CURRENT SCIENCE AND TECHNOLOGY (CST)
VOL. 02, ISSUE 1, 48 – 58
DOI: https://doi.org/10.15282/cst.v2i1.8674

ORIGINAL ARTICLE

Structure-Based Virtual Screening Towards The Discovery of Usnic Acid Derivatives as Novel mTOR Inhibitor to Treat Breast Cancer

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ABSTRACT – In this study, mTOR is chosen as the main target for breast cancer treatment. Besides, the existing drugs still pose severe side effects. Therefore, research on finding new anti-cancer agents should be done uninterruptedly. Usnic acid has been studied for its wide range of biological properties and huge potential in pharmaceutical research. A structure-based virtual screening approach is applied since it could reduce production time, cost and environmental issues. The virtual screening approach utilized in this study comprises molecular docking simulation, ADMET filtration and drug-likeness prediction. 340 usnic acid derivatives were retrieved from literature and used to build an in-house database. The resulting compounds from docking were then filtered using ADMET prediction which comprises human intestine absorption, aqueous solubility, plasma protein binding, cytochrome P450 2D6 (CYP2D6) and hepatotoxicity parameters to identify the most potent UA derivatives with favourable physicochemical characteristics. After all, the hit compound, 118, was further stimulated in order to forecast its drug-like features. The chalcone-based scaffold of 118 resembled the reported breast cancer compound's chemical structure strengthening the results obtained from this study. Thus, it is concluded that the structure-based virtual screening was an efficient and effective approach in the discovery of usnic acid derivative, 118, as a potential novel mTOR inhibitor to treat breast cancer.

ARTICLE HISTORY
Received: 26th June 2022
Revised: 21st Aug 2022
Accepted: 21st Dec 2022

KEYWORDS
Breast cancer
mTOR
ADMET
Usnic acid
Structure-based virtual screening

INTRODUCTION

The disrupted and unregulated cell division in a certain part of the body results in forming cancer cells, and breast cancer is the type of cancer that happens in the breast area (ACS, 2021). Breast cancer is the leading cancer incidence in 2020 (WHO, 2022) It has been haunting 2.26 million people in the same year. Besides, breast cancer is 100 times more common in females than males, but males tend to experience poorer results because of diagnostic delays (Muir, Kanthan, & Kanthan, 2003).

Meanwhile, according to data provided by World Health Organisation (WHO), there are more than 68 thousand people died from suffering breast cancer (IARC, 2020). Interesting enough, all the incidences of breast cancer were suffered by mere females, without any males being caught up in this disease (IARC, 2020). The prevalence of breast cancer increases as earlier the age of menarche and older the age of menopause (Collaborative Group on Hormonal Factors in Breast, 2012).

Even though the statistics had shown that breast cancer is affecting a huge number of women and causes mortalities, the situation where affordable charges of therapy remain as only chemotherapy. However, the consistent improvement for drugs never ceases to stop (Meanwell, 2016). The need for precise medication is throat-cutting. Hence, a drug targeting a specific signalling pathway is potentially better than any available drug on the market (Might & Crouse, 2022).

Drugs with significant metabolic effects have been used for targeted cancer therapy in recent years (Verges, Walter, & Cariou, 2014). Mammalian Target of Rapamycin (mTOR) has also been intriguing the scientist for its ability in regulating cancer cells (Hua et al., 2019). mTOR gene's functions are to regulate cell proliferation, lipid metabolism, and glucose metabolism in cells (Verges et al., 2014).

Natural product is a significant source of anti-cancer medications, there are numerous clinically effective anticancer medications are derived from natural product. The chemical structure of usnic acid as shown in Figure 1, is a yellow-coloured molecule with the IUPAC nomenclature [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo-furandione] and empirical formula of C18H16O7. It is found abundantly in the lichen genus Usnea and also be found in other lichens such as Alectoria and Cladonia (Ingólfsdóttir, 2002). Usnic acid possesses antiviral, antiprotozoal, anti-inflammatory, antibiotic, analgesic, and anticancer activity (Francolini, Norris, Piozzi, Donelli, & Stoodley, 2004; Mayer et al., 2005; Okuyama, Umeyama, Yamazaki, Kinoshita, & Yamamoto, 1995; Schmeda-Hirschmann et al., 2008; Sokolov et al., 2012; Vijayakumar et al., 2000). There are numerous researches on its anti-tumour effects in various types of cells, including the lung, colon, liver, gastric, and ovarian, have been conducted recently (Crawford, 2015).

