Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Fluoxetine hydrochloride loaded lipid polymer hybrid nanoparticles showed possible efficiency against SARS-CoV-2 infection

Shaymaa Elsayed Khater a, Ahmed El-khouly b,c, Hend Mohamed Abdel-Bar a,*, Abdulaziz Mohnsen Almahalli d, e, Dalia Mahmoud Ghorab d

a Department of Pharmacaceutics, Faculty of Pharmacy, University of Sadat City, Sadat City, Egypt
b Department of Organic and Medicinal Chemistry, Faculty of Pharmacy, University of Sadat City, Sadat City, Egypt
c Department of Pharmaceutical Sciences, Faculty of Pharmacy, Jerash University, Jerash, Jordan
d Department of Pharmaceutics and Industrial Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt
e School of Life and Medical Sciences, University of Hertfordshire Hosted by Global Academic Foundation, New Administrative Capital, Cairo, Egypt

ARTICLE INFO

Keywords:
COVID-19
SARS-COV-2 main protease
Drug repurposing
SSRIs
Molecular docking
Fluoxetine hydrochloride
Lipid polymer hybrid

ABSTRACT

Up to date, there were no approved drugs against coronavirus (COVID-19) disease that dangerously affects global health and the economy. Repurposing the existing drugs would be a promising approach for COVID-19 management. The antidepressant drugs, selective serotonin reuptake inhibitors (SSRIs) class, have antiviral, anti-inflammatory, and anticoagulant effects, which makes them auspicious drugs for COVID 19 treatment. Therefore, this study aimed to predict the possible therapeutic activity of SSRIs against COVID-19. Firstly, molecular docking studies were performed to hypothesize the possible interaction of SSRIs to the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-COV-2) main protease. Secondly, the candidate drug was loaded in lipid polymer hybrid (LPH) nanoparticles to enhance its activity. The studied SSRIs were Fluoxetine hydrochloride (FH), Atomoxetine, Paroxetine, Nisoxetine, Repoxetine RR, and Repoxetine SS. Interestingly, FH could effectively bind with SARS-COV-2 main protease via hydrogen bond formation with low binding energy (-6.7 kcal/mol). Moreover, the optimization of FH-LPH formulation achieved 65.1 ± 2.7% encapsulation efficiency, 10.3 ± 0.4% loading efficiency, 98.5 ± 3.5 nm particle size, and −10.5 ± 0.45 mV zeta potential. Additionally, it improved cellular internalization in a time-dependent manner with good biocompatibility on Human lung fibroblast (CCD-19Lu) cells. Therefore, the study suggested the potential activity of FH-LPH nanoparticles against the COVID-19 pandemic.

1. Introduction

COVID-19 instigate severe acute respiratory syndrome caused by infection of coronavirus 2 (SARS-CoV2) (Gil et al., 2020). The ability of both symptomatic and asymptomatic cases to spread COVID-19 infection makes it a pandemic disease in just a few months (Wu et al., 2020), which has negatively affected the global economy (Udugama et al., 2020). Consequently, more efforts are needed to find new innovative approaches that could fundamentally change our understanding and management of this disaster (Nicola et al., 2020). SARS-COV-2 consisted of a single-strand positive Ribonucleic acid (ssRNA) genome that was enveloped within a membrane and surrounded by glycoprotein spikes (S-protein) with a crown-like appearance (Liu et al., 2020). The viral cellular penetration is prompted by the interaction of the viral spike (S) glycoprotein with the angiotensin-converting enzyme 2 (ACE2) receptor (Zhou et al., 2020). Consequently, host transmembrane serine protease 2 cleaves the S protein to fuse to the host cell membrane and internalizes it via the endocytic pathway (Hoffmann et al., 2020).

Moreover, the viral genome was split by the main proteases enzymes including 3 carbon-like proteases (3CLpro) and the papain-like protease (PLpro) to yield nonstructural proteins (nsp2 – 16), which are essential for replication – transcription complex formation (V’kovski et al., 2019). Additionally, RNA-Dependent RNA Polymerase mediates the transcription and replication of the viral RNA genome through infection (Gil et al., 2020). Particularly, all SARS-CoV2 enzymes and proteins that participated in the virus life cycle could be considered as potential targets for the treatment of this crisis (Gil et al., 2020).
Up to date, there was no approved drug for COVID-19 treatment, so the repurposing of approved drugs could effectively shorten the required time and decreased the cost compared to the new drug discovery (Trezza et al., 2020). Plenty of drugs have been subjected for drug repurposing to combat COVID-19 either antiviral or non-antiviral supporting agents and miscellaneous drugs (Elmezayen et al., 2020). Molecular docking is a drug design approach used to determine the interaction of essential amino acids between the targeted protein and the candidate ligands with low binding energy (Carlesso et al., 2019). Moreover, it predicts the binding affinity and the inhibition efficiency of the candidate ligand for the targeted protein (Huang et al., 2018).

The selective serotonin reuptake inhibitors (SSRIs) are considered safe and effective antidepressant drugs that are mostly prescribed worldwide for the treatment of major depressive disorder disease (Kennedy et al., 2016). Recently, several studies examined their activity against COVID-19. Firstly, an observational study reported the potential association between the administration of certain antidepressant drugs including fluoxetine, venlafaxine, paroxetine, and mirtazapine, and the decrease in risk of infection or death in hospitalized COVID-19 patients (Hoertel et al., 2020, 2021b). And this association was confirmed by several observational studies (Diez-Quededo et al., 2021; Hoertel et al., 2021a, 2021b). Secondly, several preclinical in-vitro studies reported the potential activity of fluoxetine alone (Dechaumes et al., 2021; Zimmni et al., 2021) or in combination with antiviral drugs on COVID-19 (Schoer et al., 2021). Finally, clinical trials suggested that the antidepressant drug, fluvoxamine, can inhibit clinical deterioration in acute COVID-19 outpatients (Lenze et al., 2020; Setfél and Boulware, 2021).

The activity of antidepressant drugs on COVID-19 may be attributed to their ability to inhibit acid sphingomyelinase enzyme which decreases the release of ceramide on the epithelial cell surface (Gublin et al., 2013; Köžer et al., 2004). Consequently, they can prevent the infection of epithelial cells with COVID-19 as reported by preclinical (Carpinteiro et al., 2021, 2020) and observational studies (Hoertel et al., 2021a, 2021b). Additionally, their sigma-1 receptor agonists effect (Lenze et al., 2020; Rosen et al., 2019; Roumestan et al., 2007; Wang et al., 2019) decreases the elevated cytokine level in COVID 19 patients (Gordon et al., 2020; Hojyo et al., 2020). Furthermore, they can reduce the plasma level of numerous inflammatory mediators that are associated with COVID-19 (Köhler et al., 2018; Marín-Corral et al., 2021). Finally, their antiagulant effect (Halperin and Reber, 2007) makes them the optimum choice for COVID-19 patients who have arterial and venous thrombosis (Hamed and Hagag, 2020).

