# *In Silico* De Novo Synthesis, Screening, and ADME/T Profiling of DNA-pA104R Inhibitors as Potential African Swine Fever Therapeutics

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Abstract: African Swine Fever Virus (ASFV) is a dsDNA virus causative of the African Swine Fever (ASF) in wild and domestic hogs. ASF is characterized by hemorrhagic fever, high mortality, and transmissibility. The binding of the DNA to the pA104R protein mediates viral replication and genome packaging. In the present study, we generated nine (9) reference compounds that exhibited high docking affinities through *de novo* computer-aided drug design (CADD). These compounds were then used as query molecules to find commercially available drug-like compounds using ligand-based virtual screening (VS). We were able to retrieve 900 hit compounds that exhibited the same pharmacophoric activities. Then, these hit compounds were subjected to drug-likeness filtration to identify the most likely to be developed as commercial drugs based on established parameters. We identified sixty-two (62) drug-like molecules. Molecular docking was then performed to determine the top five compounds with the highest binding affinities against the target protein. ADME/T profiling was done on these compounds to assess their pharmacokinetic properties. Compound 8.45 performed best based on our devised scoring system. This paper shall serve as a good reference in the discovery and development of anti-ASFV therapeutics.

## 1 INTRODUCTION

The African Swine Fever Virus (ASFV) is a highly transmissible virus causative of the African Swine Fever (ASF) in wild and domestic hogs. Apart from its swift spread, ASF is characterized by high mortality rates, to which death is usually observed a week after the onset of the disease. The identification of the viral infection is of little difficulty due to the readily observable symptoms in infected pigs that include (1) high fever, (2) reduced locomotor movements, (3) lack of appetite, (4) huddling, (5) conjunctivitis, (6) diarrhea and vomiting, (7) somnolence, (8) dyspnea, (9) seizures, and (10) skin hemorrhages (Blome et al., 2020). This virus's transmission and promulgation rely on vector species such as ticks that primarily target boars found in the wilderness. ASFV has evolved from a very mild strain into a highly transmissible virus that threatens today's swine population (Chen et al., 2021). Although tremendously virulent to hogs, there is no risk of this virus being transmitted to humans and cause the same threats that it poses for pigs. The virus indirectly impacts society through the economy since the meat of the domesticated pigs is often a central ingredient in making food from all ranges of cuisine. It is therefore of great importance to develop therapeutics that could eliminate this virus. Up to this date, there is no commercially available vaccine or drug to combat ASF in infected animals. The scientific community has only relied on control

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strategies to confine the virus and prevent its further spread.

Regarding its structure, ASFV is classified as a nucleocytoplasmic large DNA virus (NCLDV), having a genome size of 180,916 base pairs (Alonso et al., 2018) and an overall virion diameter of 175-215 nm with a 70-100 nm diameter nucleoprotein core (Blome et al., 2020). The core is surrounded by: (1) an internal lipid layer, (2) an icosahedral capsid that is composed of 1892-2172 capsomeres, and (3) the dispensable lipid envelope (Alonso et al., 2018). This virus is highly stable in environmental settings and thrives in most and protein-rich areas. Furthermore, it can survive in raw meat products at variable durations. The structure and architecture of the virus have not been fully elucidated. However, the pA104R protein of the ASFV has successfully been studied (Urbano & Ferreira, 2020), and its crystallographic structure is available (Liu et al., 2020). This macromolecule is a DNA-binding protein essential for viral replication and transcription (Frouco et al., 2017b). ASFV enters the host cell through endocytosis and micropinocytosis. Then, the virus uses the pA104R to interact with the host cell's DNA, leading to the mass manufacture of the viral parts via gene editing and the completion of the replicative cycle of the ASFV (Galindo & Alonso, 2017). The structure of the pA104R is characterized as histone-like since it shares conserved sequence homology with the other histone-like proteins derived from bacteria (Frouco et al., 2017a).

In a study by Liu et al., the researchers successfully elucidated the structure and determined the binding properties of the pA104R to the host DNA (i.e., pA104R-DNA complex). Their findings illustrated the unique binding pattern of pA104R as it uses its β-ribbon arm and inserts it in the major groove of the DNA. Furthermore, the researchers evaluated the ability of the stilbene derivatives, SD1 and SD4, to inhibit viral replication by disrupting the pA104R-DNA binding in swine macrophages (Liu et al., 2020). Zhu et al. utilized protein-protein interaction (PPI) networks to determine the ASFV-interacting proteins and assessed some commercially available drugs, such as Polaprezinc and Geldanamycin, that could potentially bind to some viral proteins to inhibit the action of the ASFV (Zhu et al., 2020). A similar study by Mottola et al. helped unmask the antiviral activity of fluoroquinolones against the virus (Zhu et al., 2019). Recently, there have been explorations on the potential of antimicrobial peptides (AMPs) and their effect on porcine viruses, including their mechanism of action (Pen et al., 2020); however, the AMPs used were already existing ones, and therefore, further exploration on more efficient peptides must be done. Although the research on ASF has reached great strides in the past years, there is still no potential candidate to eliminate or inhibit the effects of the virus in question. It is therefore imperative to devise new strategies that could identify compounds that could neutralize ASFV.

