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Ultrasonic Synthesis and Preliminary Evaluation of Anticoronaviral Activity of 6,7-Dimethoxy-4-(4-(4-methoxyphenyl) piperazin-1-yl)-1-methylquinolin-1-ium Iodide

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Abstract: Quinoline scaffold is one of the most intensively utilized pharmacophores in drug design because of the variety of activities demonstrated by different quinoline-based therapeutics or drug-candidates. Herein, we describe an environmentally tolerant two-step procedure as a convenient synthetic approach to novel chloroquine and hydroxychloroquine analogues. The structures of the newly synthesized compounds are estimated by ^{1}H NMR, ^{13}C NMR, LC-MS spectrometry and IR spectroscopy.

Keywords: chloroquine; hydroxychloroquine; quinoline; anticoronaviral activity; ultrasonic treatment

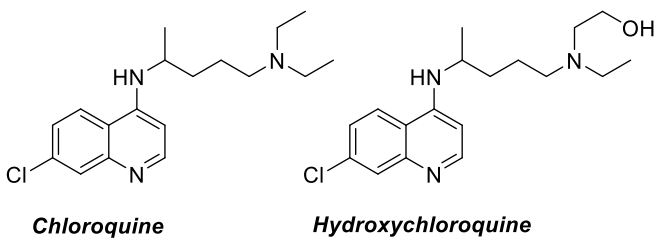
Citation: Vasilev, A.A.; Grozdanov, P.P.; Nikolova, I.; Lozanov, V.S.; Kandinska, M.I. Ultrasonic Synthesis and Preliminary Evaluation of Anticoronaviral Activity of 6,7-Dimethoxy-4-(4-(4-methoxyphenyl)piperazin-1-yl)-1-methylquinolin-1-ium Iodide. *Molbank* **2022**, *2022*, M1400. https://doi.org/10.3390/M1400

Academic Editor: Stanislav Kafka

Received: 31 May 2022
Accepted: 22 June 2022
Published: 4 July 2022

1. Introduction

Outbreaks of coronaviruses over the past 20–30 years, in particular the pandemic of the beta-coronavirus SARS-CoV-2, have been a major incentive for the scientific community not to underestimate these pathogens. Therefore, it is necessary to work hard to create new highly effective antiviral drugs for the treatment and prevention of coronavirus infections. Ones of the successful antivirals used in the early stages of the COVID-19 pandemic were the 4,7-dichloroquinoline derivatives–chloroquine and hydroxychloroquine (Figure 1). Both compounds are available as therapeutics for treatment malaria [1], rheumatoid arthritis [2], lupus [3], porphyria cutanea tarda [4]. Decreased antimalarial efficacy of chloroquine over time, its high toxicity during long-term use, the lower antiparasitic activity of hydroxychloroquine, the side effects caused by both drugs, as well as the new discovered applications of them [5,6], are the main reasons to develop novel synthetic routes to modify their structures in less toxic analogues bearing better pharmacokinetic properties [7–11].



Chloroquine ***Hydroxychloroquine***

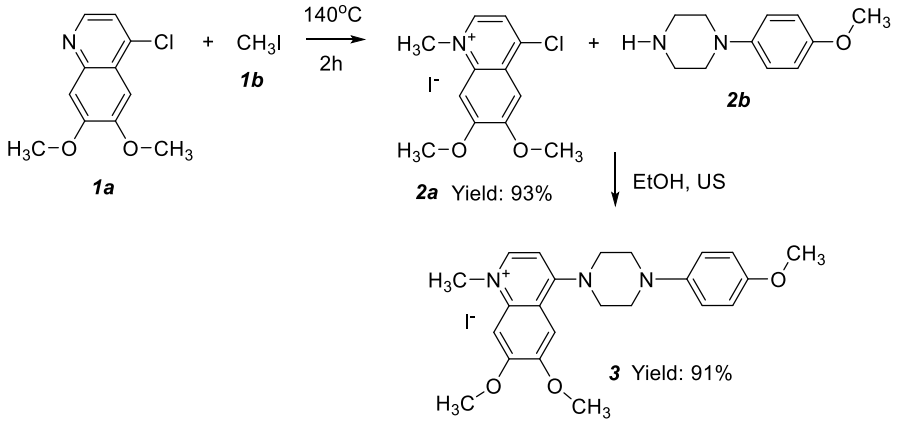
Figure 1. Chemical structures of chloroquine and hydroxychloroquine.

Since the promising in vitro efficacy of chloroquine and hydroxychloroquine against SARS-CoV-2 were firstly reported by the group of Prof. Wang [12,13], the investigations of an anticoronavirus potential of both compounds and other known therapeutics, their derivatives, are the focus of a number of research studies [14–18]. In this regard, the aim of the present study is to report an easily applicable and environmentally benign two-step synthetic approach to novel chloroquine and hydroxychloroquine analogues with combined modification of the heterocyclic pharmacophore as well as the amino substituent at 4th quinoline ring position.

