



**MOLECULAR DOCKING AND MOLECULAR
DYNAMICS STUDY ON POTENTIAL EBOLA MATRIX
PROTEIN VP40 INHIBITORS**

BY

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ABSTRACT

It is well known that Ebola virus (EBOV) causes severe haemorrhagic fever with high fatality rate. The biggest Ebola outbreak in 2014 has caused at least 11,323 deaths with more than 28,000 cases have been reported. Studies have demonstrated that EBOV matrix protein known as VP40 is crucial in the early infection stage to facilitate the transcription of the viral gene through association with ssRNA in specific manner. Two residues namely Phe125 and Arg134 were found to be important in mediating the VP40-RNA interaction. Thus, blocking this VP40-RNA interaction could interrupt EBOV life cycle in the host cell. This study aims to identify and optimise ligands that can potentially block the RNA binding site of VP40. A total of 42 previously studied ligands from literature were simulated against the RNA binding site using AutoDock Vina. The top ten ligands were used as templates for similarity search in ZINC databases using USRCAT followed by structure-based virtual screening at the RNA binding site and molecular dynamics simulation. The ADME properties of the compounds were predicted computationally and the binding free energy of the complex was calculated using molecular-mechanics Poisson Boltzmann surface area (MM-PBSA) method. Our results showed that Q-88 (ZINC ID: 1342431) turned out to be the best-docked compound with binding free energy of -97.27 kJ/mol. However, this compound gave unsatisfactory ADME properties by violating two Ghose's rule and has low GI absorption. Substituting the sulphur (S) atom with oxygen (O) in the backbone structure of Q-88 eliminated the drug-likeness violation and improved GI absorption prediction with similar binding free energy (-97.097 kJ/mol). This finding shed light on binding energies and potential modification of previously tested compounds against Ebola VP40, which could be useful to design more potent and drug-like VP40 inhibitors.

خلاصة البحث

إن من المعروف أن فيروس الإيبولا (EBOV) يسبب الحمى النزفية الحادة وبمعدل وفيات مرتفعة. قد تسبب تفشي فيروس الإيبولا في عام 2014 في وفاة 11323 شخصًا على الأقل، مع بلاغات عن أكثر من 28000 حالة. أظهرت الدراسات أن بروتين مصفوفة فيروس الإيبولا المعروف باسم VP40 بروتين مهم في المرحلة المبكرة للعدوى لتسهيل نسخ الجين الفيروسي من خلال الارتباط مع الحمض النووي الريبوزي ذي الظفيرة الواحدة (ssRNA) بطريقة محددة. يعتبر Phe125 و Arg134 من البقايا المهمة في التوسط في تفاعل مع VP40 و RNA. وبالتالي قد يؤدي وقف تفاعل VP40 و RNA إلى وقف دورة حياة EBOV في الخلية المضيفة. هدفت هذه الدراسة إلى تحديد وتحسين الروابط التي من المحتمل أن تحجب موقع ربط الحمض النووي الريبوزي في VP40. تم محاكاة 42 من الروابط سبق دراستها من المؤلفات ضد موقع ربط الحمض النووي الريبوزي باستخدام برنامج AutoDock Vina. تم استخدام الروابط العشرة الأولى كقوالب للبحث عن المتشابهات في قواعد بيانات ZINC باستخدام USRCAT متبوعة بالفحص الظاهري الهيكلي في موقع ربط RNA ومحاكاة الديناميات الجزيئية. تم التنبؤ بخصائص ADME للمركبات حسابياً وحُسبت طاقة الارتباط الحرة للمجمع باستخدام طريقة مساحة السطح الميكانيكية الجزيئية لبوزون بولتزمان (-MM (PBSA). أظهرت نتائجنا أن Q-88 (ZINC ID: 1342431) كان المركب الأفضل ربطاً بطاقة ارتباط حرة بلغت -97.27 كيلو جول/مول. ومع ذلك فقد أعطى هذا المركب خصائص ADME غير مرضية وذلك بانتهاكه لإثنين من قوانين Ghose وبسبب قيمة امتصاص GI المنخفض. أدى استبدال ذرة الكبريت (S) بالأكسجين (O) في بنية العمود الفقري لـ Q-88 إلى إزالة انتهاك مدى التشابه للأدوية وتحسين التنبؤ بامتصاص GI مع تقارب ربط مماثل (-97.097 كيلو جول/مول). تلقي هذه النتيجة الضوء على طاقات الربط والتعديل المحتمل للمركبات التي تم اختبارها سابقاً ضد VP40 الخاص بالإيبولا، والتي قد تكون مفيدة لتصميم مثبطات VP40 أكثر فاعلية وبتشابه أكبر للأدوية.

