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RESEARCH ARTICLE

Screening of terpenoids as potential therapeutics against *Zaire ebolavirus* infection through pharmacophore-based drug design [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: Ebola virus disease (EVD) has spread to various countries in the world and has caused many deaths. Five different virus species can cause EVD, but the most virulent is *Zaire ebolavirus* (EBOV). The genome of EBOV includes seven genes that encode proteins playing essential roles in the virus lifecycle. Among these proteins, VP24 plays a vital role in the inhibition of the host cells' immune system. Therefore, VP24 is a potential target for EVD therapy. In the present study, a potential inhibitor of EBOV VP24 activity was identified through pharmacophore-based drug design.

Methods: This research was a *in silico* study, using pharmacophore based molecular docking simulation to obtain inhibitor candidates.

Result: Terpenoids were used as VP24 inhibitor candidates. In particular, 55,979 terpenoids were obtained from the PubChem database. An initial screening based on the toxicity prediction test was performed with DataWarrior software: 3,353 ligands were shown to have a favorable toxicity profile, but only 1,375 among them had suitable pharmacophore features. These ligands were used for pharmacophore-based rigid and flexible molecular docking simulations with PDB ID: 4M0Q, chosen as the crystal structure of EBOV VP24. Six ligands predicted to have strong molecular interactions with EBOV VP24 underwent pharmacological property analysis through various software packages, including DataWarrior, SwissADME, admetSAR, pkCSM, and Toxtree.

Conclusions: Taxumairol V was identified as the best candidate for EVD drug therapy via EBOV VP24 inhibition based on its molecular properties, predicted molecular interactions with the target molecule, and predicted pharmacological properties.

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Keywords

Zaire ebolavirus, viral protein 24, terpenoid, pharmacophore, molecular docking simulation, ADMET.

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Introduction

The Ebola virus disease (EVD) is an often-lethal hemorrhagic fever for human and non-human primates. The World Health Organization (WHO) has declared EVD one of the global public health threats. EVD is caused by various Ebola virus species that are members of the *Filoviridae* family, some of which have spread to countries like the United Kingdom and the United States¹. Five Ebola virus species cause Ebola disease, namely Bundibugyo, Reston, Zaire, Tai Forest, and Sudan². The *Zaire ebolavirus* (EBOV) is the most virulent among them, with a mortality rate of up to 90%, and is responsible for all EVD outbreaks that have occurred worldwide since 1976, mainly in Gabon, the Democratic Republic of the Congo, and West Africa¹. The symptoms of EVD include myalgia, fever, decreased plasma volume, anorexia, hypotension, diarrhea, and mucosal bleeding in the genitourinary and gastrointestinal tracts².

EBOV's genome includes seven genes that encode as many proteins that play essential roles in the lifecycle of the virus. The seven genes are known to encode the viral proteins (VP) 24, VP30, VP35, VP40, nucleoprotein (NP), glycoprotein (GP), and L (polymerase)¹. Of importance amongst these, VP24 is responsible for the maturation of viral nucleocapsids and the regulation of viral transcription and replication. VP24 and VP40 have important roles in virus budding and assembling, the VP24 and NP have a contribution for viral budding and releasing³. VP24 also has a key role in the inhibition of the host cells' immune system, especially regarding interferon signaling. VP24 is able to bind karyopherin alpha, which prevents the accumulation of tyrosine-phosphorylated STAT1 (pSTAT) in the nucleus. Afterward, the binding of IFN-stimulated response elements (ISREs) does not occur and inhibits the interferon signaling system and causes inhibition of host-mediated IFN responses as the body's defense against the Ebola virus⁴. Therefore, VP24 is a potential drug target in the treatment of EVD⁵.

Until now, no drugs have been developed to treat EVD, and potential candidates are still in the development stage. Computer-aided drug discovery and development (CADD) is an efficient approach to identifying compounds characterized by high affinity and selectivity for macromolecular targets and beneficial pharmacokinetic and pharmacodynamic properties⁶. Use of CADD enables the screening and prediction of the absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) of drug candidates for drug discovery and development⁷. Therefore, with CADD we were able to quickly eliminate drug candidates with low biological activity and unsatisfactory ADME-Tox properties, avoiding the implementation of the time-consuming subsequent steps of the analysis⁷. The pharmacophore features test in CADD enables identification of molecular species that are likely to display a high inhibitory affinity and interaction with macromolecules recognized as important targets for disease treatment⁸. The site of the pharmacophore is indicated with vectors and colored spheres that denote the atom's characteristics: hydrophobic, hydrogen-bond acceptor, hydrogen-bond donor, aromatic, cationic, or anionic⁹. Pharmacophore features are an advanced tool in drug discovery to screen drug candidates,

optimize lead compounds, and design new compounds. Additionally, they are commonly used as templates to screen molecules (hits) with similar chemical features⁸.