Moreover, drug loading in nanocarriers improves its therapeutic efficacy and reduces its side effects (Kumar et al., 2020). Recently, lipid polymer hybrid (LPH) nanoparticles received great attention in scientific research (Mukherjee et al., 2019). It consists of a polymeric core material, the Netherlands. Acetonitrile (HPLC grade), Ethanol absolute, Ethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from ThermoFisher Scientific, UK. Dulbecco Modified Eagles Media (DMEM), 1-glutamine, penicillin, streptomycin, and trypsin-EDTA were purchased from Gibco, Invitrogen, UK. Human lung fibroblast (CCD-19Lu) cells (catalog number ATCC® CCL-210™) were purchased from The American Type Culture Collection (ATCC, UK).

2. Materials and methods

2.1. Materials

Fluoxetine hydrochloride (FH) (Mₙ: 345.79) was kindly provided by Delta Pharma for the Pharmaceutical Industry, Egypt. Poly lactide co-glycolic acid (PLGA) 50/50 DL-lactide/glycolide with an inherent viscosity midpoint of 0.2 dl/g was kindly supplied from Purac Bio-materials, the Netherlands. Acetonitrile (HPLC grade), Ethanol absolute, Lecithin soybean (LEC) (3-α-Phosphatidylcholine ≥ 99% (TLC) lyophilized powder), Triethylamine, Fetal bovine serum (FBS), and Dime-thylsulphoxide (DMSO) were purchased from Sigma Aldrich, USA. Hydrochloric acid, potassium chloride, potassium dihydrogen orthophosphate, sodium chloride, sodium dibasic hydrogen orthophosphate, sodium hydroxide, and Tween 80 were purchased from Fluka Chemika, Switzerland. TritonX-100, 1,1’-Diocatadecyl-3,3’,3’-Tetramethyleneindocarbocyanine Perchlorate (DiI), and 3,4,5-dime-thylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from ThermoFisher Scientific, UK. Dulbecco Modified Eagles Media (DMEM), 1-glutamine, penicillin, streptomycin, and trypsin-EDTA were purchased from Gibco, Invitrogen, UK. Human lung fibroblast (CCD-19Lu) cells (catalog number ATCC® CCL-210™) were purchased from The American Type Culture Collection (ATCC, UK).
2.2.2 Preparation of lipid polymer hybrid (LPH) nanoparticles

Different LPH nanoparticles loaded with the selected drug from the docking study were prepared by a modified single-step nano-precipitation self-assembly technique (Tahir et al., 2019). Briefly, the polymer (PLGA) and the candidate drug were dissolved in acetonitrile to obtain the organic phase. LEC and tween 80 were dissolved in a 4% v/v hydroalcoholic solution, as the aqueous phase at 65 °C. Consequently, the organic phase was slowly dripped into the aqueous phase while stirring for 2 h at 65 °C. Consequently, the organic to aqueous phase ratio was kept at 1:9 v/v. The resultant LPH dispersions were centrifuged at 15000 rpm for 15 min at 4 °C. The harvested pellets were washed twice with phosphate buffer saline (PBS pH 7.4), then re-suspended in the same medium for further analysis. The fluorescently labeled optimized LPH formula was prepared by dissolving the fluorescent dye (DiI) into the lipid polymer hybrid (Tahir et al., 2019). Briefly, 1 mL of the prepared LPH dispersion (30,000 MWCO, Millipore, USA) and centrifuged at 15000 rpm for 15 min at 4 °C. The amount of free unentrapped drug in the filtrate was determined using a previously validated HPLC method (Dionex™, Thermo Scientific™, USA). A

2.2.3 Experimental design and construction of the Box-Behnken (BBD) design

LPH formulae were optimized using BBD by Design-Expert software (Design-Expert 9.0.5.2, State-Ease Inc., USA). The statistical models and the response surfaces were explored from the constructed matrix (Bachhav et al., 2017). The selected critical process parameters (CPPs) under this study were; the PLGA amount, Drug amount, and the stirring speed were coded as A, B, C respectively. There were three levels for each variable; low (-1), medium (0), and high (+1). The critical quality attributes (CQAs) were particle size (Y1), zeta potential (Y2), and encapsulation efficiency (EE%) (Y3). The quality target product profile (QTPP) of the optimized LPH formula was to achieve minimum particle size, maximum zeta potential, and maximum EE%. The determined CPPs and CQAs, as well as the required QTPP, are listed in Table 1.

Table 1

| Critical process parameters (CPPs) | Levels |
|----------------------------------|--------|
| (Coded independent variables)    | Low    | Medium | High |
| A: PLGA (mg)                     | 5      | 10     | 15   |
| B: Drug (mg)                     | 10     | 15     | 20   |
| C: Stirring speed (rpm)          | 500    | 750    | 1000 |
| Critical quality attributes (CQAs) | Constraints |
| Y1: Particle size (nm)           | Minimize |
| Y2: Zeta potential (mV)          | Maximize |
| Y3: EE(%)                        | Maximize |

a EE% = Encapsulation efficiency parentage.

by the software and the polynomial equations were authenticated. According to the highest desirability, the design space was constructed to determine the optimum CPPs required to fabricate the optimized drug-LPH with the targeted QTPP (Al-mahallawi et al., 2019).

2.2.4 In vitro characterization of the optimized LPH formula

2.2.4.1 Particle size, size distribution, and zeta potential. The dynamic light scattering (DLS) (Zeta sizer, Malvern Instruments, UK) was used to estimate the particle size, polydispersity index (PDI), and zeta potential of the optimized LPH formula. The measurements were performed using a 90° angle at 25 °C (Abd-Elsalam et al., 2018a; Ahmed et al., 2019).

2.2.4.2 Drug encapsulation and loading efficiency (EE%, LE%). The drug EE% was measured indirectly by the ultrafiltration centrifugation method (Ishak et al., 2017). Briefly, 1 mL of the prepared LPH dispersion was added to the Amicon tube® (30,000 MWCO, Millipore, USA) and centrifuged at 15000 rpm for 15 min at 4 °C. The amount of free unentrapped drug in the filtrate was determined using a previously validated HPLC method (Dionex™, Thermo Scientific™, USA). A

Fig. 1. Schematic representation of LPH nanoparticle preparation. The candidate drug and PLGA polymer were dissolved in acetonitrile. Lecithin and tween 80 were dissolved in 4% v/v hydroalcoholic solution and heated at 65 °C. The drug-polymer organic solution was slowly dripped into the hydroalcoholic lipid phase with magnetic stirring at 65 °C for 2 h. LPH nanoparticles were harvested by ultrafiltration centrifugation (15000 rpm, 15 min) at 4 °C and re-suspended in PBS pH 7.4 for further analysis.

