



HAL
open science

In silico and in vitro screening of licensed antimalarial drugs for repurposing as inhibitors of hepatitis E virus

Borris Rosnay Tietcheu Galani, Vincent Brice Ayissi Owona, Romeo Joel Guemmogne Temdie, Karoline Metzger, Marie Atsama Amougou, Pascal Dieudonné Djamen Chuisseu, Arnaud Fondjo Kouam, Marceline Ngounoue Djuidje, Cécile-Marie Aliouat-Denis, Laurence Cocquerel, et al.

► To cite this version:

Borris Rosnay Tietcheu Galani, Vincent Brice Ayissi Owona, Romeo Joel Guemmogne Temdie, Karoline Metzger, Marie Atsama Amougou, et al.. In silico and in vitro screening of licensed antimalarial drugs for repurposing as inhibitors of hepatitis E virus. In *Silico Pharmacology*, 2021, 9 (1), pp.35. 10.1007/s40203-021-00093-y . hal-03327736

HAL Id: hal-03327736

<https://hal.science/hal-03327736>

Submitted on 26 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

[Click here to view linked References](#)

Original Article

***In silico and in vitro* Screening of Licensed Antimalarial Drugs for Repurposing as Inhibitors of Hepatitis E Virus**

Borris Rosnay TIETCHEU GALANI^{1,2*}, Vincent Brice AYISSI OWONA², Romeo Joel GUEMMOGNE TEMDIE³,
Karoline METZGER⁷, Marie ATSAMA AMOUGOU^{2,4}, Pascal Dieudonné DJAMEN CHUISSEU⁵, Arnaud FONDJO
KOUAM^{2,6}, Marcelline NGOUNOUE DJUIDJE², Cécile-Marie ALIOUAT-DENIS⁷, Laurence COCQUEREL⁷ and Paul
FEWOU MOUNDIPA²

¹Laboratory of Applied Biochemistry, Department of Biological Sciences, Faculty of Science, University of
Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon

²Laboratory of Pharmacology and Toxicology, Department of Biochemistry, Faculty of Science, University of Yaounde I,
P.O. Box 812 Yaounde, Cameroon

³Laboratory of Medicinal Plants, Health, and Galenic Formulation, Department of Biological Sciences, Faculty of
Science, University of Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon

⁴Virology Unit, Research Center for Emerging and Reemerging infectious Diseases (CREMER-IMPM), Yaounde, P.O.
Box 906 Yaounde, Cameroon.

⁵Department of Medicine, Medical and Biomedical Sciences, Higher Institute of Health Sciences, Université des
Montagnes, P.O. Box 208 Bangangte, Cameroon

⁶Department of Biomedical Sciences, Faculty of Health Sciences, University of Buea, P.O. Box 63 South West Region,
Cameroon

⁷University of Lille, CNRS, INSERM, CHU Lille, Pasteur Institute of Lille, U1019-UMR9017-CIIL-Center for
Infection and Immunity of Lille, F-59000 Lille, France

Correspondence should be addressed to Borris Rosnay Tietcheu Galani, Laboratory of Applied Biochemistry,
Department of Biological Sciences, Faculty of Science, University of Ngaoundere, P.O. Box 454 Ngaoundere,
Cameroon; Email: b.tietcheu@gmail.com, borris.galani@univ-ndere/cm

Orcid: <https://orcid.org/0000-0002-4324-5951>

Acknowledgements

We are thankful to Dr. Karin Seron from the Institute Pasteur of Lille for the critical review of this manuscript. This
research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

[Click here to view linked References](#)

Manuscript title : *In silico and in vitro* Screening of Licensed Antimalarial Drugs for Repurposing as Inhibitors of Hepatitis E Virus

Abstract

Hepatitis E virus (HEV) infection is emerging in Cameroon and represents one of the most common causes of acute hepatitis and jaundice. Moreover, earlier reports showed evidence of falciparum malaria/HEV coexistence. Although the Sofosbuvir/Ribavirin combination was recently proposed in the treatment of HEV-infected patients, no specific antiviral drug has been approved so far, thereby urging the search for new therapies. Fortunately, drug repurposing offers a good alternative to this end. In this study, we report the *in silico* and *in vitro* activities of 8 licensed antimalarial drugs and two anti-hepatitis C virus agents used as references (Sofosbuvir, and Ribavirin), for repurposing as antiviral inhibitors against HEV. Compounds were docked against five HEV-specific targets including the Zinc-binding non-structural protein (6NU9), RNA-dependent RNA polymerase (RdRp), cryoEM structure of HEV VLP, genotype 1 (6LAT), capsid protein ORF-2, genotype 3 (2ZTN), and the E2s domain of genotype 1 (3GGQ) using the iGEMDOCK software and their pharmacokinetic profiles and toxicities were predicted using ADMETlab2.0. Their *in vitro* effects were also assessed on a gt 3 p6Gluc replicon system using the luciferase reporter assay. The docking results showed that Sofosbuvir had the best binding affinities with 6NU9 (-98.22 kcal/mol), RdRp (-113.86 kcal/mol), 2ZTN (-106.96 kcal/mol), while Ribavirin better collided with 6LAT (-99.33 kcal/mol). Interestingly, Lumefantrine showed the best affinity with 3GGQ (-106.05 kcal/mol). N-desethylamodiaquine and Amodiaquine presented higher binding scores with 6NU9 (-93.5 and -89.9 kcal/mol respectively vs -80.83 kcal/mol), while Lumefantrine had the greatest energies with RdRp (-102 vs -84.58), and Pyrimethamine and N-desethylamodiaquine had stronger affinities with 2ZTN compared to Ribavirin (-105.17 and -102.65 kcal/mol vs -96.04 kcal/mol). The biological screening demonstrated a significant ($P < 0.001$) antiviral effect on replication with 1 μ M N-desethylamodiaquine, the major metabolite of Amodiaquine. However, Lumefantrine showed no effect at the tested concentrations (1, 5, and 10 μ M). The biocomputational analysis of the pharmacokinetic profile of both drugs revealed a low permeability of Lumefantrine and a specific inactivation by CYP3A2 which might partly contribute to the short half-time of this drug. In conclusion, Amodiaquine and Lumefantrine may be good antimalarial drug candidates for repurposing against HEV. Further *in vitro* and *in vivo* experiments are necessary to validate these predictions.

Keywords : Hepatitis E, HEV, Virtual screening, *in vitro* screening, Antimalarial drugs

1 Introduction

Hepatitis E virus (HEV) infection is regarded as a leading cause of acute hepatitis and jaundice in the world. According to previous estimates, over 20 million infections are recorded annually, with about 3.3 million symptomatic cases (Rein et al. 2012). In 2015, the World Health Organization (WHO), reported about 44000 deaths due to HEV which represents 3.3% of the mortality attributable to all forms of viral hepatitis (WHO 2020). Other studies indicated a higher annual incidence, with 56600 (Lozano et al. 2012) and even 70000 deaths/year (Navaneethan et al. 2008).

HEV is a quasi-enveloped positive-sense RNA virus, member of the *Hepeviridae* family within the *Orthohepevirus* genus. Its genome is made of a singled-strand RNA of about 7.2 kilobases in length which possess three major and conserved open reading frames (ORFs). The ORF-1 encodes a non-structural polyprotein exerting methyltransferase, papain-like cysteine proteases, helicase, and RNA-dependent RNA-polymerase (RdRp) activities needed for the viral replication. ORF-2 encodes the viral capsid protein, and ORF-3, a small phosphoprotein palmitoylated (Gouttenoire et al. 2018) of ~13kDa involved in virion morphogenesis and release (Kenney and Meng 2019). Recently, a novel ORF-4, positioned within the ORF-1 sequence, has been identified in genotype (gt) 1 HEV strains only, and ORF-4 protein was found to stimulate the viral polymerase activity (Nair et al. 2016). To date, 8 genotypes at least have been described (Nimgaonkar et al. 2018), of which gt 1 and 2 known to only infect humans (Smith et al. 2014), while gt 3 and gt 4 fewer pathogens, are zoonotic and can both infect animals and humans (Doceul et al. 2016).

In developing countries, hepatitis E occurs as large epidemics due to poor sanitation, and pregnant women in this context are associated with high mortality rates (about 33%) (Donnelly et al. 2017). Moreover, some sporadic cases of coinfection with malaria have been reported (Aslam 2017; Turner and Ch'ng 2008). In Cameroon, proofs of HEV circulation have been documented. Amougou et al., in a prospective case-control study found a high prevalence of HEV in Cameroonian patients with hepatocellular carcinoma (HCC) compared to non-HCC patients with chronic liver disease (41.8 % vs 12.6%) (Amougou et al. 2017). A report by another group indicated the presence of HEV serologic markers in HIV-infected patients, pregnant women, and the elderly population (Modiyinji et al. 2019). A prevalence of 6.7% and 12.2% was reported for anti-HEV immunoglobulins (IgG) and IgM respectively in HIV-infected patients in Yaoundé (Wilson et al. 2020). The first studies on the molecular characterization of human HEV isolates collected in infected patients from North Cameroon revealed the occurrence of gt 1 and 3 (Modiyinji et al. 2020), confirming thereby the transmission of zoonotic strains previously identified in pigs (Modiyinji et al. 2018). Therefore, the search for efficient antivirals is needed.

1 Actually, there is no specific cure for HEV infection. Recently, the HEV 239 vaccine, called Hecolin was
2 approved in China but is still unavailable for other countries (Nan et al. 2018). Therefore, efforts are more concentrated
3 on the search for new antiviral inhibitors. The development of anti-HEV drugs has been slowed down, for a long time,
4 due to difficulties to purify the viral polymerase and replicating effectively HEV in cell culture. The current treatments
5 include the administration of the ribavirin (RBV) / pegylated interferon-alpha combination which clears the virus, at
6 80%, but the multiple side effects, failure in achieving a sustained virological response, and the emergence of viral
7 resistant-mutants, increasingly prompted the search for alternative therapies(Kinast et al. 2019). Drug repurposing or
8 repositioning is an alternative approach consisting to reuse existing drugs to treat another pathology than the primary
9 indication. This approach could be an efficient way to overcome the time limitation research and development needed to
10 design a therapeutic drug against HEV. A clear advantage of the repositioned drug over traditional drug development is
11 that since the repositioned drug has already passed a significant number of tests including clinical trials, its safety is
12 known, and the risk of failure is reduced (Kinast et al. 2019). Thanks to this advantage, drug repurposing has retained
13 the attention of the scientific community over this last decade especially in the field of viral diseases. For example,
14 Sofosbuvir, an antiviral agent approved against hepatitis C was recently found efficient in inhibiting HEV replication in
15 cell culture (Thi et al. 2016) and during clinical studies (Fraga et al. 2019), particularly when combined to RBV. Since
16 HEV infection has been also reported in malaria patients, repurposing antimalarial drugs against hepatitis E could be
17 viewed as a promising strategy. Animal models usually raised ethical concerns as well as translational questions of
18 research findings to humans. Therefore, human-based computer models appear as good alternatives as many of them
19 demonstrated higher accuracy than animal models in clinical risk prediction and pharmacological evaluation (Passini et
20 al. 2017). Previous works recently highlighted the *in silico* antiviral potential of antimalarial drugs against the
21 coronavirus disease 2019 (COVID-19) (Sachdeva et al. 2020). In this study, we report new findings on the *in silico*
22 activity of eight licensed antimalarial drugs against different HEV proteins.
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

45 **2 Material and Mehods**

46 *2.1 Drugs screened for repurposing activities*

47 We examined eight approved antimalarial drugs including Amodiaquine, Artemisinin, Chloroquine,
48 Hydroxychloroquine, Lumefantrine, Mefloquine, Quinine, and Pyrimethamin for their repurposing potential against
49 HEV infection. Sofosbuvir and Ribavirin, two antiviral drugs used against hepatitis C were selected as controls to
50 compare interaction between antimalarial and antiviral drugs. The 2D structures of all these drugs is shown in Figure 1.
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2.2 Molecular docking and evaluation of the ADMET properties

The 3D structures of the tested compounds were obtained from the PubChem database in SDF format. Files were then transformed into MDL MOL files with Open Babel. The 3D structures of viral proteins were retrieved from the protein data bank (PDB) repository. These include: the Zinc-binding non-structural protein (PDB ID: 6NU9), the cryoEM structure of HEV VLP, genotype 1 (PDB ID: 6LAT), the capsid protein ORF-2, genotype 3 (PDB ID: 2ZTN), and the capsid protein E2s domain, genotype 1 (PDB ID: 3GGQ). The FASTA sequence of the RNA-dependent RNA polymerase fragment of HEV was obtained from Uniprot (Uniprot ID: A0A2Z4GU00_HEV) and a homology modeling was done using the SWISS-MODEL program. Compounds were docked against each PDB file using the drug screening mode of the iGEMDOCK software (version 2.1) provided by BioXGEM lab. For each docking, a total of 30 conformers was used with the full set of ten compounds. The below parameters were used: population size = 200, generation = 70, and number of solutions = 3. The postanalysis method helped us to visualize and determine drug interactions. The docking scores of the predicted poses were calculated as the total energy in the binding site :

$$\text{Fitness} = \text{VdW} + \text{Hbond} + \text{Elec}$$

with the vdW term referring to van der Waal energy. Hbond and Elect terms are hydrogen bonding energy and electrostatic energy, respectively. Fully description of the iGEMDOCK scoring function is presented by Yang and Shen (Yang and Shen 2005). Protein-ligand complexes were visualized using RasMol and USCF Chimera 1.14 and the pharmacological interactions were analyzed using the IGEMDOCK post-analysis tool to detect interacting amino acids.

Besides, the pharmacokinetic properties of the different drugs were predicted using ADMETlab2.0, a free web platform available at <http://admet.scbdd.com/> and supported by the CBDD group from the Xiangya School of Pharmaceutical Sciences & Central South University. This web interface systematically evaluates absorption, distribution, metabolism, excretion (ADME) properties, and various toxicities (T) of the chemical compounds based on a comprehensive collected database consisting of 288967 entries (Dong et al. 2018). Absorption was evaluated by estimating Caco-2 and MDCK permeabilities, the interactions as substrate or inhibitor of p-glycoprotein (P-gp), the human oral bioavailability 30 % ($F_{30\%}$), and human gastrointestinal absorption (HGI). Parameters of distribution included the blood-brain barrier (BBB) penetration, plasma protein binding (PPB), and volume distribution (VD), while the metabolism consisted of analyzing the interaction with the cytochromes P450 isoforms (CYP). For the excretion, the clearance and half-life ($T_{1/2}$) of drugs were estimated. For the toxicological aspect, the heart effect was determined by measuring the ability to behave as hERG blockers. Human hepatotoxicity (H-HT), carcinogenicity, and respiratory toxicity were also examined.