Terpenoids are a group of compounds that we investigated as inhibitors of EBOV VP24 via computational pharmacophore-based drug design. Terpenoid compounds are the largest group of natural products, and they are built starting from isoprene units and synthesized through the mevalonate and non-mevalonate pathway¹⁰. Members of this compound family are classified according to the number of isoprene units that make up their structures; namely, hemiterpenoids comprise one isoprene unit and five carbon atoms, monoterpenoids are composed of two isoprene units and ten carbon atoms. Sesquiterpenoids have three isoprene units and fifteen carbon atoms, diterpenoids have four isoprene units and twenty carbon atoms, sesterterpenoids have five isoprene units and twenty-five carbon atoms, triterpenoids have six isoprene units and thirty carbon atoms, tetraterpenoids have eight isoprene units and forty carbon atoms, and polyterpenoids have more than forty carbon atoms and more than eight isoprene units¹⁰. Terpenoids exhibit unique pharmacological and biological activities, including antibacterial¹¹, anti-inflammatory¹², anticancer¹³, and antiviral¹⁴ activities.

Methods

This study was performed employing a CADD approach using the pharmacophore feature to identify pharmacological properties and inhibitory activity toward EBOV VP24. Various online and offline software packages, such as **Molecular Operating Environment** 2014.09 (MOE), **ToxTree** v2.6.13, **DataWarrior** v04.07.02, **AdmetSAR**, **pkCSM**, and **SwissADME**, were used to analyze the pharmacological properties of all ligands and to obtain the best drug candidates for anti-EVD therapy^{15,16}. **Figure 1** presents the research pipeline of this study.

Preparation of EBOV protein VP24

The 3D crystal structure of EBOV VP24 was obtained from the **RCSB Protein Data Bank** —PDB ID: **4M0Q** by Edwards *et al.*¹⁷. Data on 4M0Q were saved in .pdb format, and the file thus obtained was then opened in MOE 2014.09 for protein preparation and optimization. In this study, preparation and optimization of 4M0Q was performed using the LigX features in MOE 2014.09 with the Amber 10: EHT and solvation of the gas phase. Notably, AMBER10: EHT has been chosen as the force-field for simulations involving large biomolecules, like proteins and nucleic acids¹⁸. Then, the geometry of the protein crystal was optimized, and the energy of the protein was minimized. Finally, the optimized structure of EBOV VP24 was stored in .moe format.

Ligand preparation

Terpenoids were obtained from the **PubChem** database. They are classified as organic chemicals in the chemicals and drugs category. In the organic chemicals category, terpenoids belong to the hydrocarbon group. Data from terpenoids from several subclasses, namely cannabinoids, carotenoids, diterpenes, dolichol, gefarnates, hemiterpenes, monoterpenes, polyisoprenyl

Figure 1. Research pipeline.

phosphates, sesquiterpenes, sesterterpenes, and triterpenes, were collected. The structures of 55,979 terpenoids were saved in .sdf format.

The mentioned structures were evaluated performing the toxicity prediction test using DataWarrior v04.07.02 to identify ligands with favorable characteristics for molecular docking. Subsequently, the ligands underwent preparation and optimization with MOE 2014.09 software using modified MMFF94x as a force field and gas phase solvation. The standard ligands in this stage of the study were gossypetin and bisdemethoxycurcumin which were used to compare the characteristics of the best drug candidates for treating the EVD¹⁹. Thus, the structures of the ligands selected were saved in .mdb format.

Pharmacophore features of EBOV VP24

In drug discovery, the determination of pharmacophore features contributes to the identification of ligands that have the potential to achieve a specific desired effect on a target molecule. The pharmacophore features of EBOV VP24 were obtained through molecular docking simulations with standard ligands. The standard ligands were made to dock using the site finder feature, which enabled us to identify the binding pocket of 4M0Q/EBOV VP24. Then, the molecular interaction of standard ligands with EBOV VP24 was used to determine the protein's pharmacophore features through the pharmacophore query editor in the MOE 2014.09 software with gas phase solvation and Amber10: EHT as a force field. Finally, the pharmacophore features of 4M0Q/EBOV VP24 were saved in .ph4 format²⁰.

