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In Silico Assessment and Molecular Docking Studies of Some Phyto-Triterpenoid for Potential Disruption of Mortalin-p53 Interaction

Minh Quan Pham [1,2,*], Thuy Huong Le Thi [1,2], Quoc Long Pham [1,2], Le Thi Le [3], Huy Toan Dao [3], Thanh Le Thi Dang [4], Dung Thuy Nguyen Pham [5,6] and Hai Ha Pham Thi [5,*]

1 Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Hanoi 11517, Vietnam; thuyhuong0102sp2@gmail.com (T.H.L.T.); mar.biochem@fpt.vn (Q.L.P.)
2 Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi 11517, Vietnam
3 School of Chemical Engineering, Hanoi University of Science and Technology, Hanoi 11517, Vietnam; lethile98a10@gmail.com (L.T.L.); toandh-fct@mail.hut.edu.vn (H.T.D.)
4 Faculty of Chemistry and Environment, Thuyloi University, Hanoi 11517, Vietnam; ledtt@tlu.edu.vn
5 NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City 70000, Vietnam; pntdung@ntt.edu.vn
6 Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City 70000, Vietnam
* Correspondence: pham-minh.quan@inpc.vast.vn (M.Q.P.); pthha@ntt.edu.vn (H.H.P.T.)

Abstract: Human hepatocellular carcinoma (HCC), the most common type of liver cancer, represents the second most common cause of death from cancer worldwide. The high toxicity and side effects of some cancer chemotherapy drugs increase the demand for new anti-cancer drugs from natural products. Mortalin/mtHsp70, a stress response protein, has been reported to contribute to the process of carcinogenesis in several ways, including the inhibition of the transcriptional activation of p53. This study conducted a molecular docking study of 41 phyto triterpenes originated from Vietnamese plants for potential Mortalin inhibition activity. Nine compounds were considered as promising inhibitors based on the analysis of binding affinity and drug-like and pharmacokinetic properties.

Keywords: triterpenoid; molecular docking; p53; anti-cancer

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1. Introduction

Hepatocellular carcinoma (HCC) represents the most prevalent type of liver cancer and is ranked second for causes of cancer-related mortality worldwide, especially in Eastern Asia, where the rate is more than 100/100,000 persons [1,2]. The disease is also known for its high recurrence rate in the post-treatment period, urging for an accurate prediction of the early risk in postoperative patients. Among various mechanisms through which HCC develops, mortalin, a stress chaperone of Hsp70 family of proteins, was shown to have a close link with liver cancer incidence through the apprehension of the p53 tumor suppressor protein [3–5]. Though the complexity of p53 function in tumor suppression has been investigated recently in which it regulates both pro-oxidant and antioxidant target genes for complete opposite effects [6]. In this review, Liu et al. indicated that the stability of p53 is negatively regulated by Hsp70 chaperones due to its inhibition effect toward the p53 DNA binding domain [6]. This finding also reported in recent research published by Boysen et al. [7]. To be specific, the binding of mortalin and subsequent sequestration into p53 inhibits its transcriptional activation and leads to the controlled duplication of centrosomes. The consequence of p53 functional inactivation has been demonstrated to be twofold, including the extended longevity of normal human cells and the accelerated malignancy of cancer cells [8–11]. Therefore, intervention in the interaction between mortalin and p53 might represent a promising approach in anticancer therapies.

Triterpenes are natural alkenes found in plants, animals and are derived from fungi. The triterpene class has been shown to have many different activities, such as antiplatelet, hypoglycemia, anti-cancer, anti-HIV, immune booster, anti-inflammatory, antibacterial, insecticidal, fungicidal and anti-cancer [1,2]. Vietnam is among the countries that possess abundant plant resources, housing approximately 10,500 identified species of high-value plants and 3780 species with medicinal properties. Such diversity has recently stimulated the search for new bioactive triterpenes usable in medicinal applications. The present work conducts molecular docking studies of phyto triterpenes originating from Vietnamese plants and analyzes drug-like and pharmacokinetic properties to identify potential Mortalin-p53 binding inhibition candidates.

2. Materials and Methods

2.1. Ligand Preparation

The chemical structures of 41 phyto triterpenes were collected from published literatures for study [1,3–6] (Figure S1). To establish the chemical structures of the triterpenes, Marvin software was used. The 3D structure of compounds was built using PyMOL 1.3 [9]. The energy minimization was carried out using Gabedit 2.5.0 [10]. Open bioactivity prediction online servers Molinspiration [11] and ProTox-II [12] were utilized to evaluate drug-like properties and evaluate the acute toxicity of all research compounds.

