J Biochem Technol (2024) 15(3): 1-7
https://doi.org/10.51847/X0y0hG9jqQ
ISSN: 0974-2328

Docking Study of Licensed Non-Viral Drugs to Obtain Ebola Virus Inhibitors

Victoria Easton, Martin McPhillie*, Isabel Garcia-Dorival, John N. Barr, Thomas A. Edwards, Richard Foster, Colin Fishwick, Mark Harris

Received: 26 May 2024 / Received in revised form: 26 August 2024, Accepted: 29 August 2024, Published online: 12 September 2024

Abstract

Due to the power and high prevalence of infection and the lack of suitable medicine for the Ebola virus, research has been conducted on the discovery and introduction of anti-Ebola drugs. The purpose of the present study was to investigate the bioinformatic inhibition of Ebola virus entry and proliferation by licensed non-viral drugs. This research is a descriptive-analytical method and to carry out this investigation, first, the chemical structure of the compounds was drawn using ChemDraw Ultra 10.0 software, and then it was transferred to Hyperchem8 software to optimize the energy. Docking studies were carried out by AutoDock4.2 software and were analyzed in the final stage. The results obtained from the present study showed that the bonds involved in drug binding with receptors are hydrophobic, π-π, hydrogen bonds, and cation-π. Among all studied compounds, the best docking results were related to chloroquine, diphenoxylate, and amodiaquine drugs. These three drugs with the most negative binding energy level had a greater tendency to bind to the amino acids of the binding site of GP and VP40 proteins. The weakest docking results were related to the two drugs erythromycin and dirithromycin, because the hydrophilicity of these two drugs is very high. In general, the presence of hydrophobic parts, optimal hydrogen bonds, and tertiary amine increases the anti-Ebola potency of drugs. Based on the results obtained from bioinformatics studies, all drugs show good inhibitory effects in the receptor binding site and can be considered effective inhibitors of Ebola virus entry and proliferation.

Keywords: Ebola virus, Bioinformatic inhibition, Non-viral drugs, Infection

Victoria Easton, John N. Barr, Thomas A. Edwards, Mark Harris
School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK.
Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, UK.

Martin McPhillie*, Richard Foster, Colin Fishwick
School of Chemistry, Faculty of Mathematics and Physical Sciences, University of Leeds, Leeds LS2 9JT, UK.
Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, UK.

Isabel Garcia-Dorival
Institute of Infection and Global Health, University of Liverpool, Liverpool L3 5RF, UK.

***E-mail:** M.J.McPhillie@leeds.ac.uk

Introduction

Ebola disease is one of the deadliest viral diseases in history, which has spread in West Africa and is rapidly spreading to other parts of the world (Bandyopadhyay *et al*., 2016; Mirza & Ikram, 2016; Broni *et al*., 2023; Hayat *et al*., 2024). Ebola virus transmission is mainly through physical contact with the body fluids of infected people, which leads to the weakening of the immune system, acute hemorrhagic fever, and finally the death of the host (Basu *et al*., 2015; Cui *et al*., 2018). This virus was first recognized in Congo in 1976. The name of this virus is taken from one of the rivers of the Democratic Republic of Congo. In 1976, 284 people were infected with this disease and finally 151 people died (Li *et al*., 2015; Veljkovic *et al*., 2015; Liu *et al*., 2022). Although the Ebola virus has been detected for nearly 40 years, its recent outbreak in West Africa has created a critical situation in the region, so that in the report of March 2016, about 28,610 cases were infected and 11,315 people died due to this deadly disease. They have given up (WHO, 2016).

Ebola is a member of the Filoviridae family of the Mononegaviral order. This lipid-enveloped virus has a negative-sense linear RNA strand and seven types of proteins. This virus has a filament shape and its diameter and length are about 80 and 1200 nm respectively in a mature virion. The Ebola virus is transmitted to humans through animals such as chimpanzees, gorillas, and bats (Raj & Varadwaj, 2016; Setlur *et al*., 2017; Mohamed *et al*., 2022). Among these proteins, glycoprotein (GP) is one of the important proteins of the Ebola virus. This membrane protein is the only structural glycoprotein of Ebola. Its primary structure has 676 amino acids and is converted into two heterodimers (GP1 and GP2) by the host cell's furin enzyme, which is connected by a disulfide group. GP is located on the surface of the virus and plays the role of integrating the virus into the host cell. In this way, the virus attaches to the host cell and enters it, for this reason, it plays a very important role in the life cycle of Ebola (Setlur *et al*., 2017; Darko *et al*., 2021).