2. Results and Discussion

2.1. Synthesis

The synthetic strategy for the preparation of the target compound **3** (Scheme 1) is based on environmentally benign procedures. On the first step (Scheme 1) the starting 4-chloro-6,7-dimethoxyquinoline (**1a**) and an excess of iodomethane (**1b**) were heated in a sealed tube under argon without any solvent. The presence of an activated chlorine atom at 4th position in the quinolinium salt **2a** makes the compound unstable against moisture from one side and difficult to be purified through recrystallization from another. The chosen reaction conditions providing an inert atmosphere and avoiding the solvent usage allowed **2a** to be prepared in sufficiently high yield (93 %) and isolated in a pure enough form only by simple filtration. Therefore, the target reactant **2a** was applied on the next reaction stage without subsequent purification.



Scheme 1. Synthesis of 6,7-dimethoxy-4-(4-(4-methoxyphenyl)piperazin-1-ium-1-yl)-1-methylquinolin-1-ium iodide (**3**).

The short ultrasonic treatment of 4-chloro-6,7-dimethoxy-1-methylquinolin-1-ium iodide (**2a**) and slight excess (1.3 equiv.) of 1-(4-methoxyphenyl)piperazine (**2b**) in ethanol as a solvent affords the final compound 6,7-dimethoxy-4-(4-(4-methoxyphenyl)piperazin-1-ium-1-yl)-1-methylquinolin-1-ium iodide (**3**) in excellent yield of 91%. The successful reaction outcome confirmed that the presence of a quaternized nitrogen atom (electron acceptor) in compound **2a** allows easier substitution of the chlorine atom at the 4th position in the quinoline core, thus the prolonged heating of the reaction mixture and the need of a large excess of the nucleophilic reagent (starting amine) are avoided [19,20]. The obtained product is TLC pure, and no further purification is needed.

2.2. Cytotoxicity and Antiviral Assays

Preliminary analysis of the antiviral activity of the compound **3** against the replication of human coronavirus strain OC-43 was performed. Chloroquine and hydroxychloroquine were used as reference substances. To more accurately assess antiviral activity, and to avoid the toxic effects of substances on cells, the cytotoxic effect of compound **3** on HCT-8 cell line was determined in advance. The demonstrated cytotoxicity of **3** toward HCT-8 cell

line (CC_{50} at 399 μm) was about six times lower compared to chloroquine (CC_{50} at 65 μm) and three times lower that that of hydroxychloroquine (CC_{50} at 130 μm).

Compound **3** was assayed for in vitro antiviral activity against human coronavirus strain OC-43, and its effect on virus replication has been found. The observed activity of the tested substance **3** (IC_{50} at 3.1 μM, SI 128.7) was significantly lower compared to this of chloroquine (IC_{50} at 0.1 μM, SI 650), but remarkably higher to that demonstrated by hydroxychloroquine (IC_{50} at 100 μM, SI 1.3). The initial results obtained about biological properties of **3** are a good enough basis for further molecule optimization and synthesis of its analogues in order to enhance the antiviral activity of similar quinoline derivatives.

3. Materials and Methods

3.1. General

4-Chloro-6,7-dimethoxyquinoline (**1a**), iodomethane (**1b**) and 1-(4-methoxyphenyl) piperazine (**2b**) are commercial products (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) and were used as supplied. All the solvents are TLC grade and used without preliminary purification. Melting point of **2a** and **3** are determined on Kruess M5000 melting point meter for automatic measurements. NMR spectra (^{1}H NMR, ^{13}C NMR) were obtained on a Bruker Avance II+ NMR spectrometer operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}CNMR in DMSO-d_6 as a solvent (Supplementary Materials). The chemical shifts are given in ppm (δ) using tetramethylsilane (TMS) as an internal standard. IR spectra are obtained on Specord 71 (Carl-Zeiss, Jena, Germany) spectrometer in nujol as a solvent. Liquid chromatography mass spectrometry analysis (LC-MS) was carried out on Q Exactive® hybrid quadrupole-Orbitrap® mass spectrometer (ThermoScientific Co, Waltham, MA, USA) equipped with a HESI® (heated electrospray ionization) module, TurboFlow® Ultra High Performance Liquid Chromatography (UHPLC) system (Thermo-Scientific Co, Waltham, MA, USA) and HTC PAL® autosampler (CTC Analytics, Zwingen, Switzerland). The course of the reactions was monitored by thin layer chromatography (TLC) ALUGRAM® SIL G/UV 254-60 Macherey-Nagel plates, ready to use with thickness of the silica layer 0.2 mm.

