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.
Methylene blue inhibits replication of SARS-CoV-2 in vitro

Mathieu Gendrot\textsuperscript{a,b,c,1}, Julien Andreani\textsuperscript{c,d,1}, Isabelle Duflot\textsuperscript{c,d,1}, Manon Boxberger\textsuperscript{c,d}, Marion Le Bideau\textsuperscript{c,d}, Joel Mosnier\textsuperscript{a,b,c,e}, Priscilla Jardot\textsuperscript{c,d}, Isabelle Fonta\textsuperscript{a,b,c,e}, Clara Rolland\textsuperscript{c,d}, Hervé Bogreau\textsuperscript{a,b,c,e}, Sébastien Hutter\textsuperscript{c,d}, Bernard La Scola\textsuperscript{c,d,e,*}, Bruno Pradines\textsuperscript{a,c,d,e,*}

\textsuperscript{a} Unité Parasitologie et Entomologie, Département Microbiologie et Maladies Infectieuses, Institut de Recherche Biomédicale des Armées, Marseille, France
\textsuperscript{b} Aix-Marseille Université, IRD, SSA, AP-HM, VITROME, Marseille, France
\textsuperscript{c} IHU Méditerranée Infection, Marseille, France
\textsuperscript{d} Aix-Marseille Université, IRD, AP-HM, MEPMH, Marseille, France
\textsuperscript{e} Centre National de Référence du Paludisme, Marseille, France

**A B S T R A C T**

In December 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus diseases 2019 (COVID-19) emerged in Wuhan, China. Currently there is no antiviral treatment recommended against SARS-CoV-2. Identifying effective antiviral drugs is urgently required. Methylene blue has already demonstrated in vitro antiviral activity in photodynamic therapy as well as antibacterial, antifungal and antiparasitic activities in non-photodynamic assays. In this study, non-photoactivated methylene blue showed in vitro activity at very low micromolar range with an EC\textsubscript{50} (median effective concentration) of 0.30 ± 0.03 \( \mu \)M and an EC\textsubscript{90} (90\% effective concentration) of 0.75 ± 0.21 \( \mu \)M at a multiplicity of infection (MOI) of 0.25 against SARS-CoV-2 (strain IHUMI-3). The EC\textsubscript{50} and EC\textsubscript{90} values for methylene blue are lower than those obtained for hydroxychloroquine (1.5 \( \mu \)M and 3.0 \( \mu \)M) and azithromycin (20.1 \( \mu \)M and 41.9 \( \mu \)M). The ratios \( C_{\text{max}}/EC_{\text{50}} \) and \( C_{\text{max}}/EC_{\text{90}} \) in blood for methylene blue were estimated at 10.1 and 4.0, respectively, following oral administration and 33.3 and 13.3 following intravenous administration. Methylene blue EC\textsubscript{50} and EC\textsubscript{90} values are consistent with concentrations observed in human blood. We propose that methylene blue is a promising drug for treatment of COVID-19. In vivo evaluation in animal experimental models is now required to confirm its antiviral effects on SARS-CoV-2. The potential interest of methylene blue to treat COVID-19 needs to be confirmed by prospective comparative clinical studies.

© 2020 Elsevier Ltd and International Society of Antimicrobial Chemotherapy. All rights reserved.

1. Introduction

In December 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus diseases 2019 (COVID-19) emerged in Wuhan, China [1]. Despite containment measures, SARS-CoV-2 spread in Asia, Southern Europe, then in America and currently in Africa. Presently there is no antiviral treatment recommended against SARS-CoV-2. Different drugs or combinations have been evaluated worldwide. Identifying effective low-cost antiviral drugs with limited side effects, affordable immediately, is urgently needed, especially for emerging countries.