Recent studies have focused more on GP inhibition; because the discovery and development of these inhibitors can be considered as a drug to prevent the entry and attachment of the Ebola virus to the cell (Pleško *et al*., 2015; Darko *et al*., 2021). Another important protein of the Ebola virus, which is of great interest, is the main matrix protein (P40) (Viral matrix protein). This protein is the most abundant protein under the viral envelope and contains 326 amino acids and a molecular weight of 35 kilodaltons. This protein plays a critical role in maintaining the structural integrity and maturation

of the virion. In the life cycle of the Ebola virus, this protein plays other roles, including virion formation, regulation of viral transcription, as well as assembly and budding of mature virions. VP40, like GP, is considered a good target for anti-Ebola drugs, whose inhibitors prevent virus replication, assembly, and budding (Priya *et al*., 2015; Balmith & Soliman, 2017; Dhama *et al*., 2018). Unfortunately, there is currently no effective treatment for Ebola virus infection and it is considered a major threat to global health (Li *et al*., 2015; Veljkovic *et al*., 2015). Antibody therapy has been tested in animal models and has been used for a small number of patients, but the supply of such drugs is very limited (Balmith *et al*., 2017).

The process of re-introduction of drugs (drug repositioning) has gained much attention and importance in recent years for drug development and in the pharmaceutical industry. This process involves finding a new therapeutic indication for a licensed drug, or in other words, using known drugs and compounds to treat new diseases. This method is a very good alternative to de novo drug discovery and development methods. The significant advantage of the drug repositioning method over the development of drugs by traditional methods is that this method has passed a significant number of toxicity tests and other tests, its safety is known, and the risk of failure due to adverse toxic causes is reduced; Thus, it avoids the very high costs of synthetic drug discovery due to earlier access to pharmacokinetic, bioavailability, safety, and toxicity data. The computational method of structure-based drug design, in which small molecules are "docked" into the structure of target macromolecules and their binding at the target site is scored, is widely used in the discovery, design, and optimization of lead compounds. Examining Ebola virus inhibitors to produce effective medicine to reduce the mortality of patients and improve them is a new issue that has been studied and researched in recent years. Currently, the inadequacy of appropriate methods to identify the mechanism of action of inhibitors on Ebola virus activity and the relationship between their chemical structure and anti-Ebola effect encourages us to further investigate the mechanism of action of the compounds. The drug repositioning method is a method that has recently received much attention for the discovery of inhibitors. The drugs investigated in this study with the drug repositioning method previously on the Ebola virus have been tested to introduce new inhibitors (Madrid *et al*., 2013; Stefanik *et al*., 2020). Software and computing methods for drug design is a new methods whose emergence and popularity have been associated with the development of computing power over several decades. These methods are used along with biological experiments to communicate the structure and activity of pharmaceutical compounds, discover new compounds, predict the biological activities of compounds, and also understand reactions and biochemical processes.

In addition, with these methods, it is possible to express and predict how the compound binds to the target protein, evaluate the energy difference between different conformers of the compound, and explain the reaction mechanisms and the role and effect of different groups and substitutions in the chemical structure of the compounds and their performance. The binding method and finally the effectiveness of the compound on the target protein. So far, molecular docking simulation studies have not been conducted on these licensed drugs that have shown anti-Ebola effects about how these compounds interact with glycoprotein (GP) and main matrix protein (VP40). Therefore, in this research, to know the exact mechanism of action of drugs and to identify the amino acids involved in this process, the optimal energy of drug-receptor interaction of different drugs on GP and VP40 proteins, and the relationship between different substitutions in the chemical structure of drugs have been investigated.

Materials and Methods

To perform the docking method, AutoDock4.2 software was used to perform molecular docking. For this purpose, AutoCAD software was installed on an 8-core computer under the Windows operating system and was used to conduct this research. In this study, the interactions of 24 FDA-licensed drugs, which have already been proven to inhibit the Ebola virus through various practical investigations (Madrid *et al*., 2013), with the amino acids of the binding sites of GP and VP40 proteins using the molecular docking simulation method were analyzed. The names of drugs and their IC_{50} values are given in **Table 1**.

Table 1. Drug name and IC_{50} values against Ebola virus.