Drug discovery and development is defined as the process of identifying chemical entities that have potential therapeutic effects (Mohs & Greig, 2017). Over the years, this process has undergone radical changes with the further integration of biology, chemistry, physics, mathematics, and computer science (Umashankar & Gurunathan, 2015). The pipeline (i.e., drug discovery and development) involves a multistage process that should be strictly followed before a novel chemical entity is commercially available for public consumption (Mohs & Greig, 2017). Until the late 1980s, drug discovery was solely based on blind screening and serendipity (Kiriiri et al., 2020). This was changed upon introducing high-throughput screening (HTS) and combinatorial chemistry, allowing scientists to discover and synthesize many compounds (Umashankar & Gurunathan, 2015). However, the methods above were very costly and could be described as "brute force" approach as finding lead candidates is dependent on the initial library of compounds (Polanski, 2020). A further refinement of the pipeline has emerged with the introduction of the *in silico* approach. Such a strategy uses computational methods to predict the binding properties of the compound of interest to the biological target. The Boston Consulting Group estimated that integrating *in silico* practices in the drug discovery pipeline could save 14% of the total cost (Agarwal et al., 2017).

Herein, we identified potential drug-like compounds that can be utilized to treat African Swine Fever (ASF) mainly through the inhibition of the pA104R-DNA binding. To accomplish this, *de novo* methods and a ligand-based virtual screening approach were employed. The binding affinities of the generated and retrieved compounds were determined through molecular docking studies. Finally, the pharmacokinetic properties of the identified drug-like compounds were ADME/T studies. This study shall only entail the identification and pharmacokinetic characterization of the possible hit compounds.

# 2 METHODOLOGY

## 2.1 Protein Preparation

The crystallographic structure of ASFV pA104R in complex with dsDNA was obtained from Protein Data Bank (accession number: 6LMH). Protein visualization and refinement were conducted in the Biovia discovery studio visualizer (DASSAULT SYSTEMES, 2020). pA104R contains two chains: A and B (i.e., AHR and BDR, respectively). In this study, only the AHR was considered, and so the chain B was removed. The heteroatoms (i.e., dsDNA and water molecules) were deleted and polar hydrogen was added for the subsequent analyses. The pA104R-DNA complex active site residues were shown in Table 1.

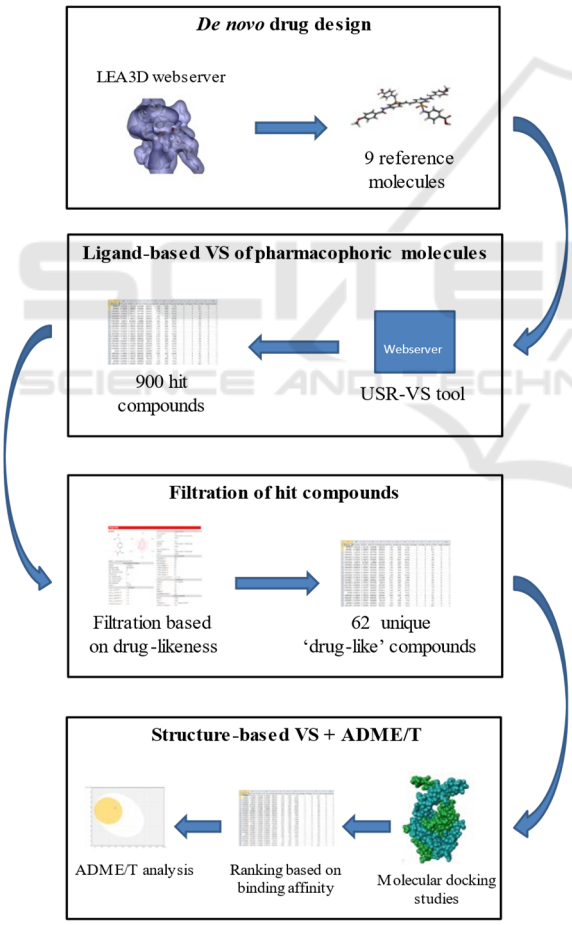


Figure 1: In silico experimental design.

## 2.2 *In Silico* De Novo Synthesis of Reference Molecules

To our knowledge, there has been no definitive report of possible drug-like DNA-binding inhibitors of the African swine fever virus. As such, we opted to perform a *de novo* drug design approach to generate potential ligands. This strategy uses computational algorithms to build molecules that exhibit specified properties. e-LEA3D (https://chemoinfo.ipmc.cnrs.fr/LEA3D/index.html) is an online tool that enables users to perform computer-aided de novo drug design efficiently. This web server creates diverse molecules through a genetic algorithm that evolves fragments based on mutation and crossover operators (Douguet, 2010).

The prepared protein structure was loaded to the server. Then, the desired molecular properties of the output molecules were selected using default parameters. e-LEA3D uses the PLANTS docking program to assign scores in the generated molecules. The parameters used in this function were as follows: binding site radius = 40, binding site residue = LYS89, weight in final score = 1. The server returned 10 'reference' compounds that have a high binding affinity towards pA104R. The experimental design employed in this study was shown in Figure 1.

