Chemistry–A European Journal
Research Article
doi.org/10.1002/chem.202103861
Chemistry Europe
European Chemical Societies Publishing
www.chemeurj.org

Anti-SARS-CoV-2 Inhibitory Profile of New Quinoline Compounds in Cell Culture-Based Infection Models

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Abstract: The presently ongoing pandemic of human SARS-CoV-2 infections (COVID-19) presents an enormous challenge in surveillance, vaccine and antiviral drug development. Here we report the synthesis of new bioactive quinoline-morpholine hybrid compounds and their virological evaluation, which proves pronounced cell culture-based inhibitory profile against SARS-CoV-2. Thus, selected quinoline compounds may suggest specific hit-to-lead development.

Introduction

Since its first identification at the end of 2019, a novel human infectious disease spread around the globe with the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). Early investigations directly pointed to the highly contagious nature of the virus that led to the disease COVID-19 (coronavirus disease 2019), at times with a serious to even life-threatening outcome, dependent on risk factors such as age, immune status and others. Human SARS-CoV-2 infection was declared a pandemic by the World Health Organization (WHO) in March of 2020.[1] Even though different types of vaccines have been newly generated and approved, until now, no effective antiviral treatment or prophylaxis has been developed, thus making the intensive investigation of novel and efficient drug candidates against SARS-CoV-2 a necessity. The most common way to provide new drugs and schemes of medication against occurring diseases, specifically within a short period of time, is the repurposing of drugs, which are already established and approved for another disease. The clinically investigated drug remdesivir (RDV, Figure 1A) was repurposed from the putative application against other viral diseases, such as Ebola virus infection, and has now been used against SARS-CoV-2/COVID-19, since the drug exhibited activity against SARS-CoV-2 in vitro as well as in preclinical and clinical investigations.[2] RDV was the first FDA-approved drug against SARS-CoV-2. However, despite promising initial data, RDV only showed poor to even a lack of efficacy in clinical studies and thus, the use in antiviral treatment of SARS-CoV-2 infection is no longer recommended.[3] In addition to RDV, the two antimalarial drugs chloroquine (CQ) and hydroxychloroquine (HCQ) (Figure 1B), have also been used in various studies to address a putative efficacy against SARS-CoV-2.[4]

The two drugs actually show, supplementary to their antimalarial activity, pronounced in vitro activities against several human pathogenic viruses, including human immunodeficiency virus type 1 (HIV-1), hepatitis C virus (HCV) and Ebola

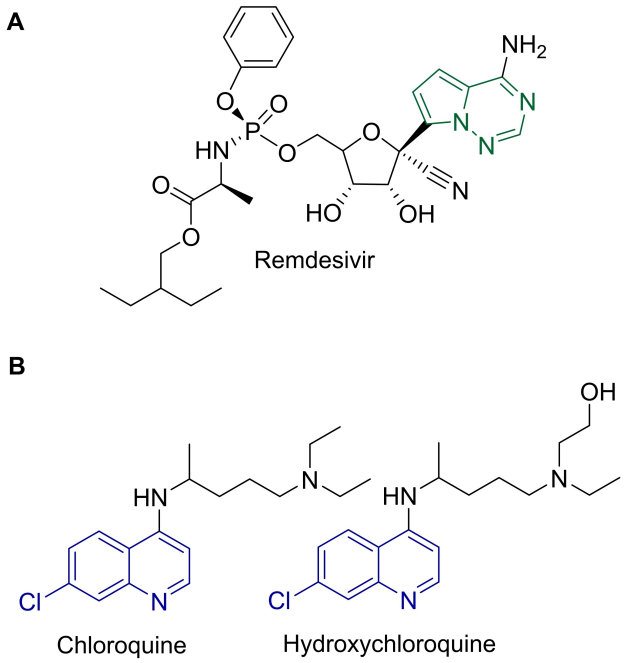

Figure 1. Structures of **(A)** antiviral remdesivir, **(B)** antimalarial/antiviral chloroquine and hydroxychloroquine.