No.	Medicine Name	Concentration (Micromolar)	Assessment Of Viral Replication	Evaluation Of Virus-Like Entry (% Activity)
1	Amlodipine	10	-	93
2	Amodiaquine	10	-	99
3	Biperiden	50	-	98
4	Carprofen	10	-	94
5	Chloroquinone	50	+	97
6	Dex brom pheniramine	50	-	85
7	D Bucain	10	-	99
8	Diphenoxylate	50	+	96
9	Diphenylpyraline	50	+	96
10	Dirithromycin	50	-	99
11	Erythromycin	10	-	96
12	Estradiol	10	-	93

13	Fluoxetine	10	-	96
14	Ketotifen	50	+	99
15	Levopropoxyphene	50	-	98
16	Mycophenolate	50	-	91
17	Oxyphencyclamine	50	-	95
18	Paroxetine	10	-	98
19	Penbutolol	10	-	98
20	Prochlorperazine	10	-	95
21	Protriptyline	10	-	83
22	Toremifene	10	-	97
23	Dipivefrine	50	-	94
24	Trihexyphenidyl	10	-	97

Molecular docking simulation was performed by AutoDock4.2 software. Genetic algorithm (GA) was used as a search algorithm by the software. The graphical program ADT (AutoDock Tools) 1.5.6 was used to prepare, perform, and analyze the molecular docking simulations. At first, the two-dimensional structures of the drugs were drawn by the ChemDraw Ultra 10.0 program and then optimized in terms of energy using Hyperchem8 software in the molecular mechanics (MM) force field and semi-empirical PM3 method and Polak-Ribire algorithm. After optimizing the ligand energy, hydrogen atoms were added to the structure of the molecule using AutoCAD Tools software. In the next step, non-polar hydrogen atoms are integrated into the corresponding carbon atom, and the electric charge of the atomic electric charge, which is experimentally calculated, and the number of degrees of freedom of the torsional angles of the ligand were calculated using AutoCAD Tools software. Finally, the ligand file was saved as pdbqt. 3D crystal structures of Ebola virus GP and VP40 proteins were downloaded from the protein database. The method of docking and analysis of conformers was based on previous descriptions (Sepehri *et al*., 2015, 2017). At first, water molecules were removed from the crystallographic structure using Notcpat++ software or Discovery Studio Viewer lite 4.0. Then, hydrogen atoms were added to the crystallographic structure using Autodoc Tools software. In the next step, the non-polar hydrogen atoms are integrated into the corresponding carbon atom and the Coleman electric charge and the solvent coverage parameters of the macromolecule are calculated and finally, the macromolecule file is saved as pdbat.

After preparing the required input files for docking (ligand macromolecule and binding map), docking studies were performed to model ligand-receptor interactions, using an algorithm called Lamarckian genetics. In the next step, based on the molecular weight of the designed ligands, a grid with dimensions of 60x60x60 angstroms along the triple axes of coordinates and the distance of the grid points is 0.573 angstroms (a quarter of the length of the simple carbon-carbon bond) which included the active site of the receptor. Was considered. The network file was saved as gpf. The parameters stored in the gpf file are available for Auto Grid calculations. After the docking operation, the results including the molecule conformations, and the types of interactions between the molecule and the protein, including hydrogen, hydrophobic, π-π interactions with the amino acids in the binding pocket of the proteins can be seen and analyzed. To obtain this information, AutoCAD Tools and 4.0 Discovery Studio Viewer lite software were used.

Results and Discussion

Due to the lack of crystallized ligands in the protein, validation of docking calculations in this study was done based on blind-docking. In this method, the whole protein is placed inside the grid box, then the placement and interactions of the ligand in the active site of the protein are compared with the results of docking the same ligand in the reported articles. Docking of the most powerful drug (Amodiaquine) into the proteins was done. By examining its results, the ligand was placed in the active position and the necessary interactions were examined according to the reported research. After several consecutive dockings, the ligand was positioned at the same site and showed similar interactions with the amino acids, so this region appeared to be the protein binding site. After the validation of the docking protocol, the examination of the docking results of these compounds showed that according to the biological results, all the drugs occupy the same space in the receptor binding site with similar binding states. Docking results are given in **Table 2**.

Table 2. Binding free energy of hydrogen bonds, hydrophobic, π-π, and cation-π interactions of drugs in VP40 protein based on molecular docking.