APPROVAL PAGE

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TABLE OF CONTENTS

| | |
|---|-----------|
| Abstract | ii |
| Abstract in Arabic | iii |
| Approval page | iv |
| Declaration | v |
| Copyright | vi |
| Acknowledgements | vii |
| List of Tables | x |
| List of Figures | xi |
| List of Symbols/units | xiii |
| List of Abbreviations | xiv |
| CHAPTER ONE: INTRODUCTION | 1 |
| 1.1 Background of The Study | 1 |
| 1.2 Problem Statement | 3 |
| 1.3 Objectives | 4 |
| 1.4 Hypotheses | 5 |
| 1.5 Significance of The Study | 5 |
| CHAPTER TWO: LITERATURE REVIEW | 7 |
| 2.1 Ebola Virus Disease and Its Pandemic | 7 |
| 2.2 EVD Treatment and Therapy | 9 |
| 2.3 Ebola Virus Life Cycle | 11 |
| 2.3.1 Virus Entry and Uptake | 12 |
| 2.3.2 Transcription and Replication | 12 |
| 2.3.3 Virion Assembly | 14 |
| 2.3.4 Viral Budding | 15 |
| 2.4 VP40 Structures | 15 |
| 2.5 VP40 Roles and Functions in Viral Life Cycle | 18 |
| 2.5.1 Viral Component's Assembly and Viral Budding | 18 |
| 2.5.2 Viral Transcription Regulation | 20 |
| 2.5.3 Cell Immune Evasion | 22 |
| 2.6 VP40 As Potential Drug Target | 23 |
| 2.7 Previously Studied Ligands As Potential VP40-RNA Inhibitors | 26 |
| 2.8 Computer-Aided Drug Design in Drug Discovery | 29 |
| CHAPTER THREE: METHODOLOGY | 32 |
| 3.1 Introduction | 32 |
| 3.1.1 Virtual Screening | 32 |
| 3.1.1.1 Molecular Docking | 32 |
| 3.1.1.2 Similarity Search Screening | 34 |
| 3.1.2 Molecular Dynamics (MD) Simulation | 35 |
| 3.2 Computer Specifications And Study Overview | 36 |
| 3.3 Receptor Preparation | 37 |

| | |
|---|------------|
| 3.4 Virtual Screening | 38 |
| 3.4.1 First Stage of Structure-based Virtual Screening | 38 |
| 3.4.2 Ligand-based Virtual Screening | 39 |
| 3.4.3 Second Stage of Structure-based Virtual Screening | 40 |
| 3.5 Ligands ADME Analysis | 40 |
| 3.6 MD Simulation of Receptor-Ligands Complex | 41 |
| 3.6.1 System Topology Preparation | 41 |
| 3.6.2 Simulation Box Setup, System Solvation and Neutralisation | 42 |
| 3.6.3 System Energy Minimisation | 42 |
| 3.6.4 System Equilibration | 42 |
| 3.6.5 Molecular Dynamics Production | 43 |
| 3.7 Simulation Analysis | 43 |
| CHAPTER FOUR: RESULTS AND DISCUSSION | 45 |
| 4.1 Virtual Screening and Molecular Docking | 45 |
| 4.1.1 First Molecular Docking Stage | 45 |
| 4.1.2 Second Molecular Docking Stage | 47 |
| 4.2 Ligands ADME Analysis | 50 |
| 4.2.1 Physicochemical properties | 51 |
| 4.2.2 Lipophilicity | 52 |
| 4.2.3 Water Solubility | 54 |
| 4.2.4 Pharmacokinetics | 56 |
| 4.2.5 Drug-likeness | 58 |
| 4.2.6 Medicinal Chemistry | 60 |
| 4.3 Molecular Dynamics Simulation of VP40-Ligand Complex | 62 |
| 4.3.1 MD Simulation of The Piperidine-based Scaffold Group | 62 |
| 4.3.1.1 RMSD and RMSF Analysis | 62 |
| 4.3.1.2 Ligand Clustering Analysis | 64 |
| 4.3.1.3 Binding Free Energy Analysis | 67 |
| 4.3.2 MD Simulation of The Pyridine-based Scaffold Group | 68 |
| 4.3.2.1 RMSD and RMSF Analysis | 68 |
| 4.3.2.2 Ligand Clustering Analysis | 70 |
| 4.3.2.3 Binding Free Energy Analysis | 74 |
| 4.3.3 S Atom Modification | 77 |
| 4.3.4 Energy Contribution of Residues to Binding | 80 |
| CHAPTER FIVE: CONCLUSION | 82 |
| REFERENCES | 84 |
| APPENDIX A: 42 PREVIOUSLY STUDIED LIGANDS IN LITERATURE | 100 |
| APPENDIX B: ENERGY MINIMISATION PARAMETERS | 109 |
| APPENDIX C: POSITION RESTRAINT/EQUILIBRATION PARAMETERS | 110 |
| APPENDIX D: MD SIMULATION PARAMETERS | 111 |
| APPENDIX E: L-38 CLUSTERING RESULT | 112 |
| APPENDIX F: ADME PROPERTIES PREDICTION FOR Q-88-O | 113 |

