In Silico Study on Interaction and Preliminary Toxicity Prediction of *Eleutherine americana* Components as an Antifungal and Antitoxoplasmosis Candidate

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Abstract: *Red bulbs of* Eleutherine americana *(Aubl.) Merr. ex K. Heyne has been known for its high content of naphthoquinones that have antifungal and antiparasitic activities. In this research,* in silico *interaction study was performed between 31 compounds reported to be found in* E. americana *with the selected target proteins for antifungal and antitoxoplasmosis activity using the molecular docking method. An ORPs (OSBP-related proteins), Osh4 (PDB ID: 1ZHX), and* N*-myristoyltransferase (Nmt, PDB ID: 1IYL) were used as the antifungal target proteins.* Toxoplasma gondii *purine nucleoside phosphorylase (TgPNP, PDB ID: 3MB8) and calcium-dependent protein kinase-1 (TgCDPK1, PDB ID: 4M84) were used as antitoxoplasmosis target proteins. Three-dimensional structures of the test compounds were made and optimized using GaussView 6.0 and Gaussian 09W. The target proteins were prepared using the Discovery Studio 2016 Program. Aquatic toxicity prediction as the preliminary assessment of the safety of the compounds was performed using ECOSAR v2.0. The results suggest that the compound having both the smallest free binding energy compared with positive control and other test compounds and low predicted toxicity is β-sitosterol with a free binding energy of −11.55 and −11.18 kcal/mol towards Osh4 and Nmt and −8.06 and −10.29 kcal/mol towards TgPNP and TgCDPK1, respectively.*

Keywords: *fungal infection; toxoplasmosis;* Eleutherine americana*; molecular docking; aquatic toxicity*

■ INTRODUCTION

Antifungal is a compound with the activity in destroying or inhibiting the growth of fungi. The development of these compounds is slower compared with antibacterial agents as the similar eukaryotic cell properties of the organisms that the developed targets are more limited [1]. The level of fungal infection has been increased in recent years, and the causes of fungal infections are also increasingly developing in other fungal species [2]. On the other hand, another emerging infection problem is related to *Toxoplasma gondii.* This parasite can have harmful effects on the fetus, children, and patients with low immune systems [3]. Antitoxoplasmosis is a group of compounds that has the activity to kill or inhibiting the growth of the parasite. Currently, first-line therapy for infection of *T. gondii* is the combination of pyrimethamine and sulfadiazine,

which work synergistically in inhibiting folic acid metabolism of the parasite [4]. However, the combination can cause severe side effects, and lifelong treatment is needed in patients with low immune systems [5]. In addition, the therapy is also contraindicated for the first trimester in pregnancy. These problems result in the demand for the discovery of antifungal and antitoxoplasmosis agents with a better spectrum of activity and safety profile.

Eleutherine americana (Aubl.) Merr. ex K. Heyne is a plant that is well distributed in South America, South Africa, and Southeast Asia. The bulb of the plant has been used in traditional medication for a long time in the treatment of heart disease, breast cancer, diabetes, and hypertension [6]. Furthermore, several kinds of research have reported the antimicrobial activity of the plant using *in silico* method [7-8]. Previous research has also reported that the *E. Americana* bulb is rich in naphthoquinones [7]. The compounds are known to have the activity as antimicrobial and antiparasitic agents [8]. In this research, *in silico* interaction study was carried out between the constituents of the plant with selected target proteins for antifungal and antitoxoplasmosis agents. Aquatic toxicity prediction was also studied to the compounds as the preliminary assessment of their safety profile.

■ EXPERIMENTAL SECTION

Materials

Three-dimensional structures of target proteins were obtained from the Protein Data Bank (https://www.rcsb.org/). The targets used for antifungal activity study were an ORPs (OSBP-related proteins), Osh4, and *N*-myristoyltransferase (Nmt). *Toxoplasma gondii* purine nucleoside phosphorylase (TgPNP) and calcium-dependent protein kinase-1 (TgCDPK1) were used in the study of antitoxoplasmosis activity (Table 1 and 2). (1-methylimidazole-2-yl)-[3-methyl-4-[3-(pyridine-3-ylmethylamino) propoxy]-1-benzo-furan-2-yl] methanone (R64) and 25-hydroxycholesterol were used as a positive control in antifungal activity study, while the positive controls used in antitoxoplasmosis activity study were immucillin-H (IMH) and 5-amino-1-tert-butyl-3-(quinolin-2-yl)-1H-pyrazole-4-carboxamide (21E). Research has shown that the NMT gene is essential for vegetative growth and survival of *Candida albicans* and *Cryptococcus neoformans* [9-10]. In addition, NMT is a promising target protein for the development of new fungicidal drugs and has a broad spectrum of antifungal [11]. Osh4 is an important antifungal target protein which plays a role in sterol membrane regulation [12].