Medicine Name	Binding Free Energy (kilocalories per mole)	Hydrogen bonds	Hydrophobic interactions	π-π interaction
Amlodipine	-6.57	His516, Asn512, Glu106, Trp104	Glu103, Trp104, Ala105, His518, Arg136, Asn514, Asn512, Trp291, Phe290, Leu547, His516, Glu106	-

Amodiaquine	-3.35	Ser316, Leu158	Glu103, Trp104, Tyr213, Tyr214, Arg136, Cys135, His516, Ala105, Phe290, Asn514, Asn512	Tyr214
Biperiden	-5.87	214Arg	Phe161, Leu158, Phe157, Glu155, Arg148, Ala156, Ser316, Lys212, Cys314, Ser319, Leu213, His315, Pro290	-
Carprofen	-7.45	Cys314, Gln155, 214Arg	Ser319, Ser316, Leu158, His315, Leu213, Lys212	-

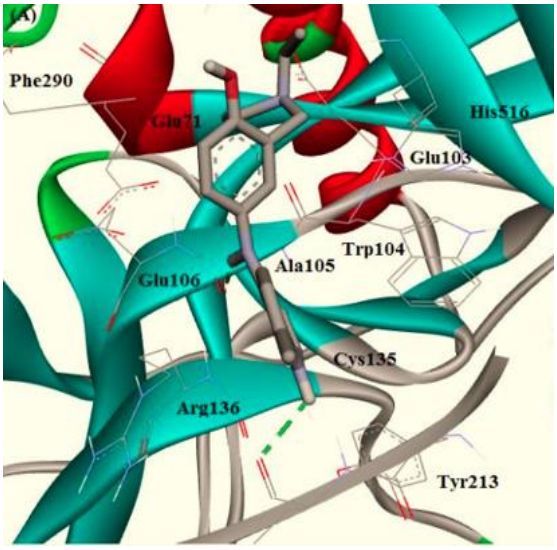
Two important criteria in determining the best-docked state are the highest (most negative) estimated free binding energy and also the most suitable interactions with the main amino acids of the active site of GP and VP40.

In this study, molecular docking simulation between GP and VP40 of twenty-four licensed drugs was performed to achieve the established connections between the drug and the receptor. In this method, the binding energy and docking energy are calculated quantitatively, each is a set of intramolecular energy, intramolecular energy, torsional free energy, and ligand internal energy, respectively (Li *et al*., 2011). The most negative $\Delta G_{binding}$ (free energy of binding), favorable interactions, and tight binding with major amino acids in the GP active site are observed for most active compounds. Among the investigated compounds, Amodiaquine, Diphenoxylate, Ketotifen, and Paroxetine showed the best docking results on the GP receptor, and their binding energy values were reported as -8.37, -9.13, -8.50, and -8.18 kcal/mol, respectively. In a study conducted in 2013 by Madrid *et al*. this drug showed the highest anti-Ebola effect in inhibiting the entry of the Ebola virus into the host cell with an inhibition percentage of 99 (Madrid *et al*., 2013); therefore, the in silico results obtained in this study are consistent with the reported in vitro results. On the other hand, two drugs, Azithromycin, and erythromycin, with binding energy equal to -4.36 and -5.89 kcal/mol, respectively, have shown the lowest binding energy among other drugs. Examining the docking of compounds on VP40, two drugs ketotifen and chloroquinone have the highest binding energy.