LIST OF TABLES

| | | |
|------------|--|----|
| Table 2.1 | VP40 Regions That Could Be Targeted for Drug Development. | 26 |
| Table 4.1 | Top Docking Result of Previously Reported Compounds. | 46 |
| Table 4.2 | Top Compounds from Virtual Screening and Molecular Docking Process. | 48 |
| Table 4.3 | Physicochemical Properties of the Top Ten Compounds and L-38. | 51 |
| Table 4.4 | Lipophilicity of the Top Ten Compounds and L-38. | 53 |
| Table 4.5 | Water Solubility of the Top Ten Compounds and L-38. | 54 |
| Table 4.6 | Pharmacokinetics Properties of the Top Ten Compounds and L-38. | 56 |
| Table 4.7 | Drug-likeness Violations and Bioavailability Score for Each Compound. | 59 |
| Table 4.8 | Medicinal Chemistry Evaluation for Each Ligand. | 60 |
| Table 4.9 | Average Binding Free Energy for Piperidine-based Ligands and L-38 in the Last 10 ns of the Simulation. | 67 |
| Table 4.10 | Average Binding Free Energy for Pyridine-based Ligands and L-38 in the Last 10 ns of the Simulation. | 74 |
| Table 4.11 | Average Binding Free Energy for Ligand Q-88-O and Q-88-C in the Last 10 ns of the Simulation. | 78 |

LIST OF FIGURES

| | | |
|------------|--|----|
| Figure 1.1 | Structure of Ebola Virus with Its Encoded Protein. | 2 |
| Figure 2.1 | Distribution of the Ebola Virus in Africa from Its Discovery in 1976 till the Largest Outbreak That Ended in 2016. | 9 |
| Figure 2.2 | An Overview of EBOV Life Cycle. | 11 |
| Figure 2.3 | VP40 Sequence. | 15 |
| Figure 2.4 | Different Form of VP40. | 16 |
| Figure 2.5 | VP40 Dimer with Its Cationic Patch and Hydrophobic Loop Residues. | 17 |
| Figure 2.6 | Truncated VP40 Monomer Structure with Its Important Residues That Involve in RNA Binding Activity. | 21 |
| Figure 2.7 | VP40 Domain Organization. | 25 |
| Figure 2.8 | Chemical Structure of Gossypetin and Taxifolin. | 27 |
| Figure 2.9 | Chemical Structure of Emodin-8-beta-D-glucoside | 28 |
| Figure 3.1 | An Example of an Equation Used to Approximate the Atomic Forces That Govern Molecular Movement. | 35 |
| Figure 3.2 | Project Workflow. | 37 |
| Figure 3.3 | The Grid Box for Molecular Docking Simulation. | 39 |
| Figure 3.4 | Criteria Used to Screen Ligands Based on Each Drug-likeness Rule. | 41 |
| Figure 4.1 | The Orientation of Ligands at the Binding Site with Its Interacting Residues. | 47 |
| Figure 4.2 | Four Compounds with Scaffold Containing Benzene, Oxadiazole, Piperidine and Carboxamide Chain. | 49 |
| Figure 4.3 | Six Compounds and L-38 with Scaffold Containing Benzene Rings, Triazole/Oxadiazole and Pyridine. | 50 |
| Figure 4.4 | RMSD of α -carbon for Piperidine-based Scaffold Group and L-38 throughout 50 ns Simulation. | 63 |

