Emetine, a potent alkaloid for the treatment of SARS-CoV-2 targeting papain-like protease and non-structural proteins: pharmacokinetics, molecular docking and dynamic studies

Mejdi Snoussia,b, Alaeddine Redissic, Amor Mosbahd, Vincenzo De Feod, Mohd Adnana, Kaiss Aouadid, Mousa Alreshidia, Mitesh Pateld, Adel Kadrih,i and Emira Noumia,j

aDepartment of Biology, College of Science, University of Hail, Ha'il, Saudi Arabia; bLaboratory of Genetics, Biodiversity and Valorization of Bio-resources, Higher Institute of Biotechnology of Monastir, University of Monastir, Monastir, Tunisia; cISBST, BVBGR-LR11ES31, Biotechpole Sidi Thabet, University of Manouba, Ariana, Tunisia; dDepartment of Pharmacy, University of Salerno, Salerno, Italy; eDepartment of Chemistry, College of Science, Qassim University, Buraidah, Saudi Arabia; fFaculty of Science of Monastir, Laboratory of Hetrocyclic Chemistry, Natural Products and Reactivity, University of Monastir, Monastir, Tunisia; gBapalal Vaidya Botanical Research Centre, Department of Biosciences, Veer Narmad South Gujarat University, Surat, India; hFaculty of Science of Sfax, Department of Chemistry, University of Sfax, Sfax, Tunisia; iFaculty of Science and Arts in Baljurashi, Al Bahah University, Al Bahah, Saudi Arabia; jLaboratory of Bioresources: Integrative Biology and Valorization, (LR14-ES06), University of Monastir, Higher Institute of Biotechnology of Monastir, Monastir, Tunisia

CONTACT Mejdi Snoussi snmejdi@yahoo.fr

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ABSTRACT The main objective of this study is to find out the anti-SARS-CoV-2 potential of emetine by using molecular docking and dynamic simulation approaches. Interestingly, molecular docking studies suggest that Emetine showed significant binding affinity toward Nsp15 (-10.8 kcal/mol) followed by Nsp12 (-9.5 kcal/mol), RNA-dependent RNA polymerase, RdRp (-9.5 kcal/mol), Nsp16 (-9.4 kcal/mol), Nsp10 (-9.2 kcal/mol), Papain-like protein (-9.0 kcal/mol), Nsp13 (-9.0 kcal/mol), Nsp14 (-8.9 kcal/mol) and Spike Protein Receptor Domain (-8.8 kcal/mol) and chymotrypsin-like protease, 3CLpro (-8.5 kcal/mol), respectively, which are essential for viral infection and replication. In addition, molecular dynamic simulation (MD) was also performed for 140 ns to explore the stability behavior of the main targets and inhibitor complexes as well as the binding mechanics of the ligand to the target proteins. The obtained MD results followed by absolute binding energy calculation confirm that the binding of emetine at the level of the various receptors is more stable. The complex Emetine-Nsp15, mechanistically was stabilized as follows: Emetine first binds to the monomer, after, binds to the second inducing the formation of a dimer which in turn leading to the formation of complex that simulation stabilizes it at a value less than 5 Å. Overall, supported by the powerful and good pharmacokinetic data of Emetine, our findings with clinical trials may be helpful to confirm that Emetine could be promoted in the prevention and eradication of COVID-19 by reducing the severity in the infected persons and therefore can open possible new strategies for drug repositioning.

Abbreviations: SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; MERS: Middle East respiratory syndrome; NSP10 to 16: A non-structural proteins 12-15; RdRp RNA: dependent RNA polymerase; HIV: human immunodeficiency virus; MHV-A59: Coronavirus Mouse Hepatitis Virus-A59; hCoV-NL: Human coronavirus-NL; hCoV-OC43: Human coronavirus OC43

1. Introduction

The current highly infectious coronavirus disease 2019 (COVID-19) pandemic caused by a novel coronavirus, SARS-CoV-2 spreads across the globe leaving a serious damage to humanity and public health (Enayatkhani et al., 2021; Enmozhi et al., 2021). The anxiety and the fear caused by COVID-19 have challenged the scientific community and researchers to develop efficient and rapid therapies to combat this fatal situation. However, there are a few new described drugs active against this morbid disease, despite some vaccines which have shown their limits vis-à-vis the new mutant strains. As of 01 May 2021, more than 152,113,721 confirmed infected cases and 3,196,379 deaths (https://www.worldometers.info/coronavirus/) has been reported around the world suggesting that there is an urgent need for efficacious therapeutic strategies that are able to block the SARS-CoV-2 viral life cycle steps involved in the synthesis of the viral components including transmission, cell binding, enzymes, replication and assembly (Elfiky, 2020; Enmozhi et al., 2021; Khan et al., 2021; Zhou et al., 2020). SARS-CoV-2 shares a 79.5% of similarity sequence to SARS-
CoV and for some encoded proteins like coronavirus main proteinase (3CLpro), RNA-dependent RNA polymerase (RdRp) and papain-like protease (PLpro), the sequence identity can exceed 96% (Liu et al., 2020; Yang & Dubick, 1980). Thus, much intensive efforts were devoted to discovering novel anti-SARS-CoV-2 (Bhardwaj et al., 2021; Gyebi et al., 2021; Kumar et al., 2020). Rosa and Santos (2020) have identified 24 clinical trials as anti-SARS-CoV-2 in which 19 medicines are in phase 1, 2, and 3 of the clinical phases as drug candidates such as human immunoglobulin, interferons, chloroquine, hydroxychloroquine, arbidol, favipiravir, remdesivir, oseltamivir, ritonavir, lopinavir, bevacizumab, methylprednisolone and Traditional Chinese Medicines (TCM). In another recent study, the authors listed 22 drugs that work against COVID-19 infection. These drugs include fingolimod, colchicine, N4- hydroxyctydidine, remdesivir, methylprednisone, oseltamivir, icatibant, parphenazine, viracept, emetine, homoharringtonine, aloxistatin, ribavirin, valrubicin, famotidine, almitrine, ampenavir, hesperidin, biorobin, cromolyn sodium, and antibodies- tocilizumab and sarilumab. Moreover, a great attention has been devoted to the secondary metabolites from medicinal plants that have been demonstrated for their antiviral bioactivities such as kaempferol, quercetin, luteolin-7-glucoside, demethoxycurcumin, naringenin, apigenin-7-glucoside, oleanuropein, curcumin, catechin, epicatechin-gallate, zingerol, gingerol, and allicin as potent inhibitor candidates for anti-COVID-19. Chandel et al. (2020) have identified 19 potent inhibitors out of thousands compounds and found Nelfinavir, Rhein, Withanolide D, Withaferin A, Enoxacin and Aloe-emodin as most appropriate inhibitors against COVID-19 Main Proteases.

