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Discovery of juglone and its derivatives as potent SARS-CoV-2 main proteinase inhibitors

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ABSTRACT

SARS-CoV-2 as a positive-sense single-stranded RNA coronavirus caused the global outbreak of COVID-19. The main protease (Mpro) of the virus as the major enzyme processing viral polyproteins contributed to the replication and transcription of SARS-CoV-2 in host cells, and has been characterized as an attractive target in drug discovery. Herein, a set of 1,4-naphthoquinones with juglone skeleton were prepared and evaluated for the inhibitory efficacy against SARS-CoV-2 Mpro. More than half of the tested naphthoquinones could effectively inhibit the target enzyme with an inhibition rate of more than 90% at the concentration of 10 μM. In the structure-activity relationships (SARs) analysis, the characteristics of substituents and their position on juglone core scaffold were recognized as key ingredients for enzyme inhibitory activity. The most active compound, 2-acetyl-8-methoxy-1,4-naphthoquinone (15), which exhibited much higher potency in enzyme inhibitions than shikonin as the positive control, displayed an IC50 value of 72.07 ± 4.84 nM towards Mpro-mediated hydrolysis of the fluorescently labeled peptide. It fit well into the active site cavity of the enzyme by forming hydrogen bonds with adjacent amino acid residues in molecular docking studies. The results from in vitro antiviral activity evaluation demonstrated that the most potent Mpro inhibitor could significantly suppress the replication of SARS-CoV-2 in Vero E6 cells within the low micromolar concentrations, with its EC50 value of about 4.55 μM. It was non-toxic towards the host Vero E6 cells under tested concentrations. The present research work implied that juglone skeleton could be a primary template for the development of potent Mpro inhibitors.

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1. Introduction

Coronavirus disease 2019 (COVID-19) is a serious infectious disease caused by a new coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2]. The rapid spread of this pneumonia disease is an ongoing global threat that generates over 197 million diagnosed cases and more than 4.21 million deaths over 233 countries and territories globally by 03 Aug 2021 [3]. Until now, no clinically specific antiviral chemotherapeutics were available to treat the disease. The approved chemotherapeutic drugs against COVID-19 included favipiravir [4], lopinavir/ritonavir [5], chloroquine/hydroxychloroquine (FDA revoked emergency use authorization for chloroquine and hydroxychloroquine on June 15, 2020) [6], and remdesivir [7,8]. All of these drugs had been developed for the treatment of other related viruses, such as SARS and MERS coronavirus, Ebola, and HIV. Their degree of efficacy in COVID-19 treatment and the side effects were still controversial issues in academia [9–12]. The monoclonal antibody therapy employing bamlanivimab and antibody combination (bamlanivimab/etesevimab) has been authorized by FDA recently. However, certain variants of SARS-CoV-2 (B.1.351 and B.1.1.248) might escape from these neutralizing antibodies [13,14]. Therefore, it is urgent to explore targeted antiviral chemotherapeutics against SARS-CoV-2.

SARS-CoV-2 virus is a positive-sense single-stranded RNA virus [15,16], and its genome is translated to two overlapping polyproteins upon entry into host cells. The two polypeptides are proteolytically processed, mainly by a 33.8-kDa virus-specific main protease (Mpro), to afford proteins with different structures and functions required for replication [17,18]. The Mpro also referred to as the 3C-like protease modified the polyproteins at no less than 11 conserved amide linkages and played a pivotal role in the replication cycle of SARS-CoV-2 in host cells. Since closely related homologues of Mpro have never been identified in host cells, the protease is identified as a potential therapeutic target for the control of virus replication [19,20].
Natural products are a wellspring of lead compounds in drug discovery [21], and several phytochemicals have been investigated for their therapeutic potentials against SARS-CoV-2 [22,23]. Previous studies conducted by Jin et al. indicated that shikonin (Fig. 1, 1) is a natural naphthoquinone isolated from Lithospermum erythrorhizon Sieb. et Zucc., was a strong inhibitor of SARS-CoV-2 Mpro with its IC50 value of 15.75 ± 8.22 μM [18]. However, due to the Michael addition of shikonin naphthazarin nucleus as electrophiles [24,25] and bioreductive alkylation of its side chain with nucleophilic biomolecules such as glutathione, proteins or DNA [26], shikonin demonstrated significant cytotoxic effects at concentrations ranging from 100 μg/mL to 10 ng/mL in vitro [27]. The toxicity of shikonin prevented its further development as an antiviral drug candidate. Therefore, rational structural modifications were essential to overcome the defects in the structure of this hallmark molecule.