2.3. RdRp Model Building with SWISS-MODEL template library

1 Template search has been conducted with BLAST and HHblits using the SWISS-MODEL template library (SMTL),
2 (last update: 2021-02-03, last included PDB release: 2021-01-29). The target sequence was searched with BLAST
3 against the primary amino acid sequence contained in the SMTL. An initial HHblits profile has been built using the
4 procedure as previously reported (Steinegger et al. 2019) followed by 1 iteration of HHblits against Uniclust30 (Mirdita
5 et al. 2016). The obtained profile has then be searched against all profiles of the SMTL. A total of 150 templates were
6 found. The porcine Aichi virus polymerase (PDB ID: 6R11) which exhibited the best sequence identity (23.64%) was
7 used as a suitable template for HEV_RdRp modeling. Models have been built based on the target-template alignment
8 using ProMod3. Coordinates that are conserved between the target and the template were copied from the template to
9 the model. Insertions and deletions were remodeled using a fragment library. Sidechains were then rebuilt. Finally, the
10 geometry of the resulting model was regularized by using a force field. In case of failure of the loop modeling with
11 ProMod3, an alternative model was built with PROMOD-II (Guex et al. 2009). The global and per-residue model
12 quality has been assessed using the QMEAN scoring function (Studer et al. 2020). The oligomeric state conservation
13 was appreciated using the GMQE score which estimates the accuracy of the tertiary structure of the resulting model.
14 The modeled protein was validated by drawing the Ramachandran plot ([https://swissmodel.expasy.org/assess/XXVhRp/
15 02](https://swissmodel.expasy.org/assess/XXVhRp/02)). The phi-psi angles of 92.45 % of amino acids were found in the favored regions. The RdRp model was also
16 checked in the ProSA-web server. The 3D analysis revealed that the model had a Z score of -0.72 (suppl. Figure 1).
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34

2.4. Replicon

35 The HEV p6GLuc replicon was constructed from the HEV genotype 3 Kernow-C1 p6 strain (Accession number
36 JQ679013.1) and was obtained from Dr S. Emerson, NIAID, NIH, Bethesda, USA. This replicon possesses a *Gaussia*
37 *Luciferase* reporter gene that substitutes the 5' part of the ORF2 gene and most part of the ORF3 gene (Emerson et al.
38 2013; Shukla et al. 2012). Thus, the p6GLuc replicon does not form viral particles and cannot infect neighboring cells
39 and the *Gaussia Luciferase* gene is transcribed by the viral replicase ORF1. Therefore, the luciferase activity is directly
40 proportional to the replication activity of the HEV p6GLuc replicon. It is also convenient for kinetics as the Luciferase
41 is secreted into the cell supernatant. A p6GLuc GAD mutant replicon in which the ORF1 polymerase active site GDD
42 was mutated to GAD to prevent any replication was used as a negative control (Emerson et al. 2013).
43
44
45
46
47
48
49
50
51
52
53
54

2.5. Capped mRNA synthesis

1 First, the plasmid DNA of the p6GLuc and p6GLuc GAD mutant replicons were linearized using the restriction enzyme
2 MluI. The restriction digestion was conducted for 2h at 37°C in 100µL reaction mix as follows: 25µg plasmid DNA,
3 10µL Cutsmart buffer 10x, 62.5µL RNase free water and 2.5µL MluI (NEB, 10,000 units/mL). Next, the DNA was
4 separated from protein by adding 50µL sodium acetate (3M, pH 5,5) and 500µL chloroform/isoamyl alcohol (96 Vol. : 4
5 Vol.) and centrifuging at 14,000rpm for 4 minutes. The supernatant was transferred, mixed with 700µL ethanol absolute
6 and incubated at -20°C for 20 minutes. The DNA pellet was vortexed and centrifuged at 14,000rpm. The pellet was
7 washed twice with 70% ethanol, dried and suspended in 25µL RNase free water. The capped mRNA of the p6GLuc
8 replicons were synthesized by in-vitro transcription of the MluI-linearized DNA according to the mMACHINE
9 mMACHINE kit (Ambion) and stored at -80°C before electroporation in PLC3 cells.
10
11
12
13
14
15
16
17
18

19 *2.6. Cell culture, electroporation and treatments*

20 PLC3 cells are a subclone of the PLC/PRF/5 (CRL-8024) hepatoma cells and were characterized as the productive cell
21 line for HEV particles by Montpellier et al. (2018). PLC3 cells were cultured in Dulbecco's modified Eagle's medium
22 (DMEM) containing 10% FBS and 1% non-essential amino acids (DMEM complete). The p6GLuc and p6GLuc GAD
23 mutant replicons were electroporated in PLC3 cells as follows. After trypsinization, cells were resuspended in DMEM
24 complete medium and washed twice in Opti-MEM medium. Three million cells were electroporated with 10µg of RNA
25 of the HEV replicon constructs and resuspended in 6mL DMEM complete medium.
26
27
28
29
30
31
32
33
34

35 *2.7. Compound Treatment*

36 The compounds Lumefantrine, Amodiaquin and **N-desethylamodiaquine** (Sigma Aldrich, Schnelldorf, Germany) were
37 diluted in DMSO at a stock concentration of 50mM. Sofosbuvir, diluted at the same concentration in DMSO, served as
38 a control of the inhibition of HEV replication. It acts as a chain terminator during replication and its antiviral potential
39 was demonstrated using a genotype 3 replicon in Huh7 and HepG2 cells (Dao Thi et al. 2016). The electroporated cells
40 were seeded in 96-well plates (20,000 cells/well) and incubated for 5 days at 37°C in a humidified atmosphere
41 containing 5% CO₂. The compounds were added at different concentrations to the electroporated PLC3 cells. The final
42 concentration of DMSO per well was 0.05% or lower. The supernatants (10µL) were sampled at 1, 3, 4 and 5 days post-
43 electroporation (dpe) and stored at -20°C until luminometer reading.
44
45
46
47
48
49
50
51
52
53
54

55 *2.8. Luciferase assay*

1 The supernatants were thawed and centrifuged at 14,000rpm for 5 minutes to remove any cell debris. Next, the samples
2 were diluted 1:100 in 1X passive lysis buffer (Promega) and 5 μ L were transferred into a white Nunc 96-well plate. At
3 1second after injection of 20 μ L of the substrate solution (Renilla Luciferase Assay System, Promega), relative light
4 units (RLUs) were acquired on a Centro Luminometer during 1 second. Experiments were repeated three times for each
5 tested compound. Means of RLUs acquired from 3 well at each time point are calculated. The results are expressed as
6 replication folds normalized to day 1 post-electroporation.
7
8
9

10 11 12 13 2.9. Cell viability Assay

14 Cell viability was determined by using the CellTiter 96[®] AQueous One Solution Cell Proliferation Assay (MTS) by
15 Promega. After aspirating the cell supernatant of the plated cells, 100 μ L of the 1x MTS solution diluted in DMEM
16 medium was added to each well. After 1-2 hours, the absorbance was read at 490nm by a Microplate Reader (BioTek).
17
18
19
20
21

22 2.10. Statistical analysis

23 Data were presented as mean \pm standard deviation (SD) of three sets of experiments. Statistical analysis was carried out
24 using the GraphPad Prism 5.0 software for windows. The comparison of means was performed using a two-way
25 analysis of variance (ANOVA) followed by Bonferroni's post hoc tests. Differences were considered significant when p
26 <0:05.
27
28
29
30
31

32 33 3 Results

34 3.1 Interaction analysis of drugs with the Zinc-binding non-structural protein (6NU9)

35 As shown by Table 1, docking results revealed that Sofosbuvir has the greatest binding affinity with 6NU9 with a
36 fitness value of -98.22 kcal/mol, followed by N-desethylAmodiaquine (-93.5 kcal/mol), Amodiaquine (-89.9 kcal/mol),
37 and Lumefantrine (-86.01 kcal/mol). The binding scores of these antimalarial drugs were greater than that of Ribavirin
38 (-80.83 kcal/mol). The top docked poses showed common van der Waal interactions with Gln91, Ser92, Thr102,
39 Tyr103, Ala104, Glu111, Arg113, Arg122, and the binding site of 6NU9 (Figure 2a-2c). Interestingly, N-
40 desethylamodiaquine and Sofosbuvir both formed 4 hydrogen bonds including one common H-bond with Thr 102
41 which seems to be an important amino acid residue in the active site. However, Lumefantrine and other ligands, lacked
42 such polar interactions.
43
44
45
46
47
48
49
50
51
52
53
54

55 3.2 Docking results of drugs with the RNA-dependent RNA polymerase of HEV

56
57
58
59
60
61
62
63
64
65

1 The RNA-dependent RNA polymerase (RdRp) of HEV is a small proteic fragment of 90 amino acid residues released
2 after processing of the non-structural ORF-1 polyprotein and which acts as a key enzyme of the replication process.

3 Docking results showed higher binding scores of RdRp with Sofosbuvir (-113.86 kcal/mol), followed by Lumefantrine
4 (-102 kcal/mol), and Amodiaquine (-93.91 kcal/mol). However, the affinity of these antimalarial drugs were greater than
5 that of Ribavirin (-84.58 kcal/mol) (Table 1). The docking poses of these drugs (Figures 2e-2f) were stabilized by the
6 same hydrophobic interactions involving 9 amino acid residues (Trp30, Lys31, Lys32, His33, Glu 36, Gly 38, Trp42,
7 Asn43 and Trp46) indicating therefore similar binding modes. However, Lumefantrine showed additionally vdW
8 contacts with Phe29 and no H-bond, while Sofosbuvir exhibited H-bonds with 3 amino acid residues (Lys31, Trp42,
9 Trp46) and Amodiaquine with only one (Lys32) (Table 3).

10 3.3 Interaction analysis of drugs with the HEV capsid proteins

11 HEV capsid proteins are proteins essential for viral entry and assembly. Compounds that inhibit or strongly
12 bind to these molecules could interfere with these processes.

13 3.3.1 Effect on the HEV VLP CryoEM structure, genotype 1 (6LAT)

14 With regard to 6LAT, the selected compounds and their binding scores are presented in Table 2. Pyrimethamine
15 exhibited a higher binding affinity (-97.99 kcal/mol) against the 6LAT target, than Sofosbuvir (-92.24 kcal/mol),
16 Lumefantrine (-90.42 kcal/mol), and Amodiaquine (-90.16 kcal/mol). However, these fitness values were lower than
17 that of Ribavirin (-99.33 kcal/mol). As shown by Figure 4a, Pyrimethamine and Ribavirin both interacted with the M and
18 S domains of the capsid protein. The strong binding of Pyrimethamine is mediated by the H-bonds with 3 amino acids
19 (Ser161, Thr272, Tyr443) and steric interactions with 5 amino acids residues (Pro159, Leu163, Leu164, Asp442, Tyr443
20 (Table 3) while that of Ribavirin, implicated 9 H-bonds with the following residues Pro142, Thr144, Ser146, Leu155,
21 Asp168, Arg322, Ser324, Thr326, Arg437, and only 4vdW contacts (Thr144, Asp168, Arg322, Arg 437). Both
22 compounds, therefore, showed low similarities in their binding pattern on 6LAT (Figure 3a). Unlike the two top
23 conformations, Sofosbuvir was found to interact with the M domain only.

24 3.3.2. Effect on the E2s domain , genotype 1 (3GGQ) and capsid protein of genotype 3 (2ZTN)

25 On docking with 3GGQ, Lumefantrine showed the most potent binding with the estimated fitness scores of -
26 106.05 kcal/mol greater than that of Sofosbuvir (-99.81 kcal/mol), and Ribavirin (-96.99 kcal/mol), followed by
27 Amodiaquine (-93.64 kcal/mol), Hydroxychloroquine (-90.55 kcal/mol), Mefloquine (-88.41 kcal/mol) Pyrimethamine
28 (-85.06 kcal/mol), Quinine (-83.61 kcal/mol), Chloroquine (-81.51 kcal/mol), and Artemisinin (-71.89 kcal/mol) (Table
29 2). The strong binding affinity of Lumefantrine was stabilized by 15 amino acid residues including one H-bond with
30 Thr489 and 14 vdW contacts with Gly486, Ser487, Thr489, Gly490, Val492, Gln531, His532, Tyr559, Asn560,
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 Asp567, Gln568, Leu570, Ile581, Ser582 residues (Table 3). However, Sofosbuvir interacted with 13 amino acid
2 residues including 6 H-bonds with Lys544, Asn560, Ser566, Asp567, Gln568, Ser582 and 7 hydrophobic contacts of
3 which 5 were common with Lumefantrine (Tyr559, Asn560, Asp567, Gln568, Ser582), explaining thereby some levels
4 of similarity in the binding mode (Figures 3b and 3c).
5

6 As far as 2ZTN is concerned, docking results indicated the highest binding scores with Sofosbuvir (-106.96
7 kcal/mol), followed by Pyrimethamine (-105.17 kcal/mol), and N-desethylamodiaquine (-102.65 kcal/mol), (Table 2).
8 These compounds stabilized the complex through polar and nonpolar interactions. As shown by Table 3, Sofosbuvir is
9 bound to the M domain of 2ZTN thanks to H-bonds formed with Arg366, Gly367, Gln420, Asp444, Gln446 and
10 hydrophobic interactions with 7 amino acids residues (Arg366, Arg399, Gln420, Gln421, Asp422, Asp444, Gln446). Its
11 binding mode is different from that of the highly interacting antimalarial drugs which exclusively targeted the P domain
12 of this capsid protein (Figures 3d and 3e). Pyrimethamine established the H-bonds with 6 amino acids (Gly543, Tyr561,
13 Asn562, Thr563, Thr564, Ser566) and vdW contacts with 11 amino acids residues (Gly543, Lys544, Leu545, Phe 547,
14 Tyr561, Asn562, Thr564, Ser566, Asp567, Thr583, Tyr584) while N-desethylamodiaquine only formed H-bonds with 3
15 amino acid residues (Tyr561, Ser 566, and Asp 567), and steric interactions with 12 amino acid residues including the
16 11 reported with Pyrimethamine plus Lys554. Therefore, both antimalarial drugs share similarities in their binding
17 modes on 2ZTN.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

33 3.4. Pharmacokinetic profiles and side effects of the screened drugs.

34 The analysis of the ADMET properties (Table 4) suggests that the drugs used have good gastrointestinal absorption in
35 general as evidenced by the $F_{30\%}$, and HGI results, and the permeability through the Caco-2 human intestinal cell lines.
36 However, the permeability of these cells to Lumefantrine, Pyrimethamine, Ribavirin, and Sofosbuvir was found lower
37 compared to other drugs. The P-glycoprotein which is a membrane protein, member of the ATP-binding cassette (ABC)
38 transporters superfamily is also known as an important mediator of the efflux of xenobiotics through cells. Several
39 drugs, including Artemisinin, Lumefantrine, Chloroquine, Hydroxychloroquine, Mefloquine, and Quinine showed a
40 high inhibitory potential on this transporter indicating thereby they might interfere with the absorption of other drugs.
41 Concerning the distribution, Lumefantrine, Chloroquine Hydroxychloroquine, and Quinine showed a low penetration
42 through the BBB, while that of Amodiaquine, Artemisinin, and Pyrimethamine were medium. However, Mefloquine
43 Ribavirin and Sofosbuvir presented a good BBB penetration. All the drugs showed a good predicted VD which is
44 comprised in the range of 0.04-20L/kg. Nevertheless, Lumefantrine, Amodiaquine, and Mefloquine displayed high
45 predicted PPB values of 99.91%, 97.33 % and 91.86%. Moreover, a good predictive clearance was observed with most
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

of the antimalarial drugs excepted Pyrimethamine, Mefloquine, and Quinine which presented values of 3.89, 2.89, and 1.89 mL/min/kg respectively. All the antimalarial drugs are able to interfere with different CYP isoforms but a high number of interactions was found with AmodiaquineLumefantrine which is both inhibitor and substrate for 4 distinct CYPs. The examination of the predicted toxicity revealed a high tendency of the antimalarial drugs to induce heart problems and hepatotoxicity excepted Amodiaquine that also demonstrated a low carcinogenic potential. However, as with other drugs, the potential side effects of these compounds on the respiratory systems is to fear.