Accordingly, emetine as an alkaloid is a protein synthesis inhibitor known for its anti-protozoan effect. It was also largely used as medication for intestinal and extra-intestinal amoebiasis and already approved for its malaria inhibition by binding to the ribosomal E site of Plasmodium falciparum (Foy, 1912; Scragg & Powell, 1966; Wong et al., 2014; Yang & Dubick, 1980). The antiviral effect of Emetine have been also examined and literature reviews demonstrated its efficacy against broad range of RNA and DNA viruses, including Newcastle disease virus, cytomegalovirus, buffalo poxvirus, Zika virus, HIV-1, rabies virus, echovirus 1, Ebola virus, bovine herpesvirus 1, metapneumovirus, pesto des petits ruminants virus, Rift Valley fever virus, herpes simplex virus-2, and influenza (Andersen et al., 2019; Khandelwal et al., 2017; MacGibeny et al., 2018; Mukhopadhyay et al., 2016; Yang et al., 2018). Recent study based on in vitro EC50 measurements with (low micro molar range) justified the inhibition effect of Emetine on hCoV-OC43, hCoV-NL43, and MHV-A59, SARS-CoV and MERS-CoV (Dyall et al., 2014; Shen et al., 2019). In the same way, achievable EC50 concentrations in the blood of emetine revealed its higher effectiveness as an anti-coronavirus agent than it is against amoebiasis (Bleasel & Peterson, 2020; Shen et al., 2019). At present and to our knowledge, there are no reports that consider the use of molecular docking analysis of emetine to target SARS-CoV-2 receptors. Therefore, the purpose of this work is carried out with the aim of investigate the possible sites of action of emetine towards the various proteins of the Sars-Cov-2 virus using in silico tools such as molecular modeling, docking and molecular dynamics techniques.

2. Material and methods

2.1. Ligand/receptors preparation and docking

Docking between nine receptors (Table 1) and emetine was carried out by using the AutoDock vina software. The autoDock tools suite 1.5.7rc1 was used to prepare ligands and receptors files (pdbqt), attribute charges and calculating the grid box (Forli et al., 2016; Sanner, 1999). The coordinate of each receptor, free or in complex with inhibitor was extracted from his crystal structure coordinate available in the protein data bank (PDB) (Berman et al., 2000). The coordinate of the spike protein of the SARS-CoV-2 was downloaded from the PDB database (PDB: 6M0J) (Lan et al., 2020). The coordinate of the RNA-dependent RNA polymerase of the SARS-CoV-2 was downloaded from the PDB (PDB: 7BTF) (Gao et al., 2020). The coordinate of the 3CL protease (3CL pro) of the SARS-CoV-2 was downloaded from the PDB (PDB: 6M2N) (Su et al., 2020). The coordinate of the NSP10 protein of the SARS-CoV-2 was downloaded from the PDB (PDB: 6W6I) (Kim et al., 2020). The coordinate of the NSP13 protein of the SARS-CoV-2 was downloaded from the PDB (PDB: 6XEZ). The coordinate of the SARS-CoV NSP14 protein of the SARS coronavirus Frankfurt 1 was used as template and downloaded from the PDB (PDB: 5NFY) (Ferron et al., 2018). The coordinate of the NSP15 endoribonuclease protein of the SARS-CoV-2 was downloaded from the PDB (PDB: 6VWW) (Kim et al., 2020). The SARS-CoV-2 NSP16 protein of the SARS-CoV-2 was downloaded from the PDB (PDB: 6YZ1) (Krafickova et al., 2020) and finally the SARS-CoV2 papain-like protease PLpro protein of the SARS-CoV-2 was downloaded from the PDB (PDB: 7NFV). The three-dimensional structure of emetine was obtained and downloaded from well-known organic compound database PubChem Compound in SDF format then converted to PDB format using the pymol tool (DeLano, 2009).

Based on the Autodock tools suite and during the preparation of the receptors, the protonation was performed by adding only polar hydrogens, and applying the Kollman charges (Kollman et al., 2000), available in the “Edit” menu of the Autodock tools suite, for the ligand a Gasteiger charges were applied. All operations were carried out based on physiological pH.

During the docking procedure, emetine was used as ligand and almost of his bonds were defined as no rotatable. All receptors were kept rigid during docking procedure. Grid maps representing the target surface in those proteins were constructed with different dimensions depending on the literature described active site of the target protein (Table 1), docking parameters corresponding to the genetic algorithm were set as defaults with ga_pop_size 150 (number of individuals in population) and ga_num_evals 25 million evaluations (maximum number of energy evaluations). The maximum number of chosen generation was 2700.
The nine three-dimensional (3D) molecular structures of the Sars-Cov2-proteins receptors free or in complex with emetine inhibitor were visualized using the molecular visualization software PyMOL (DeLano, 2009) and the interaction of the Sars-Cov-2 proteins with emetine were visualized by the Python Molecular viewer 1.5.6 (Sanner, 1999). The two-dimensional representation of all complexes was performed by Discovery Studio Visualizer 20.1.0 (Biovia, Biovia, 2017).

2.3. Molecular dynamic simulations

A molecular dynamic study was applied to the nine Sars-Cov-2 proteins targeting emetine using NAMD v.2.13 using the CHARMM36 force field to determine the consistence and the stability of the different obtained complexes and to follow the conformational changes of the complex during the supply of energy by increasing the temperature of the complex.

The charmm-gui.org module was used to build the parameters and topology files of our inhibitor emetine, which introduced in VMD software (Humphrey et al., 1996; Wong & Goscinski, 2012) to generate the coordinate files .pdb and the structure files (.psf). Another module of VMD was used to generate the solvation boxes. The structure and coordinate files were used to generate the solvated structure and coordinate files needed to initiate molecular dynamics.

The solvation model generated by VMD software, used is a cube of water encompassing the whole of the molecule or the whole of the complex. Our dynamics simulation consists of three steps. The first one is a minimization step and assessed to stabilize the complex. The last frame of this step was used to start a second one consisting on DM simulations for 140 ps. This step was performed to harmonize the water molecules with the protein structure. Only the complex which presents the strongest potential will be simulated in molecular dynamics for 120 ns (Brooks et al., 2009; Phillips et al., 2005). The time step was set to 2 fs. The simulations were performed in the NpT conditions at a constant temperature of 310 K at constant pressure 1, using the Langevin dynamics with a damping constant of 1 ps. Energy minimization was performed for 1,000 steps. The rmsd, total energy per frame and the hydrogen bond have been calculated directly through the existing commands in the analysis menu of the VMD software while the RMSF radius of gyration center of mass have been calculated through VMD using the Tk-console tool and based on scripts previously prepared.

2.4. Absolute binding energy calculations using BFEE

The absolute binding energies of the stable complex, emetine-NSP15 resulted from the MD simulations have been determined based on the same process followed by Abu-Saleh et al. (2020) using BFEE tools (Binding Free Energy Estimator). Using the equilibrated Emetine-NSP15 structure, we determined the absolute binding energy calculations using the potential of mean force (PMF) approach as follows:

\[
\Delta G_{\text{bind}}^0 = \Delta G_{\text{bind}} + \Delta G_{\text{MM}} + \Delta G_{\text{sol}} - T\Delta S
\]

Other than the absolute binding energy calculated with BFEE, we were interested in determining of the relative binding energy. This calculation was performed using an end point method called the Molecular Mechanics Poisson-Boltzmann Surface area method (MM/PBSA) through CaFE program (Calculation of Free Energy) which is an open-source program (Fu et al., 2018; Liu & Hou, 2016). In our example of MM-PBSA calculation, the outer dielectric constant was set to 80.0, the inner dielectric constant to 1.0 and the reciprocal of grid spacing of 0.5 Å was employed, while for SA calculation the surface tension value was set to 0.00542 with a surface offset of 0.92. Finally, the binding energy was summed and averaged over a set of conformations (100 snapshot) as follows (Aayush Gupta, 2020; Elmezayen et al., 2021).