3
reverse phase C18 column (150X4.6 mm, 5 μm, Hypersil ® ODS, USA) and a mobile phase consisting of acetoni tro and deionized water containing 10 mM aqueous triethylamine at a ratio of (55:45 v/v) with a flow rate of 1 mL/min at 25°C were employed. The UV detector was set at 226 nm. The calibration curve of the candidate drug in PBS (pH 7.4) in the concentration range of 1–100 μg/mL has a coefficient of determination (R²) equal to 0.9994 with a limit of detection and quantification equal to 0.5 and 1 μg/mL, respectively. Moreover, the coefficient of variation percentage ranged from 2.1% to 4.9% and the accuracy for drug determination was 1.5% to 4.6% with a mean drug recovery percentage of 97.5 ± 1.16%. The EE% and LE% of the candidate drug was calculated using the following equations:

\[
EE\% = \frac{\text{Thetotalamountofdrugindispersion} - \text{Amountofdruginthesupernatant}}{\text{Thetotalamountofdrugindispersion}} \times 100
\]

(1)

\[
LE\% = \frac{\text{MassofdrugloadedinLPH} - \text{LPHNs}}{\text{Thetotalmassofdrug} - \text{LPHNs}} \times 100
\]

(2)

2.2.4.3 Morphological studies. The morphological architecture of the optimized LPH was visualized by a transmission electron microscope (TEM) (Jeol, JEM-1230, Japan). Briefly, the examination was performed by depositing a drop of the dispersion on a carbon-coated copper grid (300-mesh) and dried for 10 min. Before imaging, one drop of 2% phosphotungstic acid was applied and dried for 5 min (Abd-Elsalam et al., 2018b). Moreover, the atomic force microscope (AFM) (Wet-SPM 9600, Scanning probe microscope, Shimadzu, Japan) was employed to visualize the topographical image and the 3D surface morphology of the optimized LPH dispersion. Briefly, one drop of the dispersion was deposited on a silicon wafer and air-dried. Consequently, it was scanned using a constant force model. A non-contact mode software was used in recording the AFM images under normal atmospheric conditions (Hamdi et al., 2020).

2.2.4.4 Differential scanning calorimetry. The physical state of the candidate drug within the optimized LPH was investigated by the Differential scanning calorimetry (DSC) (Shimadzu Scientific instrument, USA). The free candidate drug, drug-loaded LPH, and blank LPH nanoparticles were accurately weighed and sealed in aluminum pans. The analysis was performed under a nitrogen flow rate of 30 mL/minute to prevent oxidation. The DSC thermograms were recorded at a temperature range of 25°C to 200°C with a heating rate of 10°C/min (Elsherif et al., 2021; Tahir et al., 2019).

2.2.4.5 In vitro serum stability assay. The optimized LPH dispersion was incubated with 10 and 50% v/v fetal bovine serum (FBS) for 4 and 24 h at 37°C. The in vitro serum stability was evaluated by recording the change in particle size, PDI, and zeta potential (Zhao et al., 2015).

2.2.4.6 In vitro drug release. The dialysis membrane method was employed to assess the in vitro drug release from the optimized LPH formula (El-Gogary et al., 2014). Briefly, A specified volume of the optimized LPH (equivalent to 2 mg of the candidate drug) was diluted with 1 mL PBS (pH 7.4). Then it was mixed with PBS (at a final concentration of 10 and 50% v/v) and added to the dialysis membrane (cut-off: 10,000 Da). The dialysis bag was sealed and immersed in 25 mL of PBS (pH 7.4) at 37 ± 0.5°C, and 100 ± 0.1 S/min. Different aliquots (0.2 mL) were withdrawn at predetermined time intervals and replaced by fresh media. The samples were analyzed using the previously validated HPLC method. The release of free drug was performed under the same conditions as a control. Moreover, the release data were fitted into different kinetics models to determine the possible release mechanism (Pardeshi et al., 2013). Additionally, the similarity factor (f2) (Eq. (3)) was calculated to compare the different release profiles, where the two dissolution profiles were considered similar when the f2 value is ≥ 50 (Shah et al., 1998).

\[
f_2 = 50 \times \log \left[ 1 + \frac{1}{n} \sum (R_t - T_t)^2 \right]^{-0.5} \times 100
\]

(3)

Where R is the dissolution percentage of the reference (pre-change) formula at time t, T is the dissolution percentage of the test (post-change) formula at time t, and n is the number of time points (Shah et al., 1998).

2.2.4.7. Short term stability study. Briefly, the optimized LPH dispersion was initially frozen at -80°C, then primarily dried by heating the samples to -40°C at pressure 100 bar. The secondary drying step was performed at a temperature of 20°C and pressure of 20 bar. The freeze-dried optimized LPH formula was obtained after 48 h lyophilization and kept at 4°C and 25°C/60 ± 5% relative humidity (RH) (WIO, 2017) for 28 days. The change in particle size, PDI, zeta potential, and EE% was evaluated after 7, 14, 21, and 28 days as previously described (Sengel-Turk and Hascicek, 2017).

2.2.4.8. In vitro hemolytic assay. The hemolytic effect of the optimized LPH dispersion was conducted on fresh red blood cells (RBCs) of male albino rats according to the ethical committee of the faculty of the pharmacy-Cairo university with license number (PI 2077). Briefly, the blood was withdrawn on a heparinized tube from the tail vein of male albino rats (aged 2–3 months, 200 ± 10%). The blood was centrifuged at 4000 rpm for 10 min. The collected RBCs were subjected to different concentrations of optimized LPH formula (20–200 μg/mL) and incubated for 2 h at 37°C. After centrifugation of samples (4000 rpm, 5 min) at 4°C, the absorbance of the supernatant was determined at 545 nm. The negative and positive controls were prepared by incubating RBCs with PBS (pH 7.4) and 0.5% w/v Triton X-100, respectively (Hamdi et al., 2020). The percentage (%) hemolysis was calculated using the following equation:

\[
\%\text{Hemolysis} = \frac{\text{absorbance of sample} - \text{absorbance of negative control}}{\text{absorbance of positive control} - \text{absorbance of negative control}} \times 100
\]

(4)