3.5. *In vitro* effects of Lumefantrine, Amodiaquine and N-desethylamodiaquine on gt 3 HEV replicon cells

In order to validate the computational predictions, the activity of anti-malarial compounds (Lumefantrine, Amodiaquine and N-desethylamodiaquine) was tested on the efficiency of replication of the HEV p6GLuc replicon in PLC3 cells during the course of 5 days post-electroporation. The replication kinetics of the HEV p6GLuc replicon in the presence of these compounds were compared to the kinetics of (i) the untreated HEV p6GLuc as positive control, (ii) the HEV p6GLuc treated with Sofosbuvir as a known HEV inhibitor (Dao Thi et al. 2016) and (iii) the HEV p6GLuc GAD mutant as replicative-deficient negative control. Surprisingly, Lumefantrine does not seem to impact p6GLuc replication efficiency at the concentrations used (10, 5 and 1 μ M) (Figure 4a). Indeed, in the presence of 10 μ M of Lumefantrine, the p6GLuc replication efficiency is comparable to the replication fold of the untreated p6GLuc (Figure 5b). In addition, PLC3 viability was not affected at concentrations of 1, 5 and 10 μ M of Lumefantrine (Figure 5b). On the contrary, Amodiaquine drastically decreased cell viability by 49% (P<0.001) and 85% (P<0.001) at 5 and 10 μ M concentrations, respectively (Figures 4b and 5a). While the replication efficiency of PLC3 cells in the presence of these concentrations of Amodiaquine drops closer to that of sofosbuvir-treated replicons, Amodiaquine at 1 μ M appears as the most efficient concentration. Indeed, the decrease of viral replication at this concentration is not accompanied by any cytotoxicity (Figures 5a and 5b). Moreover, the N-desethylamodiaquine compound, which is the major biologically active metabolite of amodiaquine (Zhang et al. 2017), distinctly lowered the replication efficiency of the p6GLuc replicon at a concentration of 1 μ M as compared to the untreated replicon. Interestingly, at this concentration, a significant decrease (P<0.05) in replication fold of the HEV p6GLuc replicon was recorded, almost reaching 50% inhibition in comparison to the untreated p6GLuc replication efficiency (Figure 5a) and the PLC3 cells displayed close to 91% of viability (Figure 5b). However, N-Desethyl Amodiaquin was cytotoxic to PLC3 cells at concentrations of 5 and 10 μ M (Figure 5b).

4 Discussion

1 Hepatitis E is an emerging viral disease in developing countries including Cameroon where human and
2 zoonotic transmissions have been signaled (Amougou et al. 2017; Modiyinji et al. 2019; Modiyinji et al. 2018; Wilson
3 et al. 2020). In spite of recent progress in the antiviral drug development, therapeutical options against HEV are still
4 limited. Drug repurposing has been proposed recently as an innovative approach to rapidly identify efficient drugs
5 against viral diseases without the need to undergo multiple clinical trials (Kinast et al. 2019). In this study, we
6 investigated the *in silico* potential of 8 licensed antimalarial drugs against HEV, and compared it with two approved
7 anti-hepatitis C drugs (Sofosbuvir and ribavirin) which experimentally demonstrated *in vitro* effects against HEV (Thi
8 et al. 2016). Moreover, the biological effects of the best docked drugs were evaluated experimentally using gt 3 HEV
9 replicon systems.

16 Concerning the computational screening against the Zinc-binding non-structural protein (6NU9), Sofosbuvir,
17 N-desethylAmodiaquine, and Amodiaquine demonstrated the most potent affinities with fitness scores of -98.22, -93.5,
18 and, -89.9 kcal/mol respectively compared to Ribavirin (Table 1). The high score of N-desethylamodiaquine relative to
19 other drugs may be explained by similarities between the binding mode of this metabolite and that of Sofosbuvir.
20 Indeed, both compounds forms H-bond with Thr 102 (Table 3) and show hydrophobic interactions with almost the same
21 amino acid residues. First studies on the biophysical and structural characterization of 6NU9 suggested that this protein
22 might correspond to HEV protease (Proudfoot et al. 2019). Therefore, our data indicate that these drugs could inhibit
23 viral replication by highly interfering with the HEV protease. In order to check whether the affinities of these drugs
24 could be also high on other targets of viral replication, their effects were virtually screened against the RdRp. Greater
25 binding scores were found with Lumefantrine, and Amodiaquine compared to Ribavirin, but their affinities for this
26 target were still lower than that of Sofosbuvir (Table 1). Moreover, the top docked conformations were stabilized by the
27 same hydrophobic contacts (Table 3), indicating therefore that both antimalarial drugs could also highly target the RdRp
28 better than ribavirin with a binding mode similar to Sofosbuvir.

33 In order to validate these *in silico* predictions about the anti-replicative potential of these two drugs, their
34 effects were evaluated *in vitro* on PLC3 cell lines using a replicon construct (HEV gt 3 p6GLuc replicon). Our results
35 showed Lumefantrine was not active at the tested concentrations (1-10 μ M), contrary to N-desethylamodiaquine, a
36 major metabolite from amodiaquine which significantly ($P < 0.001$) decreased viral replication without affecting the cell
37 viability when tested at 1 μ M concentration (Figure 5a and 5b). The here-generated data suggest a positive correlation
38 between the *in silico* effect of N-desethylamodiaquine on HEV protease and the antiviral effect observed in cell culture,
39 considering the highest binding energy score obtained (-93.5 kcal/mol) on 6NU9, relative to other antimalarial drugs
40 and the significant viral inhibition achieved with this compound at 1 μ M concentration in p6GLuc replicon. Likewise, a

1 strong positive correlation has been observed between the great binding scores of Sofosbuvir on replication targets and
2 the experimental data. N-desethylamodiaquine could be therefore regarded as an inhibitor of the viral replication with
3 similar effects to Sofosbuvir and that strongly and preferentially targets the HEV protease with little affinity on RdRp.
4 Moreover, a moderate affinity was recorded between this compound and the HEV entry targets (Table 2). These results
5 are in line with subsequent studies which demonstrated that Amodiaquine was able to inhibit the replication of dengue
6 virus type 2 (Boonyasuppayakorn et al. 2014) and Zika virus (Han et al. 2018). Nevertheless, complementary studies
7 are required to better understand the mechanism of action of this antimalarial drug. We also found that Lumefantrine is
8 negatively correlated to viral inhibition as the lack of activity on cell models contrasts with the high binding scores
9 obtained on the viral targets, especially on RdRp. These results are also similar to that of Barger-Kamate et al who
10 found a moderate efficacy of the Lumefantrine-artemether association against the cytomegalovirus 25 in a clinical study
11 in Malian patients (Barger-Kamate et al. 2016). The lack of effect of Lumefantrine in this study might be due either to
12 the low concentrations used or to the genotype and the replicon used. Indeed, the mutations inserted in the sequence of
13 this replicon would affect the binding and therefore the susceptibility to the antimalarial drug as demonstrated in
14 previous studies with HCV-resistant mutants (Nitta et al. 2016). Furthermore, pharmacokinetic considerations are not to
15 be excluded. Biocomputational analysis of the ADMET profile of Lumefantrine revealed a low permeability through
16 biological membranes, and a higher protein-bound predictive value compared to Amodiaquine. These factors might be
17 indicative of a poor therapeutic index as earlier reported (Dong et al. 2018). In fact, previous studies demonstrated that
18 the binding of a drug to proteins in plasma may negatively influence its pharmacodynamic behavior, as it decreases the
19 concentration of the free drug to the target site (Dong et al. 2018; Smith and Waters 2019). In addition, both compounds
20 also interact with almost the same CYP isoforms but Lumefantrine seems to be a specific CYP 3A2 substrate while
21 Amodiaquine is particularly metabolized by CYPD 2D6. This difference might explain the rapid inactivation of
22 Lumefantrine and therefore the short half-time observed.

23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

HEV capsid is an important element in viral pathogenesis as it is involved in host-virus interactions, viral assembly, and immunogenicity (Liu et al. 2011). Thanks to techniques like X-crystallography and CryoEM, the capsid protein has been extensively studied and characterized into 3 structural domains including the shell domain (S domain, aa 118–317), a middle domain (M domain, aa 318–451), and a protrusion domain or E2s domain (P domain, aa 452–606) on HEV virus-like particles (HEV VLPs) purified from robust cell culture systems (Bai et al. 2020). Structure alignment with these domains revealed that M and S were highly conserved whereas the P domain was extremely variable (Zhang et al. 2018) The 6LAT protein, presented here, is an asymmetric unit of the capsid protein obtained from HEV gt1 particles. Docking results showed high binding affinities of this protein with Ribavirin and

1 Pyrimethamine on the conserved domains which are tightly associated (Figure 3a). Ribavirin was found to strongly
2 collide with the M and S domains through 9 H-bonds with Pro142, Thr144, Ser146, Leu155, Asp168, Arg322, Ser324,
3 Thr326, Arg437, and 4 vdW contacts (Thr144, Asp168, Arg322, Arg 437)while Pyrimethamine formed H-bonds with 3
4 amino acids (Ser161, Thr272, Tyr443) and steric interactions with 5 amino acid residues (Pro159, Leu163, Leu164,
5 Asp442, Tyr443 (Table 3). Studies on the functions of these domains showed that the S domain builds the integral shell
6 of the HEV particle, with a cluster of basic amino acid residues that contribute to neutralizing negative charges of the
7 HEV genomic RNA (Guu et al. 2009). Other studies indicated that the M domain also partially participated in this
8 function but its interaction with the P domain makes it essential in cell-attachment. In a previous study reported by
9 Schofield et al, (Schofield et al. 2003), it has been shown that neutralizing antibodies interfered with HEV entry steps
10 by recognizing linear epitopes located in the M domain of capsid protein. Similar findings were also reported by another
11 group (He et al. 2008), confirming thereby the possibility for Pyrimethamine to inhibit cell-attachment of gt 1 particles.
12
13
14
15
16
17
18
19
20

21 In order to confirm this antiviral potential on other genotypes, especially on zoonotic strains, antimalarial drugs
22 were screened against the ORF-2 protein, of gt3 (2ZTN). Similarly, Sofosbuvir and Pyrimethamine stood out as highly
23 interacting compounds. However, their binding patterns were strikingly different. The affinity of Sofosbuvir was still
24 located on the conserved domains through interactions with 12 amino acid residues whereas that of Pyrimethamine was
25 more oriented on the E2s domain via interactions with 17 amino acid residues including 6 in H-bond and 11 in steric
26 interactions. Most of the amino acids involved in hydrophobic interactions with Pyrimethamine was similar to that of
27 Amodiaquine. The great difference especially originated from H-bonds formed by Pyrimethamine with Gly543, Tyr561,
28 Asn562, Thr563, Thr564, Ser566. These results prove that the affinity of Pyrimethamine for the HEV capsid protein
29 changes according to mutations due to the genotypes. Besides, it also pointed out the ability of this compound to act
30 against human and zoonotic HEV strains. Several studies previously demonstrated the capacity of neutralizing
31 antibodies including 8C11 and 8G12, to bind on E2s domain (Gu et al. 2015; Tang et al. 2011; Zheng et al. 2019). It has
32 been suggested that the loop region formed by amino acid residues 550-566 and 580-593 of this domain, contained
33 hypervariable amino acids which help the viral particle to escape the antibody recognition (Liu et al. 2011). Therefore,
34 interferences with amino acids found in this region might allow decreasing the immunogenicity of the HEV particle and
35 its infectivity as well, as, these sugar-binding sites were also found to be implicated in cell-attachment (Gu et al. 2015).
36 In this study, Pyrimethamine showed hydrophobic (Tyr561, Asn562, Thr564, Ser566, Asp567, Thr583, Tyr584) and
37 hydrogen (Tyr561, Asn562, Thr563, Thr564, Ser566) interactions in E2s loops of HEV gt3, indicating it may decrease
38 the immunogenicity of these gt 3particles and their binding to the host cell.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1 The importance of dimerization of the E2s domain as a prerequisite for HEV infectivity has been previously
2 documented (Gu et al. 2015; Liu et al. 2011; Tang et al. 2011; Zhang et al. 2018). As the E2s region, seemed to be a
3 preferred target for Pyrimethamine on gt3 viral particles, all antimalarial compounds were specifically docked against
4 this domain using a gt1 viral strain. As shown by Table 2, surprisingly, Lumefantrine was the antimalarial compound
5 with the greatest binding score (-106.05 kcal/mol) followed by Sofosbuvir, and Ribavirin (-99.81 and -96.81 kcal/mol
6 respectively). However, Pyrimethamine only showed a low fitness score (-85.06 kcal/mol). The genetic diversity of
7 viral strains has certainly negatively affected the Pyrimethamine binding site on 3GGQ. In a previous study, Zheng et al
8 reported the monoclonal antibodies could bind into the E2s domain in two manners : either directly on the epitope
9 located on the top of the protrusion domain as found with 3B6 antibody or on the flanking side as shown by 8C11
10 antibodies (Zheng et al. 2019). The interaction with the epitope on the top inhibited viral attachment while preserving
11 viral integrity whereas interaction with the flanking region directly disorganized the icosahedral arrangement of capsids
12 and split the viral particles into small pieces. In an attempt to characterize the amino acids involved in the 8C11 binding
13 site using gts 1 and 4 HEV capsids, authors found that 8C11 was specific to gt 1 and that 8C11 epitope regions
14 contained Ser497 and Ala575, for gt 1 whereas gt 4 possessed Thr497 and Pro575. Ser 497 was found in H-bond with
15 8C11 (gt1) while Arg 512 is crucial in gt4 interactions (Tang et al. 2011). Our results tend to show that Lumefantrine is
16 more specific to E2s domain of gt 1 while Pyrimethamine is specific on gt 3. Moreover, Sofosbuvir, and Lumefantrine
17 share similarity in their binding patterns on the E2s region of gt1 particles as they mainly interact with the same amino
18 acid residues in antibody-binding region. Further investigations should be conducted to clarify whether this interaction
19 directly destroys the structure of HEV capsid or impede viral attachment.
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

37 **5 Conclusion**

38
39 In conclusion, among all docked antimalarial drugs, Amodiaquine, Lumefantrine, and Pyrimethamine stood out as
40 promising candidates for repurposing against HEV. Whether Amodiaquine is advantaged by the N-
41 desethylamodiaquine, its main human metabolite, which is highly active on HEV replicon systems *in vitro* and exhibits
42 a strong binding score and specificity for the Zn-binding nonstructural protein, Lumefantrine, however, seems to be
43 handicapped by pharmacokinetics constraints which limits its biological effect although having great binding scores
44 with the RdRp and E2s domain. In fact, the ADMET profile of both drugs revealed a low permeability of Lumefantrine
45 and an affinity for the CYP3A2 which could rapidly metabolize the drug and therefore inactive it, justifying thereby the
46 lack of activity on replicon systems. Unlike these drugs, Pyrimethamine seemed to collide better on HEV entry through
47 interference with the capsid protein. Further *in vivo* and *in vitro* experiments are necessary to validate the anti-HEV
48 potential of these antimalarial drugs and elucidate the mechanism of action.
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Declarations

Conflict of interest : The authors have no conflict of interest to disclose for this manuscript.