2.2. Protein Preparation

According to previous studies and of mortalin structures obtained from Protein Data Bank (RCSB PDB), there are two possible binding sites of mortalin into p53, including the peptide binding domain, described as PDB ID: 3N8E (residues 439–597) [13,14], and the residues 253–282 domain that matched with PDB ID: 4KBO [15–17]. Thus, these two models were selected for the docking study with a resolution of 2.8 Å. To determine ionization and tautomeric states of amino acid residues, the protein structure was prepared using the Graphical User Interface program named Autodock Tools (ADT) [18]. In addition, following the removal of water molecules and the addition of polar hydrogen atoms, the Kollman-united atom partial charges and salvation parameters were assigned. The process resulted in atomic coordinates of the protein in PDBQT file format for AutoGrid and AutoDock.

2.3. Validation Docking

Autodock 4.2.6 was utilized to perform interacting validation using protein models 3N8E and 4KBO. For the examination, the two known inhibitors including Withanone and Withaferin A were chosen from various studies in the literature. The two inhibitors were docked within the possible p53 binding site of Mortalin in a range of 423 to 597 residues (PDB ID: 3N8E), which include key amino acids Leu450, Ala475, Gln479, Arg513 and Glu 577, respectively [19–21]. The docking-simulated region for model PDB ID: 4KBO focus on domain 253–282 with key amino acids Asp136, Asn139, Glu270, Phe272, Asp277 and Arg284, respectively [15,19].

2.4. Molecular Docking Using AutoDock 4.2.6

A computer with configurations of Intel®CoreTM i7-9700K CPU @ 3.60 GHz, with 32 GB DDR4 RAM was used to perform docking runs. Four software packages, including PyMOL [9], Discovery Studio Visualizer [22], LigPlus [23] and Maestro [24], were used to analyze the obtained results, which describes distances of hydrogen bonds between the hydrogen and its supposed binding partner.

The operating system in which the compilation of AutoDock 4.2.6 and docking runs was carried out was Ubuntu-Linux 14.04.6 LTS. A grid box comprised of 80 × 80 × 80 points spaced by 0.130 Å was centered on the mortalin-p53 interactions site (x = −7.109, y = 35.196 and z = −2.684, respectively). The software packages that were used to prepare the binding affinity of each ligand's atom type and perform molecular docking simulation

were AutoGrid and AutoDock 4.2.6, respectively. Lamarckian genetic algorithm (LGA) was configured with following parameters: 50 runs; elitism of 1; the mutation rate of 0.02; the population size of 300; a crossover rate of 0.80; number of generations of 27,000; the energy evaluations of 50,000,000 and the root-mean-square (RMS) cluster tolerance was set to 2.0 Å in each run. From the most favored cluster, the ligand conformation for further analysis was selected on the basis of lowest free binding energy.

3. Results and Discussion

3.1. Validation Docking

Withanone and withaferin A, two structurally similar withanolides isolated from *Withania somnifera* were previously demonstrated to interact with mortalin and abrogate mortalin-p53 interactions [13,17,18,25]. In this study, they were selected as references and redocked to the p53 binding site of Mortalin on two protein models to confirm the validity of the proposed docking procedure (Table 1 and Figure 1).

Table 1. Binding energies of reference inhibitors to mortalin models 3N8E and 4KBO.

Compound Name	Dock Score (kcal/mol)		H-Bond Interacting Residues	
	3N8E	4KBO	3N8E	Ref. [19]
Withanone	−12.08	−10.40	Leu450, Gln479, Asn583	Leu450, Arg513, Asn583
Withaferin A	−10.76	−8.54	Ser473, Arg513, Asn583	Glu483, Arg513, Asn583, Gly587

