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MOLECULAR DOCKING OF COMPOUNDS FROM *Chaetomium* Sp. AGAINST HUMAN ESTROGEN RECEPTOR ALPHA IN SEARCHING ANTI BREAST CANCER

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Abstract: *A study on molecular docking-based virtual screening has been conducted to select virtual hit of compounds, reported its existence in fungal endophytes of Chaetomium sp. as cytotoxic agent of breast cancer. The ligands were docked into Human Estrogen Receptor alpha (HERα) as the protein which regulates the breast cancer growth via estradiol-estrogen receptor binding intervention. The results showed that two compounds bearing xanthone and two compounds bearing benzonaphtyridinedione scaffolds were selected as virtual hit ligands for HERα leading to the conclusion that these compounds were good to be developed as anti breast cancer.*

Keywords*: Molecular docking, Endophyte, Chaetomium, Breast Cancer*

INTRODUCTION

In family *Chaetomiaceae* (*Ascomyta*), *Chaetomium* sp. is a genus employing species broadly distributed among hundred marine and terrestrial natural products (Ferlay *et al*, 2007; Zhang *et al*, 2012). To date, about 200 compounds were reported as the chemical constituents from this genus (Li *et al*, 2011). This genus employs either bacterial or fungal endophyte which is currently being a trending topic in searching new source of biological active compounds due to its capability to be cultivated in abundance yields (Yang *et al*, 2011). This is helpful to overcome the problem in isolating a single compound from raw materials which is normally using multistep extractions as well as fractionations (Hussain *et al*, 2012).

As characterized in general natural product compounds, these endophyte from *Chaetomium* sp. also posses diverse biological activities such as antibacterial, antioxidant, anti-inflammatotry, antileishmanial and many others (Ruch, Cheng, and Klaunig, 1989). The fungal endophyte is living symbiotically in between the plant cells (Strobel, 2003). Some plants have been identified its presence of *Chaetomium* sp. are *Vinca rosea*, *Taxus braxifollia* (Zhao *et al*, 2010), *Phyllantus amarus* (Kandavel and Sekar, 2015), *Ginko biloba* (Li *et al*, 2011), and so forth. Uniquely, the chemicals contained in the endophyte often highly related with the identity compounds in the plant of origins. For example is vincristine, the chemotherapeutics for cancer which is contained in *Vinca rosea* also found in its fungal endophyte (Kumar *et al*, 2013). Likewise, taxol was characterized in the fungal endophyte of *Taxus braxifollia* (Stierle, Strobel, and Stierle, 1993).

Two mentioned plants were used clinically as the anti-cancer, a disease which is too tough to be controlled due its complicated life cycle and molecular mechanism. Breast cancer is one of the high prevalence cancers with about 1.7 million incidents annually (Lane, 2006). Therefore, the discovery and development of new anti-cancer is continuously needed especially to those resistant cancer genes.

Computational studies in a virtual identification were broadly used in the screening of natural product database as bioactive compounds. This reduces the time, budget and safety in wet lab experiment since the sample to be tested is selected based on the mathematical value of drug-receptor interaction associating with its *in*

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vivo pharmacodynamic behaviours (Kitchen *et al*, 2004). In this present study, we virtually screen 27 compounds being identified in fungal endophyte of *Chaetomium* sp. to be selected as the hit compounds by docking them into one of the protein targets in the breast cancer cell, i.e., human estrogen receptor alpha (HERα) DNA binding protein domain.

EXPERIMENTAL

The computer used in this study was a PC with Core 2 Duo processor 2.93 GHz and 2 GB of RAM. The operating system of the PC is Windows8. Docking the compounds into human estrogen receptor alpha (HERα; PDB 1G50) was carried out to predict affinity of chemical substances which are reported presenting in *Chaetomium* sp. from diverse plants. The ligands were sketched and geometrically minimized using ACD Chemsketch (www.acdlabs.com) and Marvin Sketch (www.chemaxon.com), respectively. The ligands and proteins were then prepared using AutoDockTools 1.5.6 (www.autodock.scripps.edu). The proteins were added with polar hydrogen and given by kollman charge whereas the ligands were given by gasteiger charges. The grid was centered on each protein binding site and the docking was then performed using AutoDock Vina embedded in PyRx version 8.0 (www.autodock.scripps.edu). The docking results were obeserved as free energy of binding (ΔG_{bind}) and the selected docking pose was visualised using Discovery Studio 3.5 (www.accelyrs.com).

