especially for HER2 receptors which are known not to have agonist ligands, ligand affinity is determined based on the comparison of K_i values against cocrystal ligand and HER2 antagonists that have been used clinically, in this case, lapatinib. In line with the ER and PR results, 4-Nitro-5-*O*-benzoylpinostrobin also shows the highest affinity as a HER2 antagonist. Compared to cocrystal ligand SYR127063 and lapatinib, the K_i value of 4-Nitro-5-*O*-benzoylpinostrobin has a comparison value of 24.07/0.42 = 57.31 and 1.69/0.42 = 4.02, respectively. In other words, 4-Nitro-5-*O*-benzoylpinostrobin is predicted to have an affinity 4 times higher than lapatinib. This value is relatively high, considering that lapatinib is a synthetic drug designed specifically to inhibit the receptor signal processes by the adenosine triphosphate-binding pocket of the HER2.[39] These results further emphasize the potential of 4-Nitro-5-*O*-benzoylpinostrobin as anti-breast cancer not only with overexpression of ER/PR but also HER2.

Observation of amino acid residues from docking results, as presented in Table 4, also shows impressive results. In line with the predictions of affinity obtained, the comparison of amino acid residues also showed a higher level of similarity to the antagonist compared to the agonist form in ERβ and PR but lower in ERα. These results support previous predictions that the best affinity ligand of the two receptors, 4-Nitro-5-*O*-benzoylpinostrobin, has an antagonist affinity for the two receptors. While on HER2, 4-Nitro-5-*O*-benzoylpinostrobin showed a higher level of similarity to lapatinib than SYR127063. However, the level of similarity shown is only around 50%. Interestingly, 4-Nitro-5-*O*-benzoylpinostrobin has fewer Van der Waals interactions with amino acids than lapatinib, but the affinity shown is higher. This is unique considering that usually, the number of Van der Walls interactions is directly proportional to the affinity shown,[40] especially since lapatinib has a higher molecular mass than 4-Nitro-5-*O*-benzoylpinostrobin while the comparison of the number of hydrogen bonds between 4-Nitro-5-*O*-benzoylpinostrobin and the comparative ligand shows that the test ligand has fewer hydrogen bonds than the comparable ligands, except for the antagonist form of PR and HER2.

Especially on HER2, 4-Nitro-5-*O*-benzoylpinostrobin shows a novel interaction that is not shown by lapatinib to HER2. Visual observation in Figure 2 confirms the interaction of the 4-Nitrobenzoyl group in a position not shown by either lapatinib or by 5-*O*-Benzoylpinostrobin. This is unique, considering that the benzoyl group of 5-*O*-Benzoylpinostrobin is in a very different position from the 4-Nitrobenzoyl group. The presence of a 4-nitro substituent has a significant effect on the affinity of the 5-*O*-Benzoylpinostrobin derivative against HER2, given the predicted 4-Nitro-5-*O*-benzoylpinostrobin affinity is much higher than 5-*O*-Benzoylpinostrobin.

CONCLUSION

This research has succeeded in designing the highest potential 5-*O*-Benzoylpinostrobin derivative as anti-breast cancer, 4-Nitro-5-*O*-benzoylpinostrobin, with the main target being antagonists of HER2. Besides, the compound also has the potential as an ER, especially ERβ and PR antagonist, although it is lower than HER2. Analysis of the amino acid interactions of these compounds with HER2 also shows a novel interaction of the 4-Nitrobenzoyl, which was not shown by lapatinib, thus opening the chance for the

