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In silico studies on potential MCF-7 inhibitors of alkaloid and phenolic compounds isolated from *Zanthoxylum nitidum*: a combination of molecular docking and admet analysis

Tran Thi Tuyen[1, 2], Pham Cao Bach[1], Ha Viet Hai[1], Pham Thi Hong Minh[1, 2], Nguyen Thi Hong Van[1, 2], Pham Minh Quan[1, 2, *]

[1]*Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[2]*Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[*]Emails: *pham-minh.quan@inpc.vast.vn*

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Abstract. Cancer is one of the leading cause of death worldwide that cause a burden for the health system and economic of both developed and developing countries. Tubulin is a wellknown biochemical target in the field of cancer drug discovery. The inhibition of tubulin-microtubule dynamics would result in the mitosis during cell division, thus, killing the cancerous cells. To date, there have been some tubulin targeting drugs have been developed including paclitaxel and the vinca alkaloids, however, treatments are not high ineffective and efforts to discover new molecules are still in urgent needed. In this study, computational molecular simulation and assessment of drug-like properties were used to gain insights into the binding ability of 16 alkaloid and phenolic compounds isolated from *Zanthoxylum nitidum* on tubulin protein. Among studied candidates, compounds **8** and **10** were identified as potential candidates for inhibiting the function of tubulin at the active site regarding binding affinity, dock pose and ADMET properties analysis. These findings shed light on the anti-cancer potential of compounds isolated from *Zanthoxylum nitidum*.

Keywords: Zanthoxylum nitidum, MCF-7, tubulin, anticancer, molecular docking, ADMET analysis.

Classification numbers: 1.2.1, 1.2.4.

1. INTRODUCTION

In recent decades, the increasing occurrence of degenerative diseases and drug resistance have urged scientists to discover novel drugs displaying high efficacy but low toxicity. Due to the highly toxic side effects associated with modern treatments, efforts have now shifted towards natural sources as promising pharmaceutical drugs. Alkaloid and phenolic compounds are well-known molecules distributed widely in natural resources, displaying an abundant range of

biological activities [1, 2]. They have been previously reported as the key agents responsible for the bioactivities of numerous natural extracts [3 - 5]. A number of them are considered as potent drugs since they have fewer side effects and lower resistance compared to other chemotherapeutic agents [6 - 10]. The anti-cancer potential of alkaloid and phenolic compounds have been successfully demonstrated against a wide range of cancer cell lines [11 - 15].

In this work, we investigated the inhibition of the MCF7 protein-alpha-tubulin breast tumor cells by several alkaloid and phenolic compounds isolated from *Zanthoxylum nitidum*. Microtubules are highly dynamic constituents of the cytoskeleton. They are heterodimeric polymers of α- and β-tubulin. Microtubules play an important role in a number of essential cellular functions such as cell motility, mitosis, and intracellular transport [16, 17]. The vital role of microtubules in mitosis makes them one of the prevailing targets for anti-cancer drugs. Antimitotic drugs have proven their effects in cancer treatment through interfering the microtubule dynamics which ultimately lead to cancer cell death via apoptosis [18, 19]. The efficacy of a drug also depends on its binding mode and affinity towards the target site. An *in silico* study on the binding modes of any potential drug can thus prove to be productive.

Recently, computer aided drug discovery (CADD) has been widely utilized to discover novel drugs and to investigate the mechanism behind the biological effects of potent bioactive compounds [20, 21]. In this study, molecular docking and ADMET studies have been carried out in order to discover potent MCF-7 inhibitors and also to gain better understanding of the involved anti-cancer mechanism. Firstly, in an effort to investigate the probable anti-cancer molecules, molecular docking studies were performed on tubulin. Then, the drug-likeliness of the studied compounds were evaluated by an ADMET *in silico* analysis to eliminate non-drug-like molecules. The final selection was thus based on both the potency and the pharmacokinetics of the potential drug.

2. MATERIALS AND METHODS

2.1. Ligand and protein preparation

Based on previous publications, the alkaloid and phenolic compounds isolated from *Zanthoxylum nitidum* were gathered [3, 22, 23]. Detailed structure of these compounds is presented in Supplementary information (Figure S1). The three-dimensional structures of studied compounds were prepared using MarvinSketch 19.27.0 and PyMOL 1.3.r1 [24]. The energy minimization was carried out using Gabedit 2.5.0 [25]. Colchicine, a well-known tubulin inhibitor, was chosen as reference ligand. The X-ray crystal structure of protein tubulin (PDB ID: 1Z2B) was obtained from the Protein Data Bank [26].