The research results from mechanistic investigations implied that the side chain and adjacent phenolic hydroxy group on core structure of shikonin tautomer (Fig. S1, Supplementary Information) played pivotal roles in bioreductive alkylation and conjugate addition with biocatalysts [28], which gave rise to the cytotoxicity of shikonin. Accordingly, we decided to modify shikonin skeleton through a scaffold simplification strategy to get juglone derivatives with a more appropriate scaffold in terms of improving cellular toxicity. Juglone (2) is a naturally occurring 1,4-naphthoquinone identified in Juglandaceae species, which bears a simplified shikonin core structure. It exhibited comparably low cytotoxicity against normal peripheral blood mononuclear cells with an IC50 value of more than 5 μg/mL [28]. It had been prescribed as a remedy for the treatment of a variety of skin diseases in the early 1900s [29]. The synthetic 2-methyl-1,4-naphthoquinone (menadione, 3) that served as a nutritional supplement in animal feeding was also much less toxic [30]. The results from earlier clinical investigations demonstrated that no toxic effects were observed in patients with hypoprothrombinaemia receiving menadione doses from 1 to 200 mg [31]. All of the findings mentioned above supported our initial hypothesis that scaffold simplification and modification of shikonin naphthazarin nucleus would be reasonable approaches to reduce the cytotoxicity of shikonin as a natural SARS-CoV-2 Mpro inhibitor.

As a continuing investigation of biological activities of 1,4-naphthoquinones with shikonin as a lead, and in order to contribute to the drug discovery against COVID-19, the present study afforded the discovery of juglone and its derivative as potent Mpro inhibitors of SARS-CoV-2, which are promising antiviral drug candidates in future research and development.

2. Results

Chemistry. As shown in Scheme 1, the starting material for this synthesis of 1,4-naphthoquinone (5) was 1-naphthylamine (4). The oxidation of 1-naphthylamine (4) with hydrogen peroxide and subsequent column chromatography afforded 1,4-naphthoquinone 5. Further Thiele-Winter acetoxylation of 1,4-naphthoquinone and subsequent hydrolysis of naphthalene-1,2,4-triyl triacetate (6) afforded 2-hydroxy-1,4-naphthoquinone (7) in high yield [32]. 2-Methyl-1,4-naphthoquinone (vitamin K3, 3) was obtained by the oxidation of 2-methylnaphthalene (8) using chromic anhydride in acidic conditions.

The reactions used to synthesize juglone (2) and its derivatives were outlined in Scheme 2. Juglone (2) was synthesized by the oxidation of 1,5-naphthelendiol (9) using the Freny’s salts, Soxhlet extraction, and further column chromatographic purification [33]. Juglone methyl ether (10) was obtained by the reductive methylation of juglone (1) with the presence of dimethyl sulfonate and sodium dithionate in alkali conditions and further cerium (IV) ammonium nitrate-mediated oxidative demethylation [34]. Propionyl juglone (11) and acetyl juglone (12) were prepared by the acylation of the parent compound by corresponding acid anhydride with sulfuric acid as the catalyst [35]. Acetyl juglone (12) was reduced by sodium dithionate to yield the hydroquinone intermediate. Migration of the acetyl group of hydroquinone in alkali conditions and further methylation afforded 4,8-dimethoxy-1-naphthol acetate (13) as the key intermediate [35]. Fries rearrangement of naphthol acetate (13) and subsequent CAN-mediated oxidation produced 2-acetyl-8-methoxy-1,4-naphthoquinone (15). 5,8-Dimethoxy-1,4-naphthoquinone (21) was prepared by the methylation of 1,5-naphthelenediol (9), subsequent bromination reactions, Cu(I)-catalyzed nucleophilic substitution [36], and further CAN-mediated oxidation (Supplementary Information).

7-Methyl juglone (16) and its derivatives (22–25) were prepared according to our reported procedures [37] resting on the Stobbe condensation of 2,5-dimethoxy benzaldehyde with diethyl succinate, intramolecular cyclization, reduction, acid-facilitated debenzylation and further oxidation. Plumbagin (17) was synthesized based on the reported procedure in which 1,5-naphthelenediol served as the starting point [38]. The substituted anthraquinone emodin (18), rhein (19) and aloe emodin (20) were prepared by the D-A reactions between diene and corresponding naphthoquinones [39,40].