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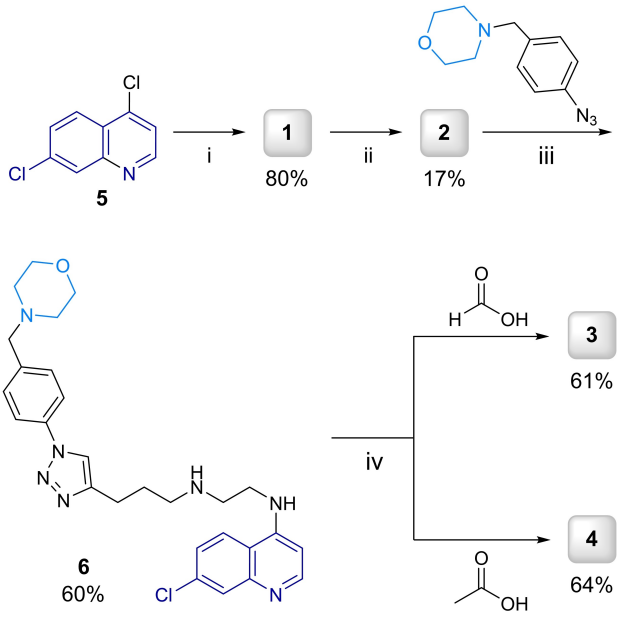
Supporting information for this article is available on the WWW under https://doi.org/10.1002/chem.202103861

virus (EBOV). Similarly, both drugs were reported to comprise activity against SARS-CoV-2,[2a,5] but neither in vitro studies nor clinical trials provided results convincing enough towards an approval for monotherapy or combination therapy together with additional antivirals.[2a,5b,6] Thus, a de novo design and synthesis of a variety of compounds, based on this chemical class, including quinoline-morpholine hybrids (Figure 2), was undertaken in order to identify new antiviral hit profiles for a putative pursuing drug development. Herein, we report the analysis of these new quinoline-based compounds and their virological evaluation in SARS-CoV-2 human and animal cell culture-based infection models[7] thereby addressing their in vitro efficacies and options of chemical hit-to-lead optimization.

Results and Discussion

Quinoline-amine **1** was prepared via a procedure recently reported by our group.[8] Starting from this point, the morpholine-containing precursor **6** was synthesized via a two-step process as we reported very recently.[9] We selected morpholine as a subunit for our new hybrids since it is a privileged structural component of many bioactive molecules and is used in a variety of drugs, both approved and experimental.[10] Furthermore, in recent reports morpholine derivatives demonstrated very promising anti-SARS-CoV-2 properties.[11]

Because of poor solubility of compound **6**, we did not consider it for biological investigations. Instead, we further functionalized it on the secondary amine moiety. Hitherto, formic/acetic acid was used in an amide bond formation reaction with HATU and DIPEA in DMF to form morpholine-containing quinolines **3** and **4** in fair yields (61% and 64%, respectively, Scheme 1). Antiviral activity of quinoline compounds **1**–**4** was assessed for SARS-CoV-2 by performing cell culture-based infection experiments with the assay systems described before.[7] For human Caco-2 cells, the yellow fluorescence protein (YFP)-expressing reporter virus SARS-CoV-2 d6-YFP was applied; for simian Vero 76 cells, the clinical isolate of SARS-CoV-2 termed MUC-IMB-1/2020. In both cases, the compounds were incubated on virus-infected cells at serial



Scheme 1. Synthesis of quinoline-based compounds (**1**–**4**): i) ethane-1,2-diamine, 80–130 °C, 4 h; ii) chloropent-1-yne, K_2CO_3, CH_3CN, 115 °C, 25 h; iii) $CuSO_4 \cdot 5H_2O$ (20 mol%), sodium ascorbate (40 mol%), THF:H_2O (1:1), r.t., 3 h, Ar; iv) HATU, DIPEA, DMF, r.t., o/n.

concentrations under identical conditions (Figure 3). As quantitative readouts of antiviral activity, on the one hand, YFP-based automated fluorometry, or on the other hand, mAb–S-/mAb J2-based (viral spike protein/viral double-strand RNA) in-cell immunofluorescence measurements were performed, respectively (Figure 3, Table 1; note that Figure 3 presents an overview of primary experimental data and Table 1 presents the data summary including calculated EC_{50}, CC_{50} and SI values). As a main result, all analyzed quinoline compounds, **1**–**4**, exerted a similar or stronger anti-SARS-CoV-2 activity than the reference drug chloroquine (CQ). The range of EC_{50} values was between 5.9 ± 3.2 μM and 18.9 ± 10.0 μM in Caco-2 cells (CQ 12.7 ± 18.7 μM), or between 1.5 ± 1.0 μM and 2.9 ± 2.5 μM in Vero 76 cells (CQ 3.1 ± 2.7 μM).