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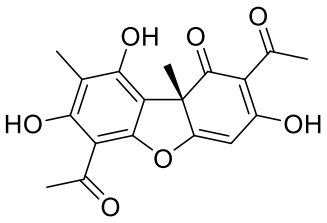


Figure 1. Usnic Acid Structure

The efforts that are required to carry out biological screening for billions of molecules remain burdensomely and as compensation, computer-aided drug discovery has become an appealing alternative. In recent years, virtual screening has emerged as a dynamic and profitable tool for searching for novel drug-like molecules or so-called hits, also methods for lead optimization in the pharmaceutical industry (Shoichet, 2004). As a result, this study has utilised this tool on usnic acid derivatives, which have been reported to have remarkable pharmaceutical properties including chemopreventive potential on cancer cell lines (Nguyen et al., 2021; Pyrczak-Felczykowska et al., 2019). We are aiming to find a potential drug with an in-house database based on usnic acid derivatives by using structure-based virtual screening. Nevertheless, the output of this study could be utilised as a potential drug candidate for chemotherapy of breast cancer.

METHODOLOGY

The basic flow of research is summarised in Figure 6. The initial step of flow is by retrieving the usnic acid derivatives database from literature. Subsequently, all the retrieved usnic acid was subjected to molecular docking to determine the best derivatives by comparing to the control, which in this study is Doxotecal. The best derivatives out of the database were then subjected to ADMET prediction. Last but not least, doing drug-likeness prediction had acquired us the lead compound of this study.

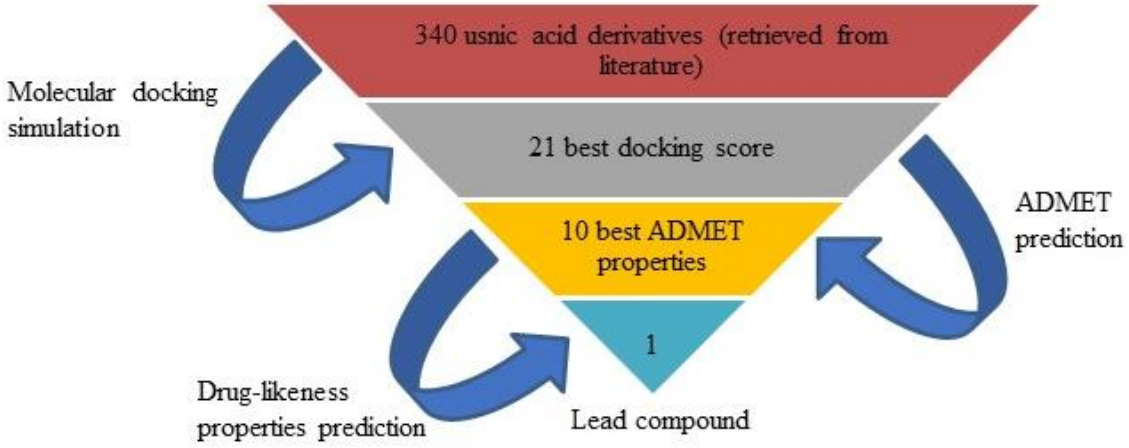


Figure 2. Flow of research employed in this study.

Ligand Preparation

Prior to virtual screening, 340 usnic acid derivatives were retrieved from PubChem and ScienceDirect to register into our in-house database. All the usnic acids that were to be subjected to the in-house database were created using the Chemdraw Professional 15.0 office for their two-dimensional (2D) structure. The three-dimensional (3D) structure was created from 2D under the CHARMm force field with Discovery Studio Client 16.1. The ligand was prepared by using the CHARMm force field in Discovery Studio Client 16.1 by the generation of low-energy ring conformations and all compounds were by default set to a pH range of 5.0-9.0 for suitable protonation state (Roney et al., 2021).