2.2.4.9. In vitro cytocompatibility assay. The in vitro cytocompatibility assay of the optimized LPH formula was assessed on human lung fibroblast (CCD-19Lu) Cells by MTT assay (Li et al., 2017). Briefly, cells were seeded in a 96-well plate (10,000 cells/well) in the DMEM culture media enriched with 100 μg/mL of streptomycin, 100 units/mL of penicillin, 1% L-glutamine, and 10% heat-inactivated FBS for 24 h at 37°C. The human lung fibroblast (CCD-19Lu) cells were then treated with serial drug concentrations ranging from 0.01 to 100 μM and incubated for 72 h. Afterward, the media was replaced with 120 μL of MTT solution for 4 h at 37°C and 5% CO2. The obtained formazan crystals were dissolved by a 200 μL of DMSO and then the absorbance was measured at 570 nm using a plate reader (ChromoMate-4300, FL, USA). The percentage of cell viability was calculated using the following equation:

\[
\%\text{Cell Viability} = \frac{\text{The absorbance of treated cells at 570 nm}}{\text{The absorbance of untreated cells at 570 nm}} \times 100
\]

(5)

2.2.4.10. In vitro cellular uptake. The flow cytometry (BD FACs Calibur™ flow cytometer, BD Biosciences) was employed to quantitatively assess the in vitro cellular uptake of the DiL-labelled optimized LPH. The human lung fibroblast (CCD-19Lu) cells were seeded into a 24-well plate (50,000 cells/well) for 24 h. Cells were treated with 50 nM of the DiL-labelled optimized LPH and incubated for 4 and 24 h. After incubation, the cells were washed twice with PBS (pH 7.4) and trypsinized for 5 min. The obtained cells were centrifuged (1750 rpm, 3 min) at 4°C and re-suspended into PBS (pH 7.4). The fluorescence intensity was measured using the FL-2 detector and the collected data were
2.2.4.11. Statistical analysis. All experiments of the present study were conducted in triplicates and the results are expressed as the mean ± SD. The difference between the two variables was compared by the Student t-test while the difference between groups was assessed by ANOVA followed by the Tukey HSD test. The SPSS 18 (Chicago, USA) was applied to assess the statistical analysis and the differences were considered significant at (p) value < 0.05.

3. Results and discussion

3.1. Molecular docking

Among several available crystal structures of SARS-COV-2 main protease in the PDB, The (5RGW) crystal structure was selected as the size of a co-crystallized ligand is quite similar to the size of the compounds to be docked and correct fitting in the pocket, i.e. ligand in (5RGK) is too small while ligand in (5R82) is out site the binding site (Fig.S2). Moreover, the docking protocol was validated by redocking the same conformer of the co-crystallized ligand with a Root Mean Square Deviation (RMSD) of 1.021 A (Fig.S3), which indicated a good solution as the calculated RMSD ≤ 2.0 Å (Gohlke et al., 2000).

Docking and molecular interaction of the selected SSRIs were studied where the best 20 modes of binding with the binding site were recorded and the binding affinity values of the best mode are shown in Table 2. According to the binding energy, the affinity of different tested SSRIs to the SARS-COV-2 main protease could be arranged as follows paroxetine > reboxetine S, S > FH > reboxetine R, R > Atomoxetine > nisoxetine. Furthermore, Fig. 2 shows the binding interactions between different SSRIs and the SARS-COV-2 main protease. Among these compounds, only FH was able to form hydrogen bonding by the interaction of the trifluoromethyl group with the binding site residues as well as hydrophobic interactions while the other compounds only were able to bind hydrophobically. Interestingly, the presence of hydrogen bonds promotes the stability of the interaction of ligands with the active sites of protein (Chen et al., 2016; Patil et al., 2010). Moreover, Fig. 3 shows the 2D and 3D ligand–protein interaction of FH with the binding site of SARS-COV-2 main protease; FH was able to form hydrogen bonding with Histine 163 (Fig. 3 C, F) and additionally sometimes can form hydrogen bonding with Serine 144 (Fig 3 E).

According to the binding affinities of the studied compounds and the ligand–protein interaction, we concluded that FH could be considered as a potential SARS-COV-2 main protease inhibitor. In this study, the suggested activity of FH on COVID-19 is based on its highest binding affinity to SARS-COV-2 main protease. On the other hand, the recent observational (Hoertel et al., 2021a, 2021b) and preclinical (Carpinteiro et al., 2020) studies suggested the activity of FH on COVID-19 based on its acid sphingomyelinase inhibitory activity that decreases the ceramide concentration on the surface of the epithelial cells and consequently prevents their infection with COVID-19 (Gulbins et al., 2013).

Additionally, FH has antiviral activity against the human enteroviruses by targeting their 2C protein as previously reported (Bauer et al., 2019; Ulberts et al., 2013). Besides, its ability to reduce Coxackievirus B4 replication (Benkahlta et al., 2018). Moreover, FH inhibited the hepatitis C virus by facilitating the interferon-α-mediated antiviral effect through the activation of c-Jun amino-terminal kinases (JNK) and signal transducer and activator of transcription (STAT)-1 (Young et al., 2014). Furthermore, FH can decrease the serum level of pro-inflammatory cytokines including IL-1B, IL-6, and TNF-α (Lu et al., 2017; Ondicova et al., 2019; Sacre et al., 2010). And consequently, FH could prevent the cytokine storm of COVID-19 that is associated with respiratory failure (SARS), organ failure, and death (Xu et al., 2020; Ye et al., 2020). Therefore, FH was selected for the forthcoming formulation study to improve its therapeutic activity against COVID-19.

3.2. Formulation and optimization of different FH loaded LPH

The candidate drug from the molecular docking study, FH, was loaded in LPH by a modified nanoprecipitation self-assembly method (Tabir et al., 2019). During this approach, the addition of the organic phase to the aqueous phase decreased the interfacial tension. Moreover, the levigation of the two phases produced turbulence which enhanced the diffusion of the water-miscible-organic solvent through the aqueous phase. Consequently, the drug and polymer migrate into the aqueous phase and form a precipitate that is considered a nucleation point for nanoparticles formation (Gutierrez et al., 2007).

The self-assembly of the lipids surrounding the produced polymeric core was derived by the hydrophobic interactions between their hydrophobic tail and the polymeric core, while their hydrophilic head would protrude to the external aqueous surrounding forming a homogenous surfactant stabilized LPH (Hadinoto et al., 2013). Based on the highest adjusted and predicted R² values with a difference below 0.2 and the least PRESS value after omitting the non-significant factors (Huang et al., 2004), the quadratic model was elected as the best fit statistical model for all responses (particle size, EE%, and zeta potential) Tables S1-S3.