2.5. Molecular mechanics Poisson-Boltzmann surface area method (MM/PBSA)

The effectiveness of ligands in inhibiting or stimulating a targeted activity is derived from their potential to bind to different receptors regulating that activity. This binding affinity is assessed by estimating the free energy of binding \(\Delta G_{\text{bind}}^0\) (Sinha et al., 2020). To explore the potential of emetine to inhibit/block Nsp15 endoribonuclease, the binding free energy was calculated from the entire MD simulation trajectory by the MM-PBSA approach. The emetine exhibits a total binding free energy that is equal \(\Delta G_{\text{bind}}^0 = -28.17\) kcal/mol, coming mainly from the contribution of Vdw energies which seems to be the most important compared to the contribution of other energies.

2.4. Adme and toxicity profiles

The physicochemical and pharmacokinetics properties of emetine were estimated using ADME (absorption, distribution, metabolism and excretion) descriptors by a SwissADME online server (http://www.swissadme.ch/; Othman et al., 2020). An online ProTox-II webserver (http://tox.charite.de/tox/; Othman et al., 2020) was also used to explore the toxicity profiles (hepatotoxicity, immunotoxicity, genetic toxicity endpoints especially cytotoxicity, mutagenicity and carcinogenicity). Values were estimated using chemical similarities between compounds with known toxic effects and the presence of toxic fragments.

3. Results and discussion

3.1. Molecular docking study: Interaction of emetine with target proteins

To elucidate the mechanism of action of the anti-SARS-CoV-2 emetine drug, we investigate all the possible binding mode
and mechanism of action of emetine. We performed a virtual screening of totality of the SARS-CoV-2 structural and non-structural proteins involved in establishing an interaction with Emetine. In fact, docking analysis was performed on the active site of nine COVID-19 protein receptors based on their binding affinities, and 2D and 3D complementarity surface. Our results showed that emetine can bind to other non-structural proteins playing an important role in the RNA synthesis and replication like NSP10, NSP15 and NSP16. The NSPs-Emetine interactions have been listed in the Supplementary material 1.

Interestingly, emetine (Figure 1A) strongly binds to tested non-structural proteins (NSPs) with the highest affinity was found with NSP15 (-10.8 kcal/mol) (Figure 1B), followed by non-structural proteins (NSPs) with the highest affinity was Supplementary material1. NSPs-Emetine interactions have been listed in the thesis and replication like NSP10, NSP15 and NSP16. The Our results showed that emetine can bind to other non-binding affinities, and 2D and 3 D complementarity surface.

Table 1. Different dimensions of the Grid box according to the active site of the target protein.

| Code       | X_Dimension | Y_Dimension | Z_Dimension | X_center | Y_center | Z_center | Spacing |
|------------|-------------|-------------|-------------|----------|----------|----------|---------|
| R1-5MO1    | 50          | 42          | 50          | -36      | 40       | 13       | 1.00 Å  |
| R2-7BF     | -47         | -01         | 49          | 42       | 42       | 42       |         |
| R3-6MN2    | 397         | 28          | 66          | 72       | 78       | 72       |         |
| R4-5NYJ    | -67         | 21          | -01         | 90       | 80       | 126      |         |
| R5-6YT     | -27         | 20          | 26          | 90       | 90       | 90       |         |
| R6-5NFY    | 123         | 112         | 117         | 40       | 40       | 54       |         |
| R7-6WWW    | 40          | 31          | -08         | 60       | 50       | 44       |         |
| R8-6YZ2    | -20         | -33         | -04         | 48       | 48       | 48       |         |
| R9-4M3     | 85          | 18          | 16          | 90       | 80       | 80       |         |

and mechanism of action of emetine. We performed a virtual screening of totality of the SARS-CoV-2 structural and non-structural proteins involved in establishing an interaction with Emetine. In fact, docking analysis was performed on the active site of nine COVID-19 protein receptors based on their binding affinities, and 2D and 3D complementarity surface. Our results showed that emetine can bind to other non-structural proteins playing an important role in the RNA synthesis and replication like NSP10, NSP15 and NSP16. The NSPs-Emetine interactions have been listed in the Supplementary material 1.

Interestingly, emetine (Figure 1A) strongly binds to tested non-structural proteins (NSPs) with the highest affinity was found with NSP15 (-10.8 kcal/mol) (Figure 1B), followed by NSP12 (-9.5 kcal/mol) (Figure 1C), NSP16 (-9.4 kcal/mol), host cell protease like TMPRSS2, etc. consisting of three S1-S2 heterodimers), emetine builds six van der Waals (Pro389, Asn33, Gln96, Gly416, Thr27, Glu23), one Pi-Anion (Asp30 (4.44 Å)) and eight alkyl/Pi-Alkyl (Thy473 (5.11 Å), Phe456 (4.93 Å), Lys26 (4.38 Å), Leu29 (4.18 Å), Val93 (4.43 Å), His34 (4.72 Å), Lys417 (4.23 Å; 4.79 Å)) interactions, respectively. Additionally, the SARS-CoV-2 main protease (3CL main protease) as the key enzyme in proteolytic processing of SARS-CoV-2 for viral replication (which was initially released by the auto-cleavage of pp1a and pp1ab and directly mediates the maturation of NSPs) bound with emetine by forming one conventional hydrogen bond (Glu166 (2.31 Å)), ten van der Waals (His163, Leu141, Asn142, Cys145, Gly143, Thr25, His41, Thr45, Met165, Leu167) and four alkyl (Pro168 (4.62 Å), Met49 (3.97 Å), Cys 44 (3.63 Å; 4.83 Å)) interactions.

This natural alkaloid is mainly isolated from Alangiaceae, Icacinaceae, and Rubiaceae plant families (Wiegrebe et al., 1984). Psychotria ipecacuanha stokes harbour the highest concentration of emetine and especially their roots from the Atlantic rain forest of South-East Brazil (Garcia et al., 2005). Previous reports have detailed the biological activities of emetine (Akinboye & Bakare, 2011). This compound is known to act as emetic and expectorant, but also able to inhibit the ribosomal protein synthesis in mammalian, yeast and plant cells based on the inhibition of the aminoacyl-sRNA transfer reaction (Grollman, 1966). Emetine is known to be used as antiparasitic (Vedder, 1912), anticancer (Van Hoose, 1912) and contraceptive (Mehrotra et al., 2004) agent.