Molecular docking simulations

A two-step molecular docking simulation was performed: rigid docking followed by flexible docking. The rigid docking process refers to the condition whereby the protein is regarded as rigid and the ligand as dynamic. Therefore, the ligand will find the most stable conformation with the protein target without the latter undergoing any changes in bond angles or bond lengths. On the other hand, in the flexible docking protocol, the conformational change that the binding site of the protein target undergoes upon docking is also taken into consideration. Ligand and protein are regarded as flexible in this case, and they will seek the conformation of the most stable ligand-protein complex to optimize the strength of the bonds between ligand and receptor²¹. In this study, the rigid and flexible molecular docking simulations were used alongside the pharmacophore features to dock the

ligands to 4M0Q. Molecular docking simulations were performed using MOE 2014.09 software with gas phase solvation and AMBER 10: EHT as a force field.

Two types of scoring functions were used in our study, namely London dG and GBVI/WSA dG. The London dG scoring function provides analytical data on hydrophobic, ionic, and hydrogen-bonding interactions and affords an estimate of the free binding energy of ligand-receptor bonds based on the given conformations²². The GBVI/WSA dG scoring function enables researchers to determine the value of refinement parameters that will afford an estimate of the ligand binding energy in each pose. Moreover, the GBVI/WSA dG scoring function displays high sensitivity to the modification of the protein environment. Therefore, docking accuracy and the strength of protein-ligand bonds can be influenced by the GBVI/WSA dG scoring function²³. Based on the results of molecular docking simulations, ligands were dropped from further analysis when they displayed values for Gibbs free binding energy ($\Delta G_{\text{binding}}$) that were smaller than those of the standard ligands and when the value of the root-mean-square deviation (RMSD) of the ligands was above 2.0 Å.

Pharmacological test

The best ligands based on the results of the previous analysis underwent pharmacological property analysis. Various online and offline software packages were used to screen the ADME-Tox properties of the ligands, such as DataWarrior, Toxtree, SwissADME, admetSAR, and pkCSM. DataWarrior was used to analyze the ligand's mutagenicity, drug-likeness, and tumorigenicity. Toxtree was used to evaluate the carcinogenicity and mutagenicity of the ligands²⁴. SwissADME was utilized to predict ligand's pharmacokinetics and physicochemical properties²⁵. Finally, the absorption, distribution, metabolism, and excretion properties of the ligands were predicted with admetSAR and pkCSM²⁶.

Results and discussion

Preparation of EBOV VP24

VP24 is an essential protein in EBOV's lifecycle. Therefore, VP24 is a potential target when trying to inhibit EBOV replication and prevent EVD progression. The protein with PDB ID: 4M0Q was chosen as EBOV VP24 crystal structure, based on the fact that this structure has also been used in other studies¹⁹. The structure of PDB ID: 4M0Q was obtained through X-ray

diffraction with a resolution of 1.921 Å. The protein's active site comprises several amino acid residues, namely Y41, G44, I45, E46, F47, D48, R154, S151, S155, L158, S225, T226, F230, and T231²⁷. In this study, all non-essential molecules in EBOV VP24 structure with PDB ID: 4M0Q, such as water, were eliminated, because their presence in the crystal structure can affect the interactions between ligand and protein²⁸. The protonate 3D step was used for the addition of hydrogen atoms to the protein structure to mimic the hydrogen atoms in protein structure that may occur in nature¹⁵.

Ligand preparation

In this study, 55,979 terpenoids were selected in the PubChem database as potential inhibitors of EBOV VP24. The ligands were screened using DataWarrior v04.07.02 to identify disqualifying properties, such as mutagenicity, tumorigenicity, having negative reproductive effects, and being an irritant, and to recognize positive traits like drug-likeness. In fact, the DataWarrior software can be used to predict reproductive effects as well as the mutagenic, irritant, and tumorigenic properties of a particular compound based on its constituent fragments. Drug candidates displaying similar or identical structural traits as compounds known to be an irritant, upon contact, can cause the human body to display dryness or reddening²⁹. In the DataWarrior software package, benzanthrone derivatives, silicon ether, and halogenated phenol ethers are regarded as irritant structural alerts whose use can lead to irritation in the human body³⁰. The tumorigenicity of drug candidates is another important trait to identify because tumorigenic compounds can induce tumor cell proliferation in human tissues³¹.

Compounds predicted to pose a risk due to their mutagenic characteristics may cause transmissible genetic damage, including gene mutations, chromosomal aberrations, and changes in the number of chromosomes³². Nitroaromatic compounds, nitrobenzene derivatives, and phenylamine derivatives are regarded as mutagenic structural alerts by DataWarrior³³. In the present study, gossypetin was predicted to be mutagenic because of its 5,8-dihydroxychromone fragment. Based on toxicity predictions

made using DataWarrior, 3,353 ligands were identified as possessing the desirable characteristics to inhibit EBOV VP24 activity. The ligands' geometries were optimized to obtain ligands having the features that can be using the MMFF94x force field with gas phase solvation¹⁵.