RESULTS AND DISCUSSION

There have been well characterized that estrogen controls the breast cancer by stimulating the expression of certain genes (Horwitz and McGuire, 1978). Estrogen receptor with its helix H12 provides the binding site for estradiol as the messenger to dimerize and expose the SF-2 region. This will attract the Co-activator to bind leading to nucleic acid transcription during the breast cancer cell replication. The side chain containing ASP351 is crucial to antagonism.

The antagonist must contain an amine group of the correct basicity such that it ionizes and forms the interaction with ASP351, and it must be of the correct length and flexibility to place the amine in the correct position for binding (Patrick, 2013). Several crystal structures of human estrogen receptor α ligand-binding domain (hERα LBD) complexed with agonist or antagonist molecules have previously been solved. The binding site was characterized where in estradiol bound to, by interacting with GLU353, ARG394, PHE404 and HIS524 via electrostatics, pi-pi and hydrogen bon interaction, respectively (Eiler *et al*, 2001) The binding of estradiol as the chemical messenger is presented in Figure 1.

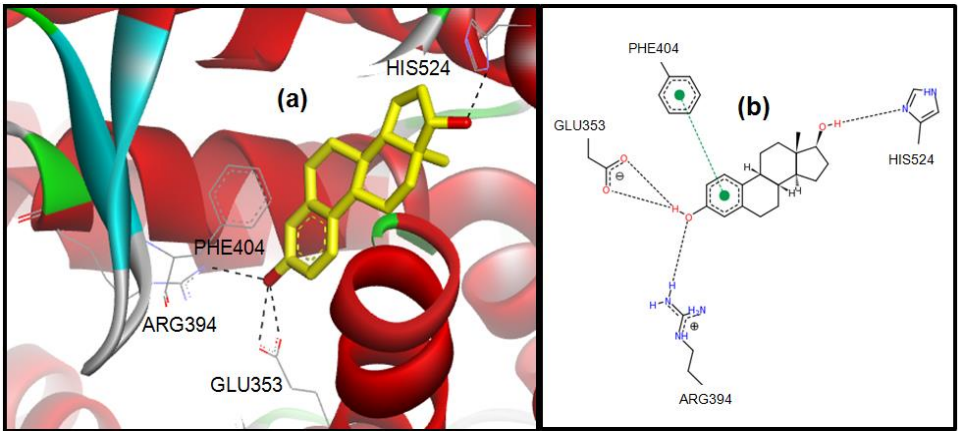
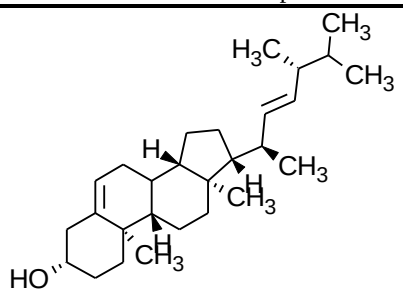
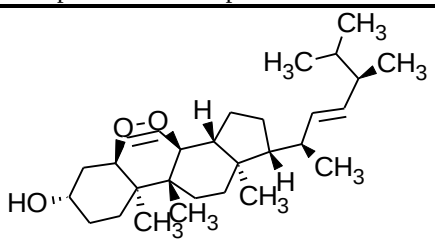
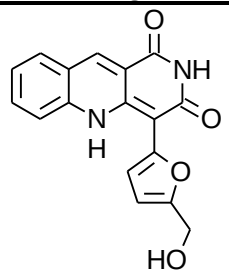
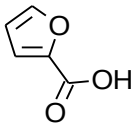
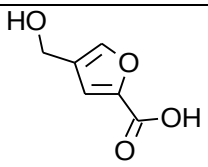
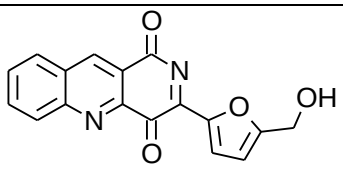
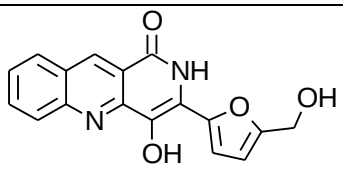
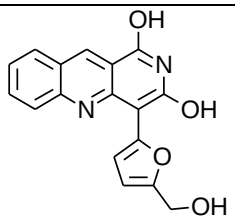
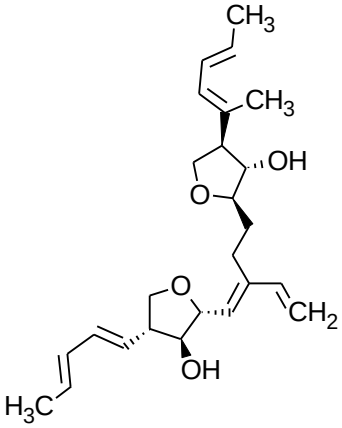
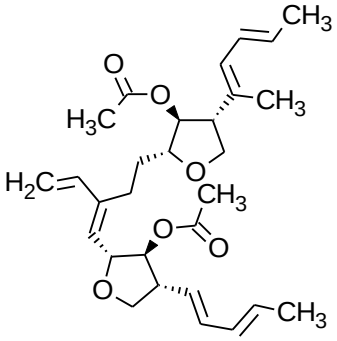
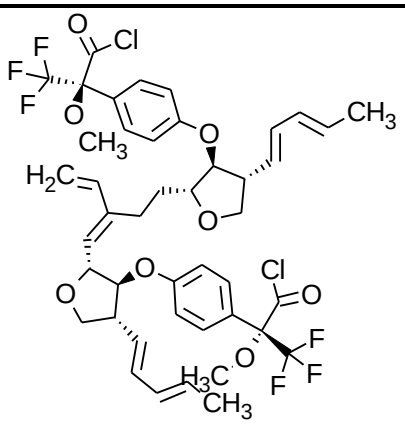