## 2.3 Ligand-Based Virtual Screening (LBVS)

The de novo approach has one major flaw: molecules generated through this strategy are hard to synthesize (Mouchlis et al., 2021). To make up for this drawback, we adapted ligand-based virtual screening that could be used to search for commercially available active compounds from several enormous libraries of molecules. This approach is usually done when there is no prior knowledge of the 3D structure of the target protein (Hamza et al., 2012). Nonetheless, ligand-based VS could also be employed when searching for new ligands with similar chemical and biological activities.

In this study, the USR-VS web server (http://usr.marseille.inserm.fr/) was used. This tool implements Ultrafast Shape Recognition (USR) and Ultrafast Shape Recognition with CREDO Atom Types (USRCAT) algorithms to screen a library for similar compounds relative to the pharmacophoric properties of the query molecule (Schreyer & Blundell, 2012). Currently, the USR-VS screening library is comprised of 23 million molecules with over 94 million low-energy conformers. To conduct the virtual screening, the structure data files (SDF) of the nine (9) reference molecules were retrieved from the e-LEA3D webserver.

The screening process was straightforward. The SDF files were uploaded into the webserver. Then, the desired scoring algorithm (i.e. USR and USRCAT) was selected. We used USRCAT since it has been reported that this algorithm outperformed USR in retrospective screening (Schreyer & Blundell, 2012). Clicking 'submit' initiates the virtual screening that will only take milliseconds. Each query (i.e. reference) molecule generated 100 hits. Once the screening was finished, the results were displayed in a separate internet tab and the SDF for each hit compound was downloaded.

## 2.4 Drug-likeness Filtration

Drug-likeness filters are important aspects of drug discovery. These parameters determined the likelihood of a compound to exhibit therapeutic effects while being biologically safe based on its molecular structure. Moreover, applying filters to a large library of compounds could eliminate the non-drug-like molecules, thus saving operation time and cost (Shen et al., 2012). In this study, a total of 900 hit compounds were identified through ligand-based virtual screening. Five drug-like filters were employed for the analysis (Table S1). The filtration process was conducted using the SwissADME website (http://www.swissadme.ch/index.php). This tool is a versatile free-of-charge webserver for determining the physicochemical properties, lipophilicity, water-solubility, pharmacokinetics, drug-likeness, and medicinal chemistry of small molecules (Daina et al., 2017).

SwissADME only accepts chemical structures in SMILES format (i.e. .smi extension). Thus, the hit compounds (i.e., in SDF format) must be converted into the acceptable file format before executing the filtration process. To do this, the OpenBabel widget of the PyRx version 0.8 (The Scripps Research Institute, 2008) was used. After the said conversion, the SMILES were uploaded to the webserver. All compounds that passed the five filtration parameters without any violations were selected for the subsequent analyses. Sixty-two (62) unique drug-like compounds were identified.

## 2.5 Molecular Docking Studies

The 62 drug-like compounds were subjected to molecular docking studies to determine their affinity for binding against pA104R. DockThor (https://dockthor.lncc.br/v2/) is a user-friendly webserver for receptor-ligand docking developed by the GMMSB group (Santos et al., 2020). This tool performs molecular docking through flexible-ligand and rigid-receptor strategies based on the MMFF94S force field (Guedes, Costa, et al., 2021). The docking procedure is a three-step process. First, the prepared protein (i.e. protonated) in PDB format was uploaded to the server. Since no cofactors were considered in this study, the 'do not use cofactor' function was selected. Then, the 62 drug-like compounds in SDF format were docking program requires the user to upload the protonated version of the ligands. For convenience, DockThor is embedded with an 'add H' function. The submitted protein and ligand structure were processed after clicking 'send'. A checkmark appeared which indicated that the input molecules were valid and recognized by the force field. The final step involves setting up the docking configuration. The user could choose from blind docking or user-defined docking. Since the binding site was already determined (Table 1), we performed user-defined docking. DockThor utilizes a genetic algorithm to determine the optimal poses for flexible ligand docking (De Magalhães et al., 2014). Furthermore, the platform allows the user to customize the algorithm parameters, but the 'standard algorithm' was selected for this study. Table S2 shows the different parameters used in the docking experiment. The webserver ranked the 62 drug-like compounds based on their binding affinities. The chemical structure (i.e., in .mol2 format) of the best docking pose for each input molecule was obtained. The 3D and 2D protein-ligand interactions were visualized using Biovia discovery studio.