Protein Preparation

On the other hand, mTOR protein complex model (PDB ID:4DRH) was retrieved from Protein Data Bank (PDB) (http://rcsb.org). The model selected was the co-crystal structure of mTOR fragment, and it comes in a co-crystalised ligand of rapamycin(März, Fabian, Kozany, Bracher, & Hausch, 2013). The protein was prepared using Chimera 1.5.3

and Discovery Studio Client 16.1. All the hydrogen atoms, missing amino acid residues, and the looped segments around the active sites of protein were all inserted into the protein model. In addition, all the water molecules around the macromolecule were removed (Roney et al., 2021).

Screening of usnic acid derivatives with molecular docking simulation

The molecular docking simulation of all the usnic acid derivatives into the binding site of the prepared enzyme was run using Biovia Discovery Studio Client 16.1 software. Molecular docking of co-crystal ligand and usnic acid derivatives on mTOR protein was accomplished in this effort with the FDA-approved drug (breast cancer) docetaxel as a control. CDOCKER algorithm was employed in predicting interaction energy in this study molecular docking. Each ligand was docked with an enzyme in ten different conformations. For each ligand, the conformation with the lowest CDOCKER interaction energy was chosen. The same molecular docking procedure was also applied to determine the CDOCKER interaction energy for Docetaxel. Thus, the CDOCKER interaction energy of each usnic acid derivative was determined and compared to that of docetaxel. Any ligand having higher CDOCKER interaction energy was chosen for amino acid interaction analysis. The top ten ligands were chosen from amino acid interaction analysis for the following steps.

Drug-likeness prediction

This step was carried out to estimate the drug-likeness of the compound. The term "drug-like" usually indicates compounds that include functional groups and/or exhibit qualities of most known drugs. This method is carried out on the ten compounds selected using Discovery Studio Client 16.1 software. The prediction was merely done by using the ADMET descriptor function in the software. Parameters such as human intestine absorption, aqueous solubility, blood-brain barrier (BBB), plasma protein binding, cytochrome P450 2D6 (CYP2D6) and liver toxicity (hepatotoxicity) were applied in this prediction. The most potent compound that was selected through the ADMET prediction is then tested with Lipinski's rule of five (www.molinspiration.com/) to examine whether the lead compound could be orally active.

RESULT

Molecular Docking Study

Molecular docking simulation was performed on 340 usnic acid derivatives and docetaxel as control of the study towards the mTOR protein. Out of the 340 derivatives, 20 of them (6, 7, 25, 63, 67, 118, 119, 120, 164, 165, 166, 192, 193, 204, 245, 256, 257, 299, 300, 301) that were having the better interaction energy with the mTOR protein than the control were chosen for further investigation. The CDOCKER interaction energy of each selected usnic acid derivative and Docetaxel was listed in Table 1.

Table 1. CDOCKER interaction energy for selected usnic acid derivatives that have better activity than the control of this study

Compound	CDOCKER interaction Energy (-kcal/mol)
docetaxel (control)	56.0624
6	72.6910
7	71.7682
15	67.3457
63	57.8503
67	60.7533
118	60.7522
119	64.2997
120	66.3383
164	57.4597
165	59.4447
166	58.6310
192	61.8293
193	60.0665

204	62.1071
245	61.2714
256	63.8673
257	68.1638
299	60.0628
300	66.4794
301	57.3104

Out of 340 usnic acid derivatives, there is more than 5 per cent of the usnic acid derivatives that has better binding affinity than the positive control docetaxel, which indicates that usnic acid is structurally potent in the inhibition of mTOR protein. On the other hand, the 20 selected usnic acid derivatives were then subjected to another screening by the comparison of amino acid interaction with the positive control (Table 2). With the aid of this screening, ten compounds were filtered out for having similar interaction as the control. The usnic acid derivatives that were chosen in this analysis were 6, 7, 15, 67, 118, 119, 120, 257, 299 and 300. Usnic acid derivative 119 was chosen for the next analysis even though it had only two contacts however, it has relatively high CDOCKER interaction eneergy as compared to other compounds that also have only two interactions, and the two amino acid residue that has interaction with 119 were also present in the interaction between docetaxel and mTOR. Other than that, usnic acid derivatives 192, 193, 204, 245 and 256 were not chosen for next analysis bcause based on the amino acid interaction, these derivatives have interactions that are not exhibit in the interaction od docetaxel. Due to the reason that we are looking for a potential drug candidate for breast cancer, we wanted the selection screening process to be as close to docetaxel as possible. All the usnic acid derivatives having a diminutive amount of interaction with the target protein mTOR have been eliminated from the selection because lesser interaction with amino acid residues can cause unstable binding.

