Discovery of 2-Phenylquinolines with Broad-Spectrum Anti-coronavirus Activity

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Cite This: ACS Med. Chem. Lett. 2022, 13, 855–864

ABSTRACT: A selection of compounds from a proprietary library, based on chemical diversity and various biological activities, was evaluated as potential inhibitors of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a phenotypic-based screening assay. A compound based on a 2-phenylquinoline scaffold emerged as the most promising hit, with EC\textsubscript{50} and CC\textsubscript{50} values of 6 and 18 \(\mu\)M, respectively. The subsequent selection of additional analogues, along with the synthesis of ad hoc derivatives, led to compounds that maintained low \(\mu\)M activity as inhibitors of SARS-CoV-2 replication and lacked cytotoxicity at 100 \(\mu\)M. In addition, the most promising congeners also show pronounced antiviral activity against the human coronaviruses HCoV-229E and HCoV-OC43, with EC\textsubscript{50} values ranging from 0.2 to 9.4 \(\mu\)M. The presence of a 6,7-dimethoxytetrahydroisoquinoline group at the C-4 position of the 2-phenylquinoline core gave compound 6g that showed potent activity against SARS-CoV-2 helicase (nsp13), a highly conserved enzyme, highlighting a potentiality against emerging HCoVs outbreaks.

KEYWORDS: SARS-CoV-2, 2-Phenylquinolines, Helicase, Repurposing, Autophagy, Pan-CoVs inhibitors

Currently, the world is in full pandemic crisis due to the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19. Since its first appearance in Wuhan, China, in December 2019, SARS-CoV-2 has rapidly spread around the globe, seriously affecting global health. On March 11, 2020, the World Health Organization declared the SARS-CoV-2 outbreak as a pandemic that had affected an estimated 250 million persons and caused more than 5 million deaths worldwide by January 2022.\textsuperscript{1,2}

SARS-CoV-2 belongs to the human coronaviruses (HCoVs), which are a large family of crown-shaped, enveloped, single-stranded, positive-sensed RNA zoonotic viruses that cause various illnesses ranging from a simple cold to a severe acute respiratory syndrome. Other members of the HCoV family include HCoV-229E and HCoV-OC43, causative agents of common cold, and, two highly pathogenic HCoVs, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-CoV-1), both responsible for previous epidemics and associated with high morbidity, clearly demonstrating the need for antiviral agents to combat CoV infections. However, the rapid resolution of these epidemics, in combination with a low worldwide spread, led to a global under-investment in CoV drug discovery. As a result, there were no appropriate antiviral drugs available at the time of the COVID-19 outbreak, which now would have paved the way for the control of this pandemic.

The development of vaccines is undoubtedly essential to contain the diffusion of the current virus, and a joint effort never seen before led to a worldwide vaccination campaign in less than 1 year after the SARS-CoV-2 outbreak. The success of this approach in reducing deaths and hospitalization is increasingly evident. However, vaccines may be less effective or even ineffective against emerging variants of SARS-CoV-2. In addition, and most importantly, it is still to be determined how long this vaccine-induced immunity will last, and at least one booster vaccination is essential. Therefore, the development of antiviral drugs targeting SARS-CoV-2 is absolutely necessary. During the lag time needed to produce a new

Received: March 18, 2022
Accepted: April 20, 2022
Published: May 3, 2022
vaccine, antiviral drugs are the only weapon to rapidly control viral infections. Moreover, the development of an antiviral therapy is of paramount importance for unvaccinated people and for the treatment of viral infections caused by emerging SARS-CoV-2 strains that evade the vaccine antibody response. Preferably, new antiviral agents should possess broad-spectrum antiviral activity against various HCoVs, since this will increase the likelihood that these drugs will be active against future outbreaks.

The quickest way to find effective COVID-19 treatment options is the drug repurposing approach, enabling the fast introduction of drugs into clinical settings. Various molecules, including many antiviral drugs, have been already evaluated in large randomized clinical trials, with the first two drugs receiving marketing approval. The monophosphoramidate nucleoside prodrug remdesivir, originally developed for Ebola virus infections, was the first drug that received emergency use authorization for the treatment of patients with severe manifestations of COVID-19. Another viral RNA polymerase inhibitor, molnupiravir, originally developed to treat influenza virus infections, has just received approval for oral use in people who have mild to moderate COVID-19 and at least one risk factor for developing severe illness.