Pharmacophore features of EBOV VP24

Pharmacophore features play a vital role in determining the optimal molecular interactions between ligand and target macromolecule that trigger or block a particular biological activity of the macromolecule. Pharmacophore features are identified through ligand-based or structure-based construction. In the ligand-based construction, the pharmacophore feature is determined by collecting a set of ligands which shows biological activity against a particular target macromolecule and extracting the chemical properties that confer upon the molecules their bioactivity. In the structure-based construction, the pharmacophore feature is determined via analysis of the possible interaction sites between ligands and macromolecular targets. Thus, pharmacophore features identified can be used in *de novo* design, virtual screening, or for the optimization of lead compounds. In pharmacophore-based virtual screening, a particular pharmacophore feature is used as a screening template to find molecules that have a similar feature as the template (hits)⁸. Therefore, based on similarities with the pharmacophore feature used as a template, the hits are expected to display the desired biological activity.

In this study, two pharmacophore features were generated based on the interaction between the standard ligands (bisdemethoxycurcumin and gossypetin) and the protein target (4M0Q EBOV VP24)^{19,34}. As shown from the structures of the ligand-target molecule complexes depicted in Figure 2, the hydrogen acceptor (acc) feature, reported in blue color, was constructed based on the interaction between standard ligands and the side chain of arginine B154. The hydrogen donor feature, reported in purple, was generated in reference to the interaction between the standard ligands and the side chain of aspartic acid A48. The pharmacophore features of EBOV VP24 were analyzed

Figure 2. Pharmacophore features of Zaire ebolavirus protein VP24 (PDB ID: 4M0Q) identified by interaction with ligands (A) bisdemethoxycurcumin and (B) gossypetin located in the viral protein's binding pocket

and validated using standard ligands. Finally, 1,375 hit ligands that match the pharmacophore features mentioned above were identified so that they could be used in molecular docking simulations. The 4M0Q EBOV VP24 protein along with the pharmacophore features is presented in [Figure 2](#).

Molecular docking simulations

In this study, molecular docking simulations were performed to assess the protein-ligand binding affinity and predict the conformations of ligand and macromolecular target in the protein-ligand complex³⁵. All of the 1,375 hit ligands identified in the previous stage of the procedure underwent a molecular docking simulation through pharmacophore-based rigid docking. In particular, a retain value of 30 was used to identify hits in the first simulation, and a retain value of 100 was used for the same purpose in the second simulation with Amber10: EHT as a forcefield and gas phase as the solvation parameter for both simulations. Of the 1,375 ligands whose structures were subjected to the pharmacophore-based rigid docking simulation, 855 were considered satisfactory as the values assigned to the relevant simulation at a minimum matched the retained value of 30. Then, these hit ligands were screened based on their RMSD value (<2 Å), leading to the selection of 651 ligands. These 651 ligands were then used in a pharmacophore-based rigid molecular docking simulation using a retain value of 100, leading to the selection of 642 ligands. Then, these ligands were screened based on their RMSD value, leading to the selection of 581 ligands. These 581 ligands were subjected to pharmacophore-based flexible docking simulations utilizing a retain value of 100. The 577 ligands were selected, and their RMSD values were evaluated, leading to the selection of 344 ligands, which were analyzed based on their $\Delta G_{\text{binding}}$ value, RMSD value, and interaction with the 4M0Q EBOV VP24 protein. In particular, ligands were selected if the value of their $\Delta G_{\text{binding}}$ was lower than the corresponding parameters calculated for the standard ligands, indicating that the relevant protein-ligand conformation was more stable than that involving the standard ligands and 4M0Q¹⁵. Rigid docking show the best performance rates between 50–75%, while flexible docking methods can enhance pose prediction up to 80–95%³⁶.

Additionally, ligands were selected if their RMSD value was below 2.0 Å since a value of this magnitude indicates that the protein and ligand conformations produced by the molecular docking simulation are highly accurate³⁷. In this study, the ability of ligands to inhibit the activity of the target protein was predicted through the pKi value. A large, positive value for pKi indicates that the protein-ligand complex has a low dissociation rate³⁸. Then, all ligands were screened based on their interaction with 4M0Q. Ultimately, six ligands were selected as the best candidates for the inhibition of 4M0Q activity, namely 3-O-acetyluncaric acid, carnosic acid, ilexgenin A, negundoside tasumatrol G, and taxumairol V, which are characterized by advantageous traits regarding $\Delta G_{\text{binding}}$, RMSD value, and strength of the protein-ligand interaction.