Table 2. Interaction of amino acid.

Compounds	Amino acid residues								
	GLN	HIS	ILE	VAL	TYR	PHE	SER	ARG	ASP
docetaxel	√ **	√ **	√ **	√ **	√ **	√ **		√ **	
6		√**	√**	√**	√**	√**	√**		√**
7		√**	√**	√**		√**			√**
15					√**	√**		√**	
63	√				√	√			
67					√**	√**			√**
118						√**	√**	√**	
119				√**				√**	
120		√**		√**		√**	√**	√**	
164			√	√					
165			√		√				
166					√	√			
192					√			√	
193								√	
204	√		√						
245					√				√
256				√		√			
257	√**			√**	√**	√**			√**
299					√**	√**	√**		√**
300		√**				√**		√**	√**
301					√				√

ADMET Properties and Drug-likeness

The basic descriptor approach in Discovery Studio 16.1 was used to evaluate the ADMET qualities of ten compounds in order to forecast their pharmacokinetics after administration and passage through the human body. Several metrics were evaluated, including plasma protein binding (PPB), atom-based log P (Alog P98), aqueous solubility, human intestinal absorption (HIA), hepatotoxicity, cytochrome P4502D6 (CYP2D6) enzyme inhibition, and polar surface area (PSA). All of the chemicals were expected to be readily absorbed by the human gut. Among the ten compounds, 118 and 119, were projected to be successfully absorbed in the human intestine based on ADMET prediction. However, 119 were not being selected from ADMET study, as for why is 119 not being selected, the reason will be listed in lateer section. The human intestinal absorption prediction has revealed that the compound 118 has pretty good intestinal absorption with polar surface area (PSA) of 123.278 Å2 and the Atom-based Log P98 (AlogP98) of 4.162, which meet the criteria of optimum cell permeability model (PSA<140 Å2 and AlogP98<5). Thus, the level of absorption was defined as very good from the prediction result (Egan, Merz, & Baldwin, 2000) (Zhang, Guo, Cui, & Qi, 2018).

The molar solubility (log(Sw)) of compound 118 is -5.921 which is falling into the low solubility level, according to the parameter determined by Discovery Studio client 16.1. Nevertheless, compound 118 has poor solubility in aqueous solution, thus larger dosage might be needed in consumption to achieve desired treatment expectation (Savjani, Gajjar, & Savjani, 2012). Even though it has a lower solubility in aqueous solution, however, it is still within the acceptable range for a drug. On the other hand, the plasma protein binding of compound 118 was predicted to be more than 90%, which indicates that only less than 10% of the drug distribution in human body (Gleeson, 2008). The information obtained also demonstrates that the lipophilicity of this drug is significant when consumed. On the other hand, the high plasma protein binding could possibly lower the toxicodynamics of 118 in human body (Miida et al., 2008). One of the main causes of high plasma protein binding might be the high molecular weight of this chemical compound which is more than 500 (Gleeson, 2008).

Additionally, there is no inhibition of cytochrome P450 (CYP450) observed, showing that these compounds could be easily metabolized by the CYP450 and hydroxylated during the early phase of metabolism. Notably, the prediction for hepatotoxicity was 0, indicating that this compound does not exhibit any hepatotoxic profile.