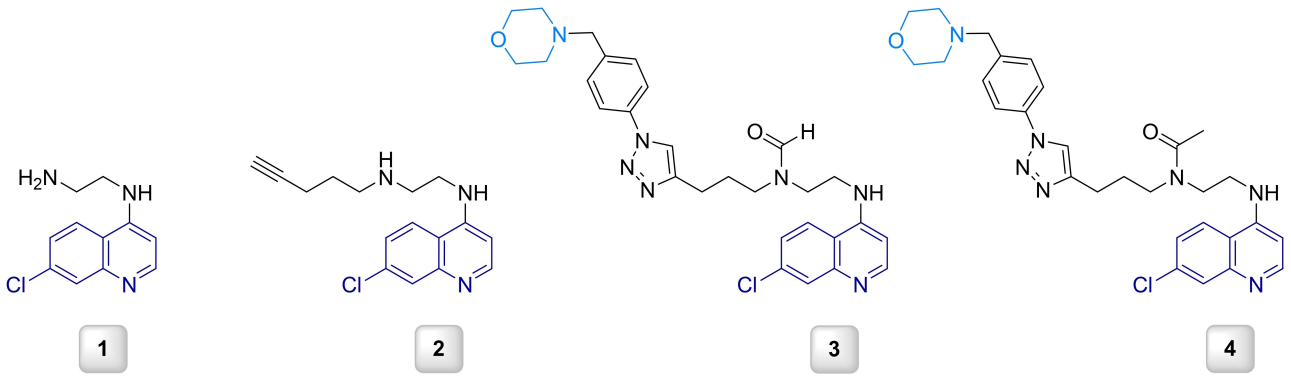


Figure 2. Quinoline-based compounds **1**–**4** designed for activity examination against SARS-CoV-2.