2.2. Docking using Autodock

Proteins and ligands were prepared for docking using AutoDock Tools 1.5.6rc3 (ADT) [27]. The small molecules including water molecules were deleted and polar hydrogen atoms and Kollman charges were added to the receptor molecule. ADT assigned the rigid roots to the ligand automatically, all other bonds were allowed to be rotatable. Since previous studies reported that the active site of tubulin was identified at the colchicine binding domain [28]. Thus, in the present study, search space was restricted to a grid box size of 55 × 70 × 56 in x, y, and z dimensions, which was centered on the colchicine binding sites of protein with x, y, and z coordinates of 117.474, 92.241 and 6.406 Å, respectively. The spacing between grid points was 0.375Å. AutoDock 4.2.6 was utilized for the molecular docking simulation. The outputs data

were analyzed using Discovery Studio Visualizer [29]. PyMOL was used to calculate the distances of hydrogen bonds as measured between the hydrogen and its assumed binding partner. The detail of molecular docking simulation is given in Supplementary information.

2.3. ADMET studies

Open bioactivity prediction online server Molinspiration and ProTox-II were utilized to evaluate the drug-like properties and the acute toxicity of all the research compounds. The admetSAR database was utilized to calculate the absorption, distribution, metabolism, elimination, and toxicity (ADMET) indexes of the studied compounds [30].

3. RESULTS AND DISCUSSION

3.1. Molecular docking studies

To explore the inhibitory potential of 16 selected ligands with protein tubulin (PDB ID: 1Z2B), docking studies were conducted using AutoDock 4.2.6.

Table 1. Binding energies of studied compounds with tubulin and experimental value IC_{50}.

Compound	ΔG_{pred} [a] (kcal/mol)	ΔG_{exp} [b] (kcal/mol)	IC_{50} (µM)
1	-11.45	-6.20	30.2 ± 2.1
2	-8.03	-5.29	> 100
3	-12.76	-6.34	24.1 ± 1.8
4	-11.78	-6.24	28.4 ± 2.0
5	-8.47	-5.20	> 100
6	-10.56	-5.98	44.1 ± 2.3
7	-7.69	-5.14	> 100
8	-13.08	-6.73	12.6 ± 2.4
9	-7.99	-5.17	> 100
10	-14.45	-9.02	0.27 ± 1.6
11	-8.56	-5.33	> 100
12	-8.85	-5.36	> 100
13	-10.17	-5.83	56.95 ± 1.7
14	-8.95	-5.83	> 100
15	-7.63	-5.35	> 100
16	-13.69	-7.39	4.1 ± 2.3
colchicine	**-9.75**	**-5.67**	**74.23 ± 1.2** [32]

[a] ΔG_{pred}: Binding free energies calculated from molecular docking simulation;

[b] ΔG_{exp}: Calculated experimental binding free energy.

Table 1 shows the docking results of the studied compounds. According to the ranking criteria of AutoDock 4.2.6, the more negative the value of docking energy, the better the binding affinity of the compound towards the targeted receptor [31]. Based on the docking validation, the obtained dock score of colchicine was -9.75 kcal/mol. Thus, any ligand whose docking energy close to or more negative than this value would be considered a potential inhibitor of tubulin. Considering the criteria mentioned above, 3 out of 16 screened compounds were identified as potential inhibitors of tubulin. Compound **10**, **16** and **8** were the top three ligands whose docking energies far exceeded that of the reference ligand (-14.45, -13.69 and -13.08 kcal/mol, respectively).