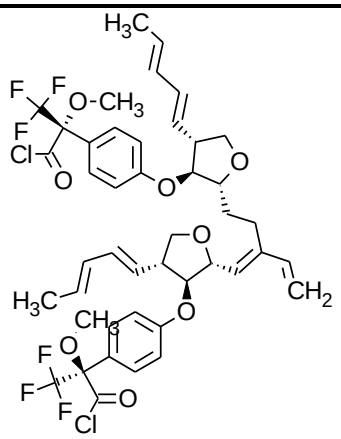
Figure 1. The chemical interaction between estradiol and HERα (a) 3D structure and (b) 2D structure. The protein was presented as ribbon and visualized using Discovery Studio 3.5 (www.accelrys.com)

Table 1. Compounds of *Chaetomium* sp. from diverse plants

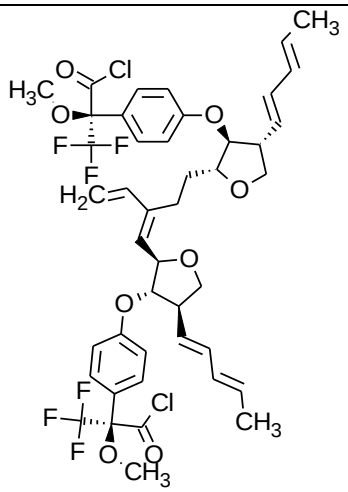
 HWergos	 HWergos58
 Latef1	 Latef2
 Latef3	 Latef4
 Latef5	 Latef6
 Marwah1	 Marwah2