3.2. Synthesis

3.2.1. Synthesis of 4-Chloro-6,7-dimethoxy-1-methylquinolin-1-ium Iodide (**2a**)

In this step, 1g (4,7 mmol) 4-chloro-6,7-dimethoxyquinoline (**1a**) and 0.83 mL (1.90 g, 13.0 mmol) iodomethane (**1b**) were placed in a 50 mL sealed tube under argon. The reaction mixture was heated at 110 °C for two hours. After cooling to room temperature, the formed precipitate was suspended in 20 mL diethyl ether, suction filtered and air dried. The reaction product was used directly on the next reaction step. Yield: 1.58 g (93%) yellow powder, m.p. = 223–224 °C. The obtained compound is TLC pure (DCM:MeOH = 4.5:0.5, R_F = 0.29). ^{1}H NMR δ = 4.10 (s, 3H, OCH_3), 4.19 (s, 3H, OCH_3), 4.57 (s, 3H, N^+CH_3), 7.65 (s, 1H, Ar), 7.69 (s, 1H, Ar), 8.24 (d, 1H, Ar, ^3J = 6.5 Hz), 9.19 (d, 1H, Ar, ^3J = 6.5 Hz). ^{13}C NMR δ = 157.5 (Cq), 152.8 (Cq), 148.4 (Cq), 146.2 (Cq), 137.5 (Cq), 123.8 (CH), 120.8 (CH), 103.7 (CH), 100.2 (CH), 58.1 (CH_3), 57.2 (CH_3), 45.9 (CH_3). IR ν (cm^{-1}) = 1605, 1550, 1500, 1450, 1370, 1350, 1280, 1250, 1195, 1170, 1110, 1030, 950, 850, 820.

3.2.2. Synthesis of 6,7-Dimethoxy-4-(4-(4-methoxyphenyl)piperazin-1-yl)-1-methylquinolin-1-ium Iodide (**3**)

In this step, 0.15 g (0.41 mmol) **2a** and 0.1 g (0.53 mmol) **2b** were mixed in a 20 mL vial with cap. Then, 7 mL Ethanol was added and the reaction mixture was sonicated for 20 min. The resulting orange precipitate was suction filtered, washed with two portions of 10 mL ethanol and dried in a desiccator. Yield: 0.21 g (91%) rusty orange powder, m.p. = 350 °C with decomposition. The obtained compound is TLC pure (DCM:MeOH = 4.5:0.5, R_F = 0.40). ^{1}H NMR δ = 3.32 brs (4H, CH_2), 3.70 s (3H, OCH_3), 3.85 brs (4H, CH_2), 4.00 s (3H, OCH_3), 4.09 s (3H, OCH_3), 4.27 s (3H, N^+CH_3), 6.88 d (2H, ^3J = 9.0 Hz, CH(Ph)), 6.99 d (2H, ^3J = 9.0 Hz, CH(Ph)), 7.27 d (1H, ^3J = 7.0 Hz, CH(Quin)), 7.38 s (1H, CH(Quin)), 7.43 s (1H,

CH(Quin)), 8.70 d (1H, ^{3}J = 7.0 Hz, CH(Quin)). ^{13}C NMR δ = 159.7 (Cq), 155.3 (Cq), 153.8 (Cq), 149.1 (Cq), 145.0(CH), 137.3 (Cq), 118.2 (2CH), 115.5 (Cq), 114.9 (2CH), 106.6 (CH), 105.6 (CH), 99.8 (CH), 57.3 (CH_3), 56.6 (CH_3), 55.7 (CH3), 51.7 ($2CH_2$), 49.9 ($2CH_2$), 43.4 (CH_3). ^{13}C DEPT 135 δ = 145.0 (CH), 118.2 (2CH), 114.9 (2CH), 106.6 (CH), 105.6 (CH), 99.8 (CH), 57.3 (CH_3), 56.6 (CH_3), 55.7 (CH_3), 51.7 (CH_2), 49.9 ($2CH_2$), 43.4 (CH_3). IR ν (cm^{-1}) = 1650, 1540, 1500, 1450, 1380, 1220, 1020. LC-MS (m/z): 394.21429, calculated for $C_{23}H_{28}N_3O_3^+$: 394.21.

3.3. Cytotoxicity Assay and Antiviral Assay against Human Coronavirus OC-43 (HCoV-OC43) (ATCC: VR-1558)

Human colon carcinoma (HCT-8) cells were purchased from the American Type Culture Collection (ATCC). Permanent HCT-8 [HRT-18] (ATCC-CCL-244, LGC Standards, Teddington, UK) were maintained at 37 °C and 5% CO_2 using sterile RPMI 1640 (Roswell Park Memorial Institute Medium, ATCC-30-2001) supplemented with 0.3 g/L L-glutamine (Sigma-Aldrich, Darmstadt, Germany), 10% horse serum (ATCC-30-2021), 100 UI penicillin and 0.1 mg streptomycin/mL (both Sigma-Aldrich).