Currently, tubulin binding molecules are considered as one of the most important classes of anticancer agents. They interfere with the normal functioning of tubulin assembly or disassembly by suppressing microtubule dynamics, thus inducing cell cycle arrest at the G2/M phase and activating signals for apoptosis. Therefore, cytotoxic activity of the studied compounds on MCF-7 cell line from literatures was also retrieved to better understand the effects resulting from the functional inhibition of tubulin caused by potential compounds (Table 1) [3, 22, 23]. Using Cheng-Prusoff's formula [33], the K_i inhibition constant was calculated as follows:

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{K_m}} = \exp\left(\frac{\Delta G}{RT}\right) \rightarrow IC_{50} = \exp\left(\frac{\Delta G}{RT}\right) + \exp\left(\frac{\Delta G}{RT}\right) \times \frac{[S]}{K_m}$$

Assuming the IC_{50} value was equal to K_i, the experimental binding free energy could be derived from the aforementioned formula as follows: $\Delta G_{exp} = RTln(K_i) = RTln(IC_{50})$ where R = 1.987 × 10^{-3} (kcal/K*mol); T = 300 (K) and inhibition constant K_i was measured in moles. Energy was measured in kilocalories per mole. As a result, ploting of experimental binding free energies against the computed values gave a relative high correlation coefficient of R^2 = 0.82, suggesting that this computational model could be useful in the prediction of potential compounds with inhibitory activity against tubulin protein (Figure 1).

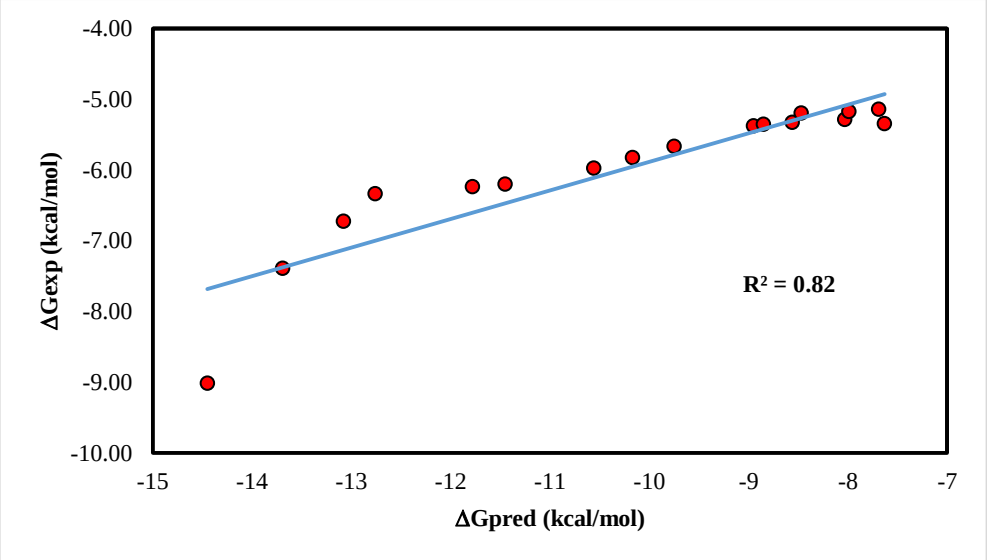


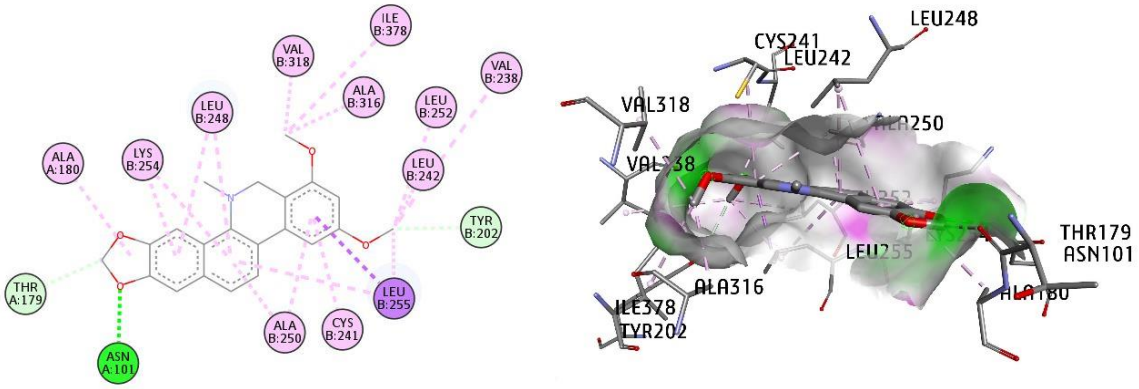
Figure 1. Correlation between experimental and computational binding free energies.