Enzyme inhibition. Using a fluorescently labeled short peptide containing Q-S scissile bond, the inhibitory activity of the prepared quinones against Mpro of SARS-CoV-2 were evaluated according to the reported procedure [17]. Firstly, we tested the enzymatic inhibition rate of several naturally occurring naphthoquinones (juglone 2, 7-methyl juglone 16, lawsone 7, plumbagin 17 and shikonin 1), 9,10-anthraquinones (emodin 18, rhein 19 and aloe emodin 20) and the synthetic vitamin K3 (3) in the first library of compounds against SARS-CoV-2 Mpro at the concentration of 10 μM. The results from primary screening indicated that most of the natural quinones were ineffective, with the inhibition rate of less than 10% at 10 μM (Table S1, Supplementary Information). Vitamin K3 (3) with the inhibition rate of 12.7% was also inactive. The natural naphthoquinone shikonin, which had been identified as one of the

Fig. 1. The chemical structure of shikonin (1), juglone (2) and menadione (3).
strong Mpro inhibitors in previous studies (IC$_{50}$ = 15.75 ± 8.22 μM) [17], was employed as the positive control. It demonstrated moderate inhibitory effects towards the target enzyme at the concentration of 10 μM. In the first library of naphthoquinones, juglone (2) and 7-methyl juglone (16) exhibited the strongest inhibition with the completely loss of the hydrolytic efficacy of Mpro. The two natural naphthoquinones were employed as the lead compounds for further structural modifications.

In the second library, the derivatives of juglone (2) and 7-methyl juglone (16) were produced by the addition of a few groups on their naphthoquinone scaffold and modifications on the phenolic hydroxyl group on the B-ring. The enzyme inhibition rate of compounds in the second library was displayed in Table S2. The results implied that almost all of the derivatives in the second library maintained the high inhibitory potency of juglone under concentrations of both 10 μM and 1 μM. At the concentration of 0.1 μM, a few analogues exhibited much higher potency as compared with the parent compounds (2 and 16). Then, the compounds with an enzymatic inhibition rate of more than 25% at the concentration of 0.1 μM entered the IC$_{50}$ value screening (Table S3).

As shown in Table S3, within the tested synthetic 1,4-naphthoquinones as strong Mpro inhibitors, 2-acetyl-8-methoxy-1,4-naphthoquinone (15) was characterized as the most potent inhibitor against the target enzyme with its IC$_{50}$ value of 72.07 ± 4.84 nM, which was comparable to the recently reported IC$_{50}$ value of a short peptide as SARS-CoV-2 Mpro inhibitor (IC$_{50}$ = 53 ± 5 nM) [17]. The 1,4-naphthoquinone (5) and propionyl juglone (11) have also been identified as potent inhibitors with IC$_{50}$ values of 110.13 ± 7.04 and 129.77 ± 0.45 nM, respectively. 7-Methyl juglone ethyl acetate (23) and its benzyl ether (25) exhibited much higher IC$_{50}$ values than propionyl juglone did.

Structure-activity relationship studies. In the first library of compounds (Table S1), the natural naphthoquinone juglone (2) exhibited potent inhibitory effects against the target SARS-CoV-2 Mpro with the inhibition rate of 99% at the concentration of 10 μM. By contrast, lawsone (7) as its analogue did not exhibit any detectable inhibition against Mpro under the same concentration. The sharp decrease in the activity should be ascribed to the
transposition of the B-ring hydroxyl group to the quinone ring. Compared with juglone, plambagin (17) with an additional methyl group on A-ring was also inactive towards M\textsuperscript{pro}. Similarly, the synthetic vitamin K\texttextsuperscript{3} (3) with a methyl group on the quinone ring displayed the enzymatic inhibition rate of only 12.7% as compared with the negative control. The elimination of the A-ring methyl group of vitamin K\texttextsuperscript{3} led to a significant increase in the efficacy in diminution of SARS-CoV-2 M\textsuperscript{pro} activity, because emodin (18), rhein (19) and aloe emodin (20) as derivatives of 7-methyl juglone showed the inhibition rate of less than 10%. The increased steric hindrance of the annulated phenyl ring caused detrimental effects on binding 7-methyl juglone with the target enzyme.