3.2.1 Effect of different critical process parameters on particle size (Y₁)

The fabricated FH-LPH had a particle size ranging from 110.8 nm to 240.5 nm as shown in Table 3. All formulae were monodispersed systems with PDI values<0.3 (Danaei et al., 2018). Eq. (6) described the effect of various significant CPPs on the particle size as following:

$$Y_1 = +214.46 + 38.49A + 7.62B - 5.26C + 17.88AB + 7.94AC - 17.55A^2 - 38.88B^2$$

(Eq. 6)

The regression coefficients of all assessed CPPs and the ANOVA results were listed in Table S4 and S5 respectively. Based on Eq. (6) and Fig. S4, it is clear that FH-LPH particle size was positively correlated with PLGA amount (A), and drug amount (B). The increase in polymer and drug amounts increased the viscosity of the organic phase, decreased its evaporation rate, and consequently produced a large particle size (Ravi et al., 2015). Furthermore, this viscous dispersion decreased the impact of the shear force of stirring and would prevent the breaking of the produced droplets into smaller ones (Gajra et al., 2016). Additionally, the positive coefficient of the interaction (AB) between PLGA amount and drug amount indicated the enhanced effect of both variables on the viscosity of the organic phase yielding LPH with a large particle size (Fig. 4A).

On the other hand, the FH-LPH particle size was negatively correlated with stirring speed (C) (Fig. S4). As the increase in stirring speed generates high mechanical and hydraulic shear that produces nanoparticles of small particle size (Maleki Dizaj et al., 2016). But the positive coefficient of the interaction (AC) between PLGA amount and the stirring speed indicated the prime effect of PLGA amount on the particle size of LPH, and the particle size was significantly increased (P < 0.05) (Fig. 4B).

| Ligand      | Binding free energy (kcal/mol) | Targeted amino acid | Total hydrogen bond number |
|-------------|---------------------------------|---------------------|---------------------------|
| Fluoxetine  | −6.7                            | Histidine 163       | 2                         |
| Hydrochloride| −6.4                            | Serine 144          |                           |
| Atomoxetine | −6.1                            | −                   | −                         |
| Nisoxetine  | −5.7                            | −                   | −                         |
| Paroxetine  | −7.3                            | −                   | −                         |
| Reboxetine R R| −6.5                         | −                   | −                         |
| Reboxetine S S| −6.8                         | −                   | −                         |

Table 2: The docking interaction parameters of the selected selective serotonin reuptake inhibitors with the SARS-CoV2 main protease.
3.2.2. Effect of different critical process parameters on FH EE% ($Y_2$)

The obtained FH-LPH had a drug EE% varied between 41.5% and 73.1% as shown in Table 3. The influence of different significant CPPs on FH EE% could be depicted in Fig. S5 according to the following equation:

$$Y_2 = + 54.54 + 7.11A + 3.11B + 5.63C + 1.95AB + 5.67BC + 8.38A^2 - 3.27B^2$$  (7)

Table S6 shows the regression coefficients values of each of the CPPs with a statistical significance value ($P < 0.05$) and Table S7 shows the ANOVA results. Eq. (7) shows a positive correlation between all factors’ effects and EE% as shown in Fig. S5. The PLGA amount (A) had the highest positive significant effect on the drug EE% ($P < 0.05$) because it formed a large core that incorporates large amounts of the drug (Gajra et al., 2015). Moreover, the higher PLGA amount increased the viscosity of the organic phase and produced a large particle size, which would prevent the production of porous particles with the acquirement of a longer diffusion path for the drug that enhances the EE% (Lalani et al., 2012). These results were in agreement with Hamdi et al. and Tahir et al., who reported that the increase in PLGA amount increased EE% of entecavir and doxorubicin respectively (Hamdi et al., 2020; Tahir et al., 2019).

Additionally, the rise in drug amount (B) (a hydrophilic drug, pKa = 10, Log p = 1.2) (Kwon and Armbrust, 2008) would compensate for its leakage from the prepared nanoparticles and significantly increased its EE% ($P < 0.05$) (Fig. S5) (Song et al., 2008). Furthermore, the rise in stirring speed (C) significantly increased the FH EE% (Fig. S5) ($P < 0.05$). During the nanoprecipitation technique, nanoparticles are formed by the interfacial phenomena, as the interfacial turbulence caused convection effects. So the solvent transport produced a physicochemical instability and formed local regions of supersaturation. Moreover, the presence of turbulence for a definite time might promote the evaporation of the organic solvent and prevents drug leakage (Salatin et al., 2017).

The positive coefficient of the interaction (AB) between PLGA amount and drug amount indicated the significant ($P < 0.05$) synergistic effect of their interaction on the FH EE% (Fig. 5A). As the (AB) interaction is associated with the formation of large particle sizes (Fig. 4A), which enhances the encapsulation of large amounts of the drug (Gajra et al., 2015). Moreover, the positive coefficient of the interaction (BC) between drug amount and the stirring speed indicated the significant ($P < 0.05$) synergistic effect of their interaction on FH EE% (Fig. 5B).

3.2.3. Effect of different critical process parameters on zeta potential ($Y_3$)

Table 3 shows that the fabricated FH-LPH had a zeta potential ranging from −5.5 mV to −15.3 mV. It is to be noted that the absolute values of zeta potential will be used in discussion to avoid misperception (Aziz et al., 2019). The influence of different significant CPPs on zeta...
potential could be depicted from Fig. S6 according to the following equation:

\[ Y_3 = +9.34 - 1.05A - 0.85B - 0.58AB - 1.72BC - 0.91A^2 + 1.39B^2 + 0.49C^2 \]  

(8)

Table S8 shows the regression coefficient values of each of the CPPs with a statistical significance value \((P < 0.05)\). Moreover, the ANOVA results demonstrated the significant effect of the CPPs \((P < 0.05)\) on FH-LPH zeta potential Table S9.

Eq. (8) and Fig. S6 illustrated that the absolute value of zeta potential was negatively correlated with PLGA amount \((A)\) and drug amount \((B)\). On the contrary, it was positively correlated with the stirring speed \((C)\). The cationic nature of FH might be responsible for the significant decrease in the absolute value of zeta potential \((P < 0.05)\) (Pham et al., 2018). Furthermore, the increase in PLGA amount is associated with higher EE% of the drug (Fig. S5) and consequently, the absolute value of zeta potential was significantly decreased \((P < 0.05)\).

On the contrary, the rise in stirring speed \((C)\) significantly decreased the particle size of FH-LPH \((P < 0.05)\) and this might be associated with a high absolute value of zeta potential (Ding and Kan, 2017).

Additionally, the negative coefficient of both \((AB)\) interaction between PLGA amount and drug amount and \((BC)\) interaction between drug amount and stirring speed showed a significant antagonistic effect of their interactions on the absolute value of zeta potential \((P < 0.05)\) (Fig. 6A and B), respectively. As either \((AB)\) or \((BC)\) interactions associated with higher EE% of the drug (Fig. 5).