Funding : No funding has been received to conduct this study.

Availability of Data and Material : The data used in this study will be made available upon reasonable request from the corresponding author

Ethical Approval : This manuscript does not include any human participants or animals.

References

Amougou AM, Atangana PJA, Noah Noah D, Moundipa PF, Pineau P, Njouom R. Hepatitis E virus infection as a promoting factor for hepatocellular carcinoma in Cameroon: Preliminary Observations. *Int. J. Infect. Dis.* [Internet]. Elsevier B.V.; 2017 Nov 1 [cited 2020 Oct 12];64:4–8. Available from: <https://pubmed.ncbi.nlm.nih.gov/28847760/>

Aslam SM. Case Study An Interesting Case Of Complicated Falciparum Malaria And Hepatitis E Virus Co-Infection. *Int. J. Clin. Cases Investig.* 2017;6(3):35–7.

Bai C, Cai J, Han P, Qi J, Yuen KY, Wang Q. The crystal structure of the emerging human-infecting hepatitis E virus E2s protein. *Biochem. Biophys. Res. Commun.* [Internet]. Elsevier Ltd; 2020;532(1):25–31. Available from: <https://doi.org/10.1016/j.bbrc.2020.07.074>

Barger-Kamate B, Forman M, Sangare CO, Haidara ASA, Maiga H, Vaidya D, et al. Effect of artemether-lumefantrine (Coartem) on cytomegalovirus urine viral load during and following treatment for malaria in children. *J. Clin. Virol.* [Internet]. Elsevier B.V.; 2016 Apr 1 [cited 2020 Oct 20];77:40–5. Available from: </pmc/articles/PMC4792724/?report=abstract>

Boonyasuppayakorn S, Reichert ED, Manzano M, Nagarajan K, Padmanabhan R. Amodiaquine, an antimalarial drug, inhibits dengue virus type 2 replication and infectivity. *Antiviral Res.* [Internet]. Elsevier; 2014 [cited 2020 Oct 15];106(1):125–34. Available from: </pmc/articles/PMC4523242/?report=abstract>

Dao Thi VL, Debing Y, Wu X, Rice CM, Neyts J, Moradpour D, et al. Sofosbuvir Inhibits Hepatitis e Virus Replication in Vitro and Results in an Additive Effect When Combined with Ribavirin. *Gastroenterology* [Internet]. W.B. Saunders; 2016 Jan 1 [cited 2021 Mar 28];150(1):82-85.e4. Available from: <http://dx.doi.org/10.1053/j.gastro.2015.09.011>

Doceul V, Bagdassarian E, Demange A, Pavio N. Zoonotic hepatitis E virus: Classification, animal reservoirs and transmission routes. *Viruses.* 2016;8(10):1–24.

Dong J, Wang N-N, Yao Z-J, Zhang L, Cheng Y, Ouyang D, et al. ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. *J. Cheminform.* [Internet]. BioMed Central Ltd.; 2018 Dec 26 [cited 2021 Mar 29];10(1):29. Available from: <https://jcheminf.biomedcentral.com/articles/10.1186/s13321-018-0283-x>

Donnelly MC, Scobie L, Crossan CL, Dalton H, Hayes PC, Simpson KJ. Review article: hepatitis E—a concise review of virology, epidemiology, clinical presentation and therapy. *Aliment. Pharmacol. Ther.* 2017;46(2):126–41.

Emerson SU, Nguyen HT, Torian U, Mather K, Firth AE. An essential RNA element resides in a central region of hepatitis E virus ORF2. *J. Gen. Virol.* [Internet]. Microbiology Society; 2013 Jul 1 [cited 2021 Mar 28];94(PART7):1468–76. Available from: </pmc/articles/PMC3709636/>

Fraga M, Gouttenoire J, Sahli R, Chtioui H, Marcu C, Pascual M, et al. Sofosbuvir add-on to ribavirin for chronic hepatitis e in a cirrhotic liver transplant recipient: A case report. *BMC Gastroenterol.* [Internet]. BioMed Central Ltd.;

2019 May 24 [cited 2020 Oct 13];19(1):76. Available from:
<https://bmcgastroenterol.biomedcentral.com/articles/10.1186/s12876-019-0995-z>

1
2 Gouttenoire J, Pollán A, Abrami L, Oechslin N, Mauron J, Matter M, et al. Palmitoylation mediates membrane
3 association of hepatitis E virus ORF3 protein and is required for infectious particle secretion. *PLoS Pathog.* [Internet].
4 Public Library of Science; 2018 Dec 1 [cited 2020 Oct 23];14(12). Available from:
5 <https://pubmed.ncbi.nlm.nih.gov/30532200/>
6

7 Gu Y, Tang X, Zhang X, Song C, Zheng M, Wang K, et al. Structural basis for the neutralization of hepatitis E virus by
8 a cross-genotype antibody. *Cell Res.* [Internet]. Nature Publishing Group; 2015 May 7 [cited 2020 Oct 16];25(5):604–
9 20. Available from: [/pmc/articles/PMC4423075/?report=abstract](https://pubmed.ncbi.nlm.nih.gov/25542200/)
10

11 Guex N, Peitsch MC, Schwede T. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-
12 PdbViewer: A historical perspective. *Electrophoresis.* 2009;30:S162–73.
13

14 Guu T, Liu Z, Ye Q, Mata D, Li K, Yin C, et al. Structure of the hepatitis E virus-like particle suggests mechanisms for
15 virus assembly and receptor binding. *Proc. Natl. Acad. Sci. U. S. A.* 2009;106:12992–12997.
16

17 Han Y, Mesplède T, Xu H, Quan Y, Wainberg MA. The antimalarial drug amodiaquine possesses anti-ZIKA virus
18 activities. *J. Med. Virol.* [Internet]. John Wiley and Sons Inc.; 2018 May 21 [cited 2020 Oct 15];90(5):796–802.
19 Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25031>
20

21 He S, Miao J, Zheng Z, Wu T, Xie M, Tang M, et al. Putative receptor-binding sites of hepatitis E virus. *J. Gen. Virol.*
22 [Internet]. Microbiology Society; 2008 Jan 1 [cited 2020 Oct 16];89(1):245–9. Available from:
23 <https://www.microbiologyresearch.org/content/journal/jgv/10.1099/vir.0.83308-0>
24

25 Kenney SP, Meng XJ. Hepatitis E virus genome structure and replication strategy. *Cold Spring Harb. Perspect. Med.*
26 2019;9(1):1–18.
27

28 Kinast V, Burkard TL, Todt D, Steinmann E. Hepatitis E virus drug development. *Viruses.* 2019;11(6):1–16.
29

30 Liu Z, Jane Y, Zhang J. Structure and Function of the Hepatitis E Virus Capsid Related to Hepatitis E Pathogenesis. In:
31 Mukomolov DS, editor. *Viral Hepat. - Sel. Issues Pathog. Diagnostics* [Internet]. In Tech; 2011. p. 141–52. Available
32 from: [http://www.intechopen.com/books/viral-hepatitis-selected-issues-of-pathogenesis-and-diagnostics/structure-and-](http://www.intechopen.com/books/viral-hepatitis-selected-issues-of-pathogenesis-and-diagnostics/structure-and-function-of-the-hepatitis-e-virus-capsid-related-to-hepatitis-e-pathogenesis)
33 [function-of-the-hepatitis-e-virus-capsid-related-to-hepatitis-e-pathogenesis](http://www.intechopen.com/books/viral-hepatitis-selected-issues-of-pathogenesis-and-diagnostics/structure-and-function-of-the-hepatitis-e-virus-capsid-related-to-hepatitis-e-pathogenesis)
34
35

36 Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes
37 of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010.
38 *Lancet* [Internet]. Lancet Publishing Group; 2012 Dec 1 [cited 2020 Oct 12];380(9859):2095–128. Available from:
39 <https://pubmed.ncbi.nlm.nih.gov/23245604/>
40

41 Mirdita M, von den Driesch L, Galiez C, Martin MJ, Söding J, Steinegger M. Uniclust databases of clustered and
42 deeply annotated protein sequences and alignments. *Nucleic Acids Res.* 2016;45:D170–D176.
43
44

45 Modiyinji AF, Amougou-Atsama M, Monamele CG, Nola M, Njouom R. Seroprevalence of hepatitis E virus antibodies
46 in different human populations of Cameroon. *J. Med. Virol.* [Internet]. John Wiley and Sons Inc.; 2019 Nov 1 [cited
47 2020 Oct 12];91(11):1989–94. Available from: <https://pubmed.ncbi.nlm.nih.gov/31297845/>
48

49 Modiyinji AF, Atsama MA, Monamele GC, Nola M, Njouom R. High seroprevalence of hepatitis e among pigs suggests
50 an animal reservoir in Cameroon. *J. Infect. Dev. Ctries.* [Internet]. Journal of Infection in Developing Countries; 2018
51 Aug 1 [cited 2020 Oct 13];12(8):676–9. Available from: <https://jidc.org/index.php/journal/article/view/10310>
52

53 Modiyinji AF, Rivero-Juarez A, Lopez-Lopez P, Atsama MA, Monamele CG, Nola M, et al. First molecular
54 characterization of the hepatitis E virus in humans in Cameroon: Confirmation of the HEV outbreak in Touboro, North-
55 Cameroon. *J. Med. Virol.* [Internet]. J Med Virol; 2020 [cited 2020 Oct 12]; Available from:
56 <https://pubmed.ncbi.nlm.nih.gov/32639604/>
57
58
59
60
61
62
63
64
65

1 Montpellier C, Wychowski C, Sayed IM, Meunier JC, Saliou JM, Ankavay M, et al. Hepatitis E Virus Lifecycle and
2 Identification of 3 Forms of the ORF2 Capsid Protein. *Gastroenterology* [Internet]. W.B. Saunders; 2018 Jan 1 [cited
3 2021 Mar 28];154(1):211-223.e8. Available from: <http://www.gastrojournal.org/article/S0016508517361796/fulltext>
4
5 Nair VP, Anang S, Subramani C, Madhvi A, Bakshi K, Srivastava A, et al. Endoplasmic Reticulum Stress Induced
6 Synthesis of a Novel Viral Factor Mediates Efficient Replication of Genotype-1 Hepatitis E Virus. *PLoS Pathog.*
7 [Internet]. Public Library of Science; 2016 Apr 1 [cited 2020 Oct 13];12(4). Available from:
8 <https://pubmed.ncbi.nlm.nih.gov/27035822/>
9
10 Nan Y, Wu C, Zhao Q, Sun Y, Zhang YJ, Zhou EM. Vaccine development against zoonotic hepatitis E virus: Open
11 questions and remaining challenges [Internet]. *Front. Microbiol.* Frontiers Media S.A.; 2018 [cited 2020 Oct 23].
12 Available from: </pmc/articles/PMC5827553/?report=abstract>
13
14 Navaneethan U, Al Mohajer M, Shata MT. Hepatitis E and pregnancy: Understanding the pathogenesis [Internet]. *Liver*
15 *Int.* NIH Public Access; 2008 [cited 2020 Oct 12]. p. 1190–9. Available from: [/pmc/articles/PMC2575020/?](/pmc/articles/PMC2575020/?report=abstract)
16 <report=abstract>
17
18 Nimgaonkar I, Ding Q, Schwartz RE, Ploss A. Hepatitis E virus: advances and challenges. *Nat. Rev. Gastroenterol.*
19 *Hepatol.* [Internet]. Nature Publishing Group; 2018 Feb 22 [cited 2020 Oct 23];15(2):96–110. Available from:
20 <http://www.nature.com/articles/nrgastro.2017.150>
21
22 Nitta S, Asahina Y, Matsuda M, Yamada N, Sugiyama R, Masaki T, et al. Effects of resistance-associated NS5A
23 mutations in hepatitis C virus on viral production and susceptibility to antiviral reagents. *Sci. Rep.* [Internet]. Nature
24 Publishing Group; 2016 Oct 5 [cited 2021 Mar 28];6(1):1–9. Available from: www.nature.com/scientificreports
25
26 Passini E, Britton OJ, Lu HR, Rohrbacher J, Hermans AN, Gallacher DJ, et al. Human in silico drug trials demonstrate
27 higher accuracy than animal models in predicting clinical pro-arrhythmic cardiotoxicity. *Front. Physiol.* 2017;8(SEP):1–
28 15.
29
30 Proudfoot A, Hyrina A, Holdorf M, Frank AO, Bussiere D. First Crystal Structure of a Nonstructural Hepatitis E Viral
31 Protein Identifies a Putative Novel Zinc-Binding Protein. *J. Virol.* [Internet]. American Society for Microbiology; 2019
32 Apr 24 [cited 2020 Oct 14];93(13). Available from: <https://pubmed.ncbi.nlm.nih.gov/31019049/>
33
34 Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST. The global burden of hepatitis E virus genotypes 1 and 2
35 in 2005. *Hepatology* [Internet]. *Hepatology*; 2012 Apr [cited 2020 Oct 12];55(4):988–97. Available from:
36 <https://pubmed.ncbi.nlm.nih.gov/22121109/>
37
38 Sachdeva C, Wadhwa A, Kumari A, Hussain F, Jha P, Kaushik NK. In silico Potential of Approved Antimalarial Drugs
39 for Repurposing Against COVID-19 . *Omi. A J. Integr. Biol.* 2020;24(10):568–81.
40
41 Schofield DJ, Purcell RH, Nguyen HT, Emerson SU. Monoclonal antibodies that neutralize HEV recognize an antigenic
42 site at the carboxyterminus of an ORF2 protein vaccine. *Vaccine.* 2003;22(2):257–67.
43
44 Shukla J, Saxena D, Rathinam S, Lalitha P, Joseph CR, Sharma S, et al. Molecular detection and characterization of
45 West Nile virus associated with multifocal retinitis in patients from southern India. *Int. J. Infect. Dis.* [Internet].
46 Elsevier; 2012 Jan 1 [cited 2021 Mar 28];16(1):e53–9. Available from:
47 <http://www.ijidonline.com/article/S120197121100213X/fulltext>
48
49 Smith DB, Simmonds P, Jameel S, Emerson SU, Harrison TJ, Meng XJ, et al. Consensus proposals for classification of
50 the family Hepeviridae. *J. Gen. Virol.* 2014;
51
52 Smith SA, Waters NJ. Pharmacokinetic and Pharmacodynamic Considerations for Drugs Binding to Alpha-1-Acid
53 Glycoprotein [Internet]. *Pharm. Res.* Springer New York LLC; 2019 [cited 2021 Mar 30]. p. 30. Available from:
54 </pmc/articles/PMC7089466/>
55
56 Steinegger M, Meier M, Mirdita M, Vöhringer H, Haunsberger SJ, Söding J. HH-suite3 for fast remote homology
57 detection and deep protein annotation. *BMC Bioinformatics.* 2019;20(473).
58
59
60
61
62
63
64
65

Studer G, Rempfer C, Waterhouse AM, Gumienny G, Haas J, Schwede T. QMEANDisCo - distance constraints applied on model quality estimation. *Bioinformatics*. 2020;36:1765–71.