## 2.6 ADME/T Studies

Compounds must undergo ADME/T studies to determine their pharmacokinetic properties and safety level. The 62 drug-like compounds and the 9 reference molecules were then subjected to ADME/T studies. To do so, we used pkCSM (biosig.unimelb.edu.au/pkcsm/prediction), a web-based tool commonly used to calculate the pharmacokinetic properties of small-molecule drugs, such as the compounds involved in this study. This application allows for the fast development of predictive models of central ADMET properties via graph-based signatures (Pires et al., 2015). Since the pkCSM webserver only accepts entries in the SMILES format, we first converted the available files to the .smi format via the OpenBabel widget of the PyRx software, similar to the earlier method. Subsequently, the compounds were uploaded to the web-based server for the prediction of their pharmacokinetic properties

# 3 RESULTS AND DISCUSSION

## 3.1 The pA104R: A Therapeutic Target for ASF

DNA packaging is a vital process in the life cycle of double-stranded DNA (dsDNA) viruses. Packaging proteins bind with the DNA and initiates conformational changes that cause it to bend and be organized into densely packed chromatin structures (Urbano & Ferreira, 2020). Failure of these proteins to promote condensation and packaging will inevitably cause DNA damage, ultimately leading to apoptosis (J. Y. J. Wang, 2001). Therefore, targeting the proteins involved in the said process is an attractive approach to design therapeutics against viruses. DNA-packaging proteins have been reported for a wide range of organisms. For instance, the packaging proteins in bacteria are the histone-like proteins belonging to the HU/IHF superfamily (Swinger & Rice, 2004). In relevance to the ASFV, p10 and pA104R are the major DNA-packaging proteins in mature ASFV (Andrés et al., 2002). However, a more recent study using small interfering RNA (siRNA) has shown that pA104R has a profound role in DNA replication, transcription, and packaging of ASFV (Frouco et al., 2017a).

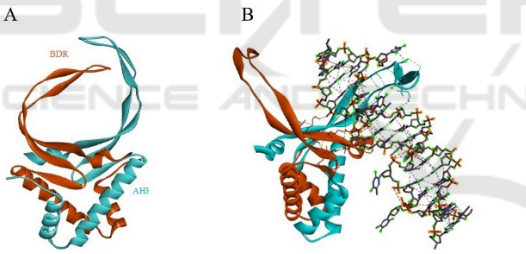


Figure 2: The structure of (A) DNA-pA104R complex (PDB accession code:6LMJ) and (B) apo-pA104R (PDB accession: 6LMH).

pA104R is a homodimer that significantly resembles other bacterial HU/IHF homologs (Liu et al., 2020). The crystallographic structures of the apo-pA104R and DNA-bound pA104R were shown in Figure 2. The protein folds into two domains, namely the "body" AHR(i.e., alpha-helical region) and the "arms" BDR (i.e., β-strand DNA binding region). As shown in Fig 2B, the DNA interacts predominantly with pA104R via the BDR arm. The surface of this region is saturated with positively charged, making it an attractive binding site for the negatively charged DNA molecule (Liu et al., 2020). Thus, the subsequent analyses were simplified by focusing on the BDR. To design an effective inhibitor, the key amino acid residues within the binding site must be identified. Thus, the active site amino acid residues in the DNA-pA104R complex were determined using the Biovia Discovery Studio (Table 1). As hypothesized, the key amino acid residues in the BDR arm are mostly positively charged at physiological pH. HIS78, LYS89, and LYS91 are all positively charged. Meanwhile, PRO80 is an aliphatic amino acid making it nonpolar and hydrophobic. This residue interacts with the DNA strand, but the nature of its interaction is not electrostatic since it is nonpolar at physiological pH. Further studies are encouraged to uncover the linkages between this residue and the target DNA strand.

Table 1: Interacting amino acid of DNA-pA104R complex. Only the AAs in the BDR were considered.

| pA104R domain | Active residues |
|---|---|
| AHR | LYS 63 |
| | LYS98 |
| | ARG100 |
| | LEU102 |
| | LYS103 |
| BDR | HIS78 |
| | PRO80 |
| | LYS89 |
| | LYS91 |

## 3.2 De Novo CADD of ASFV DNA Binding Inhibitors

There is only a handful of literature dedicated to searching for possible DNA-binding inhibitors in AFSV. Liu et al. reported that SD1 and SD4 (i.e., stilbene derivatives) had inhibitory effects on the DNA-pA104R binding. Such results were evident by their ability to repress the ASFV replication (Liu et al., 2020). To our knowledge, these are the only molecules known to have therapeutic potential against African swine fever. It is of great importance to increase the number of viable ligands. *De novo* computer-aided drug design (CADD) approach in drug discovery enables the generation of novel ligands based on defined scoring functions (Douguet, 2010). To that end, e-LEA3D, a *de novo* drug design tool, was employed in this study. This program uses a genetic algorithm that evolves molecular fragments and optimizes the combination of these fragments (Douguet et al., 2005). Once a library of optimized molecules is generated, they are assigned a score based on docking fitness calculated by the PLANTS program. As shown in Table 2, e-LEA3D generated nine molecules. ref1 has the highest score (i.e., 91.25%), implying that this compound has the best docking conformation from all the generated molecules using the program's algorithm. However,

this does not bear weight on the binding affinity of the ligand to pA104R since its primary purpose is to rank the solutions.