Plasma products can transmit a wide range of pathogens by transfusion. Methylene blue, a synthesised thiazine dye, is known to be effective in photodynamic therapy against microbes and particularly viruses. Methylene blue is able to intercalate into viral nucleic acid when illuminated with visible light and prevents transmission of pathogens. Illumination of methylene blue inactivated Zika, yellow fever, dengue, chikungunya and Ebola viruses and Middle East respiratory syndrome coronavirus in plasma [2–5]. Methylene blue also demonstrates antiviral activities without photocactivation. Methylene blue inhibited in vitro colistin-resistant strains of *Acinetobacter baumannii*, *Mycobacterium ulcerans*, *Mycobacterium* spp. and *Candida albicans* [6–8]. Methylene blue was also effective in vivo against Buruli ulcer in experimental *M. ulcerans* infection in mice [7]. Additionally, methylene blue inacti-
vated hepatitis C virus in transplant organ perfused with methylene blue [9]. The most studied effects of methylene blue are those on malaria.

In 1891, methylene blue was first used to effectively treat two patients with uncomplicated malaria [10]. In the 2010s, methylene blue showed effective in vitro activity in the nanomolar range against *Plasmodium falciparum* strains [11–14]. Methylene blue showed a protective effect against cerebral malaria in a murine model infected with *Plasmodium berghei* [15–17]. Methylene blue showed several benefits when used as a partner in triple combination with artesinin-based combination therapy in uncomplicated *falciparum* malaria in children [18].

Taken together, these reports suggest that methylene blue may have antiviral effects against SARS-CoV-2. Therefore, in this study the activity of methylene blue was assessed in vitro against a clinically isolated SARS-CoV-2 strain and was compared with the activity of hydroxychloroquine and azithromycin, which have already been evaluated in vitro and in vivo in humans [19–22].

2. Materials and methods

2.1. Antimalarial drugs, virus and cells

Methylene blue (methylthioninium chloride; Proveblue®) was provided by Provepharm SAS (Marseille, France). Stocks solutions of hydroxychloroquine (Sigma, St Louis, MO, USA) and methylene blue were prepared in water, and azithromycin (Sigma) was prepared in methanol. All stock solutions were then diluted in Minimum Essential Medium (MEM) (Gibco, Thermo Fisher) to achieve seven final concentrations ranging from 0.1–100 μM. A clinically isolated SARS-CoV-2 strain (IHUMI-3) [23] was maintained in production in Vero E6 cells (American Type Culture Collection ATCC® CRI-1586™) in MEM with 4% of fetal bovine serum (FBS) and 1% glutamine (complete medium).

2.2. Cytotoxicity assay

In vitro cell viability evaluation using the Vero E6 cell line was performed according to the method described by Mosmann with slight modifications [24]. Briefly, 10³ cells in 200 μL of complete medium were added to each well of 96-well plates and were incubated at 37 °C in a humidified 5% CO₂ atmosphere. After 24 h of incubation, 25 μL of complete medium and 25 μL of each concentration of methylene blue, hydroxychloroquine or azithromycin were added and the plates were incubated for 48 h at 37 °C. After removal of the supernatant, 100 μL of MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] (Sigma-Aldrich, France) solution (0.5 mg/mL in MEM without FBS) were then added to each well. Cells were incubated for 2 h at 37 °C. Following incubation, MTT solution was removed and 100 μL of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Plates were then shaken at 700 rpm for 10 min at 37 °C. The absorbance was measured at 570 nm using a Tecan Infinite F200 Microplate Reader. DMSO was used as a blank. The 50% cytotoxic concentration (CC₅₀) was calculated with an inhibitory sigmoid Emax model, which estimated the CC₅₀ through non-linear regression by using a standard function of the R software (ICEstimator v.1.2; http://www.antimalarial-icestimator.net). The CC₅₀ value was the mean of six different experiments.