1 Tang X, Yang C, Gu Y, Song C, Zhang X, Wang Y, et al. Structural basis for the neutralization and genotype specificity
2 of hepatitis E virus. *Proc. Natl. Acad. Sci. U. S. A.* [Internet]. National Academy of Sciences; 2011 Jun 21 [cited 2020
3 Oct 16];108(25):10266–71. Available from: /pmc/articles/PMC3121834/?report=abstract

4 Thi DVL, Debing Y, Wu X, Rice CM, Neyts J, Moradpour D, et al. Sofosbuvir Inhibits Hepatitis e Virus Replication in
5 Vitro and Results in an Additive Effect When Combined with Ribavirin. *Gastroenterology* [Internet]. Elsevier, Inc;
6 2016;150(1):82-85.e4. Available from: <http://dx.doi.org/10.1053/j.gastro.2015.09.011>

7 Turner J, Ch'ng CL. Acute hepatitis E with coexistent plasmodium falciparum infection in a patient with a history of
8 foreign travel. *Am. J. Case Rep.* 2008;9:252–4.

9 WHO. Hepatitis E: Key facts [Internet]. 2020. Available from:
10 <https://www.who.int/news-room/fact-sheets/detail/hepatitis-e>

11 Wilson A, Ndam N, Nsangou O, Njoya M, Njouom R, Kowo M, et al. Seroprevalence of Hepatitis E Virus Infection and
12 Factors Associated in HIV Infected Patients in Yaoundé (Cameroon). *Open J. Gastroenterol.* 2020;10:181–6.

13 Yang J-M, Shen T-W. A pharmacophore-based evolutionary approach for screening selective estrogen receptor
14 modulators,. *Proteins Struct. Funct. Bioinformatics.* 2005;59:205–20.

15 Zhang S, Qu C, Wang Y, Wang W, Ma Z, Peppelenbosch MP, et al. Conservation and variation of the hepatitis E virus
16 ORF2 capsid protein. *Gene. Elsevier B.V.*; 2018 Oct 30;675:157–64.

17 Zhang Y, Vermeulen NPE, Commandeur JNM. Characterization of human cytochrome P450 mediated bioactivation of
18 amodiaquine and its major metabolite N-desethylamodiaquine. *Br. J. Clin. Pharmacol.* [Internet]. Blackwell Publishing
19 Ltd; 2017 [cited 2021 Mar 28];83(3):572–83. Available from: /pmc/articles/PMC5306493/

20 Zheng Q, Jiang J, He M, Zheng Z, Yu H, Li T, et al. Viral neutralization by antibody-imposed physical disruption. *Proc.*
21 *Natl. Acad. Sci. USA.* 2019;116(52):26933–40.

Table 1: Docking scores of drugs on HEV protease (6NU9) and polymerase (RdRp)

Ligand	Energy ^a (kcal/mol)	VDW ^b (kcal/mol)	Hbond ^c (kcal/mol)	Elec ^d (kcal/mol)
Zn-binding non structural protein of HEV (6NU9)				
Amodiaquine	-89.9	-76.56	-12.84	0
N-desethylamodiaquine	-93.5	-77.03	-16.46	0
Lumefantrine	-86.01	-86.01	0	0
Chloroquine	-81.08	-73.87	-7.2	0
Mefloquine	-76.42	-63.61	-12.81	0
Hydroxychloroquine	-76.37	-60.94	-15.43	0
Quinine	-75.64	-60.63	-15.01	0
Pyrimethamine	-63.59	-50.66	-12.93	0
Artemisinin	-63.44	-50.7	-12.74	0
Sofosbuvir	-98.22	-78.04	-20.17	0
Ribavirin	-80.83	-53.13	-27.71	0
RNA-dependent RNA polymerase of HEV				
Amodiaquine	-93.91	-89.25	-4.67	0
N-desethylamodiaquine	-86.02	-80.02	-6	0
Lumefantrine	-102	-102	0	0
Chloroquine	-86.63	-86.63	0	0
Mefloquine	-86.76	-77.71	-9.05	0
Hydroxychloroquine	-83.02	-77.53	-5.49	0
Quinine	-84.11	-75.74	-8.38	0
Pyrimethamine	-73.88	-57.57	-16.31	0
Artemisinin	-74.05	-52.27	-21.79	0
Sofosbuvir	-113.86	-101.34	-12.52	0
Ribavirin	-84.58	-54.12	-30.46	0

Table 2 : Docking scores of antimalarial drugs and anti-hepatitis C drugs on HEV capsid proteins

CryoEM Structure of HEV VLP, genotype 1 (6LAT)					
1					
2					
3					
4	Amodiaquine	-90.16	-76.95	--13.21	0
5	N-desethylamodiaquine	-84.35	-68.02	-16.32	0
6					
7	Lumefantrine	-90.42	-85.08	-5.34	0
8					
9	Chloroquine	-78.62	-67.33	-11.29	0
10					
11	Mefloquine	-85.95	-80.17	-5.77	0
12					
13	Hydroxychloroquine	-82.98	-70.38	-12.36	0
14					
15	Quinine	-78.51	-67.9	-10.62	0
16					
17	Pyrimethamine	-97.99	-80.45	-17.55	0
18					
19	Artemisinin	-74.31	-58.37	-15.94	0
20					
21	Sofosbuvir	-92.24	-88.74	-3.5	0
22					
23	Ribavirin	-99.33	-51.8	-47.52	0
24					
HEV capsid protein ORF-2, genotype 3 (2ZTN)					
25					
26	Amodiaquine	-102.06	-95.06	-7	0
27					
28	N-desethylamodiaquine	-102.65	-92.19	-10.46	0
29					
30	Lumefantrine	-89.43	-83.43	-6	0
31					
32	Chloroquine	-86.49	-80.57	-5.92	0
33					
34	Mefloquine	-91.46	-88.96	-2.5	0
35					
36	Hydroxychloroquine	-89.07	-75.73	-13.34	0
37					
38	Quinine	-98.23	-91.75	-6.48	0
39					
40	Pyrimethamine	-105.17	-74.71	-30.46	0
41					
42	Artemisinin	-81.49	-71.65	-5.92	0
43					
44	Sofosbuvir	-106.96	-70.94	-36.02	0
45					
46	Ribavirin	-96.04	-65.73	-30.31	0
47					
E2s domain, genotype 1 (3GGQ)					
48					
49	Amodiaquine	-93.64	-80.9	-12.74	0
50					
51	N-desethylamodiaquine	-84.32	-70.45	-13.87	0
52					
53	Lumefantrine	-106.05	-95.44	-10.61	0
54					
55	Chloroquine	-81.51	-78.01	-3.5	0
56					
57	Mefloquine	-88.41	-78.07	-10.34	0
58					
59	Hydroxychloroquine	-90.55	-75.16	-15.39	0
60					
61	Quinine	-83.61	-75.21	-8.4	0
62					
63	Pyrimethamine	-85.06	-55.04	-30.02	0
64					
65	Artemisinin	-71.89	-64.23	-7.67	0
	Sofosbuvir	-99.81	-78.67	-21.14	0
	Ribavirin	-96.99	-57.87	-39.11	0

Table 3 : Receptor-ligand interactions of the top docking drugs with the different HEV targets

Target		Sofosbuvir	Ribavirin	Amodiaquine	N-desethyl Amodiaquine	Pyrimethamine	Lumefantrine
6NU9	H-bond	Ser92, Thr102 , Ala105, Arg113			Gln91, Thr 102 , Tyr103, Arg122		
	VdW	Gln91, Ser92, Thr102, Tyr103, Ala104, Ala105, Glu111, Val112, Arg113, Arg122			Gln91, Ser92, Thr102, Tyr103, Ala104, Glu111, Arg113, Arg122		Gln91, Ser92, Thr102, Tyr103, Ala104, Glu111, Val112, Arg113, Arg122
6LAT	H-bond	Ser403	Pro142, Thr144, Ser146, Leu155, Asp168, Arg322, Ser324, Thr326, Arg437			Ser161, Thr272, Tyr443	Asn560
	VdW	Met350, Lys351, Phe355, Val402, Ser403, Ala404, Gly406, Glu407, Pro408	Thr144, Asp168, Arg322, Arg 437			Pro159, Leu163, Leu164, Asp442, Tyr443	Gln531, Tyr532, Tyr559, Ala565, Ser566, Asp567, Gln568, Ser582
2ZTN	H-bond	Arg366, Gly367, Gln420, Asp444, Gln446			Tyr561, Ser566, Asp567	Gly543, Tyr561, Asn562, Thr563, Thr564, Ser566	
	VdW	Arg366, Arg399, Gln420, Gln421, Asp422, Asp444, Gln446			Gly543, Lys544, Leu545, Phe547, Lys554, Tyr 561, Asn562, Thr564, Ser566, Asp567, Thr583, Tyr584	Gly543, Lys544, Leu545, Phe547, Tyr561, Asn562, Thr564, Ser566, Asp567, Thr583, Tyr584	
3GGQ	H-bond	Lys544, Asn560, Ser566, Asp567, Gln568, Ser582					Thr489
	VdW	Tyr559, Asn560, Ala565, Ser566, Asp567, Gln568, Ser582					Gly486, Ser487, Thr489, Gly490 , Val492, Gln531, His532, Tyr559, Asn560,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Asp567,
Gln568,
Leu570,
Ile581, Ser582

RdRp	H-bond	Lys31, Trp42, Trp46	Lys32	
	VdW	Trp30, Lys31, Lys32, His33, Glu 36, Gly 38, Trp42, Asn43, Trp46	Trp30, Lys31, Lys32, His33, Glu 36, Gly 38, Trp42, Asn43, Trp46	Phe29, Trp30, Lys31, Lys32, His33, Glu 36, Gly 38, Trp42, Asn43, Trp46

Table 4 : Functions and ADMET properties of the different drugs

Drugs		AMQ	ART	LUM	CHL	OH-CHL	MFQ	PYR	QUI	RBV	SOF
Functions		Antimalarial drugs								Direct-acting antiviral agents in hepatitis C treatment	
A	Caco-2 permeability	Good -4.964	Good -4.64	Low -5.34	Good -4.62	Good -4.59	Good -5.11	Low -5.47	Good -4.78	Low -5.65	Low -6.08
	MDCK permeability (cm/s)	Medium 1.4×10^{-5}	High 6×10^{-5}	High 6×10^{-5}	Medium 1.1×10^{-5}	Medium 1.1×10^{-5}	Medium 1.2×10^{-5}	High 3.9×10^{-5}	Medium 1.7×10^{-5}	Medium 1.1×10^{-5}	Medium 1.1×10^{-5}
	P-gp inhibitor	Poor	High	High	High	High	High	Poor	High	Poor	Poor
	P-gp substrate	Poor	Poor	High	High	High	Poor	High	High	Medium	Poor
	F ₃₀ %	Good	Good	Good	Good	Good	Good	Good	Good	Medium	Good
	HGI	Good	Good	Good	Good	Good	Good	Good	Good	Good	Good
D	BBB penetration	Medium	Medium	Poor	Poor	Poor	Good	Medium	Poor	Good	Good
	PPB (%)	97.33	71.64	99.91	84.03	69.60	91.86	88.83 %	83.78	15.12	37.207
	VD (L/kg)	2.4	1.34	2.99	3.2	2.25	5.34	0.79	2.32	0.6	1.09
M	CYP inhibitors	1A2,2C19,2D6,	1A2,2C19,2D6	1A2,2C19,2D6	1A2,2D6	1A2,2D6	1A2,2D6	1A2	2D6	-	
	CYP substrates	1A2, 2D6		1A2, 3A4	1A2, 2D6	1A2, 2D6	2C19, 2D6	1A2	1A2, 2D6, 2C19	-	2C9
E	Clearance (mL/min/kg)	Good 7.5	Good 10.56	Good 7.57	Good 6.16	Good 6.43	Poor 2.82	Poor 3.89	Poor 1.89	Poor 4.2	Good 6.43
	T _{1/2}	Medium	Medium	Short	Short	Short	Short	High	Short	High	High
Predicted Toxicity	hERG blockers	Poor	High	High	High	High	High	High	Medium	Poor	Poor
	H-HT	Medium	High	High	High	High	High	High	High	Medium	High
	Carcinogenicity	Low	High	Low	Low	Low	Low	High	Medium	Poor	Poor
	Respiratory Toxicity	High	High	Medium	High	High	High	High	High	Poor	Medium

AMQ : Amodiaquine ; ART : Artemisinin ; LUM : Lumefantrine ; CHL : Chloroquine ; OH-CHL : Hydroxychloroquin ; MFQ : Mefloquine ; PYR : Pyrimethamine ; QUI : Quinine ; RBV : Ribavirin ; SOF : Sofosbuvir

Figures Legends

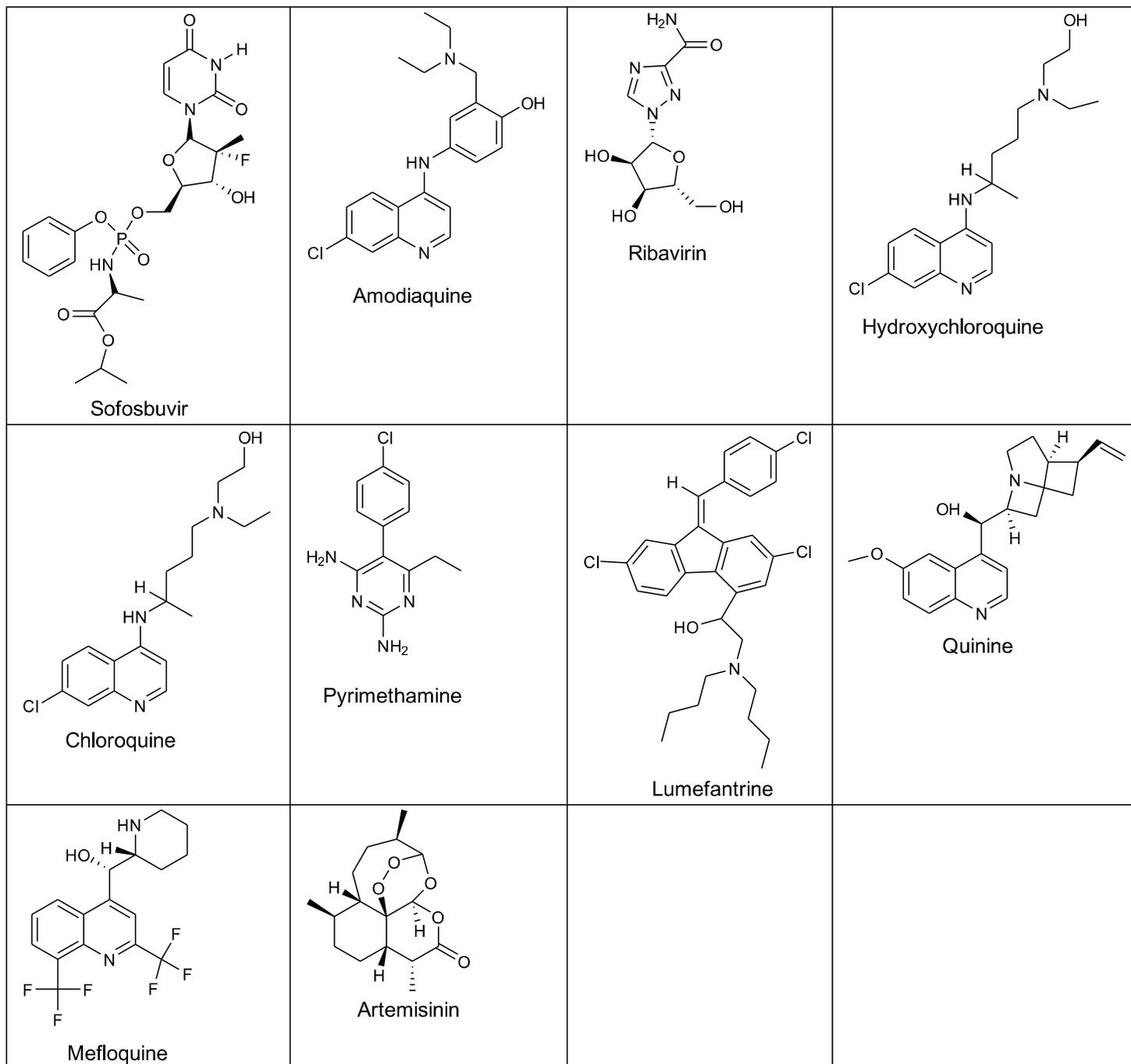
Figure 1 : 2D structures of the docked antimalarial and anti-hepatitis C drugs