Table 2: Reference molecules generated using e-LEA3D.

| Code | Molecular Formula | Weight (g $mol^{-1}$) | Score (%) |
|---|---|---|---|
| ref1 | $C_{48}H_{61}N_{10}O_{14}S_2$ | 1066.19 | 91.25 |
| ref2 | $C_{68}H_{86}N_{10}O_{17}S_3$ | 1411.66 | 87.07 |
| ref3 | $C_{43}H_{57}N_{11}O_{12}S_2$ | 984.11 | 84.89 |
| ref4 | $C_{67}H_{84}N_{10}O_{17}S_3$ | 1397.64 | 84.31 |
| ref5 | $C_{67}H_{82}N_{14}O_{17}S_3$ | 1451.65 | 83.75 |
| ref6 | $C_{55}H_{64}N_{10}O_{12}S_2$ | 1121.29 | 83.31 |
| ref7 | $C_{62}H_{82}N_{10}O_{17}S_3$ | 1335.57 | 83.29 |
| ref8 | $C_{62}H_{82}N_{10}O_{17}S_3$ | 1335.57 | 83.23 |
| ref9 | $C_{49}H_{60}N_{10}O_{16}S_3$ | 1141.25 | 82.94 |

## 3.3 Ligand-Based Virtual Screening (LBVS) of Molecules Based on Pharmacophoric Activity

The *de novo* design strategy of molecules does not guarantee their ability to be developed into therapeutic agents. As mentioned, the synthetic accessibility of the generated compounds is one of the major challenges in the *de novo* approach (Mouchlis et al., 2021). A high binding affinity serves no purpose if the molecule is hard to synthesize. To address this problem, a screening process within a library of commercially available molecules may be performed. Virtual screening (VS) of prospective drug compounds has become the norm in the early stages of drug discovery. It is often regarded as the *in silico* counterpart of the tedious and expensive high-throughput screening (HTS) (Polgar & M. Keseru, 2011).

The screening process is divided into ligand-based and structure-based approaches. The latter aims to determine the best ligand that will bind to the receptor based on surface complementarity (Maia et al., 2020). The pre-requisite for this type of analysis is the availability of the 3D protein structure. Meanwhile, ligand-based VS uses the pharmacophoric properties of a query molecule to retrieve compounds that exhibit similar biological and chemical activities from a database(Singh et al., 2021).

In this study, we applied ligand-based virtual screening to obtain commercially available molecules based on the pharmacophores of the reference compounds. USR-VS is a webserver that uses Ultrafast Shape Recognition (USR), and Ultrafast Shape Recognition with CREDO Atom Types (USRCAT) algorithms for effective pharmacophore search and retrieval (Li et al., 2016). USR predicts the molecular shape by analyzing the relative positions of bonded atoms. As implied by its name, USR enables the user to search for molecules with a similar three-dimensional shape at incredible speed. USRCAT is an extension of USR integrated with the CREDO, a structural interactomics database (Schreyer & Blundell, 2013). This algorithm works similarly with USR, but it uses pharmacophoric constraints for a more effective similarity search. Therefore, the USRCAT algorithm was used in this analysis. The nine (9) *de novo* designed compounds were used as query molecules to the USR-VS webserver. The similarity search covered 23 million molecules and 94 million low-energy conformers from the ZINC database. Each run returned 100 hit compounds. Therefore, the nine reference molecules generated 900 hits.

## 3.4 Drug-likeness Filtration of Hit Compounds

It is estimated that only 40% of hit compounds can transition from the pre-clinical to first-in-humans stage due to their poor physical and chemical properties (Venkatesh and Lipper, 2000). Drug-likeness filtration is one of the barriers a compound must overcome to advance in the late phases of drug discovery (Hu et al., 2018). This assesses the probability of a compound to be manufactured as a therapeutic drug based on some physicochemical parameters. The method of applying the drug-likeness filter has been an integral step in the drug discovery pipeline because any chemical compound may exhibit an excellent therapeutic effect. Still, not all could be transformed into viable drug.

To eliminate the hit compounds with undesirable properties, drug-likeness filtration was performed using the SwissADME webserver. This web tool has been used in 2100 *in silico* analyses (i.e., as per the number of citations of the published paper of the developers (Daina et al., 2017) ). SwissADME uses five filters to assess the drug-like properties. Violation in any of the filters (i.e., Lipinski (Lipinski, 2004), Ghose (Ghose et al., 1999), Egan (Egan et al., 2000), Veber (Veber et al., 2002), and Muegge (Muegge et al., 2001)) disqualifies the compound from further analysis. By adhering to this selection criterion, one could ascertain the excellent drug-like properties of successful compounds.

Table 3: Drug-likeness filtration of the 900 compounds. (Note: For simplification, only results from five compounds were shown).

| Compound no. | Code | Formula | No. of filter violations (i.e., Lipinski, Ghose, Veber, Egan, and Muegge) |
|---|---|---|---|
| 1 | 1.27 | $C_{23}H_{30}N_4O_2$ | 0 |
| 10 | 2.59 | $C_{19}H_{23}N_3O_3$ | 0 |
| 54 | 9.19 | $C_{20}H_{31}N_3O_2S$ | 0 |
| 58 | 9.43 | $C_{20}H_{22}N_2O_3$ | 0 |
| 62 | 9.92 | $C_{24}H_{26}N_2O_4$ | 0 |

Table 3 shows the summary of the results. After screening 900 compounds, only 62 were drug-like. This translates to a 6.89% success rate from hit identification to drug-like filtration. Lipinski's rule of five (Ro5) was primarily developed to assess the druggability of new molecular entities (Lipinski, 2000). If a molecule fails one of the parameters of Ro5, then the absorption and permeability properties are put into question. However, Lipinski et al. stated that molecules that violate at least one of the said parameters should not be necessarily removed from the selection process (Petit et al., 2012). Instead, such molecules should be given low priority in the drug discovery process. Nonetheless, satisfying the Ro5 without violation is an indicator of excellent drug-likeness.