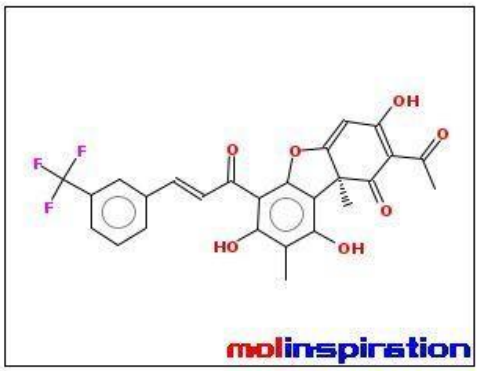
In conclusion, compound 118 is not a perfect candidate, nevertheless, it contributes good properties of what a drug candidate should be like. While comparing 118 with other 10 compounds, 118 has shown its excellency over the other usnic acid derivatives, even with little flaws such as low molar solubility and high plasma protein binding. Thus, compound 118 is selected to perform the last step of prediction which is the drug-likeness prediction. The predicted ADMET attributes of 118 are shown in Table 3.

Table 3. The ADMET profile of the selected compound from the database based on the best possible option available.

Compound	ADMET parameter							
	Human Intestinal Absorption			Aqueous Solubility		Plasma Protein Binding (PPB)	Cytochrome P450 2D6 (CYP2D6)	Hepatotoxicity
	PSA[a]	AlogP98[b]	Level[c]	log(Sw)[d]	Level[e]	Prediction[f]	Prediction[g]	Prediction[h]
6	291.987	-2.72	0	-4.377	2	0	0	0
7	288.472	-2.76	0	-3.939	3	0	0	1
15	136.089	6.209	0	-6.278	1	0	0	1
67	167.434	0.413	0	-3.649	3	1	0	1
118	123.278	4.162	0	-5.921	2	1	0	0
119	123.278	4.162	0	-5.87	2	1	0	0
120	123.278	5.104	0	-7.418	1	0	0	1
257	136.089	7.118	0	-6.316	1	0	0	1
299	214.725	-1.06	0	-3.66	3	0	0	1
300	216.361	1.619	0	-1.816	4	0	0	1

a PSA > 140: Very low absorption
b AlogP98 ≤-2.0 or ≥ 7.0: very low absorption
c Level of human intestinal absorption prediction; 0 (good), 1 (moderate), 2 (poor), 3 (very poor)
d The based 10logarithm of the molar solubility log (Sw) (acceptable drug-like compounds: $-6 < \log(Sw) \leq 0$)
e Level of aqueous solubility prediction; 0 (extremely low), 1 (very low), 2 (low), 3 (good), 4 (optimal), 5 (too soluble), 6 (warning: molecules with one or more unknown AlogP calculation)
f Prediction plasma-protein binding (0: < 90%; 1: ≥ 90%)
g Prediction cytochrome P450 2D6 enzyme inhibition (0: non-inhibitor; 1: inhibitor)
h Prediction hepatotoxicity (0: non-toxic; 1: toxic)

miSMILES: [H]c4c([H])c(C=CC(=O)c1c(O)c(C)c(O)c3c1OC2=CC(O)=C(C(C)=O)C(=O)[C@]23C)c([H])c(C(F)(F)F)c4[H]



Molinspiration property engine v2021.10

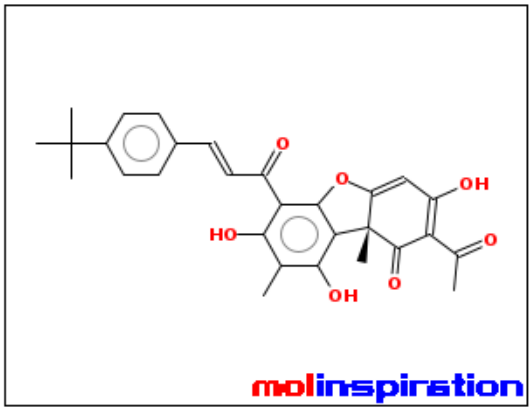
miLogP	4.44
TPSA	121.13
natoms	36
MW	500.43
nON	7
nOHNH	3
nviolations	1
nrotb	5
volume	403.49

Get data as text (for copy / paste).