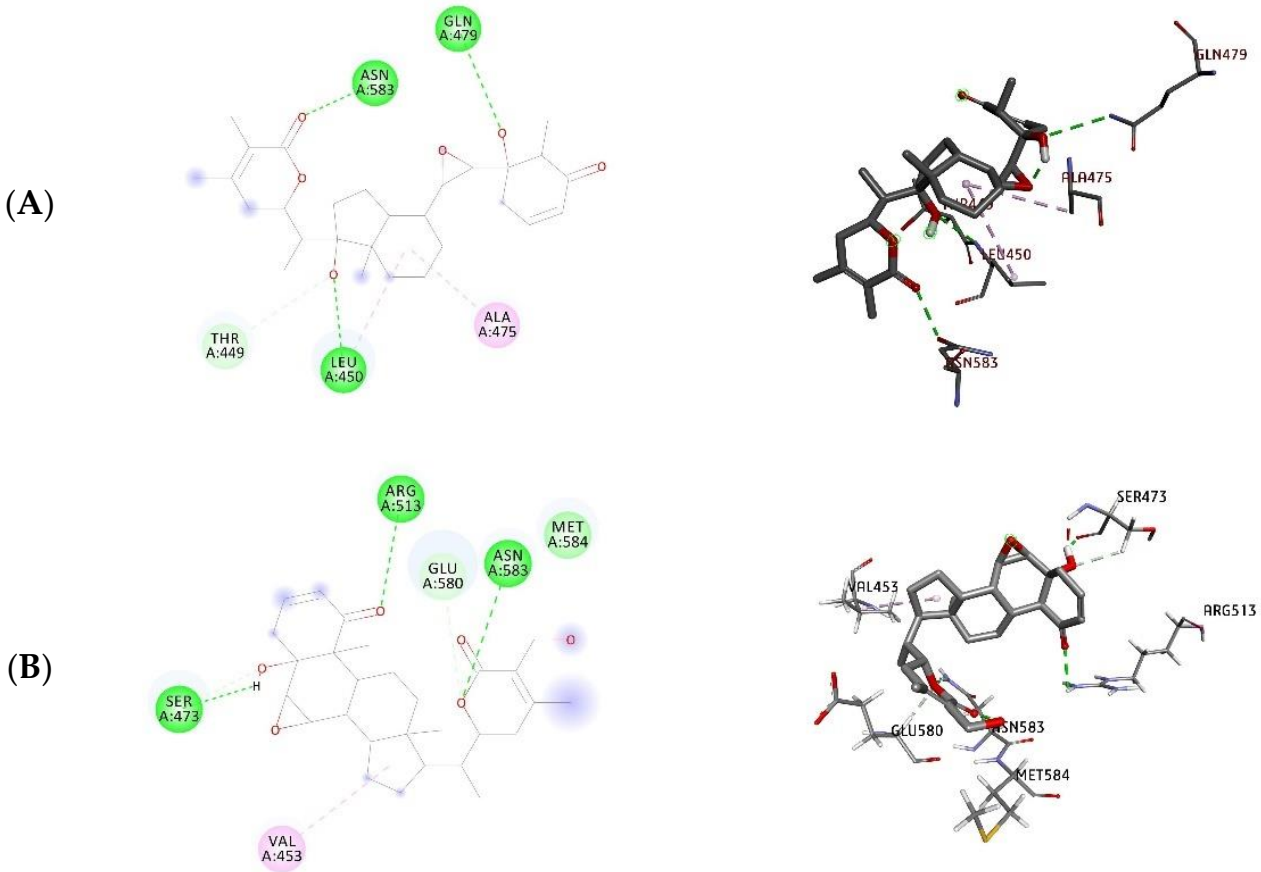

Figure 1. 2D (left panel) and stereoview (right panel) of the binding mode of withanone and withaferin A in the active site of protein Mortalin (PDB ID: 3N8E). (**A**) withanone; (**B**) withaferin A.

The validation docking (Table 1) showed that the dock scores (binding energy) of two reference inhibitors on Mortalin model 3N8E were −10.76 kcal/mol for withaferin A and −12.08 kcal/mol for withanone. Meanwhile, the binding energies on model 4KBO were −8.54 kcal/mol and −10.40 kcal/mol, respectively. Notably, the binding energies of both inhibitors to model 3N8E (439–597 sequence) were better than model 4KBO (253–282 sequence), suggesting that model 3N8E may be preferentially bound with ligands. It is indicated that (Figure 1) withanone formed three H-bond interactions with mortalin, in which Leu450 and Asn583 are two key residues also reported by Vaishnavi et al. [19]. On the other hand, withaferin A shared two common interacting hydrogen bonds with mortalin including Arg513 and Asn583 in comparison to literature [19]; Ser473 is the third H-bond that further strengthens the interaction of the inhibitor. It should be noted that Arg513 constituted the carboxy-terminus region of mortalin and played a vital role in the substrate binding domain. The interaction of potential inhibitors with these residues could deactivate the chaperone function of the protein. In general, these results suggest the reliability of the docking method, and thus, could be utilized for further investigation.