## 3.5 Molecular Docking Studies

Sixty-two (62) identified drug-like molecules underwent further screening to determine the compounds that exhibit high binding interactions with the target protein pA104R. The analysis was conducted through molecular docking, a structure-based virtual screening strategy. Molecular docking is a computational approach to screen for ligands that fit the protein's ligand-binding site with high complementarity (i.e., geometrically and energetically) (Azam & Abbasi, 2013). Docking tools use search algorithms to predict a ligand's best docking pose (Sanchez, 2013). Then, a scoring function calculates the binding free energy of the protein-ligand complex (Bissantz et al., 2000).

In this study, DockThor, a web server for highly flexible ligand-docking, was used. This tool utilizes a dynamic genetic algorithm as a search method. Such an algorithm allows the intensive survey of the energy hypersurface to generate multiple minima solutions (De Magalhães et al., 2014). DockThor uses the DockTScore as a scoring function based on the MMFF94S force field (Guedes, Barreto, et al., 2021). The scoring function considers the intermolecular interactions, torsional entropy, lipophilic interaction, polar solvation, and nonpolar solvation. As shown in Table 4, the binding affinities achieved range from -7.790 to -6.158 kcal $mol^{-1}$. Compound 8.45 ranked first with a binding affinity of -7.790 kcal $mol^{-1}$.

Table 4: Docking results of the 62 drug-like compounds. (Note: For simplification, only results from five compounds were shown).

| Rank | Compound Code | Binding affinity (kcal $mol^{-1}$) | Total energy (kcal $mol^{-1}$) | vdW Energy | Elec. energy |
|---|---|---|---|---|---|
| 1 | 8.45 | -7.790 | 12.623 | -15.214 | -11.853 |
| 2 | 2.21 | -7.705 | 36.308 | -13.926 | -11.001 |
| 52 | 2.59 | -6.817 | 18.362 | -4.727 | -19.801 |
| 51 | 9.19 | -6.841 | -1.480 | -8.951 | -16.563 |
| 62 | 6.45 | -6.158 | -7.153 | -2.613 | -19.790 |

There are two amino acids critical for the binding of 8.45 with ASFV. GLN76 (i.e., glutamine at position 76) formed three hydrogen bonds, two of which are conventional, while the remaining is a pi-donor hydrogen bond. The first hydrogen bond is formed by the interaction of the oxygen atom of the GLN76 to the hydrogen atom of the amino group in 8.45. Then, the hydrogen from the GLN76 interacts with the carbonyl oxygen of the compound. Meanwhile, the glutamine's nitrogen atom forms a pi-donor hydrogen bonding (Figure 3). HIS78 creates a pi-alkyl interaction with the said molecule. The amino acid, HIS78, is one of the active site amino acid residues (Table 1). Such interactions might explain why compound 8.45 had the highest binding affinity among all drug-like molecules that underwent molecular docking. Therefore, 8.45 could be a

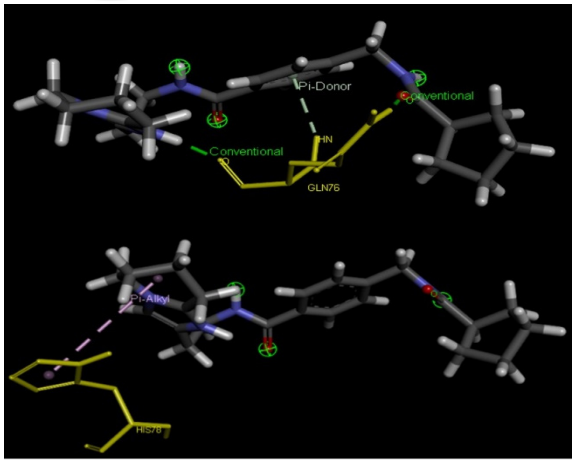


Figure 3: Molecular interactions of compound 8.45 to (top) GLN76 and (bottom) HIS78 of the ASFV.

potential DNA-binding inhibitor of pA104R solely based on the docking results. However, further tests must be conducted to determine this compound's potential as a therapeutic agent.