Figure 2: Binding interactions of the best antimalarial drugs docked against the HEV replication targets in comparison with sofosbuvir. (a) hydrophobic interactions of Sofosbuvir with the amino acid residues of the active site of 6NU9; (b) interaction of Amodiaquine with the active site of 6NU9; (c) 3D conformations of N-desethylamodiaquine (in violet) and Sofosbuvir (in blue) in the binding site of 6NU9 ; (d) 3D conformations of Lumefantrine (in blue) and Sofosbuvir (in violet) in the binding site of RdRp. (e-f) Hydrophobic amino acid residues interacting in the binding site with Sofosbuvir, and lumefantrine respectively

Figure 3 : Binding interactions of the best antimalarial drugs docked against the HEV capsid proteins in comparison with Sofosbuvir and Ribavirin. (a) Interactions of Pyrimethamine and Ribavirin with the conserved domains of the 6LAT target ; (b) 2D conformations of Lumefantrine with the E2s domain (3GGQ) showing hydrophobic interactions with Gly486, Ser487, Thr489, Gly490, Val492, Gln531, His532, Tyr559, Asn560, Asp567, Gln568, Leu570, Ile581, Ser582 ; (c) 3D conformations of Lumefantrine (in blue) and Sofosbuvir (in violet) in the 3GGQ target. (b) 2D conformations of Lumefantrine with the E2s domain (3GGQ) showing hydrophobic contacts with Gly486, Ser487, Thr489, Gly490, Val492, Gln531, His532, Tyr559, Asn560, Asp567, Gln568, Leu570, Ile581, Ser582 ; (c) 3D conformations of Lumefantrine (in blue) and Sofosbuvir (in violet) in the 3GGQ target. (d, e) 2D and 3D conformations of Lumefantrine and Sofosbuvir in 2ZTN.

Figure 4: Replication efficiency of the HEV p6GLuc replicon in the presence of Lumefantrine (a), Amodiaquine (b) and N-desethylamodiaquine (c) at the concentrations of 1, 5 and 10 μ M. The p6GLuc GAD mutant and the p6GLuc inhibited by Sofosbuvir (20uM) served as negative controls. The replication folds were normalized to 1dpe. N=3.

Figure 5: Replication efficiencies (a) and level of cell viability (b) in PLC3 replicon cells treated with the compounds Sofosbuvir (20uM), Lumefantrine, Amodiaquine, and N-desethylamodiaquine at 5dpe. The replication efficiencies were plotted as percentages of the untreated p6Gluc replication fold. N=3. Viability cell was depicted as intensity of the optic density (OD) at 490 nm.



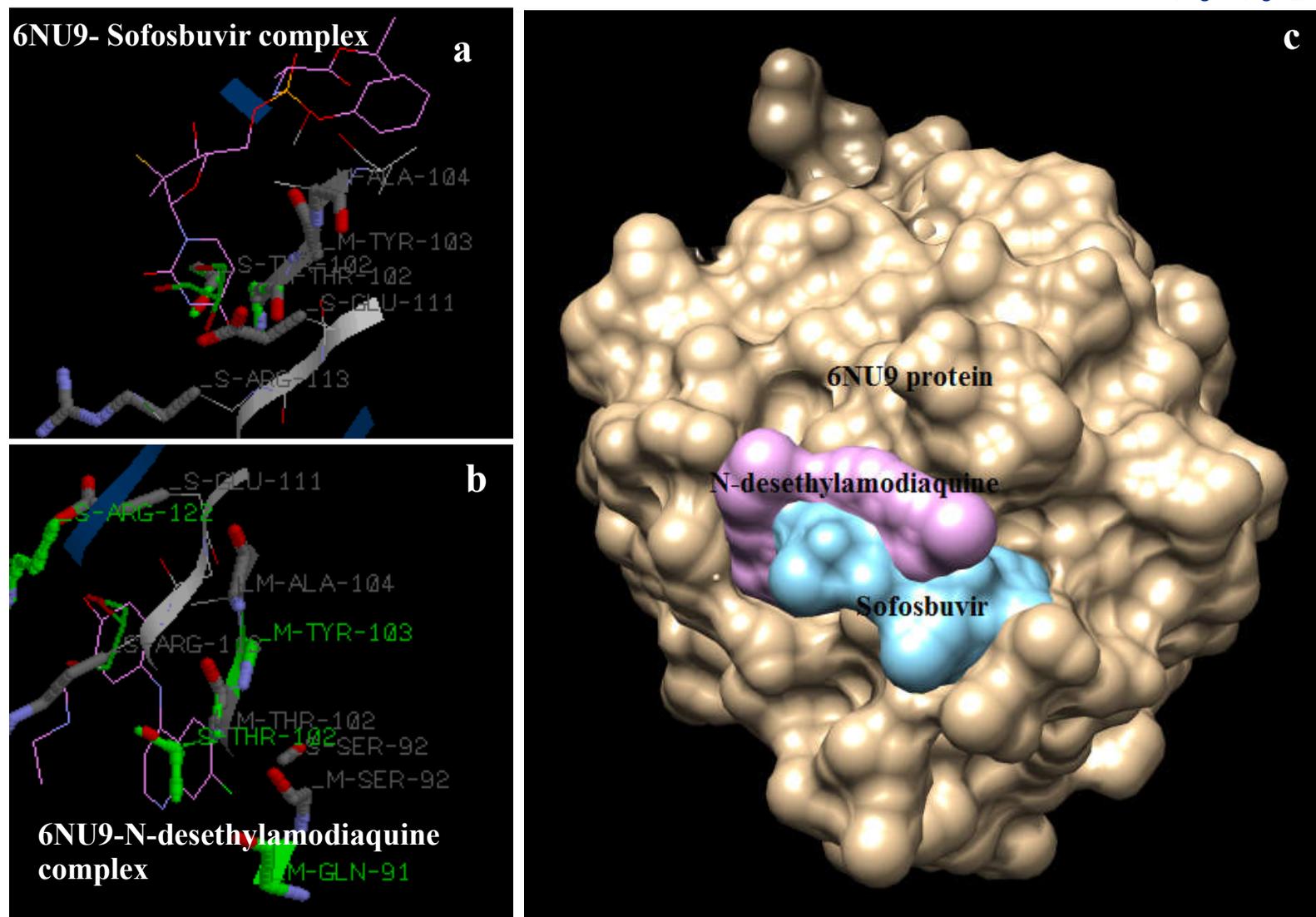


Figure 2

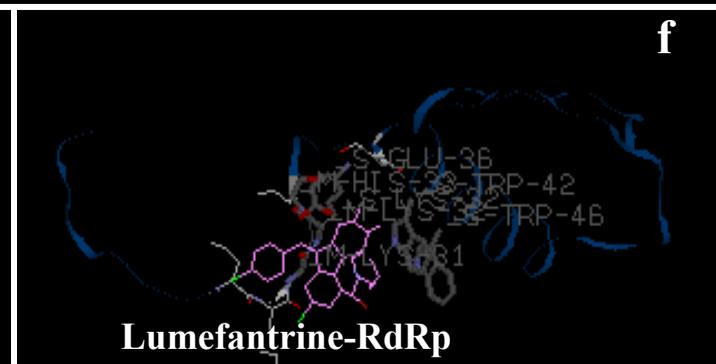
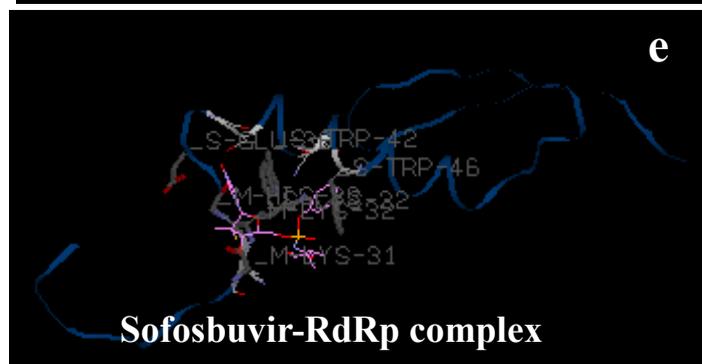
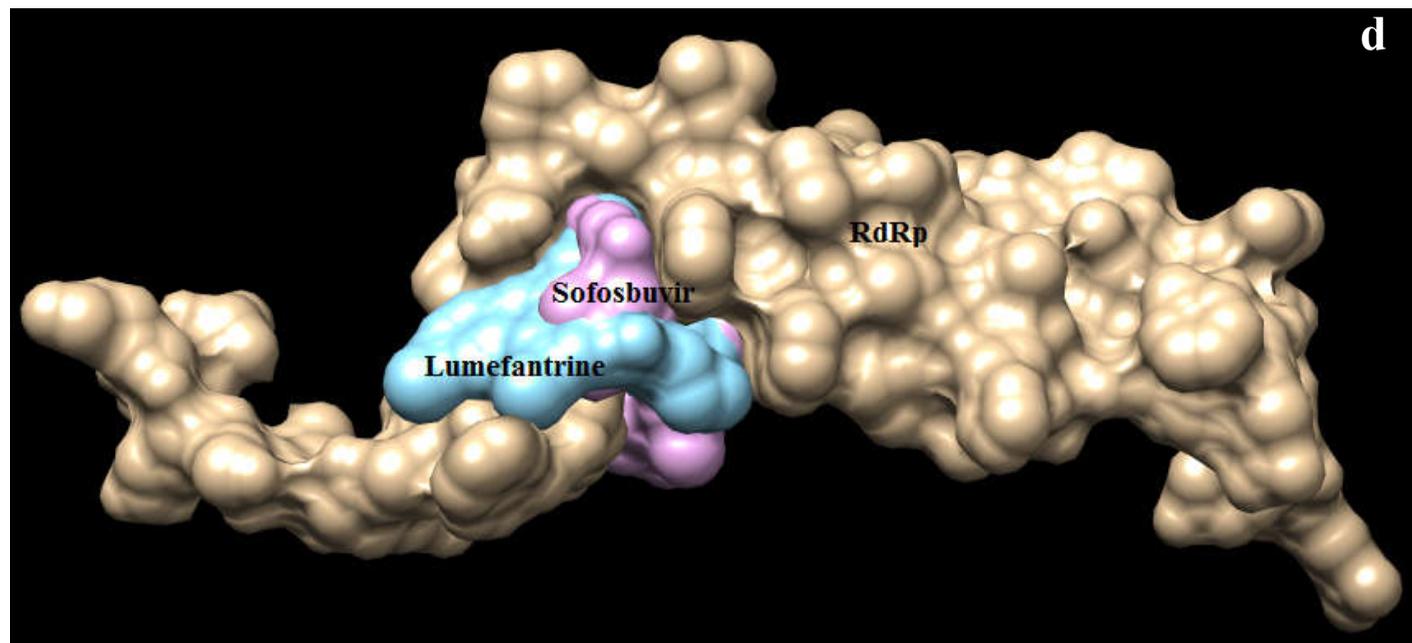


Figure 2 (next and end)

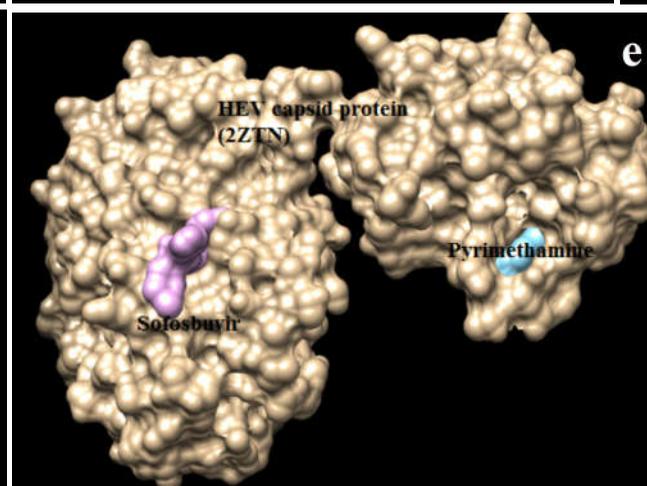
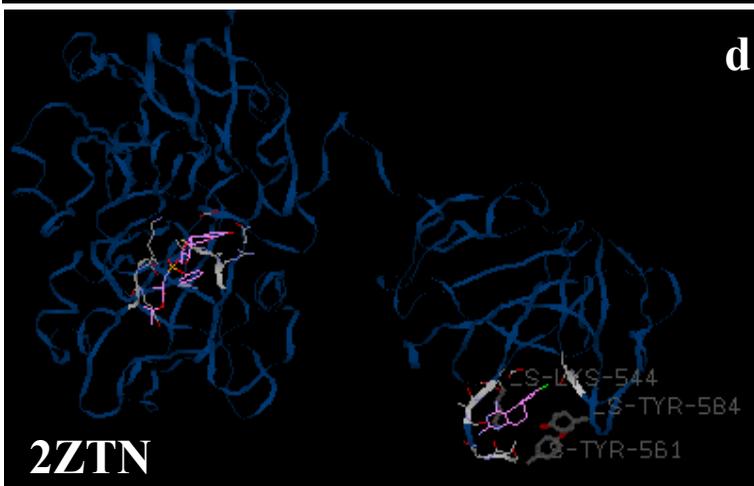
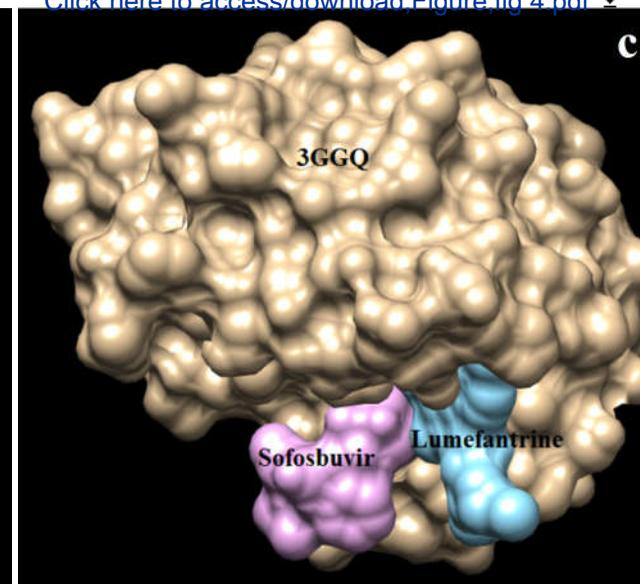
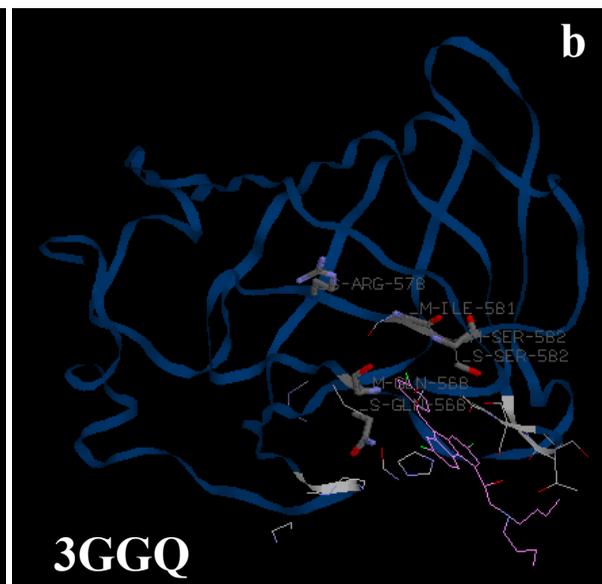
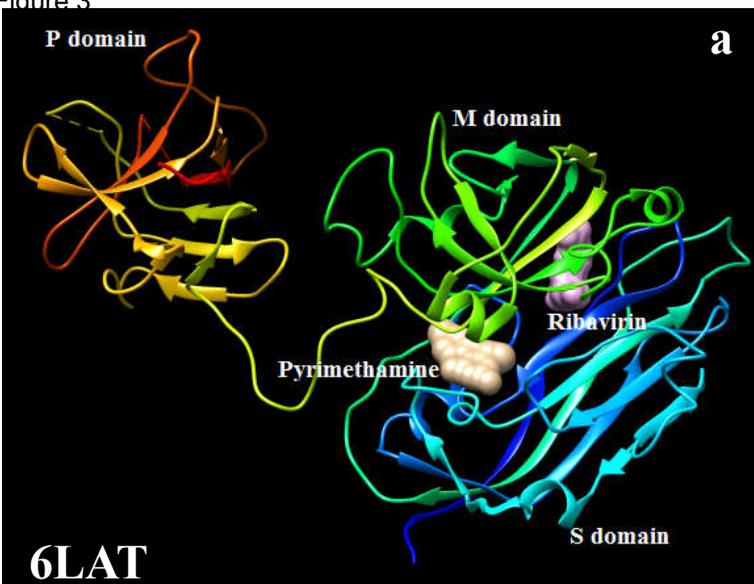