Over the years, our laboratory has worked extensively on the design and synthesis of biologically active compounds, resulting in an in-house library that mainly consists of structurally diverse, small heterocyclic molecules. Notably, this library is rich in agents with proven activity against various viruses such as the human immunodeficiency virus, the hepatitis C virus, flaviviruses, human cytomegalovirus, and the influenza virus.

In this Letter, our efforts toward finding novel anti-SARS-CoV-2 agents, based on a first set of proprietary compounds followed by ad hoc synthesis, are described. In addition, preliminary mode-of-action studies were performed in order to elucidate the molecular target of the identified compounds.

**SARS-CoV-2 Screening—Hit Identification.** In a first round of screening, approximately 100 compounds from our internal library were selected, based on chemical diversity and being endowed with various biological activities (see list of compounds in Table S1). These were evaluated for their antiviral activity against SARS-CoV-2, using SARS-CoV-2-infected VeroE6 cells constitutively expressing an enhanced green fluorescent protein (EGFP), allowing for high-content imaging readout of the virus-induced cytopathic effect. The cytotoxicity of the compounds in uninfected VeroE6 cells was determined in parallel. GS-441524, the parent nucleoside analogue of remdesivir, and chloroquine (CQ) were included as positive controls and reference compounds. From this initial screening, two structurally related hits were identified (Figure 1). The 2-phenylquinolone derivative WRNA10, originally designed as an HIV-1 TAR RNA binder, showed an EC$_{50}$ value of 10 μM and CC$_{50}$ = 40 μM, while the 2-phenylquinoline analogue PQQ40 (1a) was endowed with EC$_{50}$ and CC$_{50}$ values of 6 μM and 18 μM, respectively.

Compound 1a belongs to a large series of variously functionalized 2-phenylquinolines (2-PhQs) that have been developed as S. aureus NorA efflux pump inhibitors, with compounds that are able to restore very efficiently the ciprofloxacin antibacterial activity coupled with a good safety profile and metabolic stability.

Based on the easier synthetic feasibility and availability of more analogues within the proprietary library, the focus was directed toward hit compound 1a.

**Hit Exploration.** The two hits that emerged from this primary screening shared a very similar quinoline core functionalized with a 2-phenyl ring. Of note, only one other compound based on 2-PhQ was initially assayed, the 4-hydroxyquinoline 1 (Table 1), which was completely inactive, highlighting the importance of the basic side chain in imparting antiviral activity. Based on these preliminary insights, a new set of analogues of hit 1a was selected from the in-house library. In parallel, new congeners were designed and synthesized in order to complete the structure–activity relationship (SAR) study.

All the quinolines studied, either available from the library (1b–h, 2a, 3b, 4g, 5a,gi, 6a,gi, 5a,k, 9a,g, 11l, 13a, 14k) or synthesized ad hoc (5fj, 6fjm–m, 7a,j,k, 8j, 9fj, 10a, 12a, 13a, 14k) (Table 1), were decorated at the C-4 position with various moieties with different basicity, such as (cyclo)alkylamines and aromatic amines, but also bulkier substituents such as a dimethoxytetrahydroisoquinoline or a benzylpiperazine. The moieties were linked to the quinoline scaffold via an ether linker, mostly represented by an ethoxy linker, longer or shorter spacers were also present in a few cases. In the majority of the derivatives, the quinoline core was decorated with one or two methoxy groups at various positions. The presence of a 2-p-propoxyphenyl moiety was characteristic for all the tested compounds, with the exception of compounds 12a, 13a, and 14k.

Compounds 5fj, 6fjm–m, 8j, 9fj, and 12a (Scheme 1) were prepared by alkylation of mono- and dimethoxy-2-(4-propoxyphenyl)quinolin-4-ol scaffolds with appropriate chloroalkylamines or benzylpiperazines. The moieties were linked to the quinoline scaffold via an ether linker, mostly represented by an ethoxy linker, longer or shorter spacers were also present in a few cases. In the majority of the derivatives, the quinoline core was decorated with one or two methoxy groups at various positions. The presence of a 2-p-propoxyphenyl moiety was characteristic for all the tested compounds, with the exception of compounds 12a, 13a, and 14k.