| | | |
|-------------|---|----|
| Figure 4.5 | RMSF of α -carbon Residues for Piperidine-based Scaffold Group and L-38 throughout 50 ns Simulation. | 64 |
| Figure 4.6 | Comparison of Ligand Orientation between the Docking Position (t = 0 ns) and the Last 10 ns Cluster of the Simulation. | 66 |
| Figure 4.7 | Comparison of Average Binding Free Energy for L-38, Q-94, Q-97, Q-98 and Q-99 throughout Last 10 ns of the Simulation. | 68 |
| Figure 4.8 | RMSD of α -carbon for Pyridine-based Scaffold Group and L-38 throughout 50 ns Simulation. | 69 |
| Figure 4.9 | RMSF of α -carbon Residues for Pyridine-based Scaffold Group and L-38 throughout 50 ns Simulation. | 70 |
| Figure 4.10 | Comparison of Ligand Orientation between the Docking Position (t = 0 ns) and the Last 10 ns Cluster of the Simulation. | 71 |
| Figure 4.11 | Comparison of Ligand Orientation between the Docking Position (t = 0 ns) and the Last 10 ns Cluster of the Simulation. | 73 |
| Figure 4.12 | Comparison of Average Binding Free Energy for L-38, Q-82, Q-83, Q-88, Q-93, Q-96 and Q-100 throughout Last 10 ns of the Simulation. | 75 |
| Figure 4.13 | Chemical Structure of Ligand L-38 (Left) and Q-88 (Right). | 77 |
| Figure 4.14 | Illustration of S Atom Substitution to O and C Atom in the Q-88 Backbone Structure. | 78 |
| Figure 4.15 | Comparison of Average Binding Free Energy for Q-88, Q-88-O and Q-88-C throughout Last 10 ns of the Simulation. | 79 |
| Figure 4.16 | Ligand Position after Simulation (Clustering Result in the Last 10 ns) for A) Q-88-O (Left) and B) Q-88-C (Right). | 80 |
| Figure 4.17 | Binding Free Energy of Each VP40 Residue for Q-88, Q-88-O and Q-88-C. | 81 |
| Figure 5.1 | Proposed Chemical Structure Backbone for Further Chemical Modification and Optimisation. | 83 |

LIST OF SYMBOLS/UNITS

| | |
|----------|-------------|
| Å | angstrom |
| ns | nanosecond |
| t | time |
| α | alpha |
| β | beta |
| kcal | kilocalorie |
| kJ | kilojoule |
| mol | mole |

LIST OF ABBREVIATIONS

| | |
|------------------|--|
| EBOV | Ebola virus |
| EVD | Ebola virus disease |
| RNA | Ribonucleic acid |
| WHO | World Health Organization |
| VP30 | Viral protein 30 |
| VP35 | Viral protein 35 |
| VP24 | Viral protein 24 |
| VP40 | Viral protein 40 |
| NC | Nucleocapsid |
| NP | Nucleoprotein |
| GP | Glycoprotein |
| L | L polymerase |
| VLPs | Virus like particles |
| PIP ₂ | Phosphatidylinositol 4,5-bisphosphate |
| PS | Phosphatidylserine |
| FDA | Food and Drug Administration |
| ADME | Absorption, distribution, metabolism, excretion |
| ADMET | Absorption, distribution, metabolism, excretion, toxicity |
| MD | Molecular dynamics |
| SUDV | Sudan virus |
| BDBV | Bundibugyo virus |
| RESTV | Reston virus |
| TAFV | Tai Forest virus |
| DRC | Democratic Republic of Congo |
| USA | United States of America |
| ssRNA | Single stranded ribonucleic acid |
| RNP | Ribonucleoprotein |
| ORF | Open reading frame |
| ESCRT | Endosomal Sorting Complex Required for Transport |
| NEDD4 | Neural precursor cell expressed developmentally down-regulated protein 4 |
| TSG101 | Tumour susceptibility gene 101 |
| MVB | Multivesicular body trafficking |
| SRSs | Suppressor of RNA silencing |
| miRNA | microRNA |
| RNAi | RNA interference |
| TRBP | Transactivation response element RNA-binding protein |
| PACT | Protein activator of the interferon-induced protein kinase |
| HIV | Human immunodeficiency virus |
| HIV-GAG | HIV-group specific antigen |
| TCM | Traditional Chinese Medicine |
| CADD | Computer-aided drug design |