3.2. Drug-Like and Pharmacokinetic Properties Assessment

The drug-like properties of 41 studied phyto triterpenes 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, such as molecular weight <500 Da, number of hydrogen bond donors ≤5, number of hydrogen bond acceptors ≤10, octanol-water partition coefficient (LogP) <5 and Molar refractivity (MR) from 40–130 (Table 2) [26]. Compounds that satisfy these rules would be considered to be drug-like in nature, primarily potential oral bioavailability. The obtained results help in providing essential information regarding the development and discovery of new drugs. According to statistical results, it is believed that overly strict adherence to the rule could lead to an adverse effect of restricting diversity and reducing the similarity to natural products [27]. It is a fact that a number of natural compounds have biological activity, although they do not fullfill all the criteria in Lipinski's Rule of Five (Ro5) [28,29]; therefore, in order to increase the chance of discovering potential drugs, compounds with less than two violations of the rule will be considered for further study. On the other hand, the prediction of different molecule properties in the early stage is a vital step in the discovery and development process [29]. 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 S1).

The outcomes of Lipinski's Rule of Five assessment indicate that among the studied triterpenes, 17 candidates were determined to be favorable for oral drug development, with fewer than two violations of the conditions (Table 2). In addition, the pharmacokinetic parameters and toxicity prediction results, in combination with docking studies, contribute helpful information to the assessment of potential compounds that have inhibition ability and drug-like properties for further drug development. The calculated properties showed interesting results on the toxicity scale. From Table S1, four compounds, **16**, **17**, **19** and **21**, were classified as non-toxic (rank 6) with very high LD_{50} (5010, 5105, 5105, 9000 and 6000 mg/kg, respectively). Another three candidates were positioned at rank 5 and considered as safe, including compounds **15**, **22** and **26**. Nine compounds including **2**, **3**, **14**, **18**, **20**, **24**, **34**, **37** and **38** were classified as low-toxic (rank 4 and 3). It should be noted that all these compounds were evaluated as less toxic than that of withanone and withaferin A (rank 2). On the other hand, it is known that the biological systems are furnished with several heterogeneous phases: water, serum protein, lipid particles etc.; thus, the molecule physicalchemical properties are important to determine compound reactivity (drug transport processes and drug–receptor interaction) [30]. miLogP is an important parameter for quantitative structure-property relationship (QSPR) studies. The higher miLogP value assumes that the compounds might easily diffuse across the cell membranes. Besides, it was reported that compounds with good oral bioavailability would possess

a total polar surface area (TPSA) range from 70–140 Å^2 and 12 or fewer H-bond donors (HBD) and acceptors (HBA) [27]. In this study, following the filtered results from Ro5, most of the selected compounds (12 out of 17 compounds) except **2**, **3**, **20**, **26** and **37** were observed to have TPSA value within the range 70–140 Å^2 and miLogP from 3.36 to 6.24, which satisfies the criteria of drug-like properties and demonstrates suitability in oral drug development (Table S1).

Table 2. Drug-like properties of studied compounds according to Lipinski's Rule of Five.