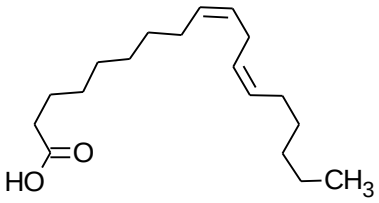
Marwah3



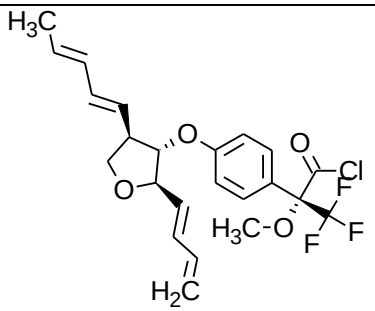
Marwah4



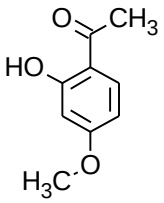
Marwah5



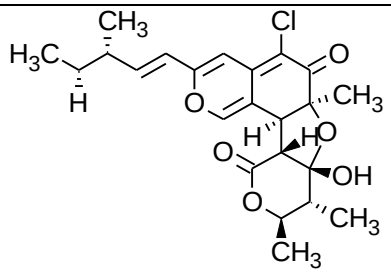
Marwah6



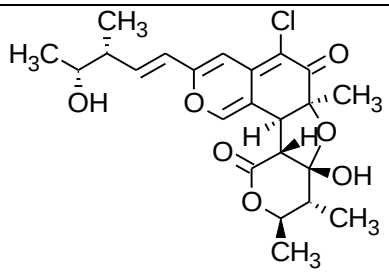
Marwah7



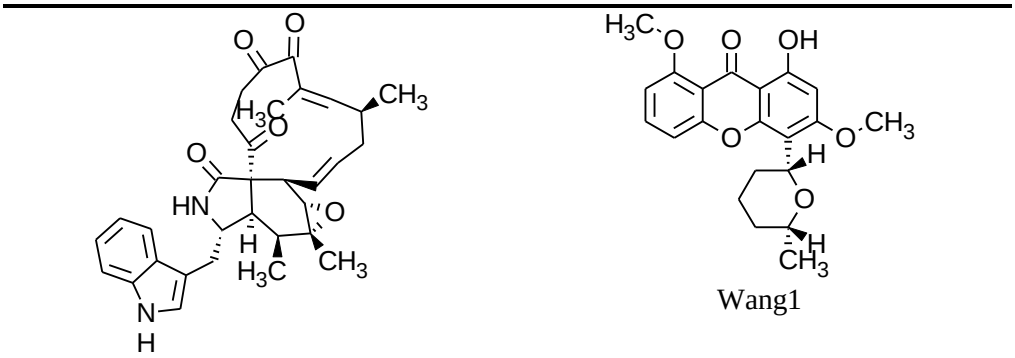
PengLi



Qin1

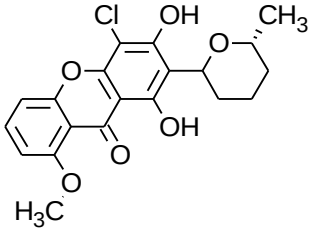


Qin2

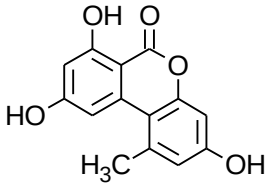


Qin4

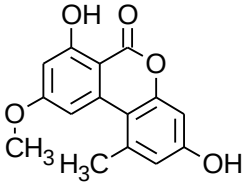
Wang1



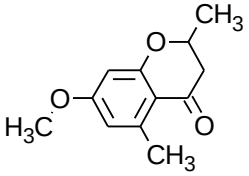
Wang2



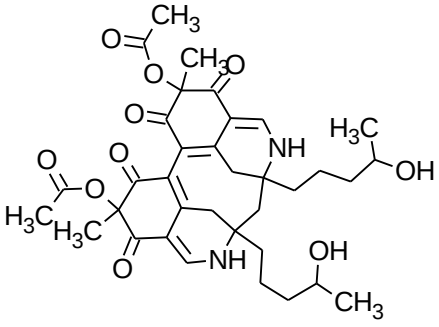
Wang3



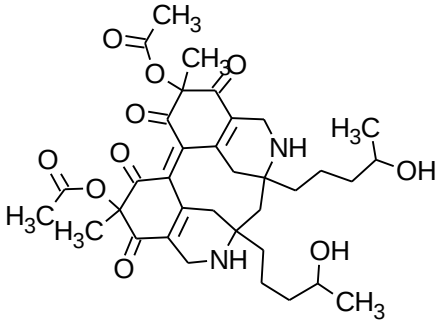
Wang4



Wang5



WP1



WP2

In searching new active ligands, a number of 27 compounds (see Table 1) from *Chaetomium* sp., was utilized as the database to be virtually screened using 1G50 as the protein target (Marwah *et al*, 2007; Abdel-Lateff, 2008; Qin et al, 2009; Li *et al*, 2011; Wang et al, 2012; Wang et al, 2015; Li *et al*, 2015;). As results, Table 2 presented the ΔG_{bind}, the amino acids which contribute to its binding via H-bonding along with its H-bond distances. The order of compounds was ranked from the lowest ΔG_{bind} to the highest one.

The ΔG_{bind} was observed at range -9.2 to -4.9 kcal/mol for virtual active compounds while inactive compounds were indicated at range -4.2 to 9.4 kcal/mol. There are six compounds found to be low in ΔG_{bind} but they are not bound to the active site as no hydrogen bond being observed in their poses. This might be due to the van der Waals contribution which is normally not as essential as

hydrogen bond. Van der Waals role in the ligand-protein binding is normally to support or stabilize the pose via dipole induced dipole such as pi-pi, pi-cation and or pi-sigma interactions. Van der waals interaction has a weak affinity, therefore, this is normally less priority in the ligand-protein interaction. Although virtual screening has been developed as a fast and accurate scoring function, however, no single scoring can satisfy the peculiarities of each target system (Li *et al*, 2009).