2.3. Antiviral activity assay

Briefly, 96-well plates were prepared with 5 × 10³ cells/mL of Vero E6 cells (200 μL well per) as previously described [20]. Methylene blue, hydroxychloroquine or azithromycin concentrations were added 4 h before infection. Vero E6 cells were infected with SARS-CoV-2 strain IHUMI-3 at a multiplicity of infection (MOI) of 0.25. At 48 h post-infection, replication was estimated by RT-PCR using a SuperScript™ III Platinum™ One-Step Kit w/ROX (Invitrogen) after extraction with a BioExtracT® SuperBall® Kit (Biosellar, Dardilly, France). The primers used have been described previously [25]. The EC₅₀ (median effective concentration) and EC₉₀ (90% effective concentration) were calculated with an inhibitory sigmoid Emax model, which estimated the EC₅₀ and EC₉₀ through non-linear regression using a standard function of the R software (ICEstimator v.1.2). EC₅₀ and EC₉₀ values were the mean of six different experimentations.

2.4. Data analysis and interpretation

The selectivity index (SI) was estimated for each drug as the ratio of CC₅₀/EC₅₀. The expected maximum blood concentration (Cmax) was estimated from the literature for each drug at doses commonly administered in oral malaria treatment and for methylene blue at intravenous (i.v.) doses used for US Food and Drug Administration (FDA) and European Medicines Agency (EMA)-approved methemoglobinemia treatment. The ratios Cmax/EC₅₀ and Cmax/EC₉₀ were estimated to determine whether the effective concentration in plasma to cure SARS-CoV-2 is achievable in humans. If data on drug accumulation in the lung were available, the ratios Clung/EC₅₀ and Clung/EC₉₀ were calculated.

3. Results

The CC₅₀, EC₅₀, EC₉₀ and SI for each drug are presented in Table 1. Methylene blue and hydroxychloroquine showed EC₅₀ and EC₉₀ values in the low micromolar range (Table 1). The EC₅₀ and EC₉₀ values for methylene blue were lower than those obtained for hydroxychloroquine and azithromycin. The ratios Cmax/EC₅₀ and Cmax/EC₉₀ in blood for methylene blue were estimated at 10.1 and 4.0, respectively, following oral administration and at 33.3 and 13.3 following i.v. administration (Fig. 1).

4. Discussion

Methylene blue showed in vitro activity at very low micromolar range with an EC₅₀ of 0.30 ± 0.03 μM and an EC₉₀ of 0.75 ± 0.21 μM at a MOI of 0.25 (SI > 333) (Table 1). The EC₅₀ and EC₉₀ values for methylene blue are lower than those obtained for hydroxychloroquine and azithromycin. Azithromycin demonstrated low in vitro efficacy against SARS-CoV-2 when used alone but potentiated the effects of hydroxychloroquine in combination [20]. Oral uptake of 325 mg of methylene blue led to a Cmax in blood of 0.97 μg/mL (± 3 μM) and an elimination half-life (t½) of 14.9 h [26]. A methylene blue dose of 2 mg/kg i.v. showed a Cmax of 2.917 μg/mL (± 10 μM) [27]. The ratios Cmax/EC₅₀ and Cmax/EC₉₀ for methylene blue were estimated at 10.1 and 4.0 for the oral route and 33.3 and 13.3 for the i.v. route, respectively. Methylene blue EC₅₀ and EC₉₀ values are consistent with concentrations observed in human blood. Approximately 3–5% of methylene blue per gram of lung was found

| Drug               | EC₅₀ (μM) | EC₉₀ (μM) | CC₅₀ (μM) | SI     |
|--------------------|----------|----------|----------|--------|
| Methylene blue     | 0.30 ± 0.03 | 0.75 ± 0.21 | >100 | >333 |
| Hydroxychloroquine | 1.5 ± 0.3  | 3.0 ± 1.9  | 20.4 ± 1.4 | 13.6  |
| Azithromycin       | 20.1 ± 4.5 | 41.9 ± 18.0 | >100 | >5   |
after i.v. methylene blue injection but the methylene blue concentration decreased rapidly below 0.1% after 10 h [28]. In comparison, oral uptake of 400 mg of hydroxychloroquine led to a $C_{\text{max}}$ of 1.22 μM [29]. Hydroxychloroquine accumulated 30 times more in the lungs than in blood [30]. The azithromycin $C_{\text{max}}$ ranged from 0.18–0.4 μg/mL of blood (0.22–0.51 μM) after the last dose of oral administration of 500 mg once daily for 3 days or after a single dose of 500 mg [31–33]. These doses led to a $C_{\text{max}}$ in the lung ranging from 8–9 μg/g (10–12 μM) [31,32]. The $C_{\text{max}}$ expected in the lung was below the $EC_{50}$ and $EC_{90}$. However, due to potentiation of the antiviral effects when azithromycin is combined with hydroxychloroquine, azithromycin can be used in vitro at lower concentrations (5 μM and 10 μM) [20]. These concentrations are compatible with expected concentrations in the lungs.