Table 1. Three-dimensional structures of proteins used in antifungal activity study

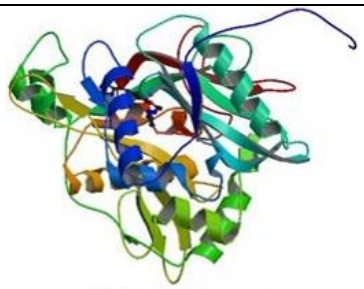
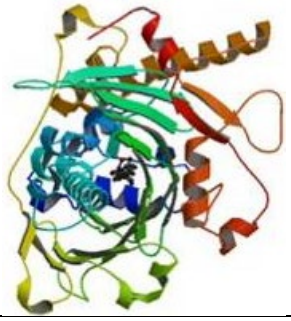
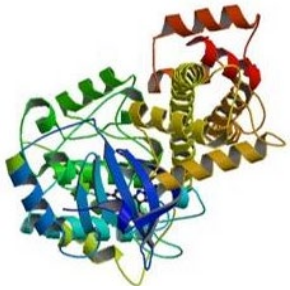
Target Name	Structure	Reference
N-myristoyltransferase (PDB ID: 1IYL)		[14]
Osh4 (PDB ID: 1ZHX)		[15]

Table 2. Three-dimensional structures of proteins used in antitoxoplasmosis activity study

Target Name	Structure	Reference
Purine nucleoside phosphorilase (PDB ID: 3MB8)		[13]
Calcium-dependent protein kinase-1 (PDB ID: 4M84)		[5]

For antitoxoplasmosis target proteins, PNP plays an important role in the rescue pathway of nucleotides, and the structure of PNP enzymes in *T. gondii* is different from the structure of PNP in mammals [13]. CDPK1 has a function in the invasive and release of *T. gondii* from its host. In addition, this enzyme is only found in plants and Apicomplexa, but it is not found in humans and animals [5]. The test compounds were 31 molecules that have been reported as the chemical constituents of *E. Americana* (Table 3).

Procedure

Geometry optimization of the test compounds and preparation of the targets

Three-dimensional structures of 31 test compounds were built using GaussView® and optimized using Gaussian® with Density Functional Theory (DFT) B3LYP method and 6-31G as the basis set. Target protein preparation, including removal of the ligand and water molecules, were carried out using Discovery Studio 2016. Hydrogen atoms were added to the proteins using AutoDock 4.2.6.

In silico *interaction study*

In silico, an interaction study was carried out by conducting molecular docking of the test compounds with each target protein using AutoDock 4.2.6. The docking procedures were validated before being used for the test compounds using the root mean square deviation (RMSD) value of the ligand's coordinates after redocking compared with the initial position before being extracted from the protein files. The value of not larger than 2 Å is considered as the acceptance criteria, which indicate that the ligands are back to their original position using the docking procedures [25]. Parameters, such as free energy of binding, inhibition constant and the interaction between the compounds and the residues of the target, were analyzed from the docking results. The data were also compared for the test compounds and the positive controls.

Toxicity prediction using ECOSAR v2.0

The test compound with the best parameter was then subjected to aquatic toxicity prediction using ECOSAR v2.0 [26]. The prediction is including their toxicity towards organisms such as fish, daphnid, and green algae. The procedures involve the submission of SMILES notation of the compounds that were being studied. The results were the value of LC_{50} for fish and daphnid or EC_{50} for green algae for acute toxicity and ChV (chronic value) for the three organisms for the chronic toxicity.