In further virtual hit selection, the priority goes to the ligands which have lower ΔG_{binds} and similar binding modes with the reference (Yang *et al*, 2013). A diverse hydrogen bond interaction was demonstrated by all ligands fit into the active site with the corresponding amino acid residues such as GLU323, PRO324, PRO325, LEU345, LEU346, GLU353, TRP393, ARG394 and LYS449. However, among those mentioned amino acid residues, interactions with GLU353 and ARG394 were the most prevalent and it was suggested to be important as these interaction were also possessed by the reference ligand. Therefore, compounds having interaction with these types of residue were proposed to be the virtual hits ready for further optimization. Figure 2 illustrated the superimposition of 27 ligands in the active site of 1G50, whereas Figure 3 illustrated the ligands-protein interaction of some representative virtual hit compounds.

Table 2. The $\Delta G_{binding}$ of 27 ligands were docked into hERα LBD (PDB 1G50).

Ligands	ΔG_{bind} (kcal/mol)	Amino acid residues	H-bond distance (Å)
Wang3	-9.2	ARG394	1.98
Wang4	-8.8	GLU353, ARG394	1.84, 1.90
Latef1	-7.8	NHB	NA
Latef6	-7.8	NHB	NA
Latef4	-7.7	PRO324, ARG394, LYS449	2.44, 2.19, 1.90
Latef5	-7.6	GLU323, ARG394, LYS449	2.27, 2.14, 2.41
Wang5	-7.2	NHB	NA
HWergos58	-6.8	TRP393	2.12
Marwah6	-6.8	NHB	NA
Marwah5	-6.6	ARG394, LYS449	2.07, 2.29
HWergos	-6.5	NHB	NA
Marwah4	-6.5	TRP393, ARG394, LYS449	2.10, 1.69-1.87, 2.49
Wang1	-6.5	NHB	NA
WP2	-6.5	LEU345, LEU346, ARG394	1.95, 2.49, 1.59
Marwah2	-6.3	TRP393, LYS449	2.20, 1.80
Wang2	-6.1	LYS449	2.20
PengLi	-5.9	PRO325, ARG394	2.10, 1.86
Marwah1	-5.8	TRP393	2.20
Marwah7	-5.7	TRP393, LYS449	1.93, 2.39
Latef3	-5.5	ARG394, LYS449,	2.02, 2.22
Qin1	-5	TRP393	1.78
Latef2	-4.9	ARG394, LYS449	2.42, 2.05
Qin2	-4.2	NHB	NA
Marwah3	0.7	NHB	NA
Qin3	3.3	NHB	NA
WP1	6.1	NHB	NA
Qin4	9.4	PRO325	1.98

NHB = No Hydrogen bond; NA = Not Applicable

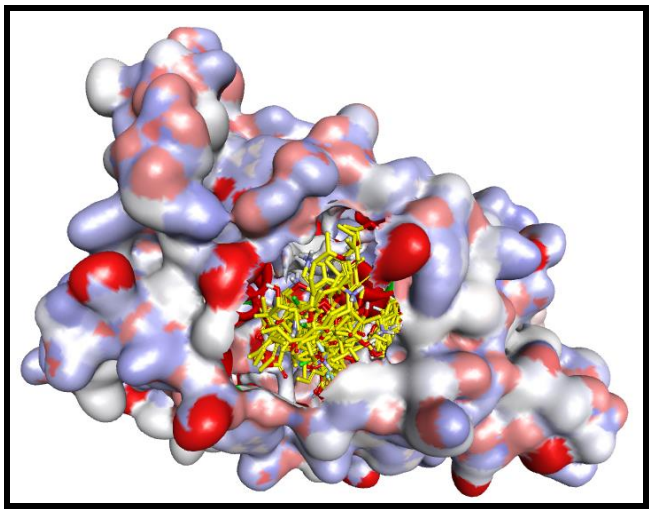


Figure 2. The superimposition of 27 ligands from *Chaetomium* sp. docked to the active site of HERα (PDB 1G50). The protein was presented as surface and visualized using Discovery Studio 3.5 (www.accelrys.com).