The methylated 7-methyl juglone led to a drop in the activity at low concentrations, and 7-methyl juglone methyl ether (22) did not show any inhibitory effects towards the target enzyme at the concentration of 0.1 \textmu M. Protection of the phenolic hydroxyl group with a methoxy methyl ether moiety also had deleterious effects since the M\textsuperscript{pro} inhibition rate of compound 24 was lower than 5% at concentrations of less than 1.0 \textmu M. By contrast, both acylation and benzylation of the phenolic hydroxyl group of 7-methyl juglone caused an increase in potency at the concentration of 0.1 \textmu M. The benzylation compound 25 exhibited an IC\textsubscript{50} value of 160.68 \pm 17.83 nM towards the target enzyme (Table S3), which was less than three-quarters of the value for the acylated derivative 23 (IC\textsubscript{50} = 220.90 \pm 14.03 nM).

**Molecular docking.** In order to gain an insight into the binding interaction of investigated naphthoquinones with SARS-CoV-2 M\textsuperscript{pro} enzyme, we performed molecular docking studies based on the crystal structures of M\textsuperscript{pro} in complex with the peptide-like inhibitor N3 (PDB ID: 6LU7). As shown in Fig. 3, both juglone (a), propionyl juglone (b) and 2-acetyl-8-methoxy-1,4-naphthoquinone (c) tightly fit the active site cavity of the enzyme. Juglone was bound to the target enzyme with the calculated binding energy of \(-8.6771\) kJ/mol. The docking results indicated that the C(1) carbonyl group formed a hydrogen bond with Gly\textsuperscript{143} amino acid residue. Another hydrogen bonding interaction between the phenolic hydroxyl group and Glu\textsuperscript{166} was also observed.

The molecular docking study of propionyl juglone with the crystal structure of M\textsuperscript{pro} (Fig. 3, b) showed that this ligand fit well into the substrate binding site of M\textsuperscript{pro} enzyme. Propionyl juglone was bound to the target enzyme with the calculated binding energy of \(-17.3199\) kJ/mol, which was the lowest value for all of the predicted 30 binding models in MOE molecular docking. The oxygen atom of the C(4) carbonyl group underwent simultaneous H-bonding interactions with the backbone NH of Gly\textsuperscript{143} and the hydroxyl group of Ser\textsuperscript{144}.

Fig. 2. SARS analysis of juglone and its derivatives as SARS-CoV-2 M\textsuperscript{pro} inhibitors.

2-Acetyl-8-methoxy-1,4-naphthoquinone (15) as the most
potent inhibitor against the target enzyme in the study was also bound to the substrate binding site of Mpro (Fig. 3, c). The C(4) carbonyl group was oriented towards the imidazole moiety of His41 with the formation of a hydrogen bonding interaction. The oxygen atom on the acetyl substitution also hydrogen bonded with the backbone of NH of Gly143. The methoxy group of compound 15 was placed towards Glu166, and there was an H-bonding interaction between the oxygen atom in the methoxy group and NH in the amide backbone of Glu166. The tight binding interaction between 2-acetyl-8-methoxy-1,4-naphthoquinone (15) and the target enzyme should explain its potent inhibitory activity against the enzymatic activity of Mpro.

Cytotoxicity of Juglone and its derivatives. The ideal antivirus agents were those ones that acted by inhibiting viral replication, but without cytotoxicity towards host normal cells [41]. Therefore, juglone and its derivatives as SARS-CoV-2 Mpro inhibitors were initially tested for their cytotoxic activity against human normal fibroblast HFF-1 cells using the standard MTT assay. As presented in Table S4, the naturally occurring juglone (2), 7-methyl juglone (16), and shikonin (1) exhibited potent growth inhibition towards the proliferation of HFF-1 cells with their IC50 values of less than 5 μM. The methylation and acylation of the phenolic hydroxyl group of juglone led to a minor decrease in cytotoxicity. Propionyl juglone (11) as a potent Mpro inhibitor was also toxic towards normal HFF-1 cells. It possibly underwent hydrolysis catalyzed by cytoplasmic enzymes to afford juglone (2) as a cytotoxic metabolite (Fig. 4). By contrast, the absence of the B-ring hydroxyl group of juglone caused a significant decrease in toxicity, because 1,4-naphthoquinone (5) exhibited a much higher IC50 value towards the normal HFF-1 cells.