ID	Compound Name	MW	HBD	HBA	LogP	MR (cm^3/mol)
1	3-oxo-threo-23,24,25-trihydroxytirucall-7-ene	474	3	4	5.97	137.60
2 *	23,24,25,26,27-pentanorlanost-7,9(11)-dien-3b,22-diol	358	2	2	4.72	109.02
3 *	23,24,25,26,27-pentanorlanost-8-en-3b,22-diol	374	2	2	5.51	112.06
4	23-hydroxyursolic acid	472	3	4	6.27	134.39
5	28-formyloxy-3ß-hydroxy-urs-12-ene	470	1	3	7.32	138.32
6	Ailanaltiolide A	630	3	9	3.87	172.39
7	Ailanaltiolide B	630	3	9	3.87	172.39
8	Ailanaltiolide C	612	3	9	3.44	167.28
9	Ailanaltiolide D	626	2	9	3.8	172.04
10	Ailanaltiolide E	594	2	8	4.28	165.57
11	Ailanaltiolide F	586	1	9	3.19	154.90
12	Ailanaltiolide G	598	3	8	3.58	165.93
13	Ailanaltiolide H	522	4	6	4.54	146.84
14 *	Ailanaltiolide I	486	2	5	4.27	136.62
15 *	Ailanaltiolide J	430	1	4	5.3	119.37
16 *	Ailanthusin B	480	2	5	3.75	136.66
17 *	Ailanthusin A	480	2	5	3.75	136.66
18 *	Ailanthusin C	482	2	5	4.43	134.73
19 *	Ailanthusin D	498	4	6	3.81	137.71
20 *	Ailanthusin E	362	3	4	4.11	97.33
21 *	Ailanthusin F	492	4	5	4.19	140.53
22 *	Ailanthusin G	490	3	5	4.75	137.98
23	Arjunic acid	502	4	5	5.92	140.16
24 *	Belamchinenin A	500	2	5	3.91	147.25
25	Bourjutinolone A	472	2	4	5.47	135.68
26 *	Cabralealactone	414	0	3	6.46	117.91
27	Corosolic acid	472	3	4	6.38	134.48
28	Euscaphic acid	488	4	5	5.18	135.70
29	Kadsuphilactone B	482	1	5	5.04	135.03
30	Maslinic acid	472	3	4	6.52	134.10
31	Oleanolic acid	470	2	3	7.89	137.25
32	Pomolic acid	472	3	4	6.13	134.24
33 *	Schinalactone D	514	1	5	6.19	144.80
34 *	Schinchinenlactone A	494	0	6	2.91	136.25
35	Schisanlactone C	480	1	5	4.33	138.80
36	Schisanlactone I	486	1	5	5.22	139.30
37 *	Schisanlactone J	478	0	5	4.08	138.26
38 *	Ursolic acid	456	2	3	7.33	123.02
39	Tormentic acid	488	4	5	5.18	135.70
40	Lanost-8-en-3b,22S,23S-triol	458	3	3	6.19	137.60
41	Schisphendilactone B	494	0	6	3.34	136.74
	Withanone	470	2	6	3.49	124.51
	Withaferin A	470	2	6	3.35	124.46

* Compound with less than two violations of Lipinski's Rule of Five.

3.3. Docking Studies

To explore the inhibition potential of 41 selected ligands with protein Mortalin (3N8E model), AutoDock 4.2.6 was utilized for docking studies. Table 3 showed the dock score of the studied compounds.

Table 3. The docking score of studied compounds on protein Mortalin (PDB ID:3N8E).

Compound ID	Dock Score (kcal/mol)	Compound ID	Dock Score (kcal/mol)
Withanone	−12.25	14	−9.71
21	−11.84	30	−9.67
22	−11.77	40	−9.55
19	−11.63	18	−9.54
9	−11.49	3	−9.53
1	−11.27	Withaferin A	−9.51
34	−11.19	8	−9.48
37	−11.18	5	−9.42
12	−11.03	35	−9.34
38	−10.73	36	−9.31
16	−10.65	24	−9.29
29	−10.44	2	−8.85
7	−10.39	25	−8.72
10	−10.36	27	−8.65
33	−10.35	23	−8.54
15	−10.34	20	−8.51
11	−10.31	31	−8.47
13	−9.97	32	−8.33
41	−9.95	4	−8.18
6	−9.93	39	7.70
26	−9.83	28	−7.35
17	−9.80		

According to docking validation, the obtained dock scores of withanone and withaferin A were −12.25 kcal/mol and −9.51 kcal/mol, respectively. Thus, any ligand whose docking energy falls within this range or more negative would be considered as a potential inhibitor of Mortalin. It is indicated from Table 4 that 26 out of 41 screened compounds were identified as potential inhibitors of Mortalin. Compounds 21, 22, 19, 9 and 1 are the top five ligands whose docking energies greatly exceeded those of reference compounds (−11.84, −11.77, −11.63, −11.49 and −11.27 kcal/mol, respectively). The remaining 22 compounds had dock scores ranging from −9.53 kcal/mol to −11.19 kcal/mol, which match the selection criteria.

The cytotoxic activity of the tested compounds on HepG2 from literature was retrieved to gain better insight into the effects resulting from the disruption of moratlin–p53 interaction caused by potential compounds (Table 4) [1,3–8].

Using Cheng–Prusoff's formula [31], the Ki inhibition constant is calculated as follows:

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

Assuming that the IC_{50} value equals 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 are measured in moles. Energy is measured in kilocalories per mole. As a result, the plotting of experimental binding free energies against the computed values gave a relative high correlation coefficient of R^2 = 0.85 which suggesting the reliable of the docking study (Figure 2). It is also interesting to note that, among studied compounds, ursolic acid has been reported

for its significant effect against HepG2 cell lines using mouse xenograft tumor models [32]. According to Liu et al. [33], ursolic acid could suppress HepG2 tumor growth in a time-dependent manner when tested at a concentration of 2.6μmol in 100 μL per day. These data further suggest the potential activities of this class of compound.