| | |
|---------|--|
| PDB | Protein Data Bank |
| 3D | Three dimensional |
| QSAR | Qualitative structure-activity relationship |
| 2D | Two dimensional |
| USR-VS | Ultrafast Shape Recognition – Virtual Screening |
| USRCAT | Ultrafast Shape Recognition with CREDO Atom Types |
| WDI | World Drug Index |
| ATB | Automated Topology Builder |
| GROMACS | Groningen Machine for Chemical Simulation |
| SPC/E | Extended simple point charge |
| MM-PBSA | Molecular mechanics Poisson-Boltzmann surface area |
| PSA | Polar surface area |
| GI | Gastrointestinal |
| BBB | Blood brain barrier |
| P-gp | Permeability glycoprotein |
| CNS | Central nervous system |
| PAINS | Pan assay interference compounds |
| RMSD | Root mean square deviation |
| RMSF | Root mean square fluctuation |
| Ala | Alanine |
| Arg | Arginine |
| Asn | Asparagine |
| Gln | Glutamine |
| Gly | Glycine |
| His | Histidine |
| Ile | Isoleucine |
| Leu | Leucine |
| Lys | Lysine |
| Phe | Phenylalanine |
| Pro | Proline |
| Thr | Threonine |
| Tyr | Tyrosine |

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Ebola virus disease (EVD) is a disease that caused by the Ebola virus (EBOV). It is formerly known as Ebola haemorrhagic fever, a severe disease that was first appeared in 1976 in two concurrent outbreaks; one was in Nzara, South Sudan and the other one was in Yambuku, Democratic Republic of Congo (WHO, 1976). EVD is transmitted to people from wild animals and then spreads in the human population through human-to-human transmission. In 2014, the disease appeared in West Africa and is known as the largest and most complex Ebola outbreak compared to the previous outbreak. During that time, EVD has been spread between countries including Liberia, Nigeria and USA (WHO, 2017).

According to Feldmann & Geisbert (2011), EBOV is a lipid-enveloped filamentous virus that affects human and non-human primates causing severe haemorrhagic fever with the fatality rate up to 90%. EBOV harbours a negative-sense RNA genome that encodes seven proteins namely nucleoprotein (NP), viral protein 30 (VP30), viral protein 35 (VP35), L-polymerase (L) protein, viral protein 24 (VP24), viral protein 40 (VP40) and transmembrane glycoprotein (GP) (Figure 1.1). NP, VP30, VP35 and L protein constitute the nucleocapsid (NC) while GP is accounted for the entry of virions into the host cell. Meanwhile, VP24 is a minor protein which is also crucial for the assembly of NC and VP40 is the viral matrix protein that regulates viral budding and NC recruitment, as well as virus structure and stability (Booth, Rabb, & Beniac, 2013; Olejnik, Ryabchikova, Corley, & Mühlberger, 2011).