Among these six inhibitor candidates, there are three ligands which fulfill the topological polar surface area (TPSA) value

(in excess of 140 Å)³⁹. Increased permeation rate are correlates to decreased polar surface area⁴⁰. Carnosic acid displayed the lowest RMSD value among the six selected terpenoids, although standard ligand gossypetin had an even lower RMSD value than carnosic acid. Negundoside had the lowest $\Delta G_{\text{binding}}$ value and the highest pKi value, indicating its superior ability to inhibit the activity of EBOV VP24. [Table 1](#) summarizes the molecular properties of the six ligands just discussed and of the two standard ligands.

In this study, the molecular interactions between the target protein and ligand identified by molecular docking simulations were analyzed to quantify the ability of each ligand to interact with the target protein. All ligands were found to interact with the target protein's active site. Ilexgenin A displayed the highest number of molecular interactions with the target protein; it displayed interactions with six active-site amino acid residues of EBOV VP24, namely glycine B44, isoleucine B45, glutamine B46, aspartic acid A48, arginine A154, and phenylalanine A230. Negundoside is the second-best ligand in terms of the number of interactions with the target protein. This ligand is involved in hydrogen-binding interactions with five amino acid residues in the protein's active site: lysine A39, glutamic acid A46, aspartic acid A48, arginine A154, and phenylalanine A230.

Tasumatrol G is involved in three hydrogen-binding interactions with amino acid residues in the protein's active site: aspartic acid A48, arginine A154, and alanine A229. Taxumairol V and carnosic acid are each involved in three hydrogen-binding interactions with EBOV VP24. Taxumairol V is involved in hydrogen-binding interactions with three amino acid residues located in the active site of EBOV VP24, namely aspartic acid A48, arginine A154, and arginine B154. Meanwhile, the carnosic acid showed have hydrogen-bonding interactions with aspartic acid A48, arginine A154, and arginine B154. The 3-O-acetyluncaric acid showed the lowest number of hydrogen-binding interactions with the target protein. [Table 2](#) depicts the structures of selected simulated ligand–4M0Q complexes and the interactions that characterize them.

Pharmacological tests

Pharmacological tests were carried out to predict the absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) properties of the six selected terpenoid ligands, and to identify the ligand among them with the potential to be used as a drug in EVD therapy (full data for the final 6 ligands is available as underlying data⁴¹). The prediction of ADME-Tox properties was performed using various software packages because each of them evaluates different traits in the pharmacological tests. In this study, the pharmacological tests were performed with software packages Toxtree, swissADME, pkCSM, and admetSAR.

Toxtree analysis was implemented to determine the mutagenic potential regarding *Salmonella typhimurium* TA100, potential carcinogenic based on the quantitative structure–activity relationship (QSAR), genotoxic carcinogenicity, and non-genotoxic carcinogenicity of the ligands. [Table 3](#) summarizes the results of the pharmacological tests performed with Toxtree.

Table 1. Molecular properties of the six best EBOV VP24 terpenoid inhibitor candidates and the two standard ligands.

Ligand Name	Flexible Docking		MW	pKi	TPSA
	$\Delta G_{\text{binding}}$	RMSD			
*Bisdemethoxycurcumin	-6.560	1.862	308.332	4.779	74.60
*Gossypetin	-7.266	0.435	318.236	5.293	147.68
3-O-acetylluncaric acid	-8.009	1.169	472.556	5.834	130.36
Carnosic acid	-8.706	0.689	443.566	6.342	110.70
Ilexgenin A	-8.009	0.855	500.673	5.834	120.72
Negundoside	-9.861	1.123	495.455	7.183	195.27
Tasumatrol G	-9.704	0.842	630.684	7.069	175.12
Taxumairol Vv	-9.252	1.021	415.460	6.739	186.12

*Standard ligand; RMSD: root-mean-square deviation; MW: molecular weight.

According to this analysis, 3-O-acetylluncaric acid, taxumairol V, ilexgenin A, and negundoside were not potential carcinogenic, mutagenic, genotoxic carcinogenic, and non-genotoxic carcinogenic compounds. Carnosic acid exhibited the characteristics of a non-genotoxic carcinogen and a mutagen. Genotoxic carcinogens can interact directly with DNA, thus, producing DNA adducts

and DNA damage. Non-genotoxic carcinogens, on the other hand, do not interact directly with DNA, but they can damage cellular structures and change cell proliferation rates⁴². The Toxtree software comprises several structural alerts for non-genotoxic carcinogens, including the presence of thiocarbonyl, halogenated benzene, and halogenated dibenzodioxin groups⁴³. Carnosic acid

Table 2. Structures of and molecular interactions within selected ligand–4M0Q complexes.