3.2.4. Design space and optimization

Design space was constructed to optimize all the investigated CPPs to fulfill QTPP criteria; minimum particle size, maximum zeta potential (absolute value), and maximum EE% (Fig. S7-S9). Based on the highest desirability (combined value 0.902), one formula was selected as a checkpoint to authenticate the obtained statistical models. Table 4 and Fig. S10-S12 illustrate its composition, where the optimum level for each factor was 5 mg, 20 mg, and 1000 rpm for PLGA amount (desirability 1), drug amount (desirability 1), and the stirring speed (desirability 1), respectively. Moreover, the comparison of the observed and predicted values of particle size \((Y_1)\) (desirability 1), EE% \((Y_2)\) (desirability 0.697), and zeta potential \((Y_3)\) (desirability 1) was shown in Table 4 with a % predicted error ranged between 0.3 and 3.8%. The small values of the % predicted error verified the applicability of the generated models to optimize FH-LPH (Abdelbary and Aboughaly, 2015). So, the optimized formula, referred to as OFH-LPH was selected for further studies.

Fig. 3. (A), (B), and (C): PyMol 3D and LigPlot 2D schematic diagrams of protein–ligand interactions of the best mode of binding of Fluoxetine hydrochloride. (E) and (F): LigPlot 2D schematic diagrams of protein–ligand interactions of the subsequent best modes of binding of Fluoxetine hydrochloride. The interactions shown are those mediated by hydrogen bonds and by hydrophobic contacts. Hydrogen bonds are indicated by dashed lines between the atoms involved, while hydrophobic contacts are represented by an arc with spokes radiating towards the ligand atoms they contact. The contacted atoms are shown with spokes radiating back.

S.E. Khater et al.
Table 3
Experimental design matrix of the critical process parameters and the related critical quality attributes.

| Run | Critical process parameters (CPPs) | Critical quality attributes (CQAs) |
|-----|----------------------------------|-----------------------------------|
|     | A PLGA (mg) | B Drug (mg) | C Stirring speed (rpm) | Y1 Particle size (nm) | Y2 EE (%) | Y3 Zeta potential (mV) |
| 1   | 10          | 10          | 15                   | 750                  | 215.2±2.4 | 53.1±3.5 | 9.3±1.1 |
| 2   | 10          | 10          | 10                  | 1000                 | 164.8±2.2 | 47.2±3.7 | 15.3±1.4 |
| 3   | 15          | 20          | 750                   | 211.4±3.5 | 72.7±4.1 | 7.2±1.2 |
| 4   | 10          | 15          | 750                   | 215.2±3.7 | 55.5±3.2 | 9.3±1.6 |
| 5   | 5           | 5           | 15                   | 1000                 | 145.6±1.8 | 60.5±3.5 | 11.8±1.4 |
| 6   | 15          | 15          | 1000                   | 240.5±2.2 | 73.1±2.8 | 10.2±1.1 |
| 7   | 10          | 15          | 750                   | 213.2±3.1 | 55.7±3.7 | 9.4±1.4 |
| 8   | 10          | 15          | 750                   | 213.5±3.4 | 53.1±4.5 | 9.4±1.6 |
| 9   | 10          | 15          | 750                   | 215.2±2.6 | 55.6±3.4 | 9.3±1.2 |
| 10  | 10          | 20          | 1000                 | 179.8±1.7 | 65.1±3.6 | 10.5±1.4 |
| 11  | 5           | 15          | 500                   | 172.5±2.4 | 50.7±3.3 | 8.2±1.7 |
| 12  | 5           | 20          | 750                   | 110.8±1.8 | 52.5±4.2 | 10.4±1.2 |
| 13  | 10          | 10          | 500                   | 175.4±3.3 | 46.3±4.4 | 8.5±1.1 |
| 14  | 15          | 15          | 500                   | 235.8±3.7 | 62.4±3.4 | 5.5±1.5 |
| 15  | 5           | 10          | 750                   | 130.4±2.8 | 50.5±4.6 | 11.3±1.4 |
| 16  | 15          | 10          | 750                   | 169.5±2.2 | 62.9±4.1 | 10.4±1.8 |
| 17  | 10          | 20          | 500                   | 189.1±3.4 | 41.5±3.8 | 10.6±1.5 |

* Measured by DLS.
\( \text{Y} \) Calculated as a percentage of initial drug added, determined indirectly by HPLC.
\( \text{Y} \) Expressed as mean ± SD (n = 3).

3.3. In vitro characterization of the selected OFH-LPH

3.3.1. Loading efficiency percentage (LE%)

Table 4 revealed that the LE% of the OFH-LPH was 10.3 ± 0.4%. This result was in agreement with the LE% values of different drugs in LPH nanoparticles as amphotericon B ≈ (9%) (Asthana et al., 2015), for ropinirole hydrochloride (9.9 – 12.5) (Pardeshi et al., 2013), and etoposide (8–10%) (Duan et al., 2017).

3.3.2. Morphological studies

The TEM image of the OFH-LPH demonstrated spherical non-aggregated particles with a white polymeric core surrounded by a gray lipid shell (Fig. 7A) (Mandal et al., 2016). The particles have a size range of 100–120 nm which was consistent with the DLS measurements. Furthermore, the 2D and the 3D structure of the OFH-LPH nanoparticles showed scattered particles with a particle size consistent with both DLS and TEM (Fig. 7-B, C).

3.3.3. Differential scanning calorimetry

The DSC thermograms of FH, blank LPH, and OFH-LPH are illustrated in Fig. 7D. According to the FH thermogram, FH has a crystalline nature with a melting point at 160 °C as previously reported (Silva et al., 2007). The endothermic peak of FH was completely disappeared in the DSC thermogram of OFH-LPH indicating the transformation of the drug into the amorphous state as a result of its encapsulation in the LPH nanoparticles (Ishak et al., 2017; Mandal et al., 2016). Moreover, the PLGA polymer prevents the recrystallization of the drug during the preparation of LPH nanoparticles (Mandal et al., 2016). Additionally, both blank LPH and OFH-LPH thermograms represent an endothermic peak at 50 °C which is correlated to the glass transition temperature of PLGA (Ishak et al., 2014) which indicated a negligible effect of formulation procedures on PLGA (Sanna et al., 2015).

3.3.4. In vitro serum stability assay

Fig. 8A-C shows the effect of serum incubation on particle size, PDI, and zeta potential of OFH-LPH. The OFH-LPH was stable in 10% v/v FBS with a non-significant effect on particle size, PDI, and zeta potential at all tested time points (P > 0.05). Moreover, the presence of 50% v/v FBS non-significantly affects the particle size, PDI, and zeta potential of OFH-LPH (P > 0.05) after 4 h incubation. But after 24 h incubation, there was a significant increase in the particle size, PDI, and zeta potential of OFH-LPH (P < 0.05).