As indicated form Table 2, all three potential compounds exhibited hydrogen bond interactions with essential residues in the active site of protein tubulin. Specifically, the number of hydrogen bonds formed between the targeted protein and compounds **8**, **10** and **16** were 1, 2 and 5, respectively.

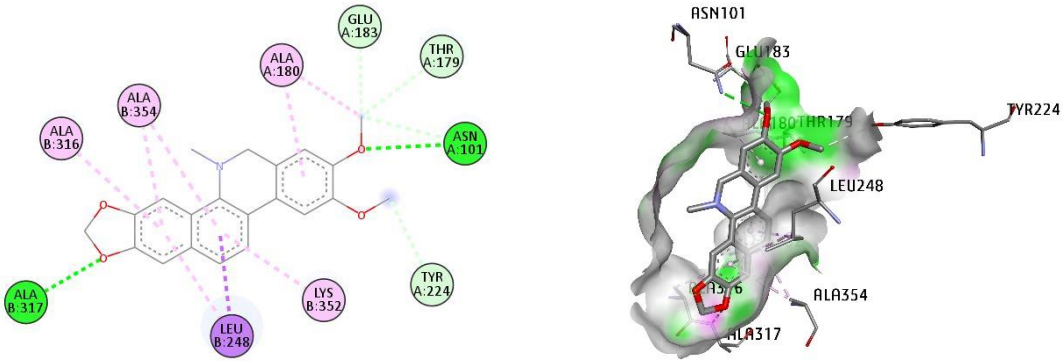
Table 2. Docking results of potential inhibitors with protein tubulin.

Compound	No. of hydrogen bonds	Interacting residues
10	2	Asn101; Thr179; Ala180; Glu183; Tyr224; Leu248; Ala316; Ala317; Lys352; Ala354
16	5	Asn101; Thr179; Val181; Val238; Cys241; Ala250; Leu255; Met259; Ala316; Ala317; Lys352
8	1	Asn101; Thr179; Ala180; Val238; Cys241; Leu242; Leu248; Ala250; Leu252; Lys254; Ala316; Val318; Ile378
colchicine	2	Asn101; Thr179; Ala180; Cys241; Leu248

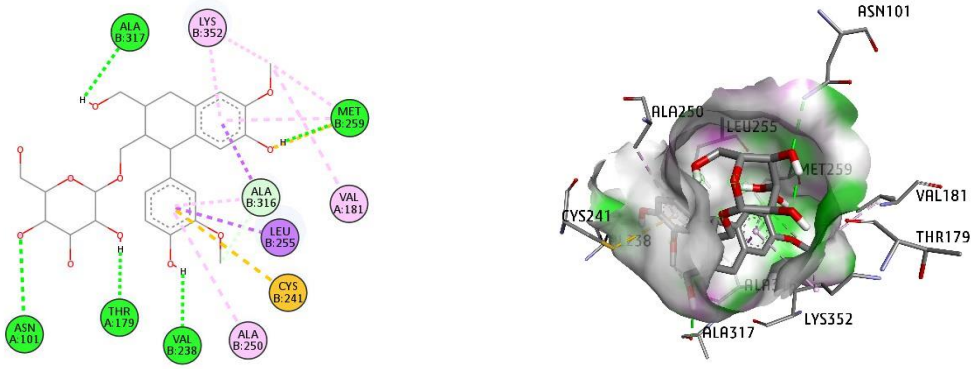
The dock pose of compound **10** in the active site of tubulin showed 2 hydrogen bonds with the key residues Asn101 and Ala317. The interaction was further stabilized with hydrophobic interactions at residues Thr179; Ala180; Glu183; Tyr224; Leu248; Ala316; Lys352; Ala354. In the dock pose of compound **16**, hydrophobic interactions were observed as contributed by Val181; Cys241; Ala250; Leu255; Ala316; Lys352. In addition, a total of 5 H-bonds were created towards Asn101; Thr179; Val238; Met259 and Ala317. In the case of compound **8**, amino acid Asn101 was recorded as the key residue involved in hydrogen bonds formation. Also, the hydrophobic contacts were constituted from the interaction with Thr179; Ala180; Cys241; Leu248.