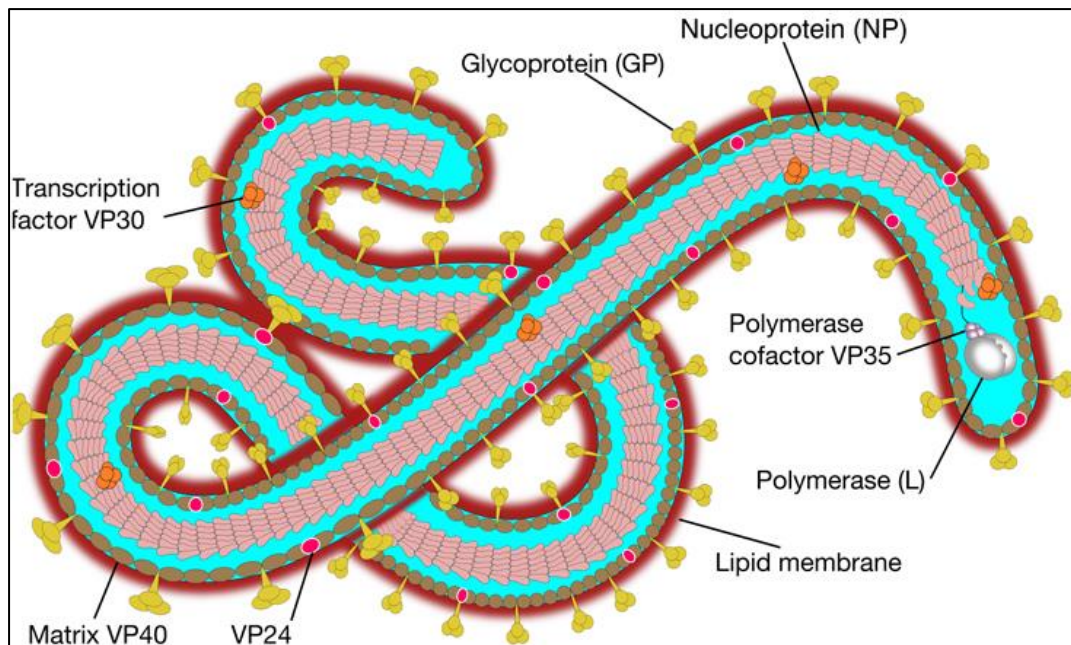


Figure 1.1 Structure of Ebola Virus with Its Encoded Protein. Adapted from SIB SWISS Institute of Bioinformatics (2014).

Jasenosky et al. (2001) stated the importance of VP40 in EBOV which appears equivalent to matrix proteins of other viruses that are able to induce virus-like particles (VLPs) formation. They also found that VP40 is able to bind to cellular membranes in a hydrophobic manner. Another study by Dessen et al. (2000) showed that the X-ray crystal structure of VP40 from EBOV at 2.0 Å resolution consisted of two domains with unique folds. The C-terminal domain has large hydrophobic patches that may involve in the interaction with lipid bilayers for membrane binding while N-terminal domain consists of six antiparallel β -strands and important for VP40 oligomerisation.

Many studies have been carried out to understand more about the interaction of VP40 with the plasma membrane. VP40 has been reported to be associated with negatively charged lipids such as phosphatidylinositol 4,5-bisphosphate (PIP₂) and phosphatidylserine (PS) (Adu-Gyamfi et al., 2015; K. A. Johnson et al., 2016). It has

been hypothesised that the VP40 dimers undergo major structural rearrangement once it interacts with the negatively charged lipids at the plasma membrane and oligomerise into hexameric structures (Bornholdt et al., 2013; Soni et al., 2013). Recent computational study showed that VP40 interact with the negatively charged membrane via two flexible loops that consist of several lysine residues (GC, Gerstman, & Chapagain, 2017).

Although VP40 is important for membrane interaction, this protein is also shown to be responsible for viral replication by involving in RNA binding through the N-terminal domain. Two amino acid residues namely, Arg134 and Phe125 were shown to be the important residues involved in the RNA-binding (Gomis-Rüth et al., 2003). Mutation on these residues did affect the RNA-binding process (Thomas Hoenen et al., 2005). Bornholdt et al. (2013) stated that VP40-RNA binding is an important process to facilitate the transcription of the viral gene in the early infection stage. These studies implied that VP40 plays a vital role in Ebola virus life cycle, thus this protein will be a good target for the drug development to treat the disease.

1.2 PROBLEM STATEMENT

To date, there is no FDA-licensed drug is currently available to solve EVD and most of the potential drugs to treat EVD are yet to be used in human clinical trials (Yuan, 2015). Currently, a trial vaccine called rVSV-ZEBOV is being used to control the Ebola virus outbreak in DRC as the vaccine showed effectiveness in protecting people from EVD during a trial in Guinea in 2015 (Henao-Restrepo et al., 2015). Besides that, many virtual screening studies has been carried out to identify potential lead ligands to block the RNA-binding site on VP40 (Abazari et al., 2015; Karthick et al., 2016; M Alam El-Din et al., 2016; Mirza & Ikram, 2016; Raj & Varadwaj, 2016;

Shah et al., 2015), however, none of the potential ligands has been tested or validated in experiment study. Therefore, there is room for improvement in the optimisation of the potential ligands through computational approach for better binding at the inhibition site. Computer-aided drug design has become a valuable approach in identifying potential compounds for the drug development (Katsila et al., 2016). For example, in the quest of identifying a small-molecule inhibitor for the TGF β -1 receptor kinase, two different research groups used different approach where one at the Eli Lilly used conventional lab approach while another group at Biogen Idec applied the *in silico* method. The *in silico* approach turned out to produce similar result of potential compound as being found experimentally in the lab (Sawyer et al., 2003; Shekhar, 2008; Singh et al., 2003). Besides that, several drugs have been successfully discovered and optimised using computational approach such as Captopril, Dorzolamide and Saquinavir (Talele, Khedkar, & Rigby, 2010). Therefore, this study focused on improving existing ligands' binding at the VP40-RNA binding site via molecular docking and molecular dynamics simulations approach and to assess its properties for developing it further as Ebola VP40-RNA binding site inhibitor.