Table 3. Chemical constituents of *E. americana* used as the test molecules

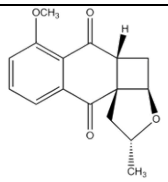
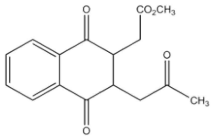
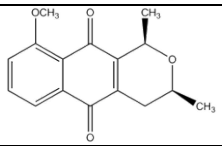
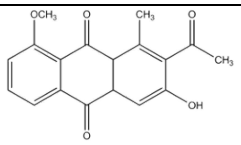
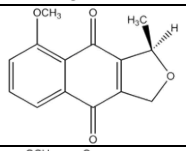
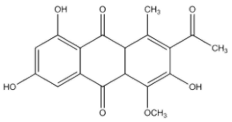
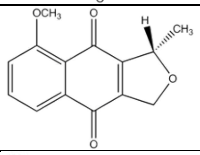
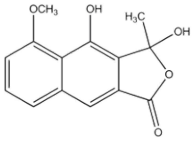
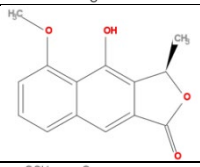
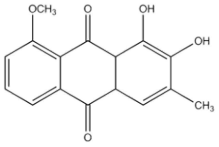
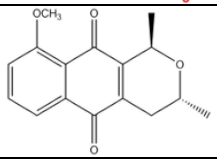
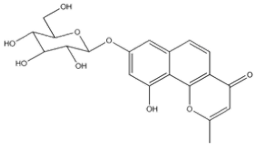
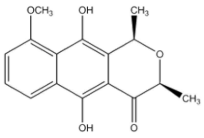
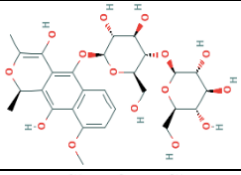
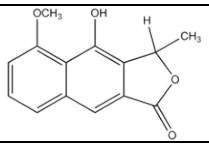
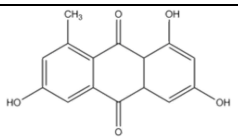
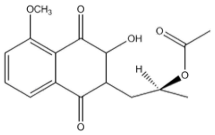
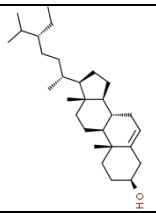
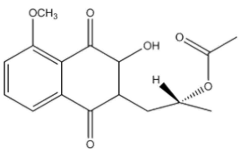
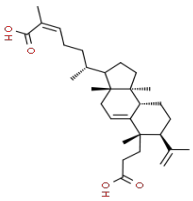
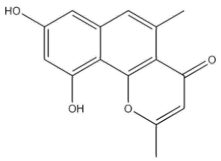
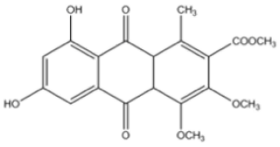
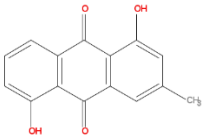
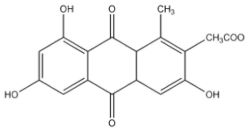
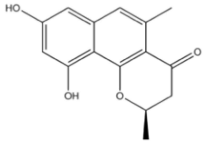
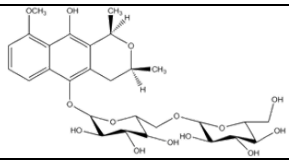
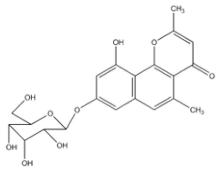
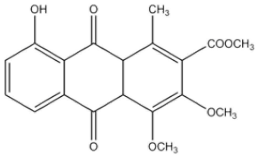
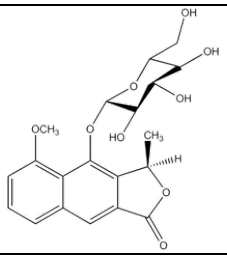
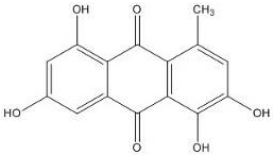
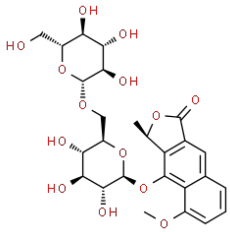
No	Compound	Structure	No	Compound	Structure
1	Elecanacin [16]		17	Eleuthinone A [22]	
2	Eleutherin [16]		18	Eleuthraquinone A [22]	
3	Eleutherinon A [17]		19	Eleuthraquinone B [22]	
4	Eleutherinon B [17]		20	Eleucanarol [22]	
5	Eleutherol [18]		21	1,2-dihydroxy-8-methoxy-3-methylanthraquinone [16]	
6	Isoeleutherin [16]		22	Eleutherinoside A [23]	
7	Hongconin [19]		23	Eleutherinoside B [23]	
8	Isoeleutherol [16]		24	1,3,6-trihydroxy-8-methylanthraquinone [16]	
9	(2S)-1-(3-hydroxy-5-methoxy-1,4-dioxo-1,2,3,4-tetrahydro naphthalen-2-yl)propan-2-yl acetate [16]		25	β-sitosterol [16]	
10	(2R)-1-(3-hydroxy−5-methoxy-1,4-dioxo-1,2,3,4-tetrahydronaphthalen-2-yl)propan-2-yl acetate [16]		26	Kadsuric acid [20]	

Table 3. Chemical constituents of *E. americana* used as the test molecules (Continued)