Table 4. Cytotoxic activity (IC_{50}, μM) of 41 phyto triterpenes on HepG2 cell line after 48 h of incubation.

Compound ID	IC_{50} (μM)	Compound ID	IC_{50} (μM)
Withanone	5.12 ± 0.32	14	44.74 ± 0.74
21	8.43 ± 0.25	30	42.83 ± 1.78
22	6.28 ± 1.21	40	35.54 ± 1.43
19	10.67 ± 1.16	18	37.43 ± 0.68
9	12.54 ± 0.54	3	38.26 ± 1.82
1	9.12 ± 0.36	Withaferin A	33.50 ± 0.76
34	15.35 ± 0.62	8	49.23 ± 0.77
37	18.52 ± 0.87	5	48.75 ± 0.35
12	28.81 ± 0.75	35	>50
38	17.42 ± 0.91	36	>50
16	15.25 ± 1.15	24	47.33 ± 0.52
29	20.75 ± 1.64	2	>50
7	28.87 ± 0.35	25	>50
10	22.65 ± 0.63	27	>50
33	25.63 ± 0.46	23	>50
15	29.43 ± 0.68	20	>50
11	34.54 ± 0.94	31	>50
13	35.53 ± 0.49	32	>50
41	33.28 ± 0.37	4	>50
6	39.16 ± 0.22	39	>50
26	40.25 ± 0.31	28	>50
17	36.88 ± 1.52		

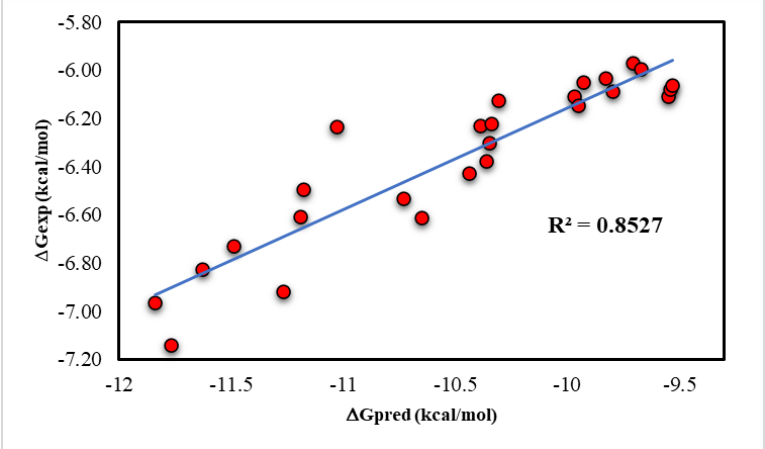

Figure 2. The correlation between experimental and computational binding free energies.

Applying Lipinski's Rule of Five and pharmacokinetic parameters in combination with obtained dock score, nine compounds were considered as promising for further drug development including compounds **14**, **16**, **17**, **18**, **19**, **21**, **22**, **34** and **38**. The stereoview of the binding mode of nine potential inhibitors of protein Mortalin is depicted in Figure S2. It was shown that the occurrence of Mortalin-p53 binding abrogation on Mortalin model 3N8E requires interaction on key amino acid residues, such as Leu450, Ser473, Gln479 and Arg513 at the active site of protein [19,20]. Arg 513, due to its position of the "latch" between the "lid and cleft regions" of mortalin, has been highlighted as the key residue determining the chaperone function of the protein [34]. As indicated from Table S2, all nine compounds exhibited hydrogen bond interactions, with essential residues in

the p53-binding site of Mortalin. Compound **21** displayed the best pharmacophore fit scores compared to the others, which formed four H-bonds with Leu450; Glu483; Arg513; Asn583 (Figure 3 and Figure S2). Compound **22** formed 3 hydrogen bonds with key residues: Glu448, Glu483 and Arg513, whereas compound **19** formed two H-bonds with Ser473—both Arg513. The obtained results suggest that interaction with these residues contribute to disrupting interaction between Mortalin and p53, thus, reactivate the function of p53. Regarding toxicity prediction (Table S1), it should be noted that, amongst 9 "hits", compounds **16**, **17**, **19** and **21** being ranked as non-toxic for human (rank 6), therefore, they could be assumed as the most suitable for further drug development. Compounds **14**, **22** and **38** also fell within the safe zone, with their rank being in the range between 4 and 5. Compounds **18** and **34** are classified as possibly toxic at high doses, thus suggesting that they are inappropriate for future drug development.