1.3 OBJECTIVES

This study was carried out to help in finding potential Ebola inhibitors by targeting the VP40-RNA binding site. Three objectives were outlined for this study which are:

- To identify potential ligands that can inhibit the VP40-RNA binding site.
- To predict the ADME (Absorption, distribution, metabolism, excretion) properties of the potential ligands.

- To investigate ligands' binding affinity at the binding sites through molecular dynamics (MD) simulation.

1.4 HYPOTHESES

To achieve the study's objectives, the research was conducted based on several hypotheses. Potential new ligands that can block the VP40-RNA binding site can be identified and optimised based on the chemical structure or scaffold of the previously studied ligands through similarity search in database and virtual screening approach. Meanwhile, the ADME properties of the potential ligands can be predicted using SwissADME, a free web tool to evaluate pharmacokinetics and drug-likeness of small molecules. Binding affinity of the ligands at the binding site can be further monitored using MD simulation tools by analysing the RMSD, RMSF, ligand clustering and binding free energy.

1.5 SIGNIFICANCE OF THE STUDY

Ever since the 2014-2016 outbreak, the calling for further research on understanding EBOV life cycle is more urgent than before. Even though the outbreaks were mainly occurred in West Africa, current mobility in term of transportation might spread the disease further from its origin (Gonzalez, Wauquier, & Vincent, 2018). The situation can be worsened in the future due to the increase in human population which eases the transmission of disease through human to human transmission. Several imported cases had occurred in the past to several countries like Russia, Nigeria, Senegal and Spain due to the travelling from the outbreak origin (Weyer et al., 2015). Since there is no approved drug or vaccine to treat the diseases till recent time, contributions on

understanding Ebola virus as well as developing potential drugs for the disease are highly pressing for safety measure purposes in future.

CHAPTER TWO

LITERATURE REVIEW

2.1 EBOLA VIRUS DISEASE AND ITS PANDEMIC

Ebola virus disease or EVD has existed for more than 40 years concentrated in the African countries. EVD is caused by Ebola virus, which is a filamentous virus belonging to Filoviridae family together with Marburg virus and Cueva virus (Kuhn et al., 2012). To date, five different species of Ebola virus have been identified namely Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Taï Forest ebolavirus (TAFV), Reston ebolavirus (RESTV) and Bundibugyo ebolavirus (BDBV). As reviewed by Weyer et al. (2015), the highest fatality rate of the disease has been associated with EBOV (90% fatality rate) followed by SUDV with the average fatality rate of 50%. BDBV is the most recent Ebola virus species that was firstly emerged in 2007 in Uganda with an average fatality rate of 30%. RESTV is the only species of the virus that was discovered outside African continents and is considered to be non-pathogenic to human. Meanwhile, the TAFV which formerly known as Côte-d'Ivoire ebolavirus has caused only one non-fatal infection in human in 1994 but very deadly in chimpanzees. Among the Ebola virus species, EBOV is the most studied species due to its high fatality rate and occurrence.

EVD, formerly known as Ebola haemorrhagic fever was firstly appeared in year 1976-1979 period where the first outbreak occurred in Sudan, near the border with the Democratic Republic of Congo (DRC) by SUDV between June and November 1976. That particular outbreak killed 150 out of 284 victims (53% fatality rate) (WHO, 1976). The second outbreak occurred in DRC between August and

November 1976 that caused 284 deaths out of 318 victims (89% fatality rate) (Breman et al., 1978). The third outbreak emerged in 1979 between July and October in Sudan with the fatality rate of 65% (Heymann et al., 1980). After 15 years of silence, EVD re-emerged in Taï National Park, Ivory Coast in 1994 that led to the discovery of TAFV that caused massive deaths among chimpanzees (Formenty et al., 1999). Since then, EVD cases have been reported almost annually leading up to its biggest outbreak in 2014 in West Africa (Weyer et al., 2015). Prior to the 2014 outbreak, EVD outbreaks occurred randomly and rarely appeared in the same location where most affected countries were in the Central Africa namely Congo, DRC, Sudan, Gabon and Uganda (Figure 2.1).