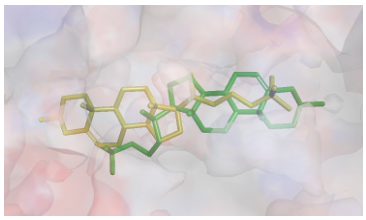
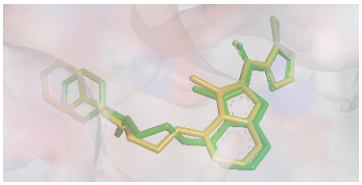
No	Compound	Structure	No	Compound	Structure
11	Eleutherinol [20]		27	6,8-dihydroxy-3,4-dimethoxy-1-methyl-anthraquinone-2-carboxylic acid methyl ester [24]	
12	1,5-dihydroxy-3-methylanthraquinone [16]		28	2-acetyl-3,6,8-trihydroxy-1-methyl anthraquinone [25]	
13	Dihydroeleutherinol [20]		29	Eleuthoside C [16]	
14	2,5-dimethyl-10-hydroxynaphtopyrone 8-O-β-glucopyranoside [16]		30	9,10-dihydro-8-hydroxy-3,4-dimethoxy-9,10-dioxo-2-anthracenecarboxylic acid methyl ester [16]	
15	Eleuthoside A [21]		31	Erythrolaccin [16]	
16	Eleuthoside B [21]				

■ RESULTS AND DISCUSSION

In silico Interaction Study

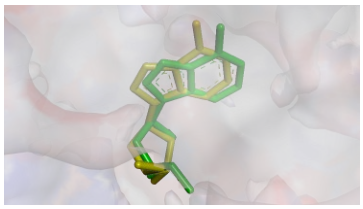
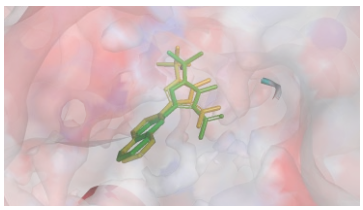
The results of the validation of the molecular docking procedures are shown in Table 4 and 5. It can be seen from the Table 4 and 5 that the docking procedures fulfill the criteria of acceptance for the value of RMSD, indicating that the positions of the ligands were not significantly changed after being used in the proposed docking procedures. The docking result of the test compound to each target (Table 6) indicates that all of them have an affinity towards the target protein with negative values of free energy of binding between them and the proteins. The characteristic compounds of *E. Americana* (compound 1, 2, 3, 4, 5 and 11) seem to have

Table 4. Result of the docking procedures validation for antifungal study

Protein	Ligand	Free binding Energy (kcal/mol)	Inhibition Constant (μM)	RMSD (Å)	Superimposed Ligand Structure*
Osh4	HC3	-11.51	3.67×10^{-3}	1.860	
Nmt	R64	-10.04	4.342×10^{-2}	0.784	

*Superimposing ligand structures before and after redocking with proposed procedures to compare both coordinates. The ligands with the original position are indicated with yellow color while the redocking results are indicated with green.

Table 5. Result of the docking procedures validation for antitoxoplasmosis study

Protein	Ligand	Free binding energy (kcal/mol)	Inhibition Constant (μM)	RMSD (Å)	Superimposed Ligand Structure*
TgPNP	IMH	-6.93	8.35	0.900	
TgCDPK1	21E	-9.04	2.351×10^{-1}	0.709	

*Superimposing ligand structures before and after redocking with proposed procedures to compare both coordinates. The ligands with the original position are indicated with yellow color while the redocking results are indicated with green.

a weaker affinity to Osh4 and Nmt, with the larger value of the energies and inhibition constants. Compound 25 has a better affinity towards both targets, compared to the positive controls used. The result was also obtained in an antitoxoplasmosis study using TgPNP and TgCDPK1 as the targets. The compound has the lower binding free energy and inhibition constant compared to the positive control and other test compounds. Compound 1, which is a typical compound in the plant, and four other compounds (compound 17, 18, 21, and 26) also have the lower value of both parameters compared to IMH in protein TgPNP. Docking results to TgCDPK1 show that no typical compound of *E. Americana* which has a better affinity than the positive control. Test compounds having that criteria are compound 25, 26 and 29, with compound 25 has the best affinity among all.

Toxicity Prediction using ECOSAR v2.0.

Aquatic toxicity prediction was carried out as the preliminary assessment of the safety of the compounds. ECOSAR itself has the maximum for the value of log K_{ow} that indicates that the compound is insoluble that it cannot