The negativity of the OFH-LPH surface (-10.5 mV) might be sufficient to prevent the adsorption of a low concentration of serum proteins (10% v/v FBS) by the electrostatic repulsion (Zhao et al., 2015). Contrarily, the presence of a high concentration of serum proteins (50% v/v FBS) for a long incubation time (24 h) might enhance the adsorption of a large number of serum proteins to the OFH-LPH surface and consequently, the particle size, PDI, and zeta potential were increased.

3.3.5. In vitro FH release

Fig. 9A illustrated the in vitro release study in PBS (pH 7.4) with/without 10% v/v, and 50% v/v FBS. FH is a hydrophilic drug that is completely diffused through the dialysis membrane after 2 h. On the contrary, FH was released from the LPH in PBS and 10% v/v FBS in a controlled release manner and released 86.6 ± 4.5% and 92.1 ± 4.2% after 24 h, respectively with almost similar release profile (J2 value of 68.1%). The controlled release pattern of LPH nanoparticles might be attributed to their architecture as they consist of a polymeric core that encapsulates the drug and a lipid shell that inhibits the drug leakage (Date et al., 2018). Besides, it reduces the water penetration to the polymeric core and consequently decreases its hydrolysis rate (Hadinoto et al., 2013). On the other hand, 50% v/v FBS increased FH release and achieved 100% release after 24 h with J2 value of 42.5%. The high

Fig. 4. Response 3D plot for the different interactions on LPH particle size (Y1). (A) The positive interaction (AB) between PLGA and drug amounts showed a positive effect on FH-LPH particle size. (B) Interaction (AC) between PLGA amount and stirring speed showed a positive effect on FH-LPH particle size.
concentration of serum proteins and lipase enzyme might induce the hydrolysis of the lipid shell and consequently enhances the polymeric core dissolution and increases the drug release (Mat Azmi et al., 2015).

The application of different kinetics models to the in vitro release data revealed that the diffusion mechanism was attained for the FH release profile in PBS pH7.4 with/without 10% v/v FBS with an $R^2$ value of 0.9969 and 0.9972, respectively. On the other hand, the FH release profile in the presence of 50% v/v FBS followed the Korsmeyer-Peppas model with an $R^2$ value of 0.9989 and a release exponent value of $n = 0.773$. Therefore, it represents a non-fickian diffusion, that resulted from the combination of both drug diffusion and polymer dissolution (Costa and Sousa Lobo, 2001; Ho et al., 2017). These results were in agreement with those of Tahir et al., who reported that the doxorubicin hydrochloride release pattern from LPH obeyed the diffusion mechanism (Tahir et al., 2019).

Table 4
The experimental and predicted particle size, EE% and zeta potential of the OFH-LPH, and the loading efficiency%.

| Parameter          | PLGA (mg) | FH (mg) | Stirring speed (rpm) | Experimental $^{\text{a,c}}$ | Predicted | %Predicted error | Loading efficiency% $^c$ |
|--------------------|-----------|---------|----------------------|-----------------------------|-----------|------------------|--------------------------|
| Particle Size $^{\text{a}}$ (nm) | 5         | 20      | 1000                 | 98.5 $\pm$ 3.5              | 98.2      | 0.3              | 10.3 $\pm$ 0.4%           |
| EE $^{\text{b}}$ (%)      | 65.1 $\pm$ 2.7 | 63.5    | 2.5                  | 10.5 $\pm$ 0.45             | 10.9      | 3.8              |
| Zeta Potential $^{\text{a}}$ (mV) |          |         |                      |                             |           |                  |                          |

$^a$ Measured by DLS.
$^b$ Calculated as a percentage of initial drug added, determined indirectly by HPLC.
$^c$ Expressed as mean $\pm$ SD ($n = 3$).
$^d$ Statistical analysis was carried out using a student’s T-test $P < 0.05$.
$^* Loading efficiency % was calculated for the optimized formula only.
3.3.6. In vitro hemolytic assay

A positive correlation between OFH-LPH concentration and hemolysis % (Fig. 9B) could be attributed to the presence of tween 80 (Ishak et al., 2017; Sun et al., 2017). However, the % hemolysis at all tested concentrations did not exceed the acceptable limit of nanocarriers (5%) according to the new consensus ASTM E2524-08-Standard test (Ishak et al., 2017). Therefore, the prepared OFH-LPH could be considered a biocompatible platform.

3.3.7. Short term stability study

The stability study of the OFH-LPH formula was performed on the freeze-dried form to decrease accumulation and avoid leakage of drug.
from the polymeric core and lipid layer during storage (Dave et al., 2017; Yuan et al., 2018). At different time intervals 7, 14, 21, and 28 days, the freeze-dried formula was redispersed in deionized water and characterized for its morphology. It has a non-significant change in its particle size, PDI, zeta potential, and EE% after 28 days of storage at 4 °C and 25 °C/60 ± 5% RH (P > 0.05) as illustrated in Table S10. Furthermore, these results were in agreement with the previous studies (Dave et al., 2017; Sengel-Turk and Hascicek, 2017).

3.3.8. In vitro cytocompatibility assay

Fig. S13 revealed that the % cell viability of the prepared OFH-LPH formula exceeded 80% at all tested concentrations. The LPH formula loaded with FH consists of biocompatible polymer (PLGA) and lipid (LEC) therefore it improved its biocompatibility to the Human lung fibroblast (CCD-19Lu) Cells.

3.3.9. Assessment of cellular uptake by flow cytometry

The cellular uptake quantification of the Dil-labeled OFH-LPH (50 nM) by the Human lung fibroblast (CCD-19Lu) cells was assessed for 4 and 24 h. Where Dil is a fluorescence lipophilic carbocyanine dye that has a high fluorescence efficiency and photo-stability (Cheng et al., 2014). Additionally, it successfully labeled different lipidic and polymeric nanoparticles (Hamdi et al., 2020; Mousseau et al., 2019; Snipstad et al., 2017). The fluorescence was highly intensified following the treatment of cells with the Dil-labeled OFH-LPH in a time-dependent manner as illustrated in Fig. 10A and B. As the longer incubation time improved the cellular uptake by cells (Hamdi et al., 2020). Moreover, the small particle size of OFH-LPH (98.5 nm) might enhance their entry to the cells via clathrin and caveolin-mediated endocytosis (Panariti et al., 2012; Thorley et al., 2014). Therefore, LPH could efficiently improve the cellular internalization of FH into Human lung fibroblast (CCD-19Lu) Cells.