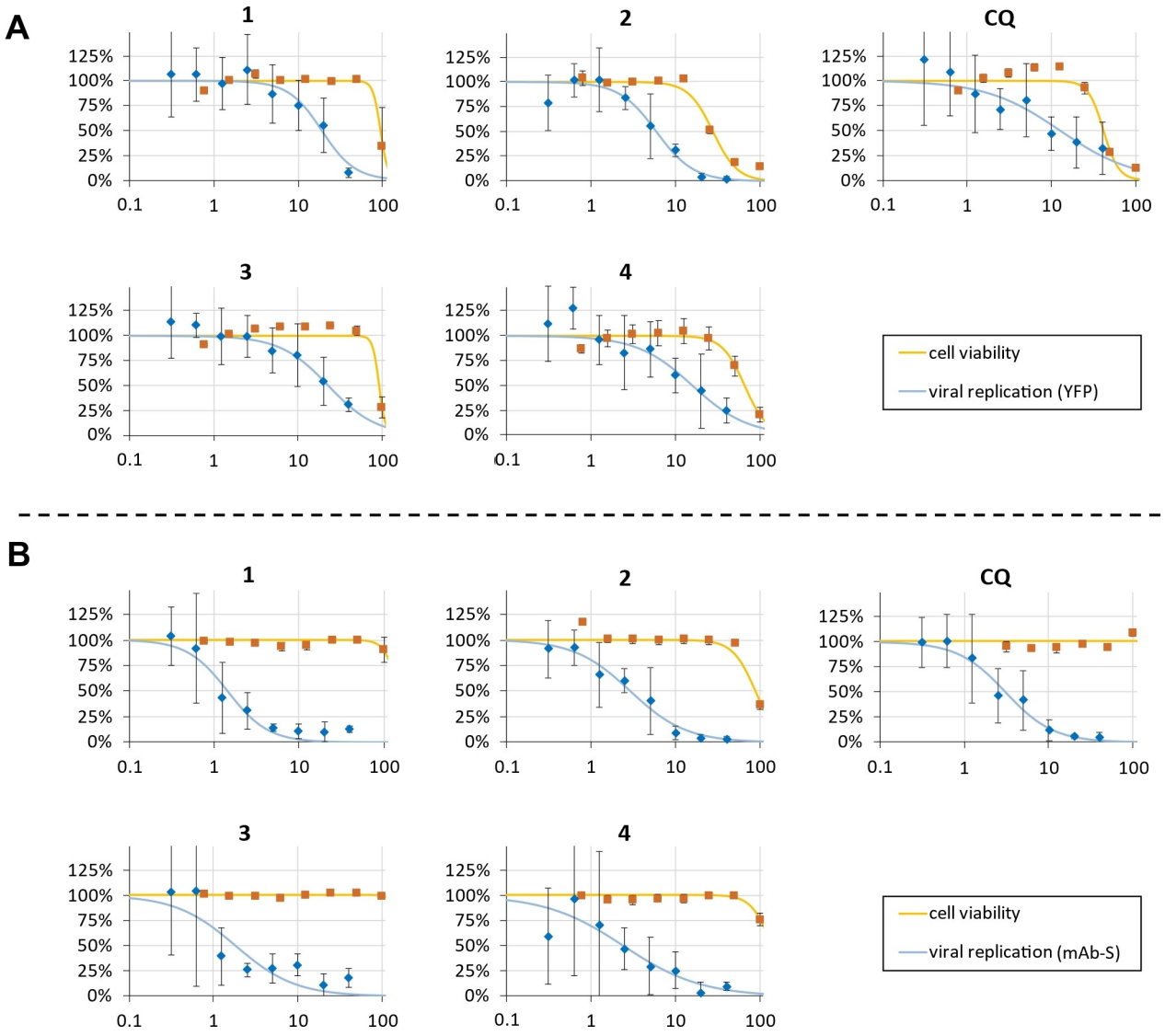


Figure 3. Primary data of the assessment of anti-SARS-CoV-2 activity in vitro. **(A)** Caco-2 cells were used for infection with the SARS-CoV-2 recombinant d6-YFP and antiviral activity was determined applying YFP-based reporter fluorometry. **(B)** Vero 76 cells were used for infection with SARS-CoV-2 isolate MUC-IMB1 and antiviral activity was determined applying mAb–S-based in-cell fluorescence staining of viral spike protein. For details of the experiments, see results and methods text described above including references.

Table 1. Antiviral activity of quinoline compounds **1**–**4** and reference compound chloroquine (CQ) analyzed for two strains of SARS-CoV-2 in two different cell types (Figure 3).[a]

	EC_{50} [μM] (Caco-2)	CC_{50} [μM] (Caco-2)	SI (Caco-2)	EC_{50} [μM] (Vero 76, mAb–S)	CC_{50} [μM] (Vero 76, mAb–S)	SI (Vero 76, mAb–S)	EC_{50} [μM] (Vero 76, mAb-dsRNA)	CC_{50} [μM] (Vero 76, mAb-dsRNA)	SI (Vero 76, mAb-dsRNA)
1	18.9 ± 10.0	93.7 ± 25.8	4.9	1.5 ± 1.0	> 100	> 66.9	5.9 ± 3.5	> 30	> 5
2	5.9 ± 3.2	27.4 ± 1.2p	4.6	2.9 ± 2.5	89.0 ± 0.3	30.7	nd	nd	nd
3	22.9 ± 12.4	92.2 ± 4.7p	4.0	1.8 ± 2.4	> 100	> 55.6	nd	nd	nd
4	15.9 ± 14.1	65.2 ± 10.7	4.1	2.4 ± 3.7	> 100	> 41.7	nd	nd	nd
CQ	12.7 ± 18.7	41.7 ± 2.5p	3.2	3.1 ± 2.7	> 100	> 32.3	2.2 ± 0.9	> 30	> 14

[a] The antiviral analysis was determined using the methodological protocols of a multi-readout assay for SARS-CoV-2 replication in cultured cells as described recently.[7b] Cell viability was measured according to standard procedures using the Neutral Red assay. The details of cell types and virus strains used, as well as the agents for detection and methodological readout systems, have been described before.[7b]