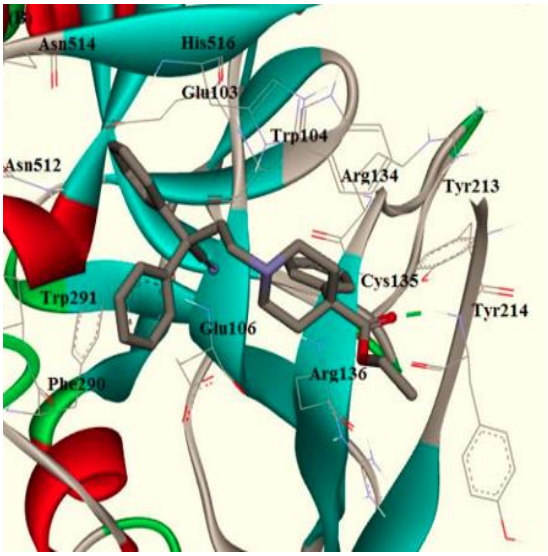
Amodiaquine drug has the main quinoline ring skeleton, which prevents the Ebola virus from entering the host cell at a concentration of 10 micromolars with 99% activity. Molecular docking studies showed that this drug binds to GP protein with hydrogen bonding and hydrophobic interactions. In addition, at the position of carbon number 4 of the quinoline ring, a bulky part is attached, and the end of this part is the amine of the third type. This part forms hydrophobic interactions with amino acids Asn512, Asn514, His516, Glu71, Glu103, Phe290, and Glu106. They also have a chlorine substitution in carbon number 7 of the quinoline ring, which increases the lipophilicity of the compound. The high lipophilicity characteristic of amodiaquine drug (CLogP equal to 5.35) can affect the hydrophobic interactions with the receptor and also, the possibility of acquiring the virus (permeability of the host cell membrane) has an effect. In addition to the mentioned interactions, this drug can create a π-π interaction, which is created between the phenyl ring attached to the 4th position of the quinoline ring and the phenyl ring of Tyr214 amino acid. It should be noted that among these four drugs, only Amodiaquine can interact. The number of rotatable bonds of Amodiaquine is equal to 6. This feature is effective on the physicochemical properties of the drug in oral administration (Veber *et al*., 2002; Refsgaard *et al*., 2005) as well as the spatial orientation of the compound in the protein binding site.

Due to the presence of hydrophobic parts (aromatic rings and aliphatic ring), diphenoxylate drug can form high hydrophobic interactions with the amino acids of the binding pocket and is a completely lipophilic compound with a CLogP equal to 5.33. The amount of lipophilicity of this drug is almost equal to the lipophilicity of Amodiaquine. In addition, this drug has 9 rotatable bonds, which has 3 more rotatable bonds compared to Amodiaquine. According to the above, the highest binding energy among all the compounds in the docking results belongs to the diphenoxylate drug (binding energy equal to -9.13 kcal/mol). Unlike amodiaquine, diphenoxylate has no π-π interaction. One of the important differences between these two drugs is the type of amine (third type amine). In cyclic diphenoxylate amine and chain amine amodiquin. The inflexibility of the tertiary amine in diphenoxylate is probably detrimental to the system, and this can be one of the reasons for the reduced anti-Ebola effect of the compound compared to amodiaquine. Compared to diphenoxylate, ketotiphene has an inflexible structure, so despite similar interactions with the amino acids of the binding site, it shows a weaker binding energy, which is probably due to the inappropriate conformer in the three-dimensional space of the pocket, weakening the interactions.

The results of docking calculations and the anti-Ebola effect of paroxetine have a good correlation with each other and both show good values. Compared to Amodiaquine, paroxetine has an anti-Ebola effect and a weaker binding energy. The reason is probably related to the higher hydrophilicity of paroxetine (CLogP equal to 4.24 vs. 5.35), the increase of hydrogen bonds with the amino acids of the receptor binding site, and the decrease in membrane permeability of this drug. The interactions of amodiaquine and diphenoxylate drugs in the GP protein binding site are shown in **Figure 1**.



a)

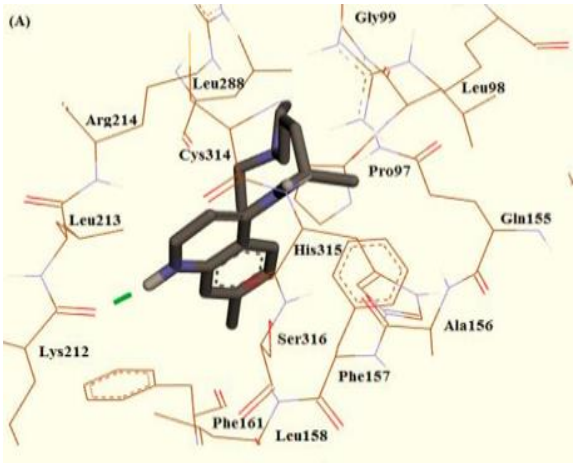


b)

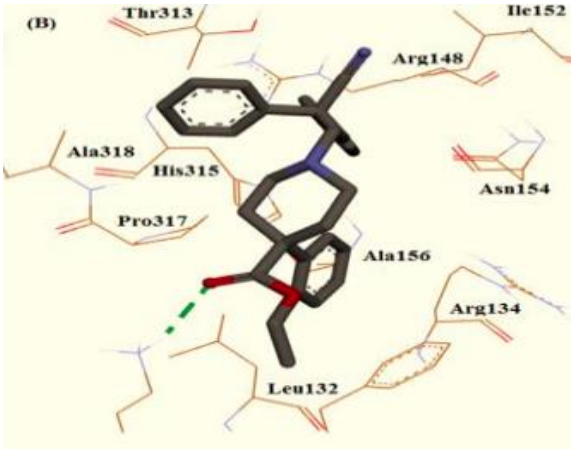
Figure 1. Investigating hydrophobic and hydrogen interactions of amodiaquine (a) and diphenoxylate (b) drugs with amino acids in the GP receptor binding site.