Table 6. Docking results of the test compounds to the target proteins

Compound	Free binding energy (kcal/mol)				Inhibition constant (μM)			
	Osh4	Nmt	TgPNP	TgCDPK1	Osh4	Nmt	TgPNP	TgCDPK1
1	−7.59	−7.61	−6.95	−6.73	2.71	2.65	8.06	11.66
2	−7.71	−7.56	−6.16	−7.12	2.22	2.89	30.75	6.09
3	−7.01	−6.68	−5.33	−6.48	7.24	12.68	124.73	17.94
4	−7.1	−6.52	−5.41	−6.31	6.2	16.54	109.16	23.89
5	−6.66	−7.04	−6.02	−6.93	13.03	6.92	38.85	8.27
6	−7.43	−7.41	−6.3	−6.83	3.58	3.69	24.17	9.85
7	−7.18	−6.67	−5.63	−6.93	5.47	12.98	74.65	8.32
8	−6.81	−7.3	−6.52	−6.68	10.24	4.43	16.51	12.72
9	−8.00	−7.28	−6.24	−7.58	1.36	4.59	26.5	2.77
10	−7.62	−7.25	−6.62	−7.38	2.58	4.86	14.01	3.91
11	−7.24	−7.18	−5.61	−6.95	4.96	5.50	76.9	8.05
12	−7.12	−6.63	−6.12	−7.14	6.09	13.7	32.66	5.83
13	−7.44	−7.37	−5.55	−7.02	3.51	3.98	85.29	7.18
14	−8.32	−8.47	−6.18	−8.41	0.800	0.621	29.73	0.686
15	−7.15	−7.54	−5.01	−7.64	5.79	2.95	212.45	2.52
16	−7.54	−7.71	−5.70	−8.58	2.95	2.22	66.69	0.513
17	−7.83	−7.79	−7.46	−7.56	1.83	1.95	3.41	2.85
18	−8.24	−8.33	−7.09	−8.00	0.911	0.781	6.4	1.36
19	−8.02	−8.54	−6.31	−8.22	1.32	0.549	23.71	0.940
20	−6.65	−6.57	−5.77	−6.22	13.28	15.35	58.48	27.39
21	−7.68	−7.35	−7.58	−7.58	2.35	4.12	2.77	2.77
22	−8.25	−7.64	−5.57	−8.03	0.894	2.52	82.53	1.3
23	−7.08	−7.44	−3.61	−6.94	6.43	3.54	$2.25.10^{3}$	8.16
24	−7.56	−7.30	−6.41	−7.80	2.85	4.45	20.13	1.91
25	−11.55	−11.18	−8.06	−10.29	$3.4.10^{-3}$	$6.4.10^{-3}$	1.24	$2,848.10^{-2}$
26	−10.11	−8.02	−7.79	−9.07	0.039	1.33	1.94	$2,238.10^{-1}$
27	−8.87	−8.65	−6.68	−7.59	0.315	0.460	12.78	2.74
28	−8.26	−8.6	−6.35	−7.59	0.876	0.495	22.11	2.73
29	−7.6	−8.52	−3.6	−9.64	2.68	0.564	2.28	$8,516.10^{-2}$
30	−9.4	−8.47	−6.4	−7.48	0.129	0.619	20.36	3.29
31	−7.15	−7.32	−5.85	−7.45	5.79	4.32	51.64	3.47
Positive controls	−11.51	−10.04	−6.93	−9.04	$3,67.10^{-3}$	$4,342.10^{-2}$	8.35	$2,351.10^{-1}$

develop the toxicity towards the test organism if it has log K_{ow} higher than the limit (Table 7). The program also has a classification of the toxicity (Table 8) that is used by the United States Environmental Protection Agency (US EPA), which consists of high, moderate, and low concern, assessed from the acute and chronic toxicity parameter [27].

The toxicity prediction result (Table 9) shows that the compound 25 and 26 have the value of log K_{ow} which are larger than the maximum limit that they are considered as '*low concern*'. Compound 1, 17, and 29 are also in the same cluster since they have LC_{50} and EC_{50} values larger than 100 mg/L and ChV value larger than 10.0 mg/L. On the other hand, compound 18 and 21 have the value of EC_{50} towards green algae smaller than 1 mg/L that these compounds are considered as '*high concern*' toxicity level.

Based on the *in silico* interaction study and the toxicity prediction test, it can be summarized that compound 25 (β-sitosterol) is the most promising compound contained in *E. americana* that predicted to be

Table 7. The maximum limit of log K_{ow} value for aquatic toxicity parameters in ECOSAR*

Fish (LC_{50}, 96 h)	Daphnid (LC_{50}, 48 h)	Green algae (EC_{50}, 96 h)	ChV
5.0	5.0	6.4	8.0

*The values are in ppm. LC_{50} indicates concentration in water that kills 50% of organism in a continuous exposure. EC_{50} is concentration that gives decrease of growth of 50% relative to the control in continuous exposure. ChV (chronic value) ia a geometric average of NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) (EPA, 2013)

Table 8. Classification of aquatic toxicity levels

High Concern	*Moderate Concern*	*Low Concern*
Any of the 3 acute values are < 1.0 mg/L, or any of the chronic values are < 0.1 mg/L	Any of the 3 acute values are between 1.0 mg/L and 100 mg/L, OR any of the chronic values are between 0.1 mg/L and 10.0 mg/L	All 3 acute values are >100 mg/L, and all three chronic values are >10.0 mg/L, or there are "No Effects at Saturation" (or NES). NES occurs when a chemical is not soluble enough to reach the effect concentration, i.e., the water solubility is lower than an effect concentration, or, for liquids, when K_{ow} criteria are exceeded for an endpoint. For solids, NES is expected if K_{ow} exceeds the specific SAR K_{ow} cutoffs, or the effective concentration is more than one order of magnitude (> 10 X) less than water solubility.