The cytotoxicity of 7-methyl juglone (16) tended to be attenuated by the benzylation of the hydroxyl group on B-ring, and the IC50 value of compound 25 was 7-fold higher than that of the parent compound 16. Lawsone (7) and vitamin K3 (3) with a substituent on the quinone ring displayed almost no cytotoxic effects on HFF-1 cells [IC50 > 50 μM]. The electron donating effects and the steric hindrance of the group adjacent to the quinoidal carbonyl group prevented Michael addition of the quinone ring with nucleophilic biomolecules. 2-Acetyl-8-methoxy-1,4-naphthoquinone (15) was also much less toxic towards normal HFF-1 cells with its IC50 value of 41.2 μM. The presence of the acetyl moiety on A-ring prohibited the generation of ROS species and nucleophilic conjugate additions of quinone moiety with nucleophiles. Due to its strong inhibitory potency towards SARS-CoV-2 Mpro and low cytotoxic profile, it entered further in vitro antiviral activity evaluations.

Antiviral activity. The antiviral activity of compound 15 to inhibit SARS-CoV-2 replication in vitro was conducted according to the reported procedures [18]. 2-Acetyl-8-methoxy-1,4-naphthoquinone (15) exhibited antiviral activity at concentrations of more than 1 μM, with the half-maximal effective concentrations (EC50) of 4.55 μM. The result indicated that the quinone (15) possibly penetrate cellular membranes and inhibit the target viral Mpro enzyme. The results from cytotoxicity evaluations implied that the compound was much less toxic than juglone towards normal HFF-1 cells. At the concentration of less than 20 μM, it didn't affect the growth of host Vero E6 cells (Fig. 5, b, cell viability of more than 90%). Balb/C mice that received the preparation of the target compound (Fig. S2, 100 mg/kg, p.o., on every the other day, 10 times...
in 20 days) did not show any obvious toxicity symptoms like reduced activity, hypothermia, or body shivering. Meanwhile, compound 15 induced no animal deaths and only caused a minor body weight loss as compared with control animals after a total treatment of 10 times in 20 days. As a potent Mpro inhibitor with antiviral activity, the juglone derivative 15 deserved further in vivo antiviral activity evaluation in future studies.

2.1. Discussion and future perspectives

Herein, we have described the discovery of juglone and its derivatives as potent Mpro inhibitors against SARS-CoV-2. Earlier chemical investigations disclosed the presence of juglone as a bioactive ingredient in *Exocarpium juglandis Immaturum*, a traditional Chinese medicine used to treat psoriasis, ichthyosis, sores, and furuncles in the Orient [42]. It has also historically been used in European folk medicines as a remedy for parasites, ringworm, and other fungal infectious diseases [43]. The research results from previous investigations demonstrated that the natural naphthoquinone juglone was active against the animal Vesicular Stomatitis Virus [44] and it could potently reactive latent HIV-1 in the bcl-2-transduced primary CD4+ T cell model [45]. The exact mechanism by which juglone acts against virus infections, however, still remains unclear. In our studies, this naphthoquinone was characterized as a potent inhibitor against SARS-CoV-2 Mpro by a high-throughput screening assay. It completely inactivated the main protease at the concentration of 1 μM. 3C-like proteases (Mpro in coronavirus), which belong to the cysteine protease family with a chymotrypsin-like fold, have been widely characterized in positive-sense single-stranded RNA viruses. In addition, 3C-like proteases shared several general similarities in substrate specificity and also inhibitor effectiveness [46]. Therefore, the structural features of juglone as a non-peptide inhibitor might act as a valuable scaffold for further anti-coronavirus drug design. Additionally, the results of our study also provided one explanation of the antiviral molecular mechanism of juglone.

Since the cleavage of viral proteins by specific proteases was crucial at post-entry stage in virus replication cycles, the SARS-CoV-2 Mpro was an attractive target for selective chemotherapeutic attack. The identified phytochemicals as Mpro inhibitors included glycosylated flavonoids [23,47], the diterpene andrographolide [48], the coumarin isopimpinellin [23], the naphthoquinone shikonin [18], and the alkaloid thalimonine [49]. However, most of these inhibitors were characterized in virtual screening. Data from in vitro evaluations were essential to confirm the potential of these phytochemicals in enzymatic inhibition.