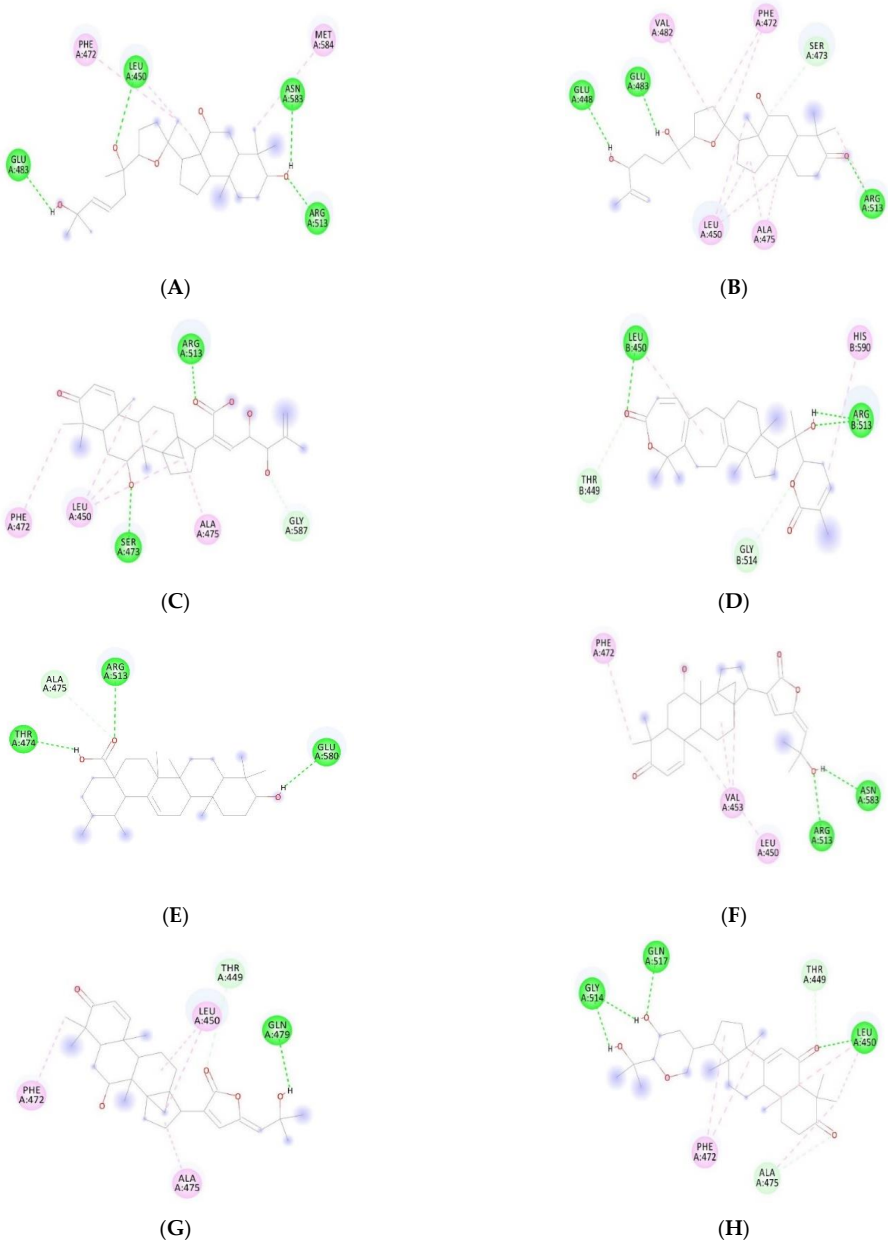

Figure 3. *Cont.*

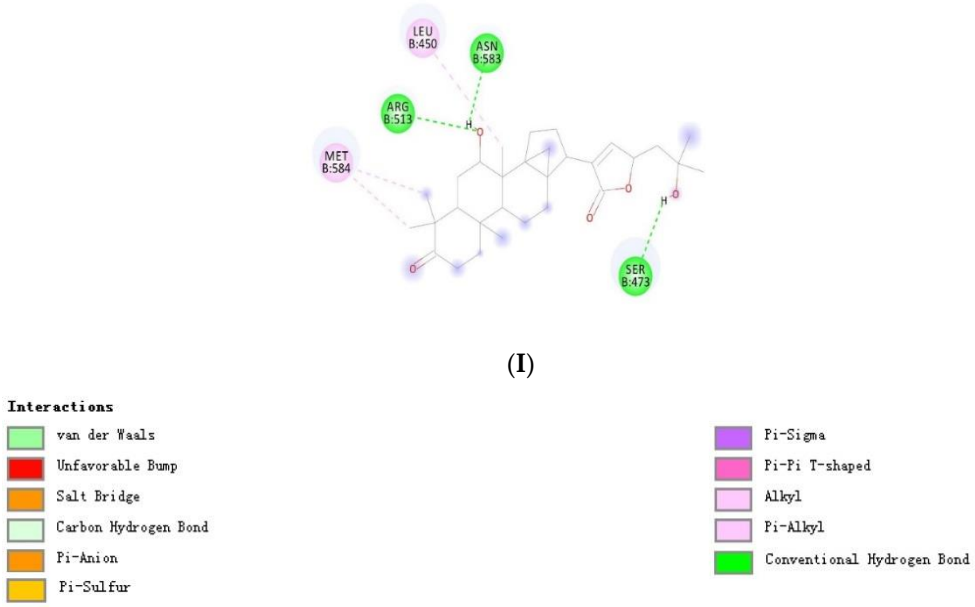

Figure 3. Hydrogen bonding patterns of potential Mortalin (PDB ID: 3N8E) inhibition compounds. (**A**) Compound 21; (**B**) Compound 22; (**C**) Compound 19; (**D**) Compound 34; (**E**) Compound 38; (**F**) Compound 16; (**G**) Compound 17; (**H**) Compound 14; (**I**) Compound 18.

In addition, the total polar surface area (TPSA) map of nine potential triterpenes was simulated. There are two factors that define the lipophilic character of a molecule, including hydrophobicity and polarity, which make the molecule cross or irreversibly damage the cellular membrane. It was found that, in Figure S3, compounds **14**, **17** and **18** with low cytotoxicity activity exhibit fewer polar areas than the one with high cytotoxicity effects. Compounds **19** and **21** with the highest TPSA values (115.05 and 90.15 $Å^2$, respectively) are observed to have more polar areas than the others and correlate with its low IC_{50} value (10.67 ± 1.16 and 8.43 ± 0.25 μM).

4. Conclusions

In this study, the computational molecular simulation and assessment of drug-like properties were used to gain insight into the interaction of 41 phyto triterpenes on the p53 binding site of protein Mortalin. The obtained results show that 9 out of 41 studied compounds, including ailanaltiolide I, ailanthusin A, ailanthusin B, ailanthusin C, ailanthusin D, ailanthusin F, ailanthusin G, schisanlactone C and ursolic