Interestingly, this alkaloid is known to act as antiviral agent (Deng et al., 2007; Low et al., 2009). In fact, Deng et al. (2007) have demonstrated that emetine inhibits poxvirus replication by blocking virion assembly. It was also reported that emetine inhibits dengue virus (DENV, single positive-stranded RNA virus of the family Flaviviridae, genus Flavivirus) by affecting the viral RNA synthesis or the viral protein translation pathways. Also, the production of positive-strand and negative-strand DENV RNA was significantly reduced by emetine (Low et al., 2009). Valadão et al. (2015) tested the effect of emetine on HIV-1. The results obtained showed that this plant alkaloid inhibits the HIV-1 replication by interacting with reverse transcriptase enzyme.

Table 1. Different dimensions of the Grid box according to the active site of the target protein.

| Code       | X_Dimension | Y_Dimension | Z_Dimension | X_center | Y_center | Z_center | Spacing |
|------------|-------------|-------------|-------------|----------|----------|----------|---------|
| R1-5MO1    | 50          | 42          | 50          | -36      | 40       | 13       | 1.00 Å  |
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| R8-6YZ2    | -20         | -33         | -04         | 48       | 48       | 48       |         |
| R9-4M3     | 85          | 18          | 16          | 90       | 80       | 80       |         |
residues Asn180, Arg105, and Phe185 in the papain-like protein sites. This compound can form 3-H bonds with Tyr273, Leu270, and Val330, two $\pi$-cation reaction with Ser325 and Phe275, two hydrophobic reactions with Leu329 and Pro328 in the RNA-dependent RNA polymerase (RdRp) sites (Yu et al., 2020). Additionally, Sinha et al. (2020) reported many molecules as targets for PLpro inhibitors including antiviral, antibacterial, muscle relaxant, anti-tussive, and natural molecules. Ribavirin, an antiviral drug, was reported to bind to PLpro with low binding energy (score = $-38.58$), especially by hydrogen bonds and hydrophobic interactions. Sinha et al. (2020) reported several molecules able to bind to the RdRp, a key enzyme for the viral replication and transcription. In fact, itraconazole, novobiocin, cortisone, silybin, rosmarinic acid, magnolol, and idarubicin, can bind to the RdRp with mf-scores lower than $-110$. Indeed, natural plant molecules like betulonal, gnidicin, and gniditrin presented high affinity to RdRp.

**Table 2.** Binding affinities and interacting active site residues of emetine with NSP15 protein. Binding affinity measured in kcal/mol.

| Ligand/Receptor | Binding energy (kcal/mol) | 2D interactions | Van der Waals: | Carbon Hydrogen Bond: | Alkyl: |
|-----------------|---------------------------|-----------------|----------------|-----------------------|--------|
| Emetine/NSP15   | -10.8 kcal/mol            |                 | Thr31, Glu45, Asn30, Val52, Ile28, Ile27, Asn30 | Asn29(A) (3.72), Asn29(B) (3.47) | Lys47 (5.35), Val52 (5.19) |

![Figure 1. 2D molecular structure of emetine (1 A) and 3D structure of emetine in complex with SARS-CoV-2 NSP15 (1B), and NSP12 (1 C) proteins.](image)
Among the NSPs proteins, NSP15 seems to play a powerful role in suppressing the type I IFN (IFN-α/β)-associated innate immune response by infecting macrophages, thus avoiding detection of viral mRNA by double stranded RNA sensor. The highest binding affinity was recorded with NSP15, one of the processing endoribonucleases that preferentially cleaves 3’ of uridines. Many antibacterial, antiviral, and anti-inflammatory drugs were reported to bind to these non-structural proteins (Mukhopadhyay et al., 2016; Sinha et al., 2020).

Recently, lymecycline, cefsulodine, rolitetramycine, itraconazole, saquinavir, dabigatran, canrenoic acid, and many flavonoids were predicted to highly bind to this helicase with low binding mf-scores (Mukhopadhyay et al., 2016).

### 3.2. Molecular dynamics simulation

To predict the interactional stability of complex and conformational of proteins following ligand binding, a molecular dynamics analysis was performed. Once the 70,000 calculation cycles were completed, 140 frames which correspond to 140 ps were obtained for all of the different complexes and proteins alone. From these results and using the VMD software, the calculation of the RMSD and the RMSF was considered (Supplementary data S1).

RMSF was measured relative to the carbon atom of each amino acid of the two proteins and the RMSF plot was used to visualize fluctuations at the different residues. The superposed RMSF plots of the different complexes shows a similar appearance to those of the proteins alone in a range of variation less than 3 Å while the different superposed RMSD curves show similar shapes for some complexes by comparing them to the proteins alone such as emetine-spike protein, emetine-NSP13, emetine-NSP15, emetine-papain-like protein, emetine-NSP12, emetine-NSP10 and emetine-NSP16.

On the other hand, at the level of the Emetine-3CL pro and Emetine- NSP 14 complexes, a decrease in the RMSD variations is noted, these results suggest that the binding of Emetine at the level of the various receptors is stable. Since the curves are increasing until the end of the simulation and do not present a stable plateau, a simulation in the order of ns for the emetine-NSP 15 complex was necessary to decide on the efficiency of Emetine compared to the protein NSP15. The number of calculation cycles has been increased to 60 million cycles which corresponds to 900 frames for 120 ns (Figure 2). From these results and using the same method mentioned above, the Root Mean Square Deviation (RMSD), the Root Mean Square Fluctuation (RMSF) and the radius gyration (Rg) plots were obtained and a hydrogen bond formation during the DM simulation was assessed as well as center of mass, absolute energy, and relative energy estimation.

A first observation is noticed at the level of the superposition of the RMSF (Figure 2) curve of the protein alone and of the complex where the protein alone has a range of variation of 10 Å up to 26 Å compared to the complex whose values are much lower, stable and vary between 10 Å and 14 Å.

Overlaying the RMSD (Figure 3) traces allowed us to show the stability of the complex by comparing it to the protein alone. In fact, the RMSD values of the protein alone gradually increase to stabilize at 25 Å with a transition period corresponding to a conformational change which lasts 4.958 ns (starts at 98 ns and ends at 103 ns) whose values reach 30 Å, while at level of the complex we observe that during the whole simulation the RMSD values are stable in a variation range of 4 Å-5 Å. Over than 120 ns, the complex presents a transition period which lasts 7.236 ns, starts at 121.538 ns and ends at 128.774 ns with RMSD values in the order of 21 Å. Additionally, during this transition phase and at the graphic level, it was possible to determine the mechanics of binding of the ligand to the target protein.

We could distinguish the presence of the ligand far from the protein (Figure 4A) then its binding to a non-specific site (Figure 4B) afterwards the ligand changes its binding site to attach in the place where it presents the weakest binding energy and therefore the highest affinity and for this reason Emetine first binds to the monomer (Figure 4C) and then binds to the second causing the formation of a dimer leading to the formation of complex (Figure 4D) and from this step the simulation stabilizes at a value less than 5 Å. Thus, and based on all these results we can judge the effectiveness of emetine to be fixed to different targets and its high stability.