Get 3D geometry BETA

Figure 3. Drug likeness result of 118. The results were obtained from molinspiration.com.

miSMILES: CC(=O)C4=C(O)C=C3Oc2c(C(=O)C=Cc1ccc(C(C)(C)C)cc1)c(O)c(C)c(O)c2[C@@]3(C)C4=O



Molinspiration property engine v2021.10

miLogP	5.27
TPSA	121.13
natoms	36
MW	488.54
nON	7
nOHNH	3
nviolations	1
nrotb	5
volume	438.38

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Figure 4. Drug likeness result of 118. The results were obtained from molinspiration.com.

On one hand, the term "drug-likeness" refers to a stable equilibrium of molecular properties that affect the pharmacodynamics and pharmacokinetics of molecules, thus, affecting their absorption, distribution, metabolism, and excretion (ADME) in the human body. On the other hand, permeability and bioavailability of membranes are usually associated with fundamental chemical properties such as logP, molecular weight (MW), topological polar surface area (TPSA), or the number of hydrogen bond acceptors and donors in a molecule. While lipophilicity is associated with toxicity, which fits with the idea that lipophilic binding is nonspecific, whereas polar binding is associated with specificity and thus selectivity. This indicates that drugs with a MilogP value greater than 5 and a TPSA value greater than 752 may cause significantly more harm to human body (Jagadish, Soni, & Verma, 2013).

Based on the ADMET results we have obtained from Discovery Studio 16.1, we have determined two compounds, 118 and 119 which are having better ADMET properties than the other 8 compounds tested. However, based on the result from molinspiration (Figure 3), we have found out that 119 has milogP greater than 5, which we have mentioned, when milogP is greater than 5, it will cause significantly more harm to human body. Therefore, we have excluded 119 from our selection and have selected 118 as our lead compound.

Referring to Figure 2, 118 had a milogP value of 4.44, which was less than 5.00, and a TPSA of 121.13, which was greater than 752 but less than 1402. This indicates that the target molecule appears to be safe to consume and has a highly anticipated oral bioavailability. Besides, 118 has a molecular weight (MW) of 500.43 Da, unfortunately, it violates one of Lipinski's rules of five with MW less than 500 Da. However, it happens that some of the FDA-approved drugs also have a molecular weight greater than 500 Da, such as Everolimus and Doxorubicin which are commonly used in the

treatment of breast cancer (NCI, 2021). Thus, 118 could still have the potential in becoming a drug-candidates for breast cancer. The log P value of a substance, which is the logarithm of its MW between n-octanol and water, is a well-established indicator of its hydrophilicity (Kwon, 2001). The number of rotatable bonds in the selected compound was 5, which was within the acceptable range of 5-10. As a result, their conformational stability is minimal. Additionally, the number of hydrogen bond donors (total number of NH and OH) in 118 was 3, which is less than 5 as indicated in Lipinski's rule of five, while the number of hydrogen bond acceptors (sum of N and O) was 7, which is also less than 10 as indicated in Lipinski's rule of five. This indicates that the number of hydrogen bond donors and acceptors is within the acceptable range according to Lipinski's rule of five. Last but not least, the number of atoms within 118 is 36, which falls within the range of 20-70 which is considered acceptable for a drug candidate. Overall, 118 has violated only one rule, which is having its MW over 500 Da. Only one violation of Lipinski's rules of five indicates that the drug is acceptable for oral consummation.

Lead compound

Upon screening and filtering through the 340 usnic acid derivative database, 118, as presented in Figure 4 was identified as the lead compound of this study. The first layer of filter which applied the use of molecular docking simulation has revealed the CDOCKER interaction energy of 118 (-60.7522 kcal/mol) when binding towards mTOR protein, together with the other 19 usnic acid derivatives that have better interaction energy than docetaxel.

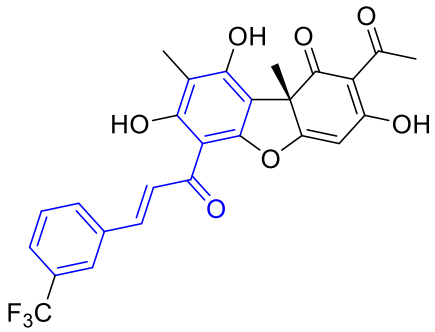

Figure 5. Chemical structure of 118 as a lead compound for inhibition of mTOR in this study. The highlighted structure is identified as a chalcone.