Table 9. Results of prediction of aquatic toxicity

Compound	Molecular weight (g/mol)	Solubility in water (mg/L)	Log K_{ow}	Organism					
				Fish		Daphnid		Green algae	
				LC_{50}, 96 h	ChV	LC_{50}, 48 h	ChV	EC_{50}, 96 h	ChV
1	272.3	728.72	1.62	487.69	45.75	267.44	23.67	172.52	41.81
17	274.28	15,384.22	0.06	498.69	56.38	1,276.11	1,524.82	738.63	100.82
18	312.32	172.81	2.08	5.61	1.48	14.56	0.74	0.4	2.7
21	286.29	871.62	1.44	7.93	3.59	22.15	1.5	0.67	4.82
25	414.72	0	9.65	0	0	0	0	0	0
26	470.7	0	9.28	0	0	0	0	0.01	0.02
29	598.61	6,124.03	-1.16	12,177.76	873.82	1,264.8	79.72	950.84	950.88

*The values are in ppm

the inhibitor of Osh4, Nmt, TgPNP, and TgCDPK1 with better affinity compared to the positive control and other constituents of the plant. In a previous study, it also has been reported that the compound has the activity as an antifungal in an in vitro study [28-29].

Study of Interaction of Compound 25 towards the Target Proteins

Fig. 1 shows the two-dimensional diagram of the interaction between compound 25 and the positive controls with Osh4 and Nmt. The molecular docking result in Fig. 1(a) shows that the compound 25 forms 8 van der Waals interactions with the residues GLN96, ARG100, GLU107, LYS108, LYS109, ASN165, PRO198, and VAL213 of Osh4. Twenty-four of alkyl interactions with the residues TRP10, PHE13, LEU24, LEU27, ALA29, ILE33, LEU39, PHE42, TYR97, PRO110, ILE167, PHE171, LEU177, VAL179, LEU201, ILE203, ILE206 and PRO211, and a hydrogen bond with the residue GLN181 of the target. On the other hand, it is indicated in Fig. 1(b) that the positive control form a similar interaction with the target which consists of 12 van der Waals interactions with TRP10, ALA29, PRO30, LEU39, PHE42, GLU107, LYS108, ILE167, LEU201, ILE206, PRO211 and