Docking results showed that the main interactions of ketotifen drug are hydrophobic and it only can form a hydrogen bond, that's why it is second in terms of binding energy after diphenoxylate. In addition, this drug has a completely inflexible structure (no rotatable bonds); therefore, due to the absence of rotatable bonds and the presence of cyclic third-type amine, compared to amodiaquine, it shows a weaker anti-Ebola effect.

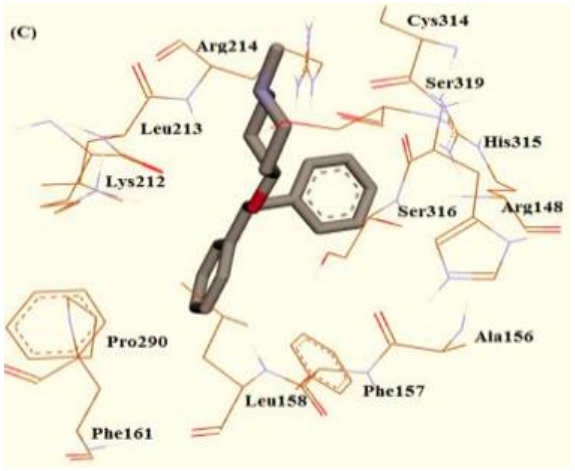
Among the selected drugs, only four drugs, chloroquine, diphenoxylate, diphenylpyraline, and ketotifen, were tested to inhibit Ebola virus proliferation and gave positive results. Other compounds were either not tested or had no effect. Since VP40 protein is one of the most important targets of virion replication. Therefore, the docking of these drugs on this protein was investigated. The investigation of the docking of these four drugs in the VP40 receptor showed that they all show hydrophobic interactions with the hydrophobic cavity of the active site of the protein **(Figure 2)**. Two drugs, chloroquine, and diphenoxylate, in addition to hydrophobic interactions with amino acids of the binding site, form a hydrogen bond with amino acids 212Lys and 127Lys, respectively. The drug diphenylpyraline is placed in the second row, which only shows hydrophobic interactions with amino acids of the active site and lacks hydrogen bonds **(Figure 2, Table 2)**.



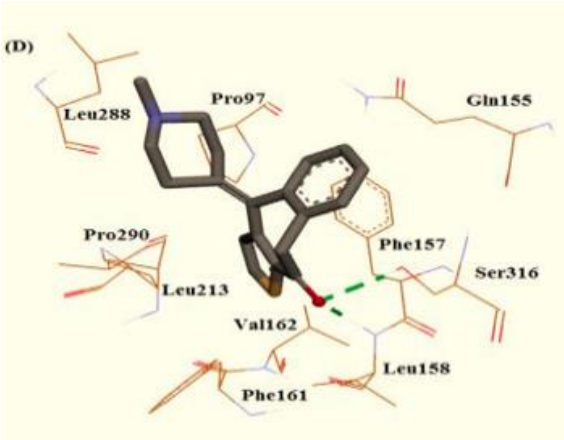
a)



b)



c)



d)

Figure 2. Investigation of the amino acids involved in the formation of hydrophobic and hydrogen bonds (hydrogen bonds are shown with green lines) with the drugs chloroquine (a), diphenoxylate (b), diphenylpyraline (c) and ketotifen (d) in the binding site of the VP40 receptor.

Ketotifen shows the lowest $\Delta_{Gbinding}$ in docking results with the VP40 receptor. Docking studies showed that placing some objections on certain parts of these drugs creates hydrophobic connections or hydrogen bonds between the compound and the receptor, which increases the potency of the compounds. The investigation of the structure-activity relationship (SAR) of these compounds in VP40 protein and GP protein showed that the presence of aromatic rings and hydrophobic parts, tertiary amine, rotatable bonds, and optimal hydrogen bonds increase the anti-Ebola power of the compound. It can be concluded that the formation of optimal hydrogen bonds and high lipophilicity of the compound to increase hydrophobic interactions are important factors in inhibiting the entry and proliferation of the Ebola virus. These compounds can be introduced as a new drug for the treatment of Ebola virus infection.