The calculation of the radius of gyration (Rg) (Figure 5) offers an advanced information on the compactness of a protein or a receptor in general and allows the determination of its folding properties, low Rg values prove a tight structural conformation while high Rg values with a jump are a sign of a tense and unstable conformation (Lobanov et al., 2008; Mosquera-Yuqui et al., 2020). Rg as the root-mean-square distance of all electrons from their center of gravity is used to assess the folding of regular 2D structures into 3D protein structure. Comparing the two Rg plots, Rg of the free protein plot shows values varying between 32 Å and 44 Å sign of a high degree of freedom compared to the Rg plot of the protein in complex with emetine which shows a stable curve with ranged from 33 Å to 34 Å (1 Å variability) results in a rigid structure with a minimal degree of freedom and high compactness.

From these results, it can be concluded that the emetine guides the folding behavior of the protein which provides a higher level of compactness leading in a higher conformational stability throughout the simulation. It should also be noted that the conformational behavior of the ligand was checked, the values were stable and varying between 5 Å and 5.5 Å indicating the rigidity of the ligand in its docked position (Figure 5).

Hydrogen bonds play an important role in the formation of secondary and tertiary structural motifs in proteins. The formation of a hydrogen bond between a ligand and protein promotes the degree of interaction between the two entities over time, the higher the number of hydrogen bonds the stronger the interaction is (Menendez et
In this study, the total number of hydrogen bonds was calculated per frames for the whole simulation. In the figure 6, a difference can be seen in the interval (400 frames-600 frames) where the number of hydrogen bonds at the protein in complex is clearly higher compared to the free protein. For more details, the average number of hydrogen bonds per frames was calculated for the protein in complex as well as for the free protein. A difference of three hydrogen bonds (173 hydrogen bonds/frame for the protein in complex compared to 170
hydrogen bonds/frame for the free protein). These H-bonds play an important role in forming the ligand-protein complex. The graphical difference and the difference of three hydrogen bonds in the average can only be derived from the formation of the emetine-protein complex and the affinity of the ligand towards the protein.

To enhance the hit identification, the absolute binding energy of the stable emetine-NSP15 complex (form MD

![Figure 4. Mechanic of binding of emetine to the target protein (NSP-15). (A): Emetine far from the target protein, (B): Emetine bounded to non-specific site, (C): emetine bounded to the monomer, and (D): emetine forming a complex (dimer).](image)

![Figure 5. Analysis of Rg of NSP15 free and complexed protein with emetine at 120 ns MD simulations.](image)
simulations) has been predicted (Figure 7). The different contributions from the course variables and the final standard binding energy ($\Delta G^{\text{bind}}$) of emetine to the SARS-CoV-2 NSP15 protein are summarized in Table 3.

Results showed that emetine bind spontaneously to the SARS-CoV-2 NSP15 with $\Delta G^{\text{bind}} = -24.01$ kcal/mol, suggesting its potency to battle the SARS-CoV-2 by blocking NSP15.

As previously described, the total energy per frame was calculated using VMD (Figure 7). In fact, VMD allowed us to get an idea of the energy variation of the complex through the monitoring of the electrostatic energy and the Vdw energy. The total energy plot shows a fluctuation during the first 350 frames to stabilize until the end of the simulation in an energy plateau (-45/-55 kcal/mol) with an average energy value of -42.51 kcal/mol. In addition, the center of mass (Com) of the ligand and the protein was calculated in relation to the frames and used to determine the distance between Com of the ligand and Com of the protein. The plot of the curve shows stability through the simulation at values close to 10 Å, no jumps or abrupt increases were observed, indicating no detachment of the ligand or conformational changes in the protein.

The superposition of the two plots allowed us to better judge the stability of the complex where we notice that the two curves present a similar aspect with the same variation zone and the same stability level. In fact, as the distance between the two centers of mass increases, the energy value of the binding force of the ligand to the protein decreases and vice versa. This is observed in the fluctuation reported during the first 300 frames and then once the distance between the two centers stabilizes, the energy stabilizes. From these results, it can be concluded that there is an inversely proportional relationship between the binding energy force and the distance between the two Com's (Figure 8).

The MM-PBSA is one of the trusted and widely used approaches that combine the molecular mechanics and continuum solvent models to calculate the $\Delta G^{\text{bind}}$ of small molecules. Comparing the relative binding energy $\Delta G^{\text{bind}}$ (MM/PBSA) = -28.17 kcal/mol (Table 4) with the absolute energy $\Delta G^{\text{bind}}$ (BFEE) = -24.01 kcal/mol we notice the existence of 4.16 kcal/mol difference where the MM/PBSA approach describes a lower energy value hence possess strong binding affinity, although the values exposed by the two approaches are not so divergent, meaning that the reliability of the docking results was confirmed by MD and MM/PBSA approaches. The existence of this energy difference is probably due to the algorithmic level of the software where the two

Figure 6. Analysis of the total number of H-bond count throughout the simulation of NSP15 free (A) and complexed protein with emetine at 120 ns MD simulations (B). The difference in the number of hydrogen bonds formation between the complexed form and the free form throughout the simulation is shown in C.
Figure 7. Graphical representation of the total energy per frames shown in blue as the sum of the electrostatic energy (black) and the van der Waals energy (brown).

Table 3. Absolute binding energy calculations of the complex emetine-NSP15.

| Absolute binding energy calculations of emetine-NSP15, $\Delta G_{\text{bind}}^0$ (kcal/mol) |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| $\Delta G(\text{site, } c)$                  | -1.53           | $\Delta G(\text{site, polarTheta})$ | -0.38           | $(1/\beta) \ln(\beta)$ |
| $\Delta G(\text{site, eulerTheta})$          | -0.60           | $\Delta G(\text{site, polarPhi})$   | -0.13           | $\Delta G(\text{bulk, C})$ |
| $\Delta G(\text{site, eulerPhi})$            | -0.27           | $\Delta G(\text{site, polarPhi})$   | -0.24           | $\Delta G(\text{bulk, O})$ |

$\Delta G_{\text{bind}}^0 = -24.01$ kcal/mol

Figure 8. Graphical superposition of the trace presenting the distance between both centers of mass (protein and ligand) during 120 ns (blue) with the variation of the total energy calculated previously (brown).
approaches do not use the same input variables which results in different output variables. Comparing our results to the work done by Parida et al. (2021) based on exploiting the potential of somniferin, a dimeric alkaloid isolated from *Withania somnifera*, to combat SARS-CoV-2 having NSP15 as target. The authors found that somniferin exhibited a relative binding energy of −79.263 kJ/mol (-18.94 kcal/mol) which was significantly lower than the results obtained from the calculation of the relative binding energy of emetine suggesting that emetine has a greater affinity than somniferin towards NSP15, involved to combat SARS-CoV-2.