4. Conclusion

In this study, we investigate the binding affinity of different SSRIs drugs to the SARS-COV-2 main protease to assess their potential therapeutic activity against COVID-19. Among the studied drugs, FH had a promising SARS-COV-2 main protease inhibitory activity with a binding energy of (−6.7 kcal/mol) and a hydrogen bonding formation with histidine163 and serine 144 amino acids. Moreover, the encapsulation of FH in the LPH nanoparticle improved its EE%, promote its biocompatibility, and enhance its cellular uptake by the Human lung fibroblast (CCD-19Lu) cells. Therefore the fabricated FH-LPH formula represents a promising therapy for the COVID-19 pandemic. Additionally, these results along with the prior works on FH, reinforce the scientific presumption of FH efficacy in the COVID-19 pandemic, and that large-scale phase 3 trials testing FH and FH-LPH are urgently needed.
Ravi, P.R., Vats, R., Dalal, V., Gadekar, N., Aditya, N., 2015. Design, optimization and evaluation of poly-e-caprolactone (PCL) based polymeric nanoparticles for oral delivery of Iopinavir. Drug Dev. Ind. Pharm. 41, 141–160. https://doi.org/10.3109/03639045.2015.857101.

Rosen, D.A., Seki, S.M., Fernández-Castaneda, A., Beiter, R.M., Eccles, J.D., Woodfolk, J. A., Gautier, A., 2019. Modulation of the sigma-1 receptor (SIG1R) pathway is beneficial in preclinical models of inflammation and sepsis. Sci. Transl. Med. 11, eaau5266. https://doi.org/10.1126/scitranslated.aau5266.

Roumestan, C., Michel, A., Bichon, F., Portet, K., Detoc, M., Henriquet, C., Jaffuel, D., Mathieu, M., 2007. Anti-inflammatory properties of desipramine and fluoxetine. Respir. Res. 8, 35. https://doi.org/10.1016/j.art.2006.12.004.

Sacre, S., Medghalchi, M., Gregory, B., Brennan, F., Williams, R., 2010. Fluoxetine and riposono reuptake inhibitor fluoxetine inhibits replication of human enteroviruses B and D by targeting viral protein 2C. Antimicrob. Agents Chemother. 57, 1952–1956. https://doi.org/10.1128/AAC.02884-12.

Skelton, S., Kadhiresan, P., Malenckij, A., Honopak, A., Osborne, M., Li, Y.V.C., Chen, H., Mubarak, S., Gubay, J.B., Chen, W.C., 2020. Determining COVID-19: The Disease and Tools for Detection. ACS Nano 14, 3822–3835. https://doi.org/10.1021/acsnano.0c02624.

Ulferts, R., Van Der Linden, I., Tilbault, H.J., Lanne, K.H.W., Leyssens, P., Coudert, B., De Palma, A.M., Canard, B., Neys, J., Van Kuppeveld, F.J.M., 2013. Selective serotonin reuptake inhibitor fluoxetine inhibits replication of human enteroviruses B and D by targeting viral protein 2C. Antimicrob. Agents Chemother. 57, 1952–1956. https://doi.org/10.1128/AAC.02884-12.

Yakovlev, P., Gerber, M., Kley, D., Paesler, S., Braga Lagache, S., Simion, C., Portmann, J., Stalder, H., Gaschen, V., Bruggmann, R., Stoffel, M.H., Helller, M., Dijkman, R., Thiel, V., 2019. Determination of host proteins composing the microenvironment of coronavirus replicase complexes by proximity-labeling. Elife 8, 1–30. 10.7554/eLife.42037.

Wang, L., Wang, R., Liu, L., Qiao, D., Baldwin, D.S., Hou, R., 2019. Effects of SSRIs on peripheral inflammatory markers in patients with major depressive disorder: A systematic review and meta-analysis. Brain. Behav. Immun. 79, 24–38. https://doi.org/10.1016/j.bbi.2019.02.021.

WHO, 2017. Stability testing of active pharmaceutical ingredients and finished pharmaceutical products, WHO technical report series.

Wu, D., Wu, T., Liu, Q., Yang, Z., 2020. The SARS-CoV-2 outbreak: What we know. Int. J. Infect. Dis. 94, 44–48. 10.1016/j.ijid.2020.03.004.

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., Tai, Y., Bai, C., Gao, T., Song, J., Xia, P., Dong, J., Zhao, J., Wang, F.S., 2020. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir. Med. 8, 420–422. 10.1016/S2213-2600(20)30076-X.

Ye, Q., Wang, B., Mao, J., 2020. The pathogenesis and treatment of the ‘Cytokine Storm’ in COVID-19. J. Infect. 80, 607–613. https://doi.org/10.1016/j.jinf.2020.03.037.

Young, K.C., Bai, C.H., Su, H.C., Tsai, P.J., Pu, C.Y., Liao, C.S., Lin, Y.M., Lai, H.W., Chong, L.W., Tsai, Y.S., Tsao, C.W., 2014. Fluoxetine a novel anti-hepatitis C virus agent via ROS-, JNK-, and PPAR-γ-dependent pathways. Antiviral Res. 110, 158–167. https://doi.org/10.1016/j.antiviral.2014.08.002.

Yuan, Y., Chiba, P., Cai, T., Callaghan, R., Bai, L., Cole P.C., S., Cai, Y., 2018. Fabrication of poral-aloaded lipid-polymer hybrid nanoparticles and their rheval effect on drug resistance of cancer cells. Oncol Rep 40, 1055–1063. 10.1080/10212670.2016.126492.

Zhao, X., Li, F., Li, W., Wang, H., Ren, H., Chen, J., Nie, G., Hao, J., 2015. Co-delivery of HIF1α siRNA and gencatibine via biocompatible lipid-polymer hybrid nanoparticles for effective treatment of pancreatic cancer. Biomaterials 46, 13–25. https://doi.org/10.1016/j.biomaterials.2014.12.028.

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, J., Jiang, R.-D., Liu, M.-Q., Chen, Y., Shen, X.-R., Wang, X., Zheng, X.-S., Zhao, K., Chen, Q.-J., Dong, F., Liu, L.-L., Yan, B., Zhan, F.-X., Wang, Y.-Y., Xiao, G.-F., Shi, Z.-L., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273. https://doi.org/10.1038/s41586-020-2012-7.

Zimniak, M., Kirschner, L., Hilpert, H., Griger, N., Danow, D., Oberwinkler, H., Steinke, M., Sewald, K., Seidel, J., Bodem, J., 2021. The serotonin reuptake inhibitor Fluoxetine inhibits SARS-CoV-2 in human lung tissue. Sci. Rep. 11, 5890. https://doi.org/10.1038/s41598-021-85049-0.