Figure 3

Figure 4

[Click here to access/download;Figure;Figure 4.pdf](#)

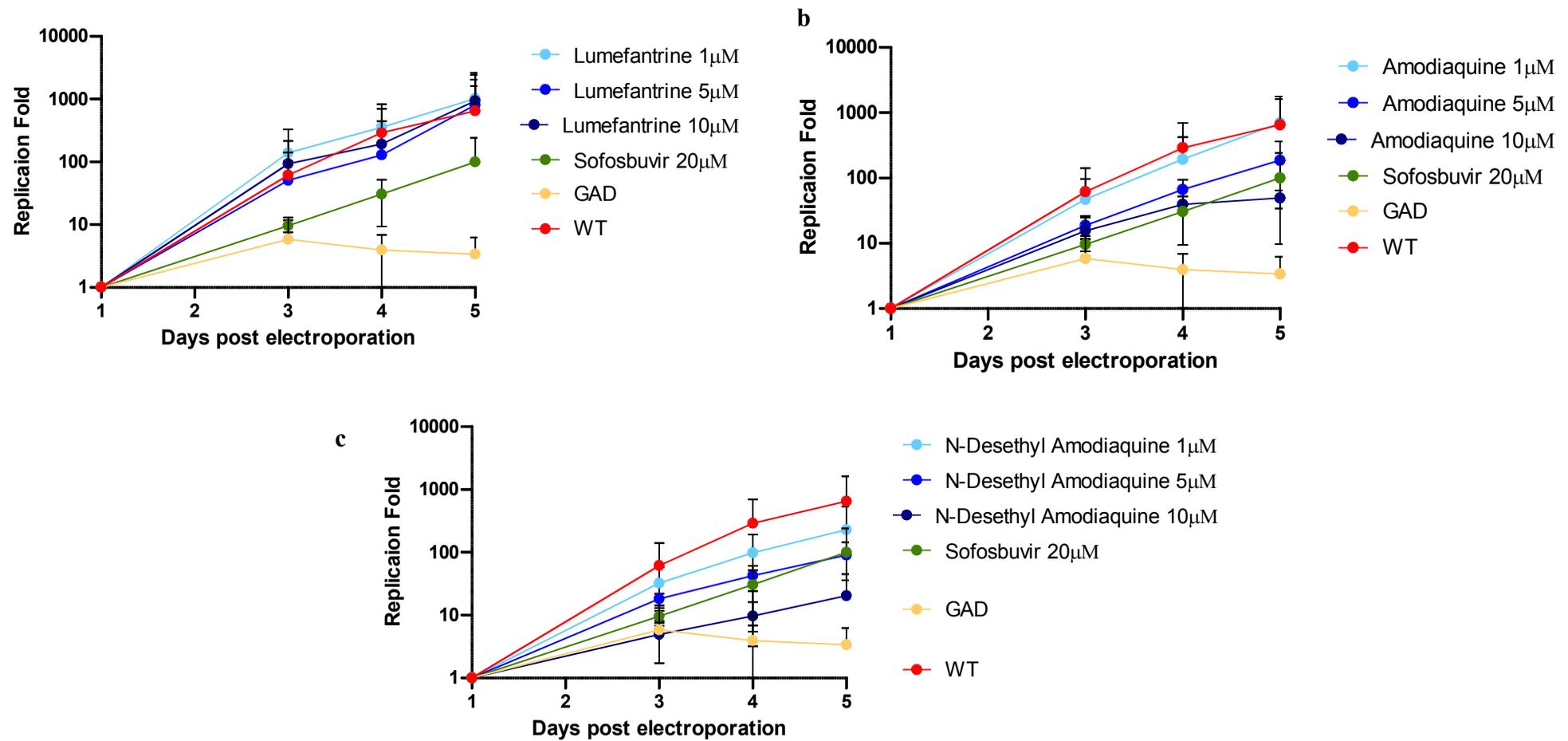


Figure 4

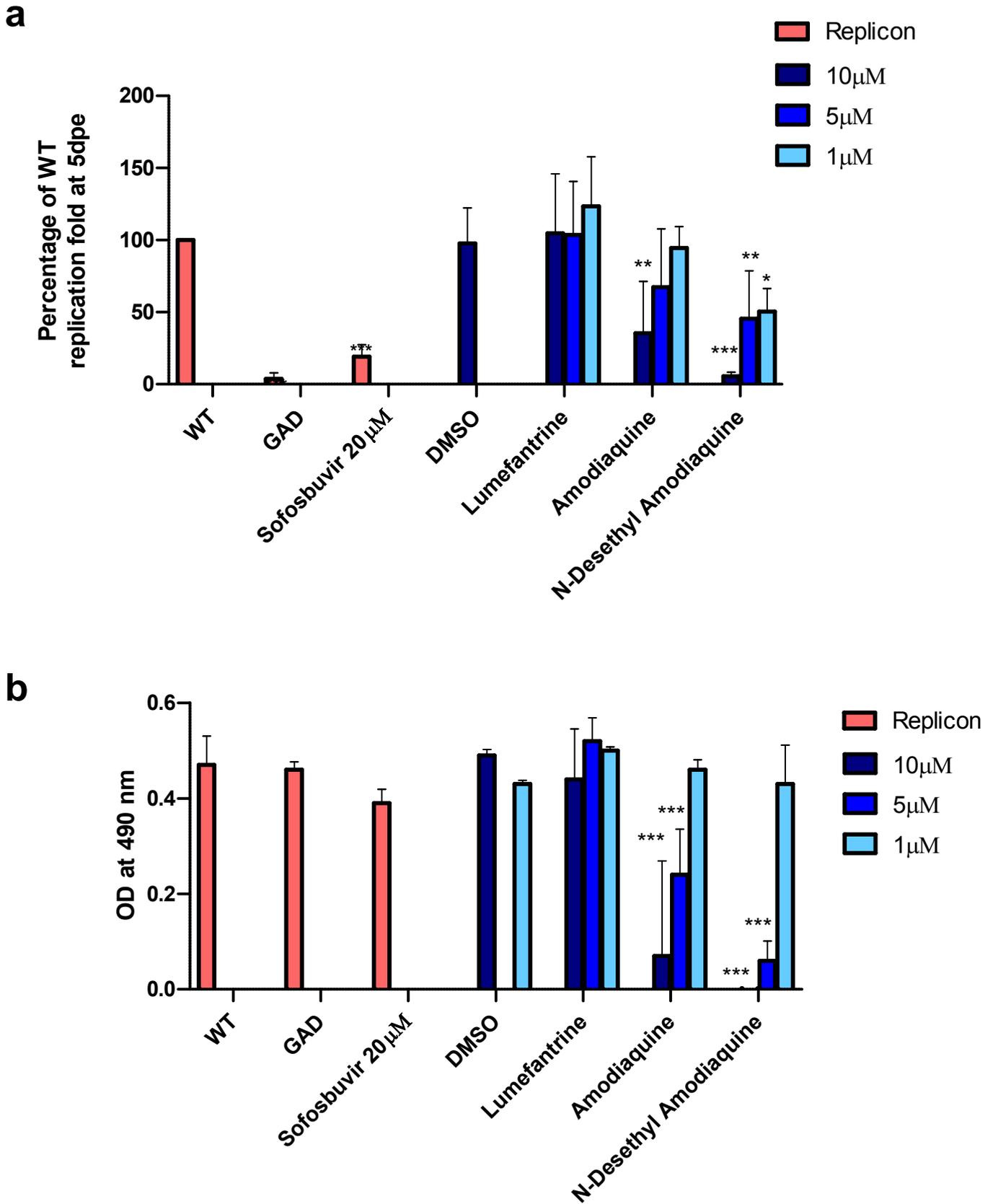
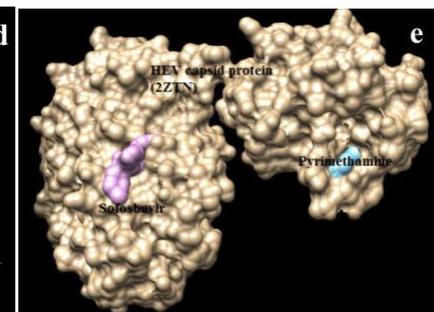
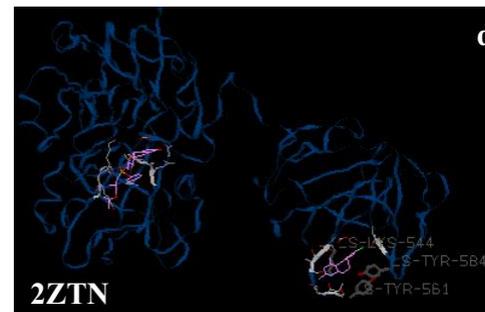
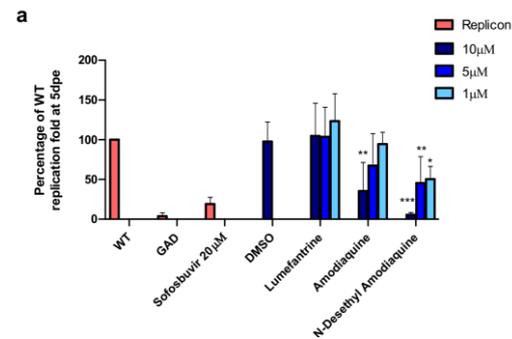
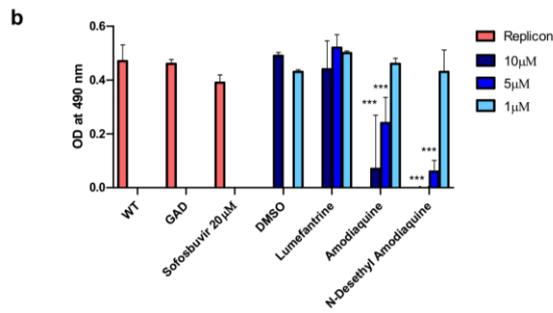
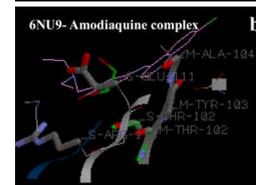
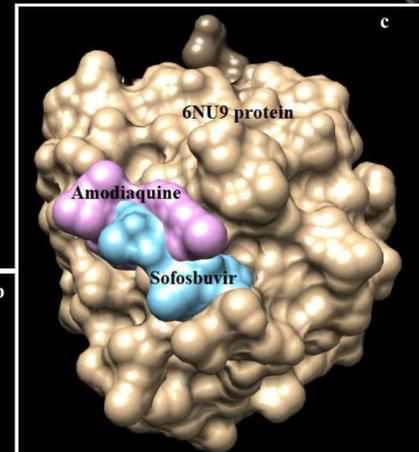
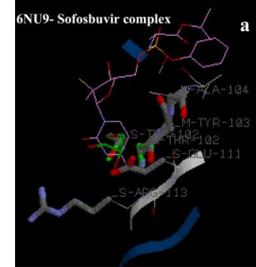
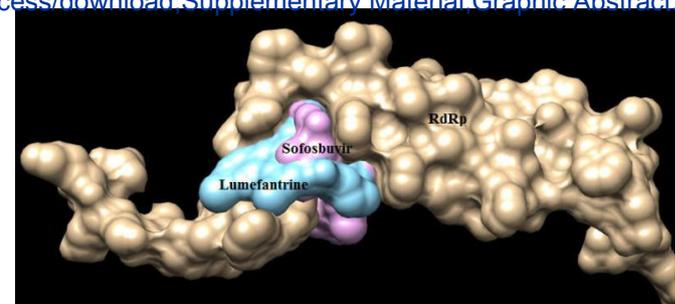
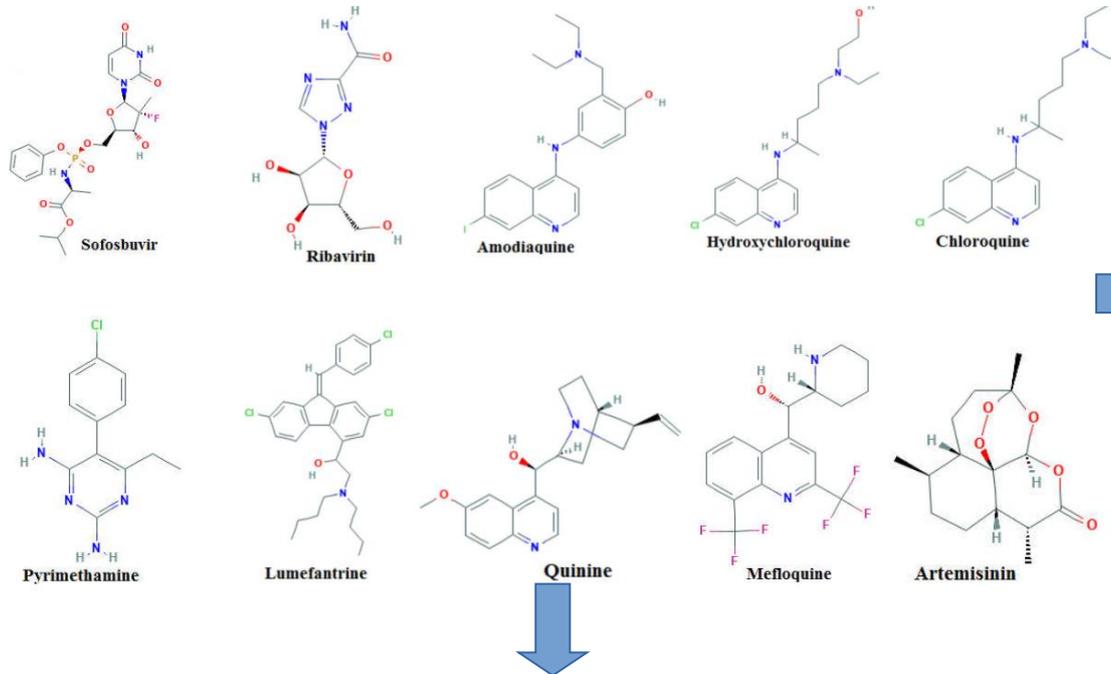
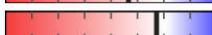
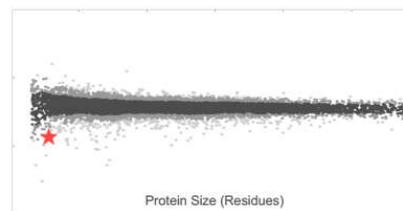
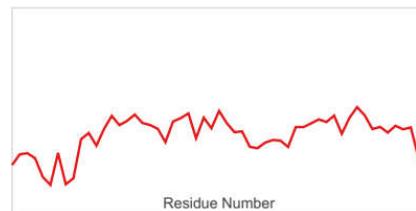