Ligand	2D Ligand–Protein Molecular Interactions in 2D	Ligand–Protein Molecular Interactions in 3D
*Bisdemethoxycurcumin
*Gossypetin
3-O-acetyluncaric acid
Carnosic acid

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Table 3. Pharmacological properties of selected ligands as determined by analysis with the Toxtree software package.

No	Ligand	Pharmacological Properties			
		Genotoxic	Non-genotoxic	Potential <i>S.typhimurium</i> TA 100 mutagenicity	Potential Carcinogenicity based on QSAR
1	3-O-acetyluncaric acid	No	No	No	No
2	Carnosic acid	No	Yes	Yes	No
3	Ilexgenin A	No	No	No	No
4	Negundoside	No	No	No	No
5	Tasumatrol G	No	Yes	No	No
6	Taxumairol V	No	No	No	No
7	*Bisdemethoxy-curcumin	No	No	No	No
8	*Gossypetin	No	Yes	No	No

*standard ligand

and tasumatrol G exhibited the characteristics of non-genotoxic carcinogens because they include n-alkyl carboxylic acid and thiourea groups within their structure, which match the software's thio-carbonyl structural alert. Thiourea is a non-genotoxic carcinogen that can cause cancer in the thyroid gland. In fact, thiourea acts as a peroxidase inhibitor in the thyroid gland, inhibiting thyroxine production. A reduced synthesis level of the thyroxine hormone causes, in turn, the pituitary gland to increase thyrotropin hormone secretion, resulting in an increased risk of thyroid cancer⁴⁴.

In this study, the potential mutagenicity of ligands was determined using the Toxtree and admetSAR software packages through the Ames test and potential *S. typhimurium* TA 100 mutagenicity test. In this test, chemicals are analyzed in terms of their mutagenicity toward *S. typhimurium*, given that bacteria belonging to this species are highly sensitive to mutagenic compounds. In particular, in Ames tests, *S. typhimurium* bacteria modified so that they cannot synthesize histidine are exposed to potentially mutagenic compounds. These compounds may in turn cause mutations in the bacterial his-operon, a type of change that would be made evident by the ability of the modified bacteria to grow in culture media lacking histidine. In the prediction of the mutagenic properties of ligands, the Toxtree software packages comprise a structural alert for mutagenic compounds based on results from Ames tests; structures that set off this alert include aromatic amines and nitroarenes⁴⁵.

Given that carnosic acid comprises an aromatic amine group, Toxtree-based analysis classified this ligand as potentially mutagenic. In fact, aromatic amines will undergo biotransformation in the human body that results in the formation of covalent bonds with guanine, which may, in turn, cause DNA mutations. Therefore, carnosic acid was removed from the list of potential drug candidates for EVD therapy. Meanwhile, based on the Ames test which was performed using admetSAR software, all the best ligands showed no Ames toxic, which means that it did not have the

potential to be mutagenic compound⁴⁶. Importantly, the results of mutagenicity analysis performed using Toxtree and admetSAR were different from each other because the two software packages use different databases to formulate predictions on the mutagenic potential of ligands.

The six EBOV VP24 inhibitor candidates and the two standard ligands underwent analysis for potential mutagenicity, acute oral toxicity, human intestinal absorption (HIA), and cytochrome P450 (CYP)-inhibiting activity using the admetSAR software package. Table 4 reports data on the relevant pharmacological properties. HIA is an important oral bioavailability indicator⁴⁷, and it is given a positive (+) value when a compound is promptly absorbed in the human intestine, or a negative (-) one, when the compound has poor absorption characteristics in the human intestine. The only negundoside displayed a negative HIA value following analysis, meaning that this ligand has poor or weak absorptivity in the human intestine. Regarding acute oral toxicity, compounds subjected to analysis may fall into four categories: category I designates highly toxic compounds, category II designates moderately toxic compounds, category III designates compounds of low toxicity, and category IV designates non-toxic compounds⁴⁸. Acute oral toxicity analysis performed with the admetSAR software package led to ilexgenine A, negundoside, and tasumatrol G being classified as highly toxic. 3-O-acetyluncaric acid, taxumairol V, and carnosic acid were instead classified as low-toxicity compounds. Ilexgenine A and negundoside were, thus, removed from the list of potential candidates for the inhibition of EBOV VP24 activity. Trasumatrol G was retained for analysis given its good molecular docking characteristics.

It is essential to identify CYP-inhibiting properties to develop a new drug. In fact, CYPs belong to an enzyme family that plays a key role in the metabolism of drugs, as its members catalyze the biotransformation of drugs in advance of their decomposition and excretion⁴⁹. The AdmetSAR software package can be used to

Table 4. Pharmacological properties were obtained by analyses performed using the admetSAR software package.