Concerning, reference drugs, is should be mentioned that both, CQ and RDV have been used in our previous studies in the context of anti-SARS-CoV-2 activity, thus showing EC_{50} values in the range of 2.7 ± 0.9 to 3.8 ± 1.7 μM and 1.7 ± 0.5 to 24.4 ± 2.5 μM, respectively, depending on the individual virus strains and readout systems.[7] In our hands, CQ represented a more reliable control compound, with a lower degree of variation, when applied in the cell culture-based in vitro studies on SARS-CoV-2, so that this reference is also preferentially given in the present report. The reason to evaluate the compounds in two different cell types by the use of various sorts of readout systems is based on our previous experience that the antiviral efficacy of individual compounds may vary substantially between the cellular models[7] (additional unpublished data), and this is seen also here, with the quinoline compounds, albeit to a minor pronounced extent. These two cell types represent different culture-based SARS-CoV-2-susceptible models of simian or human origin. Due to the published information that Vero cells lack some of the SARS-CoV-2-supportive cellular signaling pathways that are maintained on Caco-2 cells, the antiviral assessment was presented in a comparative manner. The additional use of various readouts, recently described as the SARS-CoV-2-specific multi-readout assay system (MRA), allows for an initial monitoring of the potential mechanistic mode of drug-mediated interference with the regulatory levels of viral replication.[7b] Thereby, most pronounced antiviral activity was determined for compound **2** with 5.9 ± 3.2 μM in Caco-2 and compound **1** with 1.5 ± 1.0 μM in Vero 76. The selectivity indices (SI) of quinolines ranged within much higher concentrations in Vero 76 (EC_{50} values 30.7 to >66.7) than in Caco-2 cells (4.0 to 4.9), which was in dependence of the levels of compound-induced cytotoxicity (CC_{50} values between 27.4 ± 1.2 μM and 93.7 ± 25.9 μM in Caco-2, or 89.0 ± 03 μM and >100 μM in Vero 67). In essence, these quinoline compounds show a promising potential of in vitro anti-SARS-CoV-2 activity and are considered as a pharmaceutically interesting candidate class for further development. So far, no information is given about the putative mode of antiviral action (MoA), so that distinct mechanistic analyses should be performed to address this question. Of note, compounds **1**–**4** possess a similar basic chemical framework as CQ, so that their expected mechanistic basis, in terms of antiviral MoA, might also be similar. However, due to the fact that the CQ-specific MoA has not been specifically described in detail, this issue remains speculative so far.

In this regard, it should be taken into account that the MoA analysis of these compounds may require the establishment of further tools of SARS-CoV-2-specific investigation, recombinant biological systems including quantitative reporters and drug derivatives allowing for target identification such as click-chemistry-suited compounds. Thus, this will be a challenging goal for future studies.

From the chemical point of view, quinolines provide a valuable platform for further derivatization and analyses of structure-activity relationship (SAR) in order to achieve an optimization of biological activities. At this stage, data are supporting the use of selected quinoline compounds for specific hit-to-lead development and, in the favorable case, for further advanced developmental steps using a quinoline candidate arising from this basic characterization.

Conclusion

In conclusion, new quinoline-based compounds were synthesized and investigated for their activity against SARS-CoV-2. These new compounds exerted a similar or stronger anti-SARS-CoV-2 activity (EC_{50} down to 1.5 ± 1.0 μM) than the reference drug chloroquine ($EC_{50}=3.1\pm2.7$ μM). These results provide a valuable basis for design of further quinoline-based drug candidates to treat SARS-CoV-2 infections.

Acknowledgements

We gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft (DFG, grant TS 87/23-1 for S.B.T.) and financial support from Bayerische Forschungsstiftung (grant AZ-1499-21 for M.M./Immunic). Scientific support of the M.M. laboratory antiviral research projects by Immunic AG, Hella Kohlhof and coworkers (Gräfelfing, Germany), including various long-term collaborative drug developmental activities, is specifically acknowledged. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: antiviral drug assessment · cell culture-based infection models · morpholine-quinoline hybrids · quinoline compounds · SARS-CoV-2

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Manuscript received: October 26, 2021
Accepted manuscript online: December 3, 2021
Version of record online: December 29, 2021