### 3.3. Physicochemical, pharmacokinetics and toxicological properties of emetine

To increase the success rate of emetine towards the higher phases of drug development, we are interested to elucidate its ADME and toxicity properties (Table 5). The target was firstly described by its 2D chemical structure and canonical SMILES along with the bioavailability radar to predict oral bioavailability. Thus, the in silico pharmacokinetics, drug likeness and oral toxicity profiles were estimated, and the outcomes are summarized in Table 3. As shown, Emetine was found to follow Lipinski’s rule of five, having TPSA value of 52.19 Å allowed it to be easily cross cell membranes. It was appeared to be P-glycoprotein substrate, pass BBB (blood brain barrier) and highly absorbed in gastrointestinal. With higher value of consensus lipophilicity (4.243), also confirmed its ability to permeate BBB. Emetine cannot inhibit all CYP450 isoforms which are involved in phase-I metabolism of drugs and lacking hepatotoxicity, immunotoxicity, carcinogenicity, cytotoxicity, and mutagenicity.

| Physicochemical Properties | Druglikeness | Bioavailability (Radar plot) |
|----------------------------|--------------|----------------------------|
| Molecular weight (g/mol)   | 480.64       | Lipinski                   |
| Number heavy atoms         | 35           | Ghose                      |
| Number heavy atoms         | 12           | Veber                      |
| Fraction Csp3              | 0.59         | Egan                       |
| Number rotatable bonds     | 7            | Muegge                     |
| Number H-bond acceptors   | 6            | Bioavailability Score      |
| Number H-bond donors       | 1            | Consensus log $P_{ow}$     |

**Table 5.** Physicochemical properties, druglikeness, bioavailability, pharmacokinetics, and oral toxicity parameters of emetine.

| Pharmacokinetics | Hepatotoxicity | Immunotoxicity | Carcinogenicity | Cytotoxicity | Mutagenicity |
|------------------|----------------|----------------|-----------------|--------------|--------------|
| G1 permeant      | Inactive       | Inactive       | Inactive        | Inactive     | Inactive     |
| BBB permeant     | 0.81           | 0.79           | 0.99            | 0.77         | 0.65         |

4. Conclusions

Aiming to elucidate the efficiency of repositioning of an alkaloid emetine towards developing an effective drug against SARS-CoV-2 targets, in silico pharmacokinetics, molecular docking and dynamic simulation analysis based on the binding energy and active site interactions as well as the stability of the formed complex has been applied. The obtained results successfully demonstrated the binding Emetine to all SARS-CoV-2 receptors, especially to the NSP15 viral protein indicating that emetine may be considered as a good inhibitor of SARS-CoV-2. The complex NSP15-Emetine was further analyzed for structural stability by 140 ns
molecular dynamics simulation, justifying the considerable stability of SARS-CoV-2 NSP15 docked complex with Emetine according the following binding mechanics: Emetine first binds to the monomer, after, binds to the second inducing the formation of a dimer which in turn leading to the formation of complex that simulation stabilizes it at a value less than 5 Å. Based on combinatorial molecular simulation analysis, emetine may prove more effective and specific for NSP15 targeted therapy as an attractive anti-COVID-19 clinical drug candidate in parallel with further in-vitro and in-vivo analyses that may also be taken into consideration, however, in vitro and in vivo evaluation study is required to repurpose these three drugs against 2019-nCoV.

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ORCID
Mejdi Snoussi http://orcid.org/0000-0002-2309-2601
Aldeddine Redissi http://orcid.org/0000-0001-8412-9298
Amor Mosbah http://orcid.org/0000-0001-5642-8805
Vincenzo De Feo http://orcid.org/0000-0002-1070-3207
Moh Adnan http://orcid.org/0000-0002-7080-6822
Kais Kauadi http://orcid.org/0000-0002-6814-8154
Mouss Aalirev http://orcid.org/0000-0002-3763-9698
Mitesh Patel http://orcid.org/0000-0002-9283-2124

References
Abu-Saleh, A. A. A., Awad, I. E., Yadav, A., & Poirier, R. A. (2020). Discovery of potent inhibitors for SARS-CoV-2’s main protease by ligand-based/structure-based virtual screening, MD simulations, and binding energy calculations. Physical Chemistry Chemical Physics, 22(40), 2309-2316. https://doi.org/10.1039/d0cp04326e.
Akinboye, E. S., & Bakare, O. (2011). Biological activities of emetine. The Open Natural Products Journal, 4, 8–15. https://doi.org/10.2174/1874841011040010008.
Andersen, P. I., Krpina, K., Ianevski, A., Shtaida, N., Jo, E., Yang, J., Koit, S., Andersen, P. I., Krpina, K., Ianevski, A., Shtaida, N., Jo, E., Yang, J., Koit, S., Dyall, J., Coleman, C. M., Hart, B. J., Venkataraman, T., Holbrook, M. R., Kindrachuk, J., Johnson, R. F., Olinger, G. G., Jahrling, P. B., Laidlaw, M., Johansen, L. M., Lear-Rooney, C. M., Glass, P. J., Hensley, L. E., & Frieman, M. B. (2014). Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrobial Agents and Chemotherapy, 58(8), 4885–4893. https://doi.org/10.1128/AAC.03036-14.
Elfiky, A. A. (2020). Natural products may intervene SARS-CoV-2 attachment to the host cell. Journal of Biomolecular Structure and Dynamics, https://doi.org/10.1080/07391102.2020.1761881.
Elmezayen, A. D., Al-Obaidi, A., Sahin, A. T., & Yelekci, K. (2021). Drug repurposing for coronavirus (COVID-19): In silico screening of known drugs against coronavirus 3CL hydrolyse and protease enzymes. Journal of Biomolecular Structure and Dynamics, 39(8), 1–13.
Enayatkhani, M., Hasaniazad, M., Faezi, S., Goukhlani, H., Davoodian, P., Ahmedi, N., Einakian, M. A., Karmostaji, A., & Ahmadi, K. (2021). Reverse vaccinology approach to design a novel multi-epitope vaccine candidate against COVID-19: An in silico study. Journal of Biomolecular Structure and Dynamics, 39(8), 2857–2872. https://doi.org/10.1080/07391102.2020.1756411.
Enmozioni, S. K., Raja, K., Sebastine, I., & Joseph, J. (2021). Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: An in silico approach. Journal of Biomolecular Structure and Dynamics, 39(9), 3092–3098, https://doi.org/10.1080/07391102.2020.1760136.
Ferron, F., Subissi, L., Silveira De Morais, A. T., Le, N. T. T., Sevajol, M., Gluais, L., Decroly, E., Vonhein, C., Bricogne, G., Canard, B., & Imbert, I. (2018). Reverse vaccinology approach to design a novel multi-epitope vaccine candidate against SARS-CoV-2. Proceedings of the National Academy of Sciences of the United States of America, 115(2), E162–E171. https://doi.org/10.1073/pnas.1718806115.
Ferron, F., Gluais, L., Vonhein, C., Bricogne, G., Canard, B., & Imbert, I. (2018). SARS-CoV-2 Nsp10/nsp14 dynamic complex. Worldwide Protein Data Bank. https://doi.org/10.2210/pdbsnfy/pdb.
Forlì, S., Huey, R., Pique, M. E., Sanner, M. F., Goodsell, D. S., & Olson, A. J. (2016). Computational protein-ligand docking and virtual screening with the AutoDock suite. Nature Protocols, 11(5), 905–919. https://doi.org/10.1038/nprot.2016.051.
Foy, G. (1912). Ipecacuanha and emetine. The Lancet, 180(4653), 1242. https://doi.org/10.1016/S0140-6736(04)07415-X.
Fu, H., Gumbart, J. C., Chen, H., Shao, X., Cai, W., & Chipot, C. (2018). BFEE: A user-friendly graphical interface facilitating absolute binding free-energy calculations. Journal of Chemical Information and Modeling, 58(3), 556–560. https://doi.org/10.1021/acs.jcim.7b00695.
Gao, Y., Yan, L., Huang, Y., Liu, F., Zhao, Y., Cao, L., Wang, T., Sun, Q., Ming, Z., Zhang, L., Ge, J., Zheng, L., Zhang, Y., Wang, H., Zhu, Y., Zhu, C., Hu, T., Hua, T., Zhang, B., … Ruo, Z. (2020). Structure of the RNA-dependent RNA polymerase from COVID-19 virus. (New York, NY.), 368(6492), 779–782. https://doi.org/10.1126/science.abb7498.
Garcia, R. M. A., Oliveira, L. O., Moreira, M. A., & Barros, W. S. (2005). Variation in emetine and cephaeline contents in roots of wild ipecac (psychotria ipecacuanha). Biochemical Systematics and Ecology, 33(3), 233–243. https://doi.org/10.1016/j.bse.2004.08.005.
Brooks, B. R., Brooks, C. L., Mackerrell, A. D., Nilsson, L., Pettrella, R. J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Cavas, L., Cui, Q., Dinner, A. R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., … Karplus, M. (2009). CHARMM: The biomolecular simulation program. Journal of Computational Chemistry, 30(15), 1545–1614. https://doi.org/10.1002/jcc.21287.
Chandel, V., Raj, S., Rathi, B., & Kumar, D. (2020). In silico identification of potent COVID-19 main protease inhibitors from FDA approved antiviral compounds and active phytochemicals through molecular docking: A drug repurposing approach. Preprints, 2020030349. https://doi.org/10.20944/preprints202003.0349.v1.
DeLano, W. L. (2009). PyMOL: An open-source molecular graphics tool. DeLano Scientific.
Grollman, A. P. (1966). Structural basis for inhibition of protein synthesis by emetine and cycloheximide based on an analogy between ipecac alkaloids and glutarimide antibiotics. *Proceedings of the National Academy of Sciences of the United States of America*, 56(6), 1867–1874. https://doi.org/10.1073/pnas.56.6.1867