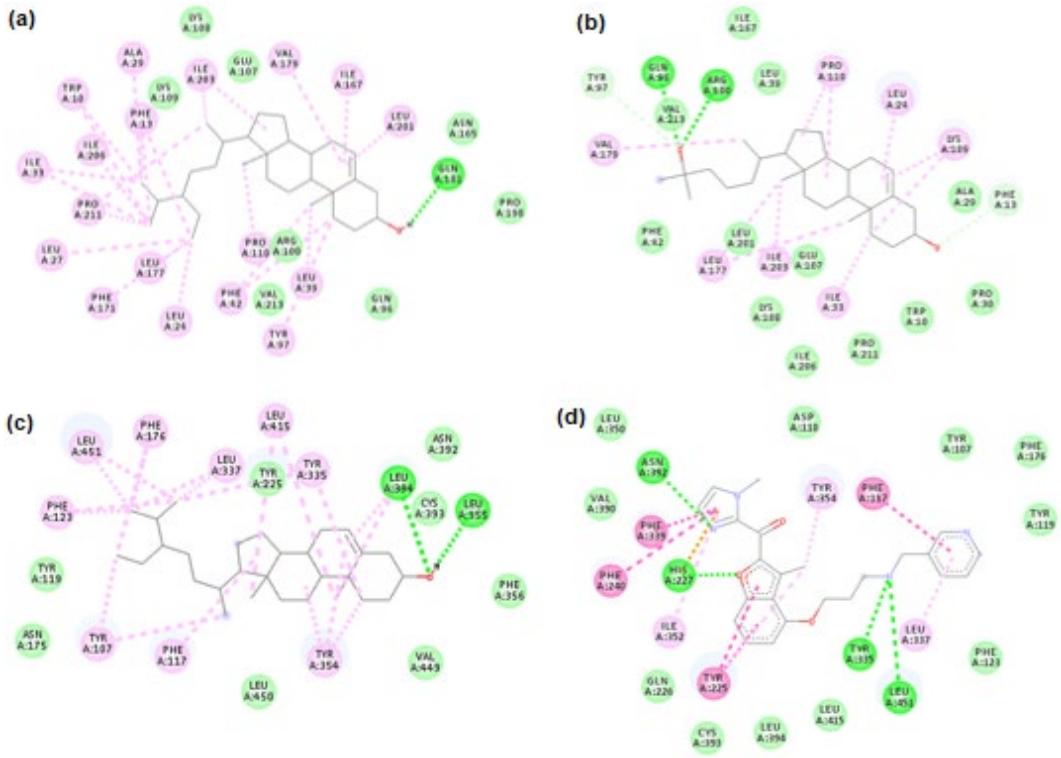


Fig 1. Two-dimensional scheme of interaction between compound 25 and the positive controls with the antifungal target proteins*: (a) compound 25 and Osh4, (b) HC3 and Osh4, (c) compound 25 and Nmt, (d) R64 and Nmt. *alkyl interaction (violet), hydrogen bond (green), van der Waals interaction (light green), pi-pi stacked/pi-pi T-shaped (magenta), pi-cation (orange), and carbon-hydrogen/pi-hydrogen donor (pseudo green)

VAL213, ten alkyl interactions with LEU24, ILE33, LYS109, PRO110, LEU177, VAL179 and ILE203, two hydrogen bonds with GLN96 and ARG100, and 2 phi and hydrogen donor interactions with PHE13 and TYR97 of the target. The same residues are found to be involved in the interaction of the protein with both compounds (PHE13, LEU24, ILE33, TYR97, PRO110, LEU177, VAL179, and ILE203). The interactions of the two ligands with Nmt are shown in Fig. 1(c) and (d). It can be summarized that compound 25 and the protein form 8 van der Waals interactions with the residues of TYR119, ASN175, TYR225, PHE356, ASN392, CYS393, VAL449 and LEU450, twenty alkyl interactions with TYR107, PHE117, PHE123, PHE176, TYR335, LEU337, TYR354, LEU394, LEU415 and LEU451, and 2 hydrogen bonds with LEU355 and LEU394 of Nmt. Meanwhile, the positive control and the protein form 11 van der Waals interactions involving residues TYR107, ASP110, TYR119, PHE123, PHE176, GLN226, LEU350, VAL390, CYS393, LEU394 and LEU415. Four alkyl interactions are also found between the compound and TYR225, LEU337, ILE352 and TYR354. Other interactions consist of 4 hydrogen bonds with HIS227, TYR335, ASN392 and LEU451, five phi-phi interactions with PHE117, TYR225, PHE240 and PHE339, and a pi-cation interaction with HIS227 of the protein. Same as before, the similar residues are also found to be involved in the interaction of the compounds with the target such as PHE117, TYR335, LEU337, TYR354, and LEU451. The previous study suggested that the residues of PHE117 and TYR354 are involved in the inhibition of the target by ligands [14]. From the diagrams, it can be seen that more alkyl interaction formed by compound 25 and the targets may be the reason for its better affinity parameter in the docking result.

Two-dimensional diagram of interactions between compound 25 and the positive control used with TgPNP and TgCDPK1 is shown in Fig. 2. Fig. 2(a) and (b),