For the synthesis of 5,8-dimethoxy-2-(4-propoxyphenyl)-quinoline derivatives 7a,j,k, the key intermediate 23 was prepared as shown in Scheme 2. Acrylate 20 was reacted with 2,5-dimethoxyniline 21 in the presence of catalytic p-TsOH in dry benzene, affording aminoacrylate 22 that was heated in a mixture of diphenyl and diphenyl oxide (Dowtherm A) at 240 °C, yielding the 5,8-dimethoxyquinoline 23. The subsequent reaction with various chloroalkylamines furnished the target compounds 7a,j,k.

The synthesis of the trimethoxy derivative 10a is depicted in Scheme 3. Intermediate 26, which was obtained by reacting p-propoxybenzoyl chloride 24 with 3,4,5-trimethoxyniline 25, was subjected to a Friedel–Crafts acylation with acetyl chloride and SnCl$_4$ in dry CH$_2$Cl$_2$ to give compound 27. Cyclization of 27 in the presence of t-BuOK in t-BuOH
Table 1. Structure, Anti-SARS-CoV-2 Activity, and Cytotoxicity of 2-PhQs

| Compd | R          | \( \text{EC}_{50} \) (pM) | \( \text{CC}_{50} \) (pM) | SI  |
|-------|------------|---------------------------|---------------------------|-----|
| 1     | -          | >100                      | NT                        | -   |
| 1a'   |            | 6.5 ± 0.05                | 21.2 ± 4.2                | 3.3 |
| 1b'   |            | >100                      | NT                        | -   |
| 1c'   |            | >100                      | NT                        | -   |
| 1d'   |            | >100                      | NT                        | -   |
| 1e'   |            | >100                      | NT                        | -   |
| 1f'   |            | 11.9 ± 5.5                | > 100                     | > 8.4|
| 1g'   |            | > 100                     | NT                        | -   |
| 1h'   |            | >100                      | NT                        | -   |
| 2a'   |            | >100                      | NT                        | -   |
| 3a'   |            | >100                      | NT                        | -   |
| 4a'   |            | >40                       | NT                        | -   |
| 5a'   |            | 9.1 ± 3.05                | > 100                     | > 11.0|
| 5f    |            | 35.9 ± 32.2               | > 100                     | 2.8 |
| 5g'   |            | > 100                     | > 100                     | -   |
| 5i'   |            | 7.1 ± 4.2                 | 30.3 ± 0.8                | 4.3 |
| 5j    |            | > 100                     | 30.8 ± 0.1                | -   |
| Compd | R | \(^a \text{Anti-SARS-CoV-2 activity EC}_{50} \text{(nM)}\) | \(^b \text{VeroE6 cells IC}_{50} \text{(nM)}\) | \(^c \text{SI}\) |
|-------|---|-----------------|-------------|-----|
| 6a'   |   | > 100           | 6.71±3.5    | -   |
| 6f    |   | 7.9 ± 5.6       | > 100       | > 12.7 |
| 6g'   |   | 8.8 ± 5.6       | > 100       | > 11.4 |
| 6j    |   | > 100           | 16.1 ± 17.0 | -   |
| 7a    |   | 4.6 ± 0.2       | 24.6 ± 0.1  | 5.3 |
| 7j    |   | 11.3 ± 2.1      | 89.8 ± 2.8  | 7.9 |
| 7k    |   | 2.7 ± 1.9       | 23.8 ± 5.7  | 8.8 |
| 8a'   |   | 5.1 ± 1.4       | 70.3 ± 23.7 | 13.8 |
| 8j    |   | 9.6 ± 2.2       | 12.9 ± 4.8  | 1.3 |
| 8k'   |   | 2.6 ± 1.4       | 11.1 ± 1.3  | 4.3 |
| 9a'   |   | 13.0 ± 10.0     | > 100       | > 7.7 |
| 9f    |   | > 100           | > 100       | -   |
| 9g'   |   | > 100           | > 100       | -   |
| 9j    |   | 5.9 ± 2.4       | > 100       | > 16.9 |
| 10a   |   | 8.3 ± 1.8       | 23.8 ± 8.3  | 2.9 |
| 11P   |   | > 100           | NT          | -   |
| 12a   |   | > 100           | 64.7 ± 29.8 | -   |
Table 1. continued