Conclusion

The purpose of the present study was to investigate the bioinformatic inhibition of Ebola virus entry and proliferation by licensed non-viral drugs. The results obtained from the present study showed that the bonds involved in drug binding with receptors are hydrophobic, π-π, hydrogen bonds, and cation-π. Among all studied compounds, the best docking results were related to chloroquine, diphenoxylate, and amodiaquine drugs. These three drugs with the most negative binding energy level had a greater tendency to bind to the amino acids of the binding site of GP and VP40 proteins. The weakest docking results were related to the two drugs erythromycin and dirithromycin, because the hydrophilicity of these two drugs is very high. In general, the presence of hydrophobic parts, optimal hydrogen bonds, and tertiary amine increases the anti-Ebola potency of drugs. Based on the results obtained from bioinformatics studies, all drugs show good inhibitory effects in the receptor binding site and can be considered effective inhibitors of Ebola virus entry and proliferation.

Acknowledgments: None

Conflict of interest: None

Financial support: None

Ethics statement: None

References

Balmith, M., & Soliman, M. E. (2017). Potential Ebola drug targets–filling the gap: A critical step forward towards the design and discovery of potential drugs. *Biologia*, *72*(1), 1-13.

Balmith, M., Faya, M., & Soliman, M. E. (2017). Ebola virus: A gap in drug design and discovery-experimental and computational perspective. *Chemical Biology & Drug Design*, *89*(3), 297-308.

Bandyopadhyay, B., Verma, N., & Chouhan, U. (2016). In Sillico analysis of newly identified potential drug lead compound against VP40 for the treatment of Ebola virus infection. *International Journal of Advanced Biotechnology and Research*, *7*(4), 1357-1365.

Basu, A., Mills, D. M., Mitchell, D., Ndungo, E., Williams, J. D., Herbert, A. S., Dye, J. M., Moir, D. T., Chandran, K., Patterson, J. L., et al. (2015). Novel small molecule entry inhibitors of Ebola virus. *The Journal of Infectious Diseases*, *212*(suppl_2), S425-S434.

Broni, E., Ashley, C., Adams, J., Manu, H., Aikins, E., Okom, M., Miller III, W. A., Wilson, M. D., & Kwofie, S. K. (2023). Cheminformatics-based study identifies potential Ebola VP40 inhibitors. *International Journal of Molecular Sciences*, *24*(7), 6298. doi:10.3390/ijms24076298

Cui, Q., Cheng, H., Xiong, R., Zhang, G., Du, R., Anantpadma, M., Davey, R. A., & Rong, L. (2018). Identification of diaryl-quinoline compounds as entry inhibitors of Ebola virus. *Viruses*, *10*(12), 678. doi:10.3390/v10120678

Darko, L. K., Broni, E., Amuzu, D. S., Wilson, M. D., Parry, C. S., & Kwofie, S. K. (2021). Computational study on potential novel anti-Ebola virus protein VP35 natural compounds. *Biomedicines*, *9*(12), 1796. doi:10.3390/biomedicines9121796

Dhama, K., Karthik, K., Khandia, R., Chakraborty, S., Munjal, A., Latheef, S. K., Kumar, D., Ramakrishnan, M. A., Malik, Y. S., Singh, R., et al. (2018). Advances in designing and developing vaccines, drugs, and therapies to counter Ebola virus. *Frontiers in Immunology*, *9*, 1803. doi:10.3389/fimmu.2018.01803

Hayat, M., Gao, T., Cao, Y., Rafiq, M., Zhuo, L., & Li, Y. Z. (2024). Identification of prospective Ebola virus VP35 and VP40 protein inhibitors from Myxobacterial natural products. *Biomolecules*, *14*(6), 660. doi:10.3390/biom14060660

Li, F., Li, X., Liu, X., Zhang, L., You, L., Zhao, J., & Wu, H. (2011). Docking and 3D-QSAR studies on the Ah receptor binding affinities of polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans

(PCDFs). *Environmental Toxicology and Pharmacology*, *32*(3), 478-485.