Methylene blue showed low cytotoxicity in vitro against Vero E6 cells with $CC_{50} > 100$ μM. The SI as a ratio of $CC_{50}/EC_{50}$ was estimated to be >333. The present $CC_{50}$ of hydroxychloroquine with an SI of ~13 against Vero E6 cells was higher than previously reported $CC_{50}$ values, ranging from >50 μM to 250 μM against Vero E6 cells [19,34] or >500 μM in Felis catus whole fetus-4 cells [35]. Azithromycin also showed low cytotoxicity against Vero E6 cells with $CC_{50} > 100$ μM and SI > 5. The $CC_{50}$ for azithromycin was consistent with previous data (>130 μM) [34]. Methylene blue showed low cytotoxicity but predominantly the higher SI.

Although methylene blue is on the list of drugs potentially dangerous for patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, no association between methylene blue and severe haemolysis has been detected after oral administration [36]. Additionally, the i.v. route for methylene blue has been granted a marketing authorisation in Europe in 2011 and in the USA in 2016 for the treatment of acquired methemoglobinemia based upon a confirmed positive benefit/risk ratio in this pathology.

5. Conclusion

Methylene blue showed high in vitro antiviral effective activity against SARS-CoV-2 with an $IC_{50} (0.3$ μM) and $IC_{90} (0.75$ μM) compatible with oral uptake and i.v. administration. This in vitro activity is higher than those obtained with drugs that have been evaluated in clinical trials worldwide such as hydroxychloroquine (1.5 μM), azithromycin (20.1 μM), remdesivir (23 μM), lopinavir (26.6 μM) or ritonavir (>100 μM) [37]. We propose that methylene blue is a promising drug for the treatment of COVID-19. In vivo evaluation in animal experimental models is now required to confirm its antiviral effects against SARS-CoV-2. The potential interest of methylene blue to treat COVID-19 needs to be confirmed by prospective comparative clinical studies.

Acknowledgment

The authors thank Provepharm for providing the methylene blue (ProveBlue®).

Funding: This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the program «Investissements d’avenir» [reference ANR-10-IAHU-03].

Competing interests: BLS and BP are associated as co-inventors with Provepharm in the patent EP 20305425.9 (30/04/2020) but have no financial interest with the subject matter; MB received a PhD grant supported by L’Occitane Society. Provepharm or the funders had no role in the design of the study, in the collection, analysis or interpretation of the data, in the writing of the manuscript or in the decision to publish the results. All other authors declare no competing interests.

Ethical approval: Not required.