development of new HER2 antagonists with 4-Nitro-5-*O*-benzoylpinostrobin as the template. This study shows that the novel 5-*O*-Benzoylpinostrobin derivative, specifically 4-Nitro-5-*O*-benzoylpinostrobin, is very feasible to be synthesized and tested in the *in vitro* stage with appropriate cancer cell lines.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Tan BC, Tan SK, Wong SM, Ata N, Rahman NA, Khalid N, *et al.* Distribution of flavonoids and cyclohexenyl chalcone derivatives in conventional propagated and *in vitro* derived field-grown *Boesenbergia rotunda* (L.) mansf. Evid Based Complement Alternat Med 2015;2015:451870.
2. Gómez-Betancur I, Pereañez JA, Patiño AC, Benjumea D. Inhibitory effect of pinostrobin from *Renealmia alpinia*, on the enzymatic and biological activities of a PLA2. Int J Biol Macromol 2016;89:35-42.
3. Jadaun A, Subbarao N, Dixit A. Allosteric inhibition of topoisomerase I by pinostrobin: Molecular docking, spectroscopic and topoisomerase I activity studies. J Photochem Photobiol B 2017;167:299-308.
4. Massaro CF, Katouli M, Grkovic T, Vu H, Quinn RJ, Heard TA, *et al.* Anti-staphylococcal activity of C-methyl flavanones from propolis of Australian stingless bees (*Tetragonula carbonaria*) and fruit resins of *Corymbia torelliana* (*Myrtaceae*). Fitoterapia 2014;95:247-57.
5. Wangkangwan W, Boonkerd S, Chavasiri W, Sukapirom K, Pattanapanyasat K, Kongkathip N, *et al.* Pinostrobin from *Boesenbergia pandurata* is an inhibitor of ca2+-signal-mediated cell-cycle regulation in the yeast *Saccharomyces cerevisiae*. Biosci Biotechnol Biochem 2009;73:1679-82.
6. Abdelwahab SI, Mohan S, Abdulla MA, Sukari MA, Abdul AB, Taha MM, *et al.* The methanolic extract of *Boesenbergia rotunda* (L.) mansf. And its major compound pinostrobin induces anti-ulcerogenic property *in vivo*: Possible involvement of indirect antioxidant action. J Ethnopharmacol 2011;137:963-70.
7. Le Bail JC, Aubourg L, Habrioux G. Effects of pinostrobin on estrogen metabolism and estrogen receptor transactivation. Cancer Lett 2000;156:37-44.
8. Sukardiman, Charisma D, Plumeriastuti H, Arifianti L. Anticancer effect of pinostrobin from (*Kaempferia pandurata* Roxb) in Benzo(a)pyrene induced fibrosaroma in mice. E J Planta Husada 2014;2:44-6.
9. Junior WA, Gomes DB, Zanchet B, Schonell AP, Diel KA, Banzato TP, *et al*. Antiproliferative effects of pinostrobin and 5,6-dehydrokavain isolated from leaves of *Alpinia zerumbet*. Rev Bras Farmacogn 2017;27:592-8.
10. Atun S, Arianingrum R. Anticancer activity of bioactive compounds from *Kaempferia rotunda* rhizome against human breast cancer. Int J Pharmacogn Phytochem Res 2015;7:262-9.
11. Guo Z. The modification of natural products for medical use. Acta Pharm Sin B 2017;7:119-36.
12. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, *et al.* Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv 2015;33:1582-614.
13. Wang TY, Li Q, Bi KS. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian J Pharm Sci 2018;13:12-23.
14. Poerwono H, Sasaki S, Hattori Y, Higashiyama K. Efficient microwave-assisted prenylation of pinostrobin and biological evaluation of its derivatives as antitumor agents. Bioorg Med Chem Lett 2010;20:2086-9.
15. Fadilah F, Andrajati R, Yanuar A, Arsianti A. *In vitro* anticancer activity combination of eugenol and simple aromatic benzoate compounds against human colon HCT-116 cells and WiDr cells. J Pharm Sci Res 2017;9:637-41.
16. Akyuz L, Duman F, Kaya M. Encapsulation of flurbiprofen by chitosan using a spray-drying method with *in vitro* drug releasing and molecular docking. Turk J Pharm Sci 2017;14:34-9.
17. Siswandono S. Kimia Medisinal 2. 2nd ed. Surabaya: Airlangga University Press; 2016. p. 472.
18. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, *et al.* Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes Dis 2018;5:77-106.
19. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, *et al.* AutoDock4 and autoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009;30:2785-91.
20. Pratama MR, Sutomo S. Chemical structure optimization of lupeol as ER-A and HER2 inhibitor. Asian J Pharm Clin Res 2018;11:298-303.
21. O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR, *et al.* Open babel: An open chemical toolbox. J Cheminform 2011;3:33.
22. Pratama MR, Gusdinar T. Between artemisinin and derivatives with neuraminidase: A docking study insight. Asian J Pharm Clin Res 2017;10:304-8.
23. Forli S. Charting a path to success in virtual screening. Molecules 2015;20:18732-58.
24. Arba M, Yamin Y, Ihsan S, Tjahjono DH. Computational approach toward targeting the interaction of porphyrin derivatives with Bcl-2. J Appl Pharm Sci 2018;8:60-6.
25. Megantara S, Iwo MI, Levita J, Ibrahim S. Determination of ligand position in aspartic proteases by correlating tanimoto coefficient and binding affinity with root mean square deviation. J Appl Pharm Sci 2016:6:125-9.
26. Castro-Alvarez A, Costa AM, Vilarrasa J. The performance of several docking programs at reproducing protein-macrolide-like crystal structures. Molecules 2017;22:E136.
27. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: A review. Biophys Rev 2017;9:91-102.
28. Abdullah EC. E-pharmacophore mapping combined with virtual screening and molecular docking to identify potent and selective inhibitors of P90 ribosomal S6 kinase (RSK). Turk J Pharm Sci 2016;13:241-8.
29. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ, *et al.* Computational protein-ligand docking and virtual drug screening with the auto dock suite. Nat Protoc 2016;11:905-19.
30. Natesan S, Subramaniam R, Bergeron C, Balaz S. Binding affinity prediction for ligands and receptors forming tautomers and ionization species: Inhibition of mitogen-activated protein kinase-activated protein kinase 2 (MK2). J Med Chem 2012;55:2035-47.
31. Neshich IA, Nishimura L, de Moraes FR, Salim JA, Villalta-Romero F, Borro L, *et al.* Computational biology tools for identifying specific ligand binding residues for novel agrochemical and drug design. Curr Protein Pept Sci 2015;16:701-17.
32. Miller RL, Thompson AA, Trapella C, Guerrini R, Malfacini D, Patel N, *et al.* The importance of ligand-receptor conformational pairs in stabilization: Spotlight on the N/OFQ G protein-coupled receptor. Structure 2015;23:2291-9.
33. Ravindranath PA, Forli S, Goodsell DS, Olson AJ, Sanner MF. AutoDockFR: Advances in protein-ligand docking with explicitly specified binding site flexibility. PLoS Comput Biol 2015;11:e1004586.
34. Sliwoski G, Kothiwale S, Meiler J, Lowe EW Jr. Computational methods in drug discovery. Pharmacol Rev 2014;66:334-95.
35. Ng HW, Zhang W, Shu M, Luo H, Ge W, Perkins R, *et al.*

Competitive molecular docking approach for predicting estrogen receptor subtype α agonists and antagonists. BMC Bioinformatics 2014;15 Suppl 11:S4.
36. Chang M. Tamoxifen resistance in breast cancer. Biomol Ther (Seoul) 2012;20:256-67.
37. Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F. Estrogen receptors alpha (ERα) and beta (ERβ): Subtype-selective ligands and clinical potential. Steroids 2014;90:13-29.
38. Peng J, Sengupta S, Jordan VC. Potential of selective estrogen receptor modulators as treatments and preventives of breast cancer. Anticancer Agents Med Chem 2009;9:481-99.
39. Li J, Wang H, Li J, Bao J, Wu C. Discovery of a potential HER2 inhibitor from natural products for the treatment of HER2-positive breast cancer. Int J Mol Sci 2016;17:E1055.
40. Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, *et al.* Insights into protein-ligand interactions: Mechanisms, models, and methods. Int J Mol Sci 2016;17:E144.