Ebola outbreak in 2014 was caused by Zaire species that affected three countries; Guinea, Liberia and Sierra Leone. In March 2014, World Health Organization (WHO) declared the emergence of the outbreak in Guinea which could be the biggest and fatal outbreak in Ebola history (WHO, 2014). Throughout two years, 28,646 EVD cases were reported in the three countries, with 11,323 deaths. Besides that, several travel-associated cases had been reported during that time in Nigeria, USA and Spain but only with small casualty (WHO, 2016). The 2014-2016 Ebola outbreaks occurred in major urban areas while previous Ebola outbreaks were occurred in rural and secluded areas. In August 2018, an EVD outbreak was declared by the Ministry of Health of the Democratic Republic of the Congo that took place in North Kivu Province. By March 2019, almost 1000 cases were confirmed with 621 deaths (WHO, 2019).

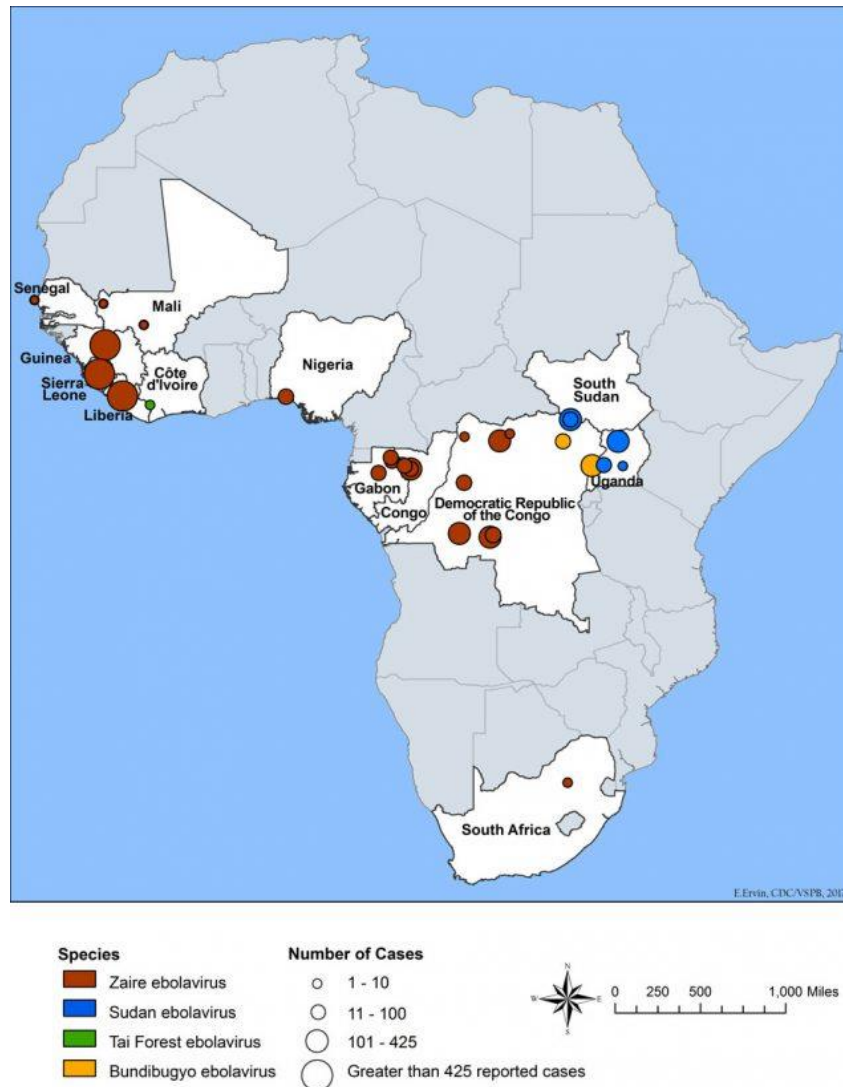


Figure 2.1 Distribution of the Ebola Virus in Africa from Its Discovery in 1976 till the Largest Outbreak That Ended in 2016. Most of the Outbreaks Were Associated with Zaire and Sudan Species.

Adapted from <https://www.cdc.gov/vhf/ebola/history/distribution-map.html>

2.2 EVD TREATMENT AND THERAPY

Currently, there are no approved treatments or medications for EVD around the world. Current treatment of EVD includes the administration of supportive care and treatment strategies like rehydration with intravenous or oral fluids (Geenen et al., 2014). However, due to the severity of the 2014-2016 Ebola outbreak, WHO has allowed several experimental medicines and vaccines to be used to combat the