## 3.6 ADME/T Profiling

Determination of pharmacokinetic characteristics is one of the most critical steps to ensure that the drug being developed is safe to be administered during animal and clinical trials. The results for the ADME/T of the unique compounds with the highest binding affinities are shown in Table 5. For the absorption parameters, it can be observed that the intestinal absorption% for the drug-like compounds have a very high positive value, ranging from 69.985% to 95.646%. Such results match the existing literature since adhering to Lipinski's Rule of Five entails that the drug-like compounds are likely to be well-absorbed in the intestine (Zhao et al., 2001). Further supporting this idea, the range derived for the human intestinal absorption (HIA) % was above the optimum level of 30%, as shown by Wang (2016) (N. N. Wang et al., 2017). All the values for skin permeability of both the unique compounds indicate skin permeability because all the values were lower than -2.5 (Hassan et al., 2018). The results are favorable since they signify that the drugs can be applied through skin contact and promote the elimination of these drugs to prevent the accumulation of chemicals in the body (Osborne & Musakhanian, 2018). This finding provides an alternative route of administration for the proposed compounds.

Caco-2 permeability is considered the final absorption parameter. It makes use of the Caco-2 cell, or the human colon adenocarcinoma, to model the intestinal absorption of many compounds (Matsumoto et al., 2018). The Caco-2 permeability values for the unique group were varying. The unique and reference groups yielded acceptable results as all values were above the reported threshold for optimum Caco-2 permeability value (i.e., -5.15) (N. N. Wang et al., 2016). This finding reinforces the results given by the HIA% that the compounds under study have adequate intestinal absorption.

We observed that the $VD_{ss}$ values of the unique group varied but were negative for all reference compounds for the distribution parameters. A higher $VD_{ss}$ value entails better distribution into the tissues than in the plasma (Yates & Arundel, 2008). Compounds 2.2 and 9.19 had unfavorable $VD_{ss}$ values because they were close to the minimum range (i.e., -0.15) (Firdausy et al., 2020). On the other hand, the drug compounds 8.45, 2.21, 8.40, 7.21, and 2.59 displayed moderate $VD_{ss}$ values because their values were between the range reported (i.e., -0.15 to 0.45) (Firdausy et al., 2020).

The blood-brain barrier permeability was varying for the unique group but all negative for the reference compounds. Nevertheless, all the unique compounds were unable to penetrate the blood-brain barrier (i.e., < 0.3) (Firdausy et al., 2020). Such a result is a positive indication since the expected target of the compounds is not found within the brain. Regarding CNS permeability, compound 7.21 can effectively penetrate the central nervous system (i.e., > -2), whereas the remaining unique compounds could only poorly penetrate the CNS (i.e., < -3) (Pires et al., 2015). However, even if the CNS permeability values were favorable for all the compounds, they would still not penetrate the CNS due to the blood-brain barrier (Carpenter et al., 2014).

The metabolism of the compounds being studied was dictated by their capacity to become either CYP2D6 or CYP3A4 (i.e., the two main subtypes of cytochrome P450) inhibitors (Firdausy et al., 2020). All of the unique compounds were not CYP2D6 inhibitors. Meanwhile, compounds 2.2, 8.40, and 7.21 were known to be CYP3A4 inhibitors. A negative result from these tests could suggest the excellent metabolism of the proposed drug-like compounds in the human body; the presence of inhibitors poses a threat for the body since decreased metabolism leads to the accumulation of the compounds and will thus increase the toxicity of that potential drug (Niel et al., 1992).

For the excretion parameter, total clearance was considered. This parameter measures the compound's ability to be cleared from all tissues (i.e., the combination of renal and hepatic clearances. The CLtot values for compounds considered were within the range -0.278 to 1.449 log ml $min^{-1}$ $kg^{-1}$. It was found that the highest total clearance was achieved by compound 8.45, which suggests that it has the highest bioavailability (Firdausy et al., 2020). Meanwhile, compounds 7.21 and 9.19 had negative values indicating their poor systemic clearance.

Finally, the toxicity of the proposed drugs was evaluated. The Ames test is a preliminary evaluation to determine the mutagenicity of drug candidates (Mortelmans et al., 2016). Based on the results, only compound 2.2 was characterized as a mutagen. There is a high correlation between carcinogenicity and mutagenicity (ca. 90%). This indicates that compound 2.2 could induce mutations leading to cancer (Mortelmans et al., 2016). It is therefore essential to perform other tests to determine its genotoxicity.

Table 5: ADME/T Results of the unique (i.e., drug-like) group. The top 5 compounds based on their binding affinities from the molecular docking studies were used. Compounds 9.19 and 2.59 were also included for the ADME/T profiling due to their low hepatoxicity.