Gupta, A. (2020). Profiling molecular simulations of SARS-CoV-2 main protease (Mpro) binding to repurposed drugs using neural network force fields.

Gyebi, G. A., Ogunro, O. B., Adegunloye, A. P., Ogungbemi, O. M., & Afolabi, S. O. (2021). Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CLpro): An in silico screening of alkaloids and terpenoids from African medicinal plants. *Journal of Biomolecular Structure and Dynamics*, 39(9), 3396–3408. https://doi.org/10.1080/07391102.2020.1764868

Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD: Visual molecular dynamics. *Journal of Molecular Graphics*, 14(1), 33–83. https://doi.org/10.1016/0267-8556(96)00018-5

Islam, R., Parves, M. R., Paul, A. S., Uddin, N., Rahman, M. S., Al Mamun, A., Hossain, M. N., Ali, M. A., & Halim, M. A. (2021). A molecular modeling approach to identify effective antiviral phytochemicals against the main protease of SARS-CoV-2. *Journal of Biomolecular Structure and Dynamics*, 39(9), 3213–3224. https://doi.org/10.1080/07391102.2020.1769733

Khan, M. T., Ali, A., Wang, Q., Irfan, M., Khan, A., Zeb, M. T., Zhang, Y.-J., Chinnasamy, S., & Wei, D.-Q. (2021). Marine natural compounds as potent inhibitors against the main protease of SARS-CoV-2. A molecular dynamic study. *Journal of Biomolecular Structure and Dynamics*, 39(10), 3627–3637. https://doi.org/10.1080/07391102.2020.1769733

Khandelwal, N., Chander, Y., Rawat, K. D., Riyesh, T., Nishanth, C., Sharma, K., Khan, M. T., Ali, A., Wang, Q., Irfan, M., Khan, A., Zeb, M. T., Zhang, Y.-J., Chinnasamy, S., & Wei, D.-Q. (2021). Marine natural compounds as potent inhibitors against the main protease of SARS-CoV-2. A molecular dynamic study. *Journal of Biomolecular Structure and Dynamics*, 39(10), 3627–3637. https://doi.org/10.1080/07391102.2020.1769733

Kollman, P. A., Massova, I., Reyes, C., Kuhn, B., Hsu, S., Chong, L., Lee, M., Lee, T., Duan, Y., Wang, W., Donini, O., Cieplak, P., Srinivasan, J., Case, D. A., & Cheatham, T. E. (2000). Calculating structures and free energies of complex molecules: Combining molecular mechanics and continuum models. *Accounts of Chemical Research*, 33(12), 889–897. https://doi.org/10.1021/ar000033

Krafcikova, P., Silhan, J., Nencova, R., & Boura, E. (2020). Structural analysis of the SARS-CoV-2 methyltransferase complex involved in RNA cap biosynthesis. *Journal of Biomolecular Structure and Dynamics*, 18(26), 6111–6121. https://doi.org/10.1080/07391102.2020.1772108

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., & Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*, 581(7807), 215–220. https://doi.org/10.1038/s41586-020-2180-5

Liu, W., Morse, J. S., Lalonde, T., & Xu, S. (2020). Learning from the past: Possible urgent prevention and treatment options for severe acute respiratory infections caused by 2019-nCoV. *ChemBioChem: A European Journal of Chemical Biology*, 21(5), 730–738. https://doi.org/10.1002/cbic.201728938.xv1

Liu, H., & Hou, T. (2016). CaFE: A tool for binding affinity prediction using end-point free energy methods. *Bioinformatics (Oxford, England)*, 32(14), 2216–2218. https://doi.org/10.1093/bioinformatics/btw215

Lobanov, M. Y., Bogatyrevsna, N. S., & Galizitskaya, O. V. (2008). Radius of gyration as an indicator of protein structure compactness. *Molecular Biology*, 42(4), 623–628. https://doi.org/10.1134/S1068356208040195

Low, Y. J. S., Chen, K. C., Wu, I. C., Mah-Lee Ng, M., & Chu, H. J. J. (2009). Antiviral activity of emetine dichloride against dengue virus infection. *Journal of Antivirals & Antiretrovirals*, 1(2), 62–71. https://doi.org/10.4172/jaa.1000009

MacGibeny, M. A., Koyuncu, O. O., Wirblisch, C., Schnell, M. J., & Enquist, L. W. (2018). Retrograde axonal transport of rabies virus is unaffected by interferon treatment but blocked by emetine locally in axons. *Plos Pathogens*, 14(7), e1007188.