References

[1] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:365–9.
[2] Fyk J, Markis DC, Hobson-Peters J, Prow NA, Watterson D, Hall RA, et al. Dengue and chikungunya viruses in plasma are effectively inactivated after treatment with methylene blue and visible light. Transfusion 2016;56:2278–85.
[3] Faddy HM, Fyk JJ, Hal RA, Young PR, Reichenberg S, Tolksdorf F, et al. Inactivation of yellow fever virus in plasma after treatment with methylene blue and visible light and in platelet concentrates following treatment with ultraviolet C light. Transfusion 2019;59:2223–7.
[4] Wang Y, Ren K, Liao X, Luo G, Kuntakis K, Leetraakool N, et al. Inactivation of Zika virus in plasma and derivatives by four different methods. J Med Virol 2019;91:2059–65.
[5] Eickmann M, Gravemann U, Handke W, Tolksdorf F, Reichenberg S, Müller TH, et al. Inactivation of Ebola virus and Middle East respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively. Transfusion 2018;58:2202–7.
[6] Gadel D, Tatman Orkun M, Akcakı A. In vitro activity of methylene blue and eosin methylene blue agor on colistin-resistant A. baumannii: an experimental study. J Med Microbiol 2019;68:3607–13.
[7] Tian RBD, Asmar S, Navez C, Lépidi H, Drancourt M. Effectiveness of purified methylene blue in an experimental model of Mycobacterium ulcerans infection. Int J Antimicrob Agents 2017;49:290–5.
M. Gendrot, J. Andreani, I. Duflot et al.

International Journal of Antimicrobial Agents 56 (2020) 106202

[8] Pal R, Ansari MA, Saibabu V, Das S, Fatima Z, Hameed S. Nonphotodynamic roles of methylene blue: display of distinct antimycobacterial and anticanical mode of actions. J Pathog Biofilm 2018;2018:3759704.

[9] Helfritz FA, Bokova D, Vandervs W, Kukilinski N, Westhaus S, von Hohn C, et al. Methylen blue treatment of grafts during cold ischemia time reduces the risk of hepatitis C virus transmission. J Infect Dis 2018;218:1711–21.

[10] Guttman P, Ehrlich P. Ueber die Wirkung des Methyleneblau bei Malaria [About the effect of methylene blue in malaria]. Berl Klin Wochenschr 1891;28:953–6.

[11] Pascual A, Henry M, Briolant S, Charras S, Baret E, Amavicht R, et al. In vitro activity of Proveblue (methylene blue) on Plasmodium falciparum strains resistant to standard antimalarial drugs. Antimicrob Agents Chemother 2011;55:2472–4.

[12] Fall B, Camara C, Fall M, Nakoulima A, Dionne P, Diatta B, et al. Plasmodium fal- ciparum susceptibility to standard and potential anti-malarial drugs in Dakar, Senegal, during the 2013–2014 malaria season. Malar J 2015;14:60.

[13] Fall B, Madamet M, Diawara S, Briolant S, Wade KA, Lo G, et al. Ex vivo activity of Proveblue, a methylene blue, against isolated Plasmodium falciparum in Dakar, Senegal from 2013 to 2015. Int J Antimicrob Agents 2017;50:155–8.

[14] Gendrot M, Madamet M, Mosnier J, Fonta I, Amavic I, Benoit N, et al. Base-line and non-randomized control of ex vivo susceptibilities of Plasmodium falciparum to methylene blue in Africa, 2013–18. J Antimicrob Chemother 2020;75:2141–8.

[15] Dormoi J, Pradines B. Dose responses of Proveblue methylene blue in an experimental murine cerebral malaria model. Antimicrob Agents Chemother 2013;57:4080–1.

[16] Dormoi J, Briolant S, Desgrous C, Pradines B. Efficacy of Proveblue (methylene blue) in an experimental cerebral malaria model. Antimicrob Agents Chemother 2013;57:3412–14.

[17] Dormoi J, Briolant S, Desgrous C, Pradines B. Impact of methylene blue and atovastatin combination therapy on the appearance of cerebral malaria in a murine model. Malar J 2013;12:127.

[18] Mendes Jorge M, Ouermi L, Meissner P, Compaoré G, Coulbaly B, Nebie E, et al. Safety and efficacy of artemesate-amodiaquine combined with either methylene blue or primaquine in children with falciparum malaria in Burkina Faso: a randomized controlled trial. PLoS One 2019;14:e0222993.

[19] Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020;6:16.

[20] Andreani J, Le Bideau M, Duflot I, Jardot P, Rolland C, Bosberger M, et al. In vitro testing of hydroxychloroquine and azithromycin on SARS-CoV-2 shows synergistic effect. Microb Pathog 2020;145:104228.