| Code | Absorption | | | Distribution | | | Metabolism | | Excretion | Toxicity | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Intestinal abs. | Skin permeability | Caco-2 permeability | $VD_{ss}$ | BBB permeability | CNS permeability | CYP 2D6 inhibitor | CYP 3A4 inhibitor | Total Clearance | Ames Toxicity | Hepato toxicity | LD50 (mg $kg^{-1}$) |
| 8.45 | 89.927 | -2.849 | 0.794 | 0.361 | -0.569 | -2.641 | No | No | 1.022 | No | Yes | 695.29 |
| 2.21 | 91.148 | -3.022 | 0.811 | 0.395 | -0.802 | -2.653 | No | No | 0.808 | No | Yes | 911.54 |
| 2.2 | 82.316 | -2.741 | 0.158 | -0.182 | -1.053 | -2.42 | No | Yes | 0.109 | Yes | Yes | 1056.52 |
| 8.40 | 92.54 | -3.069 | 0.662 | 0.221 | -0.955 | -2.974 | No | Yes | 0.248 | No | Yes | 1081.99 |
| 7.21 | 92.942 | -3.246 | 0.703 | 0.126 | -0.183 | -1.953 | No | Yes | -0.317 | No | Yes | 793.17 |
| 9.19 | 91.444 | -3.869 | 0.878 | -0.087 | -0.375 | -2.918 | No | No | -0.278 | No | No | 390.81 |
| 2.59 | 88.437 | -2.863 | 0.987 | 0.105 | -0.836 | -2.44 | No | No | 0.438 | No | No | 2583.83 |

Meanwhile, a hepatotoxicity test was also performed to determine whether the drug could cause significant liver injury. This stage of the development process greatly impedes the translation of a substance into a commercial drug (Björnsson, 2016). Based on the results, only compounds 9.19 and 2.59 were not hepatoxic. This result signifies that these are the only compounds from the unique group that causes minimal harm to the human liver.

The final parameter considered is the rat oral acute toxicity ($LD_{50}$) of the proposed drug candidates. This parameter determines the amount of the substance that could kill 50% of the test animal population (Adamson, 2016). The higher the $LD_{50}$ value of the compound, the less toxic the substance is when taken orally by the individual. The resulting LD50 of the unique group compounds ranged from 2.121 to 2.985. Based on the Hodge and Sterner scale, all compounds except 9.19 are considered only slightly toxic, with a toxicity rating of 4 (ca. 500-5000 mg $kg^{-1}$ ) (Ahmed, 2015). Meanwhile, compound 9.19 is considered moderately toxic since its $LD_{50}$ value falls under a toxicity rating of 3 (ca. 50-500 mg $kg^{-1}$). The remaining compounds had relatively low $LD_{50}$ that is indicative of their high toxicity. Therefore, caution must be exercised when deriving the optimum dosage of these drug candidates.

To identify the most suitable compounds among the unique group, we devised a scoring system that consisted of the molecular docking rank and the ADME/T score. The top five compounds from the molecular docking studies were analyzed for ADME/T profiling. However, we also considered compounds 9.19 and 2.59 even though they ranked 51$^{st}$ and 52$^{nd}$, respectively. These two were the only non-hepatoxic compounds; therefore, we opted to include them in the scoring system. For the ADME/T scoring, each parameter violation was awarded one point. The compound with the lowest score was deemed to have the most favorable ADME/T properties. Based on the results, compound 2.59 performed best on the ADME/T studies (Table 6). Meanwhile, compound 2.2 had the most number of parameter violations.

Table 6: Summary of docking and ADME/T performance. For ADME/T profiling, violation in any of the parameters is rewarded one point. The final score is the average of the docking and ADME/T scores.

| Code | Docking Rank/Score | ADME/T Score | Final Score |
|---|---|---|---|
| 8.45 | 1 | 2 | 1.5 |
| 2.21 | 2 | 2 | 2 |
| 2.2 | 3 | 4 | 3.5 |
| 8.40 | 4 | 3 | 3.5 |
| 7.21 | 5 | 3 | 4 |
| 9.19 | 51 | 2 | 26.5 |
| 2.59 | 52 | 1 | 26.5 |

The docking score and the ADME/T score were averaged to calculate the final score. Compound 8.45 had the lowest score (ca. 1.5), signifying its excellent binding affinity against pA104R and favorable pharmacokinetic properties. Although compound 2.59 ranked first in the ADME/T studies, it fell short of its molecular docking ranking resulting in a low final score (ca. 26.5). Compounds 2.21, 2.2, 8.40, and 7.21 had relatively good final scores. Such results implied their superior properties similar to compound 8.45. Additional tests must be conducted on these compounds to determine their capabilities as therapeutic agents against ASF. Particularly, bioassays such as the haemadsorption test (HAT) are useful in exploring the efficiency of the compounds as ASFV therapeutics (Fischer et al., 2020).

# 4 CONCLUSION

In this paper, the identification and characterization of potential drug candidates for the treatment of ASF were conducted. We were able to characterize the structure of the pA104R protein with visualization software. The DNA binding site of the pA104R was determined. Nine (9) *de novo* reference compounds were generated. Of these compounds, 900 commercially available drug-like small molecules were retrieved through ligand-based virtual screening using pharmacophoric similarities.

Drug-likeness filtration was done to determine the compounds with excellent druggability properties. Sixty-two (62) drug-like compounds were subjected to molecular docking and ADME/T studies. Of these filtered drug-like molecules, compound 8.45 achieved exceptional docking rank (ca. 1) and ADME/T score (ca. 2), earning the lowest final score. The other drug-like molecules (i.e., 2.21, 2.2, 8.40, and 7.21) also performed well. Compounds 9.19 and 2.59 had the best ADME/T profile but performed poorly in the molecular docking studies. Further experiments must be performed to identify their potential as anti-ASF therapeutics.

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