Mehrotra, P. K., Kitchlu, S., Dwivedi, A., Agnihotri, P. K., Srivastava, S., Roy, R., & Bhaduri, A. P. (2004). Emetine ditartrate: A possible lead for emergency contraception. *Contraception*, 69(5), 379–387. https://doi.org/10.1016/j.contraception.2003.12.011

Menendez, C. A., Accordino, S. R., Gerbino, D. C., & Appignanesi, G. A. (2016). Hydrogen bond dynamic propensity studies for protein binding and drug design. *Plos One*, 11(10), e0165717. https://doi.org/10.1371/journal.ppat.1005717

Othman, I. M. M., Gad-Elkareem, M. A. M., El-Hassane, A., Aouadi, K., Kadrí, A., & Snoussi, M. (2020). Design, synthesis ADMET and molecular docking of new imidazole-4,5-bipyrindine-5-thione derivatives as potential tyrosyl-tRNA synthetase inhibitors. *Bioorganic Chemistry*, 102, 104105. https://doi.org/10.1016/j.bioorg.2020.104105

Parida, P. K., Paul, D., & Chakravorty, D. (2021). Nature’s therapy for COVID-19: Targeting the vital non-structural proteins (NSP) from SARS-CoV-2 with phytochemicals from Indian medicinal plants. *Phytochemistry Plus*, 11(1), 100002. https://doi.org/10.1016/j.phyplu.2020.100002

Phillips, J. C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R. D., Kale, L., & Schulten, K. (2005). Scalable molecular dynamics with NAMD. *Journal of Computational Chemistry*, 26(16), 1781–1802. https://doi.org/10.1002/jcc.20289

Rosa, S., Santos, W. C. (2020). Clinical trials on drug repositioning for COVID-19 treatment. *Revista panamericana de salud publica = Pan American journal of public health*, 44, e40. https://doi.org/10.26633/RPSP.2020.40

Sanner, M. F. (1999). Python: A programming language for software integration and development. *Journal of Molecular Graphics & Modelling*, 17(1), 57–61.

Scruggs, J. N., & Powell, S. J. (1966). Emetine hydrochloride and chloroquine in the treatment of children with amoebic liver abscess. *Archives of Disease in Childhood*, 41(12), 1136/adc.41.219.549

Shakya, A. (2020). Identification of bioactive compounds from Glycyrrhiza glabra as possible inhibitor of SARS-CoV-2 spike glycoprotein and non-structural protein-15: A pharmacoinformatics study. *Journal of Biomolecular Structure and Dynamics*, 1–15. https://doi.org/10.1080/07391102.2020.1762741

Shin, S. K., Prasad, S. K., Islam, M. A., Gurav, S. S., Patil, R. B., AlFaris, N. A., … Shoja, A. (2020). Identification of bioactive compounds from Glycyrrhiza glabra as possible inhibitor of SARS-CoV-2 spike glycoprotein and non-structural protein-15: A pharmacoinformatics study. *Journal of Biomolecular Structure and Dynamics*, 1–15. https://doi.org/10.1080/07391102.2020.1762741

Shin, S. K., Prasad, S. K., Islam, M. A., Gurav, S. S., Patil, R. B., AlFaris, N. A., … Shoja, A. (2020). Identification of bioactive compounds from Glycyrrhiza glabra as possible inhibitor of SARS-CoV-2 spike glycoprotein and non-structural protein-15: A pharmacoinformatics study. *Journal of Biomolecular Structure and Dynamics*, 1–15. https://doi.org/10.1080/07391102.2020.1762741
Su, H. X., Yao, S., Zhao, W. F., Li, M. J., Zhang, L. K., Ye, Y., Jiang, H. L., & Xu, Y. C. (2020). Identification of a novel inhibitor of SARS-CoV-2 3Clpro. Published online, https://doi.org/10.2210/pdb6m2n/pdb

Su, H. X., Zhao, W. F., Li, M. J., Xie, H., & Xu, Y. C. (2020). SARS-CoV-2 3CL protease (3CL pro) in complex with a novel inhibitor. Worldwide Protein Data Bank. https://doi.org/10.2210/pdb6m2n/pdb

Umesh, Kundu, D., Selvaraj, C., Singh, S. K., & Dubey, V. K. (2020). Identification of new anti-nCoV drug chemical compounds from Indian spices exploiting SARS-CoV-2 main protease as target. Journal of Biomolecular Structure and Dynamics, 39(9), 3428–3434, https://doi.org/10.1080/07391102.2020.1763202

Valadão, A. L. C., Abreu, C. M., Dias, J., Arantes, P., Verli, H., Tanuri, A., & de Aguiar, R. S. (2015). Natural plant alkaloid (emetine) inhibits HIV-1 replication by interfering with reverse transcriptase activity. Molecules (Basel, Switzerland), 20(6), 11474–11489. https://doi.org/10.3390/molecules200611474

Van Hoose, B. (1912). Emetine hydrochloride in malignancy. Women’s Health Medicine - Journal, 29, 102–116.

Vedder, E. B. (1912). An experimental study of the action of ipecacuanha on amoebae. Journal of Tropical Medicine and Hygiene, 15, 313–314.

Wahedi, H. M., Ahmad, S., & Abbasi, S. W. (2020). Stilbene-based natural compounds as promising drug candidates against COVID-19. Journal of Biomolecular Structure and Dynamics, 39(9), 3225–3234, https://doi.org/10.1080/07391102.2020.1762743

Wiegrebe, W., Kramer, W. J., & Shamma, M. (1984). The emetine alkaloids. Journal of Natural Products, 47(3), 397–408. https://doi.org/10.1021/np50033a001

Wong, W., Bai, X. C., Brown, A., Fernandez, I. S., Hanssen, E., Condron, M., Tan, Y. H., Baum, J., & Scheres, S. H. W. (2014). Cryo-EM structure of the Plasmodium falciparum 80S ribosome bound to the antiprotozoan drug emetine. eLife, 3(3), e03080. https://doi.org/10.7554/eLife.03080

Wong, A. K. L., & Goscinski, A. (2012). The design and implementation of the VMD plugin for NAMD simulations on the Amazon cloud. International Journal of Cloud Computing and Services Science (I-JCLOSER), 1(4), 155. https://doi.org/10.11591/closer.v1i4.1284

Yang, W. C., & Dubick, M. (1980). Mechanism of emetine cardiotoxicity. Pharmacology & Therapeutics, 10(1), 15–26. https://doi.org/10.1016/0163-7258(80)90007-8

Yang, S., Xu, M., Lee, E. M., Gorschkov, K., Shiryaev, S. A., He, S., Sun, W., Cheng, Y.-S., Hu, X., Tharappel, A. M., Lu, B., Pinto, A., Farhy, C., Huang, C.-T., Zhang, Z., Zhu, W., Wu, Y., Zhou, Y., Song, G., … Zheng, W. (2018). Emetine inhibits Zika and Ebola virus infections through two molecular mechanisms: Inhibiting viral replication and decreasing viral entry. Cell Discovery, 4, 31. https://doi.org/10.1038/s41421-018-0034-1

Yu, R., Chen, L., Lan, R., Shen, R., & Li, P. (2020). Computational screening of antagonist against the SARS-CoV-2 (COVID-19) coronavirus by Molecular docking. International Journal of Antimicrobial Agents, 56(2), 106012. https://doi.org/10.1016/j.ijantimicag.2020.106012

Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., Chen, H.-D., Chen, J., Luo, Y., Guo, H., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., … Shi, Z.-L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature, 579(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7