Figure 5



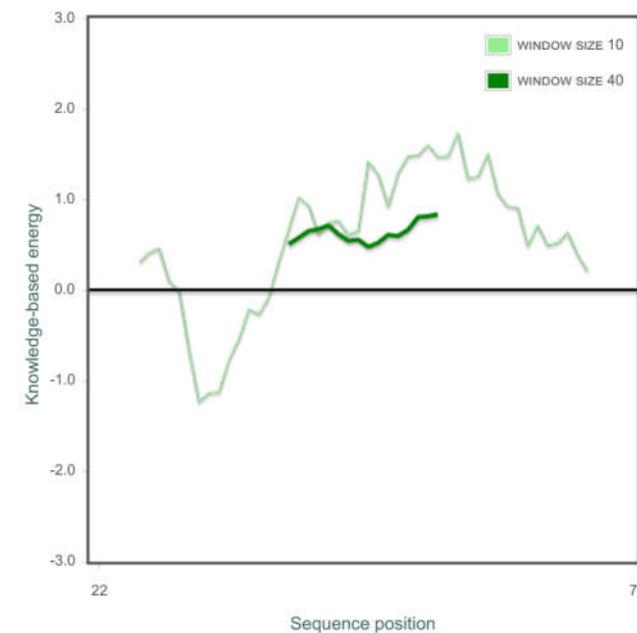
Model #02	File	Built with	Oligo-State	Ligands	GMQE	QMEAN
	PDB	ProMod3 3.2.0	monomer	None	0.18	-2.51

QMEAN		-2.51
C β		-1.34
All Atom		-0.28
solvation		-2.26
torsion		-1.54



Template	Seq Identity	Oligo-state	QSQE	Found by	Method	Resolution	Seq Similarity	Range	Coverage	Description
6r1i.2.A	23.64	monomer	0.00	HHblits	X-ray	2.63Å	0.30	22 - 76	0.61	Genome polyprotein

The template contained no ligands.



Supplementary Figure 1

Borris Rosnay Tietcheu Galani, Ph.D.
Laboratory of Applied Biochemistry
Department of Biological Sciences
Faculty of Science
University of Ngaoundere
P.O. Box 454 Ngaoundere, Cameroon
Email: b.tietcheu@gmail.com

To the Editorial Office of "*In silico Pharmacology*"

April 4^h, 2021

Subject: Cover letter for the revised version (R2) of our manuscript

Dear Editor-in-chief,

Thank you for your prompt review of our manuscript entitled : **In silico and in vitro Screening of Licensed Antimalarial Drugs for Repurposing as Inhibitors of Hepatitis E Virus.**

We wish to thank reviewer 2 for the constructive comments. We greatly appreciate the opportunity to improve our manuscript and found the suggestions very helpful.

In response to your email dated 04 April, we have made efforts to address the reviewer's concerns. N-desethylamodiaquine was docked against the different viral targets and the binding energy we found for 6NU9 and the viral polymerase allowed us to discuss the correlations between the in silico activity and the inhibitory activity observed on replicon cell lines.

The reviewers' comments have been addressed point by point and are appended to this letter. We also highlighted changes in coloring text in yellow.

We look forward to your response and hope the revisions will enable you to accept this version of our manuscript.

Thank you for your consideration of this manuscript.

Sincerely yours,

Dr. Borris Rosnay Tietcheu Galani
The Corresponding author

Responses to Reviewers comments

Comments to the author (if any):

Reviewer #2: It would be good if you can show some correlations between the docking energies and experimental data

We thank the reviewer for this particular comment. N-desethylamodiaquine was docked against the different viral targets and especially against the targets of the viral replication (6NU9 and RdRp) and the results obtained were newly discussed. We found that Sofosbuvir, Amodiaquine, and N-desethylamodiaquine were positively correlated to viral inhibition as in silico data and antiviral effect go in the same direction while Lumefantrine is negatively correlated to the viral inhibition. This was stated in the discussion section, pages 12 and 13.

Change of authorship request form (pre-acceptance)

Please read the important information on page 4 before you begin

This form should be used by authors to request any change in authorship including changes in corresponding authors. Please fully complete all sections. Use black ink and block capitals and provide each author's full name with the given name first followed by the family name.

Please note: In author collaborations where there is formal agreement for representing the collaboration, it is sufficient for the representative or legal guarantor (usually the corresponding author) to complete and sign the Authorship Change Form on behalf of all authors.

Section 1: Please provide the current title of manuscript

(For journals: Please provide the manuscript ID, title and/or DOI if available.)

(For books: Please provide the title, ISBN and/or DOI if available.)

Manuscript ID no. in case of unpublished manuscript:

INSP-D-20-00147R1

DOI in case of published manuscript:

ISBN (for books):

Title: In silico and in vitro Screening of Licensed Antimalarial Drugs for Repurposing as Inhibitors of Hepatitis E Virus

Section 2: Please provide the previous authorship, in the order shown on the manuscript before the changes were introduced. Please indicate the corresponding author by adding (CA) behind the name.

	First name(s)	Family name	ORCID or SCOPUS id, if available
1 st author	Borris Rosnay	Tietcheu Galani (CA)	https://orcid.org/0000-0002-4324-5951
2 nd author	Vincent Brice	Ayissi Owona	https://orcid.org/0000-0002-2380-527X
3 rd author	Romeo Joel	Guemmogne Temdie	https://orcid.org/0000-0002-1487-6332
4 th author	Marie	Atsama Amougou	https://orcid.org/0000-0001-6662-9045
5 th author	Dieudonne Pascal	Djamen Chuisseu	https://orcid.org/0000-0001-9787-4449
6 th author	Arnaud	Fondjo Kouam	https://orcid.org/0000-0002-2629-6394
7 th author	Marcelline	Ngounoue Djuidje	https://orcid.org/0000-0003-0031-3958
8 th author	Paul	Fewou Moundipa	https://orcid.org/0000-0002-2694-5569

Please use an additional sheet if there are more than 7 authors.

Change of authorship request form (pre-acceptance)

Section 3: Please provide a justification for change. Please use this section to explain your reasons for changing the authorship of your manuscript, e.g. what necessitated the change in authorship? Please refer to the (journal) policy pages for more information about authorship. Please explain why omitted authors were not originally included and/or why authors were removed on the submitted manuscript.

In the first version of this manuscript, our data were only based on in silico screening. After a discussion with our research group, our partners from the University of Lille 2 decided to bring us their contributions by carrying out in vitro tests with some of these antimalarial drugs on HEV replicons in order to validate the bio computational predictions. In this framework, Ms. Karoline Metzger (KM), Prof. Cécile-Marie Aliouat-Denis (MCAD), and Prof. Laurence Cocquerel made substantial contributions to the design of the protocol, the reagents acquisition, course of experiment itself, the analysis or interpretation of data of this part of our study. With their approval, their names were added to this paper for this revised version.

Section 4: Proposed new authorship. Please provide your new authorship list in the order you would like it to appear on the manuscript. Please indicate the corresponding author by adding (CA) behind the name. If the corresponding author has changed, please indicate the reason under section 3.

	First name(s)	Family name (this name will appear in full on the final publication and will be searchable in various abstract and indexing databases)
1 st author	Borris Rosnay	Tietcheu Galani (CA)
2 nd author	Vincent Brice	Ayissi Owona
3 rd author	Romeo Joel	Guemmogne Temdie
4 th author	Karoline	Metzger
5 th author	Marie	Atsama Amougou
6 th author	Dieudonne Pascal	Djamen Chuisseu
7 th author	Arnaud	Fondjo Kouam
8 th author	Marcelline	Ngounoue Djuidje
9 th author	Cécile-Marie	Aliouat-Denis
10 th author	Laurence	Cocquerel
11 th author	Paul	Fewou Moundipa

Please use an additional sheet if there are more than 7 authors.

Change of authorship request form (pre-acceptance)

Section 5: Author contribution, Acknowledgement and Disclosures. Please use this section to provide a new disclosure statement and, if appropriate, acknowledge any contributors who have been removed as authors and ensure you state what contribution any new authors made (if applicable per the journal or book (series) policy). **Please ensure these are updated in your manuscript - after approval of the change(s) - as our production department will not transfer the information in this form to your manuscript.**

State 'Not applicable' if there are no new authors.

New acknowledgements: Not applicable

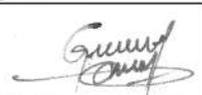
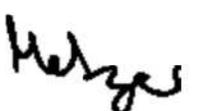
New Disclosures (financial and non-financial interests, funding): Not applicable

New Author Contributions statement (if applicable per the journal policy): Not applicable for this journal

Change of authorship request form (pre-acceptance)

Section 6: Declaration of agreement. All authors, unchanged, new and removed must sign this declaration.

(NB: Please print the form, sign and return a scanned copy. Please note that signatures that have been inserted as an image file are acceptable as long as it is handwritten. Typed names in the signature box are unacceptable.) * Please delete as appropriate. Delete all of the bold if you were on the original authorship list and are remaining as an author.

	First name	Family name		Signature	Affiliated institute	Date
1 st author	Borris Rosnay	Tietcheu Galani	I agree to the proposed new authorship shown in section 4		University of Ngaoundere Faculty of Science, Department of Biological Sciences P.O. Box 454 Ngaoundere, Cameroon	29.03.2021
2 nd author	Vincent Brice	Ayissi Owona	I agree to the proposed new authorship shown in section	 Dr OWONA AYISSI Vincent Brice	University of Yaounde I, Faculty of Science, Department of Biochemistry P.O. Box 812 Yaounde, Cameroon	30.03.2021
3 rd author	Romeo Joel	Guemmogne Temdie	I agree to the proposed new authorship shown in section		University of Ngaoundere Faculty of Science, Department of Biological Sciences P.O. Box 454 Ngaoundere, Cameroon	30.03.2021
4 th author	Karoline	Metzger	I agree to the proposed new authorship shown in section		University of Lille, CNRS, INSERM, CHU Lille, Pasteur Institute of Lille, U1019-UMR 9017-CIIL- Center for Infection and Immunity of Lille, F- 59000 Lille, France	29.03.2021
5 th author	Marie	Atsama Amougou	I agree to the proposed new authorship shown in section 4		Virology Unit, Research Center for Emerging and Reemerging infectious Diseases (CREMER-IMP), Yaounde, P.O. Box 906 Yaounde, Cameroon.	29.03.2021
6 th author	Dieudonne Pascal	Djamen Chuisseu	I agree to the proposed new authorship shown in section 4		Université des Montagnes Higher Institute of Health Sciences, PO. Box 208 Bangangte, Cameroon	29.03.2021



Change of authorship request form (pre-acceptance)

7 th author	Arnaud	Fondjo Kouam	I agree to the proposed new authorship shown in section 4		University of Yaounde I, Faculty of Science, Department of Biochemistry P.O. Box 812 Yaounde, Cameroon	29.03.2021
8 th author	Marcelline	Ngounou Djuidje	I agree to the proposed new authorship shown in section	 <small>Marcelline Djuidje Ngounou, Ph.D. Associate Professor, University of Yaounde I Faculty of Science, Yaounde, Cameroon</small>	University of Yaounde I, Faculty of Science, Department of Biochemistry P.O. Box 812 Yaounde, Cameroon	31.03.2021
9 th author	Cécile-Marie	Aliouat-Denis	I agree to the proposed new authorship shown in section 4		University of Lille, CNRS, INSERM, CHU Lille, Pasteur Institute of Lille, U1019-UMR 9017-CIIL- Center for Infection and Immunity of Lille, F- 59000 Lille, France	29.03.2021
10 th author	Laurence	Cocquerel	I agree to the proposed new authorship shown in section 4		University of Lille, CNRS, INSERM, CHU Lille, Pasteur Institute of Lille, U1019-UMR 9017-CIIL- Center for Infection and Immunity of Lille, F- 59000 Lille, France	29.03.2021
11 th author	Paul	Fewou Moundipa	I agree to the proposed new authorship shown in section 4		University of Yaounde I, Faculty of Science, Department of Biochemistry P.O. Box 812 Yaounde, Cameroon	30.03.2021

Please use an additional sheet if there are more than 7 authors.

Important information. Please read.

- Please return this form, fully completed, to Springer Nature. We will consider the information you have provided to decide whether to approve the proposed change in authorship. We may choose to contact your institution for more information or undertake a further investigation, if appropriate, before making a final decision.
- By signing this declaration, all authors guarantee that the order of the authors are in accordance with their scientific contribution, if applicable as different conventions apply per discipline, and that only authors have been added who made a meaningful contribution to the work.
- Please note, we cannot investigate or mediate any authorship disputes. If you are unable to obtain agreement from all authors (including those who you wish to be removed) you must refer the matter to your institution(s) for investigation. Please inform us if you need to do this.
- If you are not able to return a fully completed form within **30 days** of the date that it was sent to the author requesting the change, we may have to withdraw your manuscript. We cannot publish manuscripts where authorship has not been agreed by all authors (including those who have been removed).
- Incomplete forms will be rejected.