As illustrated in Figure 5, 118 formed three intermolecular hydrogen bonds with mTOR kinase. There were two hydrogen bonds between 118 and Arg 2036 of the mTOR kinase residue, with a hydrogen bond strength of 4.55 Å and 6.14 Å, respectively. Besides, the bond distance between 118 and Ser 2035 of the mTOR kinase was 3.47 Å. Furthermore, van der Waals alkyl, pi-alkyl, and salt bridge bonds were also formed during the binding simulation and had bond lengths greater than 3.00 Å.

a) b)

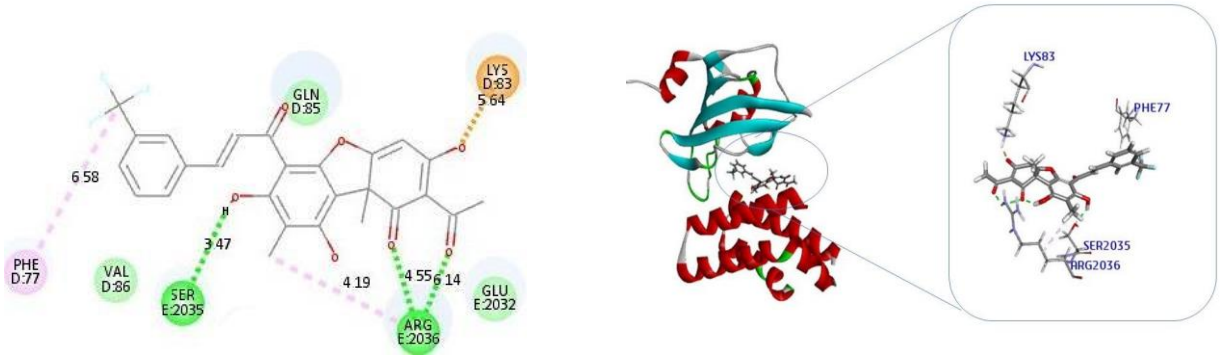

Figure 6. Molecular docking interaction between 118 and mTOR (FKBP51 domain) kinase, (a) 2D diagram; (b) 3D diagram

When we screen through the structure of 118, a chalcone structure could be spotted (highlighted structure in Figure 4). Chalcones as weandhetic analogues have intrigued researchers with their wide range of biological activities and therapeutic potential in various diseases including cancer (Karthikeyan et al., 2015). Chalcones exhibit a broad spectrum

of biological activities as a result of the inclusion of multiple functional groups (aryls, halogens, hydroxyls, carboxyls, and phenyl) that enable chalcones to attach to a variety of molecular targets and interact with other molecules as compounds [10]. According to Pyrczak-Felczykowska et al. [7], usnic acid derivatives containing this chalcone scaffold show better antiproliferative efficacy against the MCF-7 breast cancer cell line. Thus considering 118 also contains chalcone scaffold, its antiproliferation activity against breast cancer cells should be as good.

CONCLUSION

The emerging efforts for computational power had lead us here, using merely the computational resource, we are able to identify the ability of designed chemical compounds in approximately 340 usnic acid derivatives have been evaluated and predicted for some parameters and variables. All 340 usnic acid derivatives were undergone molecular docking simulation and high binding energy values which may result in the identification of novel mTOR inhibitor. Then, 20 compounds were selected from the molecular docking results based on interaction energy (-55 to -73 kcal/mol) with the target enzyme and then screened based on their interaction as compared to the breast cancer FDA drug, Docetaxel. 10 compounds were chosen and re-analyzed by subjects to ADMET prediction with human intestine absorption, aqueous solubility, plasma protein binding, cytochrome P450 2D6 (CYP2D6) and hepatotoxicity parameters and drug-likeness to obtain a lead compound, compound 118. Compound 118 has revealed the presence of chalcone. The addition of chalcone in the molecule as having the potential to treat breast cancer. However, the current virtual screening approach investigations will establish whether compound 118 is effective as novel mTOR inhibitor to treat breast cancer.

ACKNOWLEDGEMENT

This research was supported by Universiti Malaysia Pahang and FIST of University Malaysia Pahang.

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