In our studies, 2-acetyl-8-methoxy-1,4-naphthoquinone (15) exhibited the most potent inhibition against SARS-CoV-2 Mpro among the synthesized 1,4-naphthoquinones with its IC50 value in the nanomolar range. Compared with the naphthoquinone shikonin as a lead, it displayed more potent inhibitory effects against the target enzyme and showed much less cytotoxicity. The results from in vitro antiviral activity evaluation demonstrated that this inhibitor (15) effectively suppressed the replication of SARS-CoV-2 in Vero E6 cells with its EC50 value of 4.55 μM. All of these results supported that natural products and their derivatives are one of the most important sources of screening novel antiviral agents.

The data presented herein would be interpreted with emphasis, since the antiviral IC50 value of compound 15 may not reflect the results from actual in vivo experiments. Its antiviral efficacy against SARS-CoV-2 and safety should be further evaluated in adequate infected animal models. At present, the main impediment to the in vivo efficacy evaluation was the lack of generally applicable and validated animal models [50,51]. The recently established thens macaque model [52], which contributed to confirm the in vivo activity of remdesivir against SARS-CoV-2, might be used to test the treatment efficacy of other antiviral drug candidates in future. The in vitro research results confirmed by animal experiments would provide the rational basis for further research and development.

Author contributions

J. Cui and J. Jia conceived the idea. J. Cui performed the experiment and analyzed the data. J. Jia supervised the project. J. Cui and J. Jia wrote the paper.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

[1] N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G.F. Gao, W. Tan, A novel coronavirus from patients with pneumonia in China, 2019, N. Engl. J. Med. 382 (2020) 727–733.
[2] J.A. Müller, R. Groß, C. Conzelmann, J. Krüger, U. Merle, J. Steinhardt, T. Weil, L. Koecke, C.P. Bozzo, C. Read, G. Fois, T. Eiseler, J. Gehrmann, J. van Vuure, J.M. Wessbecher, M. Frick, I.G. Costa, M. Breunig, B. Grüner, L. Peters, M. Schuster, S. Liebow, T. Seufferlein, S. Stenger, A. Stenzinger, P.E. MacDonald, F. Kirchhoff, K.M.J. Sparrer, P. Walthier, H. Lickert, T.E.E. Barth, M. Wagner, J. Münch, S. Keller, A. Kleger, SARS-CoV-2 infects and replicates in cells of the human endocrome and exocrine pancreas, Nat. Metab. 3 (2021) 149–165.
[3] World Health Organization, Coronavirus Disease (COVID-19) Weekly Epidemiological Update and Weekly Operational Update, 2021, https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.
[4] V. Pilkington, T. Pepperrell, A. Hill, A review of the safety of lopinavir–ritonavir: a potential treatment in the COVID-19 pandemic? J. Virus Erad. 6 (2020) 45–51.
[5] H. Stover, Lopinavir–ritonavir in severe COVID-19, Nat. Med. 26 (2020) 465, 465.
[6] Z. Kashour, M. Riaz, M.A. Garbati, O. Aliosary, H. Tayjeh, D. Gerberi, M.H. Murad, M.R. Solhai, T. Kashour, I.M. Teyjeh, Efficacy of chloroquine or hydroxychloroquine in COVID-19 patients: a systematic review and meta-analysis, J. Antimicrob. Chemother. 76 (2021) 30–42.
[7] J.H. Beigel, K.M. Tomashek, L.E. Dodd, A.K. Mehta, B.S. Zingman, A.C. Kalil, E. Hohmann, H.Y. Chu, A. Luetkemeyer, S. Kline, D. Lopez de Castillo, R.W. Finberg, K. Dierberg, V. Tapson, L. Hsieh, T.F. Patterson, R. Paredes, D.A. Sweeney, W.R. Short, C. Read, G. Fois, T. Eiseler, J. Gehrmann, J. van Vuure, M. Frick, I.G. Costa, M. Breunig, B. Grüner, L. Peters, M. Schuster, S. Liebow, T. Seufferlein, S. Stenger, A. Stenzinger, P.E. MacDonald, F. Kirchhoff, K.M.J. Sparrer, P. Walthier, H. Lickert, T.E.E. Barth, M. Wagner, J. Münch, S. Keller, A. Kleger, SARS-CoV-2 infects and replicates in cells of the human endocrome and exocrine pancreas, Nat. Metab. 3 (2021) 149–165.
[8] Emergency Use Authorization. https://www.fda.gov/emergency-