[21] Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Sevestre J, et al. Cli- nical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: an observational study. Travel Med Infect Dis 2020;34:101663.

[22] Million M, Lagier JC, Gautret P, Colson P, Fournier PE, Amran S, et al. Early treatment of 1061 COVID-19 patients with hydroxychloroquine and azithromycin, Marseille, France. Travel Med Dis 2020;35:101738.

[23] Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hy- droxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020;56:105949.

[24] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.

[25] Amrane S, Tissot-Dupont H, Doudier B, Eldin C, Hocquart M, Mailhe M, et al. Rapid viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the infection diseases referral hospital in Mar- seille, France, January 31st to March 1st, 2020: a respiratory virus snapshot. Travel Med Infect Dis 2020;36:101632.

[26] Ash CX, Chavchich M, Birrell GW, van Breda K, Travers T, Rowcliffe K, et al. Pharmacokinetics and ex vivo antimarial activity of artemesate-amodia-quine plus methylene blue in healthy volunteers. Antimicrob Agents Chemother 2020;64.e01441–19.

[27] Center for Drug Evaluation and Research Clinical pharmacology and biopharmaceutics review(s); application number 2046300rg10000; Oc- tober 2020. http://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/ 2046300rg10000ClinPharmR.pdf [Accessed 16 October 2020].

[28] Link EM, Costa DC, Liu D, Ell PJ, Lower PJ, Spittle MF. Targeting disseminated melanoma with radiolabelled methylene blue. Acta Oncol 1996;35:331–41.

[29] Rainsford KD, Parke AL, Clifford-Rashotte M, Keen WF. Therapy and pharmacolo- gical properties of hydroxychloroquine and chloroquine in the treatment of systemic lupus erythematosus, rheumatoid arthritis and related diseases. In- flammatopharmacology 2015;23:231–69.

[30] Chhonker YS, Sleightholm RL, Li J, Oupcky D, Murry DJ. Simultaneous quanti-fi- cation of hydroxychloroquine and its metabolites in mouse blood and tissues using LC-ESI-MS/MS: an application for pharmacokinetic studies. J Chromatogr B Analyt Technol Biomed Life Sci 2018;1072:320–7.

[31] Danesi R, Lupetti A, Barbara C, Cheardi E, Chella A, Malizia T, et al. Compari- tive distribution of azithromycin in lung tissue of patient given daily oral doses of 500 and 1000 mg. J Antimicrob Chemother 2001;51:939–45.

[32] Luchii M, Damié B, Fang A, de Caprariis PJ, Mussi S, Sanchez SP, et al. Pharma- cokinetics of azithromycin in serum, bronchial washings, alveolar macrophages and lung tissue following a single oral dose of extended or immediate release formulations of azithromycin. J Antimicrob Chemother 2008;61:884–91.

[33] Davidson RJ. In vitro activity and pharmacodynamic/pharmacokinetic parame- ters of clarithromycin and azithromycin: why they matter in the treatment of respiratory tract infections. Infect Drug Resist 2019:12:585–96.

[34] Madrid PB, Panchal RG, Warren TK, Shurlle AC, Endsay AN, Green CE, et al. Evaluation of Ebola virus inhibitors for drug repurposing, ACS Infect Dis 2015;1:317–26.

[35] Takano T, Satoh K, Doki T, Tanabe T, Hochdatsu T. Antiviral effects of hydroxy- chloroquine and type I interferon on in vitro fatal feline coronavirus infection. Viruses 2020;12:578.

[36] Lu G, Naganshi M, Goldau N, Mendes Jorge M, Meissner P, Jahn A, et al. Ef- ficacy and safety of methylene blue in the treatment of malaria: a systemic review. BMC Med 2018;16:59.

[37] Chey KT, Wong AVL, Kaewpreedee P, Sia SF, Chen D, Hui KPY, et al. Remdesivir, lopinavir, emetine, and homoharringtonine inhibits SARS-CoV-2 replication in vitro. Antiviral Res 2020;178:104786.