No	Ligand	Pharmacological Properties			
		Ames-test Mutagenicity	HIA	Acute Oral Toxicity	CYP Inhibitor
1	3-O-acetyluncaric acid	No	+	III	None
2	Carnosic acid	No	+	III	CYP450 1A2, 2C9, 2C19
3	Ilexgenin A	No	+	I	None
4	Negundoside	No	-	I	None
5	Tasumatrol G	No	+	I	None
6	Taxumairol V	No	+	III	None
7	*Bisdemethoxycurcumin	No	+	III	CYP450 1A2, 2C9, 2C19, 3A4
8	*Gossypetin	No	+	II	CYP450 1A2, 3A4

Note: *Standard ligand, CYP: cytochrome P450; HIA: human intestinal absorption.

analyze the ability of ligands to act as inhibitors of several CYP isoforms that play a role in drug metabolism, namely 1A2, 2C9, 2C19, 2D6, and 3A4. In this study, carnosic acid, bisdemethoxycurcumin, and gossypetin displayed the potential to inhibit CYP 1A2 activity. Carnosic acid and bisdemethoxycurcumin displayed the potential of inhibiting the activity of CYP 2C9 and CYP 2C19. Bisdemethoxycurcumin and gossypetin displayed the potential to inhibit CYP 3A4 activity. Drugs that have the potential to inhibit the activity of various CYP isoforms may cause undesirable side effects, as they may cause drug metabolic disorders and the accumulation of potentially toxic drug metabolites⁵⁰. Therefore, it was concluded that carnosic acid, gossypetin, and bisdemethoxycurcumin were not suitable candidates for the development of drugs for EVD therapy.

Ligands' potential for hepatotoxicity and hERG I-inhibiting activity were analyzed using the pkCSM software package. Table 5 summarizes results from this analysis. The human *Ether-à-go-go* Related Gene (hERG) is a selective potassium ion channel that regulates potassium ion transfer and is expressed in various cells and tissues, including cardiac myocytes, neurons, smooth muscles, and neuroendocrine cells⁵¹. Inhibition of hERG I can extend cardiac repolarization, which may result in ventricular arrhythmias, torsades de pointes, and sudden cardiac death⁵². Terfenadine, cisapride, astemizole, and grepafloxacin are drugs known to have inhibitory effects on hERG. I, and they have been withdrawn from the market because they can cause arrhythmias⁵³. None of the ligands tested were predicted to display any hERG I-inhibiting activity. Regarding the potential for hepatotoxicity, carnosic acid was predicted to cause damage to the liver, an organ that plays an important role in the metabolism of proteins, fats, carbohydrates, and toxins⁵⁴. Therefore, carnosic acid was removed from the list of drug candidates for the inhibition of EBOV VP24 protein activity.

The predicted bioavailability and synthetic accessibility of ligands were determined through analysis performed with the swissADME software package (Table 5). Bioavailability is the fraction of a drug that can be absorbed by the body and the concentration of that drug which reach systemic circulation according to the total dose of the drug⁵⁵. The bioavailability pre-

diction score of a compound ranges from 0 to 1. If the bioavailability value of a compound is less than 0.5 (<0.5), the compound is predicted to have low bioavailability. If, on the other hand, that value is above 0.5 (> 0.5), the compound is predicted to have high bioavailability⁵⁶. Based on the results of the bioavailability test, negundoside is predicted to display the lowest bioavailability (bioavailability score: 0.11). Therefore, negundoside was removed from the list of candidate drugs that inhibit VP24 *Zaire ebolavirus* activity.

Synthetic accessibility (SA) is an important parameter in drug design because, in some cases, compounds designed computationally prove very difficult to synthesize⁵⁷. The SA score ranges from a value of 1, indicating that the compound is very easy to synthesize, to a value of 10, indicating that the compound is very difficult to synthesize²⁵. Based on the low SA score, carnosic acid is predicted to be the easiest compound to synthesize. 3-O-acetyluncaric acid and ilexgenin A have high SA scores, meaning that these ligands are predicted not to be easily synthesized.

Results from the molecular docking simulations and pharmacological tests conducted in this study indicate that taxumairol V is the best drug candidate to inhibit EBOV VP24 protein activity, based on its $\Delta G_{\text{binding}}$, RMSD value, molecular weight, predicted interaction with the target protein and predicted pharmacological properties. Taxumairol V's molecular weight is under 500 Dalton, and this ligand has a high propensity to inhibit the activity of the target protein, even though it does not have the highest pKi value among all the ligands tested. Furthermore, taxumairol V is predicted to pose no risk as a potential mutagenic, tumorigenic, carcinogenic, or irritant drug. This compound is predicted to display no inhibitory activity toward CYP, or hERG I. Taxumairol V is also predicted to display good HIA, and it is characterized by an intermediate bioavailability score. Finally, taxumairol V has an average SA score, indicating that it should be moderately easy to synthesize.