Human coronavirus OC-43 (HCoV-OC43) (ATCC: VR-1558) strain was propagated in HCT-8 cells in RPMI 1640 supplemented with 2% horse serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were lysed 5 days after infection by double freeze and thaw cycles, and virus was titrated according to the Reed and Muench formula. Virus and mock aliquots were stored at −80 °C.

3.3.1. Cytotoxicity Assay

Confluent HCT-8 monolayer cell culture in 96-well plates (Costar®, Corning Inc., Kennebunk, ME, USA) was treated with 0.1 mL/well-containing a maintenance medium that did not contain/or contained decreasing concentrations of test substances. The cells were incubated at 37 °C and 5% CO_2 for 5 days. After microscopic evaluation, the medium containing the test compound was removed, the cells were washed and incubated with neutral red, at 37 °C for 3 h. After incubation, the neutral red dye was removed and the cells were washed with PBS and 0.15 mL/well desorbing solution (1% glacial acetic acid and 49% ethanol in distilled water) was added. The optical density (OD) of each well was read at 540 nm in a microplate reader (Biotek Organon, West Chester, PA, USA). Then, 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the material that reduces cell viability by 50% compared to untreated controls. Each sample was tested in triplicate with four wells for cell culture on a test sample.

The maximum tolerable concentration (MTC) of the extracts is also determined, which is the concentration at which they do not affect the cell monolayer, and in the sample, it looks like the cells in the control sample (untreated with compounds).

3.3.2. Anticoronaviral Assay

The cytopathic effect (CPE) inhibition test was applied for assessment of antiviral activity of the tested compounds on the replication of coronavirus OC43 strain, CPE was registered by the neutral red uptake assay [21].

Confluent HCT-8 cell monolayer in 96-well plates infected with 100 cell culture infectious dose 50% ($CCID_{50}$) in 0.1 mL coronavirus OC-43 strain. After 120 min of virus adsorption, the tested compound was added in various concentrations and cells were incubated for 5 days at 33 °C for coronavirus OC-43 strain. The cytopathic effect was determined using a neutral red uptake assay and the percentage of CPE inhibition for each concentration of the test sample was calculated using the following formula:

$$\% \text{ CPE} = [OD_{\text{test sample}} - OD_{\text{virus control}}]/[OD_{\text{toxicity control}} - OD_{\text{virus control}}] \times 100$$

where $OD_{\text{test sample}}$ is the mean value of the ODs of the wells inoculated with virus and treated with the test sample in the respective concentration, $OD_{\text{virus control}}$ is the mean value of the ODs of the virus control wells (with no compound in the medium) and

$OD_{toxicity\ control}$ is the mean value of the ODs of the wells not inoculated with virus but treated with the corresponding concentration of the test compound. The 50% inhibitory concentration (IC_{50}) was defined as the concentration of the test substance that inhibited 50% of viral replication when compared to the virus control. The selectivity index (SI) was calculated from the ratio CC_{50}/IC_{50}.

4. Conclusions

In conclusion, the described two-step synthesis of 6,7-dimethoxy-4-(4-(4-methoxyphenyl) piperazin-1-yl)-1-methylquinolin-1-ium iodide (**3**) is based on easily implemented and environmentally friendly methodology, leading to high yields of the intermediate and the final product, as well as their isolation with satisfactory analytical purity, without to requiring further purification of the compounds. A preliminary evaluation of the anticoronaviral properties against Human coronavirus OC-43 of compound **3** was performed, and the results obtained confirmed its low cytotoxicity toward HCT-8 cells and its efficacy with respect to coronavirus replication. The reported synthesis is a promising and affordable approach to future transformations of 4-chloroquinoline derivatives into novel compounds with valuable antiviral potential.

Supplementary Materials: The following data are available online: ^{1}H-NMR, ^{13}C-NMR, IR spectra of **2a** and ^{1}H NMR, ^{13}C NMR, ^{13}C DEPT 135, IR and mass spectra of **3**.

Author Contributions: M.I.K. designed the synthetic route to **3**, A.A.V. performed the chemical synthesis. V.S.L. carried out LC-MS characterization of compound **3**. P.P.G. and I.N. carried out the cytotoxicity and anticoronaviral assays and their interpretation. M.I.K., A.A.V. and I.N. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by the Bulgarian National Science Fund (BNSF) project COVIDAvir (KP-06-DK3/1) from 8 December 2020.

Conflicts of Interest: The authors declare no conflict of interest.

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