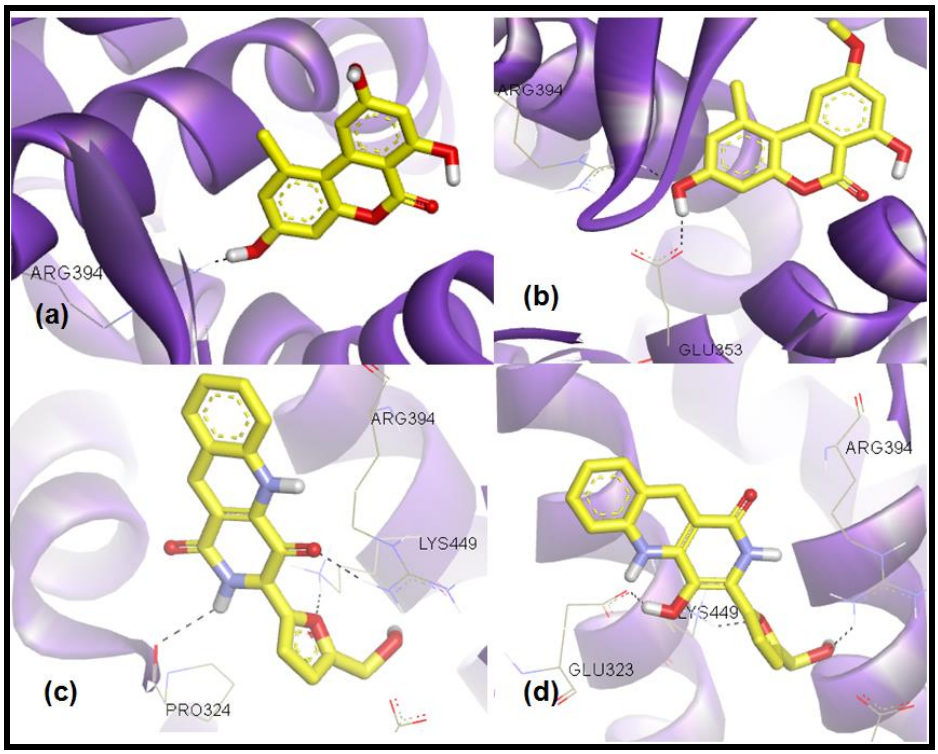


Figure 3. The ligand-binding interaction of four virtual hit compounds from *Chaetomium* sp. docked to the active site of HERα (PDB 1G50): (a) Wang3 (b) Wang4, (c) Latef4 and (d) Latef5. The protein was presented as ribbon and visualized using Discovery Studio 3.5 (www.accelrys.com).

Compounds bearing xanthone (Wang3 and Wang4) and benzonaphtyridinedione (Latef4 and Latef5) could be good scaffolds for further lead optimization of cytotoxic agent against breast cancer by targeting HERα DNA binding protein domain. Xanthone has been widely published as anticancer due to triple heteroaromatic ring which acts a DNA intercalating agent. The planar system

of heteroaromatic ring takes over the space in between two layers of nucleic acid pairs and therefore disrupts the shape of the helix. This action occurred at either minor or major groove which prevents the DNA replication as well as its transcription. On the other hand, benzonaphtyridinedione has been reported as tyrosine kinase inhibitors which play roles in signal transduction which regulates the intercellular communication, thus it is a good approach for the therapeutic intervention against pathological processes such as proliferation, inflammation and cancer.

CONCLUSIONS

Fungal endophyte has been recognized as the new source of bioactive compounds from natural products. One of genus widely cultivate this fungi is *Chaetomium* sp. spread over plant has been well known in producing high value of compounds for many pharmacological activities. The advantage of this material is its capability to be cultivated in a high yield via biotechnology process compares to the traditional fabrication that needs more plantation. In order to increase the knowledge in developing new anticancer drug from natural product, a molecular docking based virtual screening has been approached to select some hit compounds to be active as breast cancer cytotoxic agent by HERα DNA binding protein domain intervention. Compounds bearing xanthone and benzonaphtyridinedione were selected as the most active ligand against the protein target. In conclusion, *Chaetomium* sp. is strongly suggested to be developed as the source for bioactive compounds concerning on anticancer agent. However, further retrospective validation is required to have a validated protocol in virtual screening leading to the features of ligand to be suitable as HERα antagonist.

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