Conclusions

In silico approaches aid drug discovery and the development of chemical species that can achieve a desired biological effect.

Table 5. Pharmacological properties as inferred from analyses performed using pkCSM and swissADME software packages.

No	Ligand	Pharmacological Properties			
		hERG I Inhibitor	Hepatotoxicity	Bioavailability	Synthetic Accessibility
1	3-O-acetyluncaric acid	No	No	0.56	6.64
2	Carnosic acid	No	Yes	0.55	3.03
3	Ilexgenin A	No	No	0.56	6.52
4	Negundoside	No	No	0.11	5.79
5	Tasumatrol G	No	No	0.17	6.61
6	Taxumairol V	No	No	0.56	4.91
7	*Bisdemethoxycurcumin	No	No	0.55	2.59
8	*Gossypetin	No	No	0.55	3.29

Note: *Standard ligand. A yellow column head means the ligands were analyzed by pkCSM, whereas a green column head means that the ligands were analyzed by swissADME.

A total of 55,979 terpenoids were screened based on their potential toxicity, resulting in the identification of 3,353 compounds predicted to display beneficial properties. Six best drug candidates that have good molecular properties and are predicted to display strong molecular interaction with the EBOV VP24 protein were subsequently identified after molecular docking simulations were performed. These ligands were then subjected to pharmacological analyses performed using various online and offline software packages. Based on its molecular properties, predicted molecular interaction with the target protein, and predicted pharmacological characteristics, taxumairol V was identified as the best overall drug candidate to inhibit EBOV VP24 protein activity. Therefore, taxumairol V has the potential to be the basis for a drug to be developed to treat *Zaire ebolavirus*-caused Ebola disease.

Data availability

Source data

Ebola virus VP24 structure from Protein Data Bank, Accession number PDB:4M0Q: <http://identifiers.org/pdb:4M0Q>

Underlying data

Figshare: All Test Result of Molecular Docking Simulation. <https://doi.org/10.6084/m9.figshare.8275079.v1>⁴¹

This project contains the following underlying data:

- Result of Molecular Docking.xlsx (Molecular docking results)

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Daryono Hadi Tjahjono

School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia

This paper is interesting enough and has described the screening of terpenoids as potential therapeutics against *Zaire ebolavirus* infection through pharmacophore-based drug design. However, it not enough to screen molecules just using only a molecular docking.

Authors are suggested to improve (major revision) the manuscript, i.e:

1. update the references by citing the current literature;
2. conduct MD simulation for the best six molecules, and please analyze in detail the binding energy (as well as its components), residues involved in the interaction, hydrogen bond occupancy, etc.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Medicinal chemistry, molecular modeling and drug design.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 04 December 2019

<https://doi.org/10.5256/f1000research.21082.r56523>

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Ndumiso N. Mhlongo

College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa

The study identifies a potential EBOV VP24 inhibitor via pharmacophore-based virtual screening. Selected compounds were also assessed for safety properties using numerous in silico methods.

1. The work is clearly and accurately presented but it doesn't cite current literature. Only 3 literature sources were cited from 2018, and 0 citations from 2019.
2. The study design is partly appropriate and partly technically sound. This study is missing binding free energies extracted from molecular dynamics simulations of the reported systems. The reported docking results are generated from static crystal structures which is not a sound reflection of a biological system. The docking results must be correlated to the binding free energies from molecular dynamics simulations, which serve as a core validation of the docking results presented.
3. The conclusions drawn are not adequately supported by the presented results since the point raised above in 2 has not been fulfilled.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular modeling of proteins and small molecules implicated in infectious diseases; Drug Design: Homology Modeling, Molecular Docking & Virtual Screening; Protein conformation and energetics; Peptide/Protein-surface interface; Electronic molecular structure; X-ray reflection data interpretation by simulation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 09 Dec 2019

Usman Sumo Friend Tambunan

We thank Ndumiso N. Mhlongo for his review and suggestion.

We want to respond regarding his comment:

1. The writing process of this article was finished at the end of 2018, and the submission process took place in early of 2019. this explain why this article does not contain any relevant reference from 2019 publication.

2. We will add the data for the molecular dynamics simulation to explain further about the interaction, as the validation for the molecular docking simulation result.

Competing Interests: No competing interests were disclosed.

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