acid, which correspond to IDs **14**, **16**, **17**, **18**, **19**, **21**, **22**, **34** and **38**, were identified as potential candidates for inhibiting the p53 binding site of protein Mortalin through hydrogen bonds with key amino acids Leu450, Ser473, Gln479 and Arg513. In particular, ailanthusin C and schisanlactone C will need more attention in in vivo tests due to their high toxic LC_{50} prediction. These results contribute information on the interaction mechanism of potential inhibitors for further studies to develop drugs that prevent the function of Mortalin in liver cancer.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr9111983/s1, Figure S1: Structure of 41 studied phyto triterpenes, Figure S2: Stereoview of the binding mode of nine potential inhibitors of protein Mortalin (PDB ID: 3N8E). (A) Compound 21; (B) Compound 22; (C) Compound 19; (D) Compound 34; (E) Compound 38; (F) Compound 16; (G) Compound 17; (H) Compound 14; (I) Compound 18, Figure S3: Maps of total polar surface area (TPSA) of nine potential compounds showing the nonpolar area (gray white color) and polar area (red color). (A) Compound 21; (B) Compound 22; (C) Compound 19; (D) Compound 34; (E) Compound 38; (F) Compound 16; (G) Compound 17; (H) Compound 14; (I) Compound 18, Table S1: Pharmacokinetic parameters and toxicity prediction of studied compounds, Table S2: The H-bond interactions between potential compounds and Mortalin model 3N8E.

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