Li, H., Ying, T., Yu, F., Lu, L., & Jiang, S. (2015). Development of therapeutics for treatment of Ebola virus infection. *Microbes and Infection*, *17*(2), 109-117.

Liu, C. H., Hu, Y. T., Wong, S. H., & Lin, L. T. (2022). Therapeutic strategies against Ebola virus infection. *Viruses*, *14*(3), 579. doi:10.3390/v14030579

Madrid, P. B., Chopra, S., Manger, I. D., Gilfillan, L., Keepers, T. R., Shurtleff, A. C., Green, C. E., Iyer, L. V., Dilks, H. H., Davey, R. A., et al. (2013). A systematic screen of FDA-approved drugs for inhibitors of biological threat agents. *PloS one*, *8*(4), e60579.

Mirza, M. U., & Ikram, N. (2016). Integrated computational approach for virtual hit identification against Ebola viral proteins VP35 and VP40. *International Journal of Molecular Sciences*, *17*(11), 1748.

Mohamed, E. A., Abdelwahab, S. F., Alqaisi, A. M., Nasr, A. M. S., & Hassan, H. A. (2022). Identification of promising anti-EBOV inhibitors: De novo drug design, molecular docking and molecular dynamics studies. *Royal Society Open Science*, *9*(9), 220369. doi:10.1098/rsos.220369

Pleško, S., Volk, H., Lukšič, M., & Podlipnik, Č. (2015). In silico study of plant polyphenols' interactions with VP24-Ebola virus membrane-associated protein. *Acta Chimica Slovenica*, *62*(3), 555-564.

Priya, J. V., Prabha, A. S., Rajesh, S. V., Aroulmoji, V., & Gnanendra, T. S. (2015). High throughput screening for identification of potent inhibitors targeting Ebola virus major matrix protein VP40. *International Journal of advanced Science and Engineering*, *1*(4), 10-16.

Raj, U., & Varadwaj, P. K. (2016). Flavonoids as multi-target inhibitors for proteins associated with Ebola virus: In silico discovery using virtual screening and molecular docking studies. *Interdisciplinary Sciences: Computational Life Sciences*, *8*, 132-141.

Refsgaard, H. H., Jensen, B. F., Brockhoff, P. B., Padkjær, S. B., Guldbrandt, M., & Christensen, M. S. (2005). In silico prediction of membrane permeability from calculated molecular parameters. *Journal of Medicinal Chemistry*, *48*(3), 805-811.

Sepehri, S., Gharagani, S., Saghaie, L., Aghasadeghi, M. R., & Fassihi, A. (2015). QSAR and docking studies of some 1, 2, 3, 4-tetrahydropyrimidines: Evaluation of gp41 as possible target for anti-HIV-1 activity. *Medicinal Chemistry Research*, *24*, 1707-1724.

Sepehri, S., Soleymani, S., Zabihollahi, R., Aghasadeghi, M. R., Sadat, M., Saghaie, L., & Fassihi, A. (2017). Synthesis, biological evaluation, and molecular docking studies of novel 4-[4-Arylpyridin-1 (4H)-yl] benzoic acid derivatives as anti-HIV-1 agents. *Chemistry & Biodiversity*, *14*(12), e1700295.

Setlur, A. S., Naik, S. Y., & Skariyachan, S. (2017). Herbal lead as ideal bioactive compounds against probable drug targets of Ebola virus in comparison with known chemical analogue: A computational drug discovery perspective. *Interdisciplinary Sciences: Computational Life Sciences*, *9*, 254-277.

Stefanik, M., Valdes, J. J., Ezebuo, F. C., Haviernik, J., Uzochukwu, I. C., Fojtikova, M., Salat, J., Eyer, L., & Ruzek, D. (2020). FDA-approved drugs efavirenz, tipranavir, and dasabuvir inhibit replication of multiple flaviviruses in vero cells. *Microorganisms*, *8*(4), 599. doi:10.3390/microorganisms8040599

Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, *45*(12), 2615-2623.

Veljkovic, V., Loiseau, P. M., Figadere, B., Glisic, S., Veljkovic, N., Perovic, V. R., Cavanaugh, D. P., & Branch, D. R. (2015). Virtual screen for repurposing approved and experimental drugs for candidate inhibitors of EBOLA virus infection. *F1000Research*, *4*(34), 1-13.

World Health Organization. (2016). Ebola Situation Reports; Available from: http://www.who.int/csr/disease/ebola/situationreports/en/