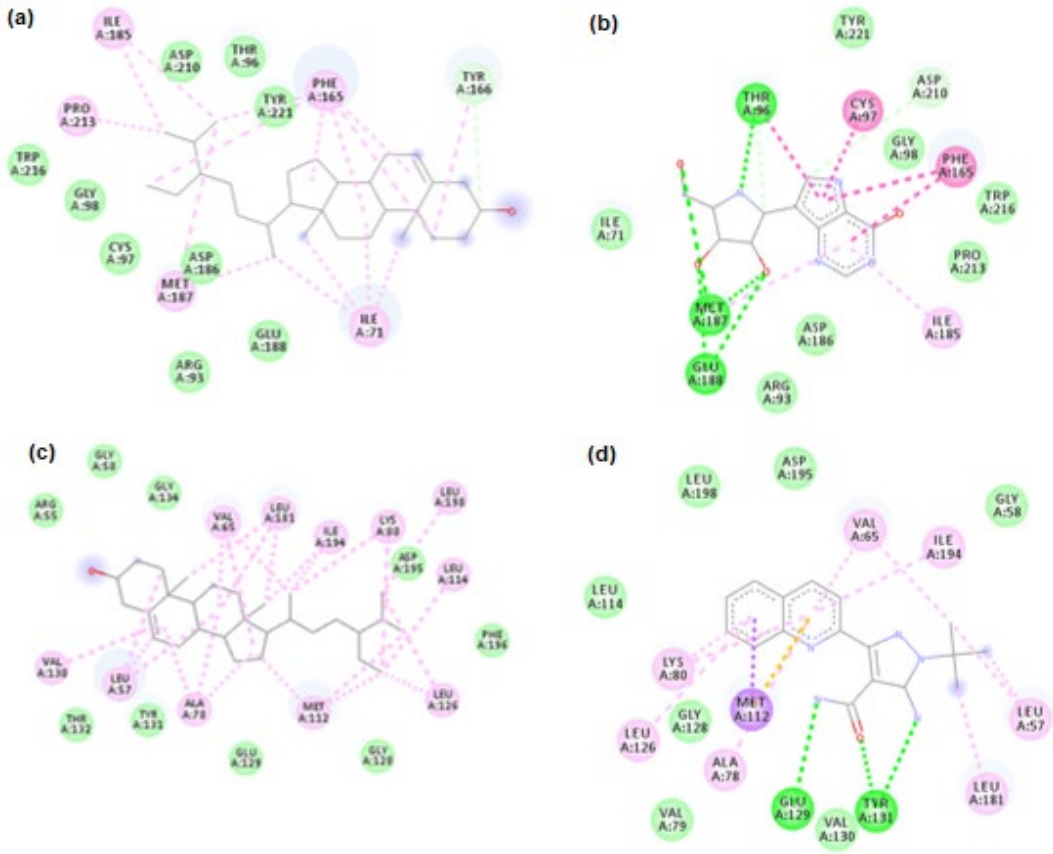


Fig 2. Two-dimensional scheme of interaction between compound 25 and the positive controls with the antitoxoplasmosis target proteins*: (a) compound 25 and TgPNP, (b) IMH and TgPNP, (c) compound 25 and TgCDPK1, (d) 21E and TgCDPK1. *alkyl interaction (violet), hydrogen bond (green), van der Waals interaction (light green), pi-pi stacked/pi-pi T-shaped (magenta), carbon hydrogen/pi-hydrogen donor (pseudo green), pi-sigma (deep purple) pi-sulfur (deep yellow)

indicating the test compound forms 9 van der Waals interactions with the residues of ARG93, THR96, CYS97, GLY98, ASP186, GLU188, ASP210, TRP216, and TYR221, sixteen alkyl interactions with ILE71, PHE165, TYR166, ILE185, MET187 and PRO213, and a carbon-hydrogen interaction with TYR166. Meanwhile, IMH and the protein TgPNP form 7 van der Waals interactions with the residue of ILE71, ARG93, GLY98, ASP186, PRO213, TRP216 and TYR221, two pi-alkyl interactions with ILE185 and MET187, five hydrogen bonds with THR96, MET187 and GLU188, two carbon-hydrogen interactions with THR96 and ASP210, and 4 pi interactions with THR96, CYS97, and PHE165. Compound 25 and TgCDPK1, as shown in Fig. 2(c) and (d), form 9 van der Waals interactions at the residues of ARG55, GLY58, GLY128, GLU129, TYR131, THR132, GLY134, ASP195 and PHE196, twenty-four alkyl interactions at LEU57, VAL65, ALA78, LYS80, MET112, LEU114, LEU126, VAL130, LEU181, ILE194, and LEU198, while the control (21E) and the protein form 7 van der Waals interactions at GLY58, VAL79, LEU114, GLY128, VAL130, ASP195 and LEU198, ten alkyl interactions at LEU57, VAL65, ALA78, LYS80, LEU126, LEU181 and ILE194, three hydrogen bonds at GLU129 and TYR131, a pi-sigma and a pi-sulfur interaction interactions at MET112. Fig. 2 shows us that several residues involved in the interaction between compound 25 and both TgPNP and TgCDPK1 have similarities with their positive controls. PHE165, ILE185, and MET187 are involved in the interaction of compound 25

and the positive control with TgPNP, while LEU57, VAL65, ALA78, LYS80, LEU126, and LEU181 are involved in the interaction of the compound and the control with TgCDPK1. Similar to the overall comparison between compound 25 and the control in antifungal interaction study, the more of the alkyl interactions formed between the compound and the target compared with its interaction with the positive control results in the better affinity of the compound 25 towards the two target proteins used in antitoxoplasmosis study.

■ CONCLUSION

The research results suggest that *E. Americana* can be proposed as the candidate of alternatives in the treatment of fungal infection and toxoplasmosis as the constituents of the plant seem to have an affinity to the target used in this study. Compound 25 (β-sitosterol) is the constituent of the plant with a better affinity compared to the positive controls. A preliminary toxicity study suggests that the compound has a low level of aquatic toxicity.

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