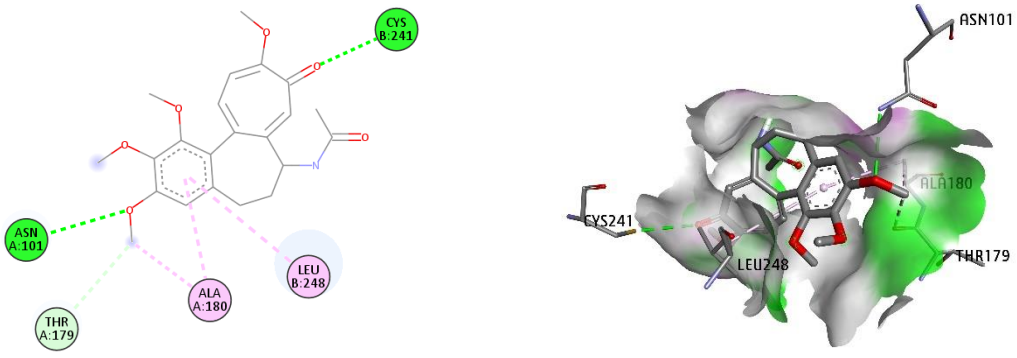
Compound **8**



Compound **10**



Compound **16**



Colchicine

Figure 2. Binding orientation of potential inhibitors in the binding site of tubulin (PDB ID: 1Z2B) suggested by molecular docking studies.

3.2. ADMET profile

The drug-like properties of 3 potential compounds were assessed by subjecting them to Lipinski's Rule of Five (Ro5), which consists of criteria that determine which compound is

considered to be drug-like in nature. For a more detailed analysis, the research compounds were then further evaluated for pharmacokinetic properties and toxicity prediction using Molinspiration and ProTox-II cheminformatic servers (Table 3).

Table 3. ADMET properties and toxicity prediction of potential inhibitors.

CP	MW	HBD	HBA	LogP	MR (cm^3/mol)	TPSA ($Å^2$)[a]	LD_{50} (mg/kg)	Toxicity prediction[b]	HIA
10	348	0	4	3.56	98.71	125.57	1000	4	0.9701
16	522	7	11	-0.15	128.64	178.53	823	4	0.5542
8	348	0	4	4.51	101.56	101.35	1500	4	0.9644
colchicine	399	1	7	2.59	106.26	83.11	6	2	0.9856

[a] Molecular total polar surface area

[b] Toxicity prediction class: 1 → 6 (High toxicity to non-toxicity)

The obtained results indicated that among the potential inhibitors, compounds **8** and **10** were determined to be favorable for oral drug development, with fewer than two violations of the conditions. The pharmacokinetic parameters and toxicity prediction results, in combination with docking studies, contributed helpful information to the assessment of potential compounds with inhibitory ability and drug-like properties for further drug development. The calculated properties showed that compounds **8** and **10** were classified as low-toxicity (rank 4) which was better than the reference drug (colchicine, toxicity rank 2). Besides, it was reported that compounds with good oral bioavailability would possess a total polar surface area (TPSA) ranging from 70 to 140 $Å^2$ and 12 or fewer H-bond donors (HBD) and acceptors (HBA) in total. In this study, according to the analytical results, both compounds **8** and **10** were observed to have TPSA values of 101.35 and 125.57, respectively, satisfying the criteria of drug-like properties and demonstrating suitability for oral drug development.

In ADMET study, one of the most important challenges facing an oral drug is its movement across the intestinal epithelial barrier that determines the rate and extent of human absorption and ultimately affects its bioavailability. According to the HIA value analysis, compounds **8** and **10** possess the value close to 1, suggesting that they are highly available to be absorbed in the human intestinal.

4. CONCLUSIONS

In this study, computational molecular simulation and drug-like properties assessment were used to gain insights into the binding ability of 16 alkaloid and phenolic compounds isolated from *Zanthoxylum nitidum* on protein tubulin. Amongst studied candidates, compounds **8** and **10** were identified as potential candidates for inhibiting the function of tubulin at the active site regarding binding affinity, dock pose and ADMET properties analysis. These findings shed light on the anti-cancer potential of compounds isolated from *Zanthoxylum nitidum*.

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CRediT authorship contribution statement. Tran Thi Tuyen, Pham Cao Bach, Ha Viet Hai: Investigation, methodology and editing manuscript. Pham Thi Hong Minh, Nguyen Thi Hong Van, Pham Minh Quan: analyze data and drafting manuscript. Pham Minh Quan: supervision and revising manuscript..

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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