| Compd | R                  | *Anti-SARS-CoV-2 activity (EC50 (μM)) | *VeroE6 cells CC50 (μM) | *SI  |
|-------|--------------------|--------------------------------------|-------------------------|------|
|       |                    |                                      |                         |      |
| 13a†  |                   | >100                                 | NT                      |      |
| 14k†  |                   | >100                                 | NT                      |      |

**6g-analogues**

|       | R                  | *Anti-SARS-CoV-2 activity (EC50 (μM)) | *VeroE6 cells CC50 (μM) | *SI  |
|-------|--------------------|--------------------------------------|-------------------------|------|
| 6m    |                   | >100                                 | >100                    |      |
| 6n    |                   | >100                                 | >100                    |      |
| 6o    |                   | >100                                 | >100                    |      |
| GS-441524 |            | 0.7 ± 0.07                          | 72.9 ± 12.2             | 104.1|
| Chloroquine |             | 10.9 ± 0.9                          | 83.4 ± 4.2              | 7.7  |

*EC50 = concentration of compound that gives 50% rescue of the virus-reduced eGFP signals as compared to the untreated virus-infected control cells. †CC50 = 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. The values represent the means ± SD of data derived from duplicate experiments. ‡SI = selectivity index calculated as the ratio between CC50 and EC50 of each compound. §Ref 17. ¶Ref 19. ․Ref 18. …Ref 20. ‧Ref 21.

Scheme 1†

Reagents and conditions: (i) 1-(2-chloroethyl)amines (for R, see Table 1), K₂CO₃, dry DMF, 80 °C, 4−24 h, 25−51%.

Scheme 2‡

Reagents and conditions: (i) p-TsOH, dry benzene, reflux, 24 h, 22%; (ii) Dowtherm A, 240 °C, 1.5 h, 52%; (iii) 1-(2-chloroethyl)-amines (for R, see Table 1), K₂CO₃, dry DMF, 80 °C, 4−24 h, 20−38%.
Scheme 3*

![Scheme 3](https://example.com/scheme3.png)

*Reagents and conditions: (i) Et$_3$N, dry THF, 0 °C → rt, 12 h, 98%; (ii) SnCl$_4$, acetyl chloride, dry CH$_2$Cl$_2$, 0 °C → rt, 24 h, 35%; (iii) t-BuOK, t-BuOH, 90 °C, overnight, 68%; (iv) 1-(2-chloroethyl)diethylamine, K$_2$CO$_3$, dry DMF, 80 °C, 3 h, 60%.

Table 2. Antiviral Activity against HCoV-229E and HCoV-OC43 and Cytotoxicity on HEL 299 Cells of Selected 2-PhQs

| compd | HEL 299 cells CC$_{50}$ (μM) | HCoV-229E EC$_{50}$ (μM) | SI$^c$ | HCoV-OC43 EC$_{50}$ (μM) | SI$^d$ |
|-------|-----------------------------|-----------------------|-------|-----------------------|-------|
| 1f    | >100                        | 61.3 ± 32.2           | ≥1.6  | 92.3 ± 11.0           | >1.2  |
| 5a    | 10.4 ± 0.8                  | 1.3 ± 0.9             | 8.2   | 0.6 ± 0.1             | 17.0  |
| 5i    | 8.0 ± 1.3                   | 0.3 ± 0.05            | 25.6  | 1.7 ± 0.2             | 4.7   |
| 6g    | 26.7 ± 4.1                  | 2.9 ± 0.05            | 9.1   | 1.5 ± 0.1             | 18.0  |
| 6f    | 35.6 ± 0.2                  | 2.2 ± 0.7             | 16.4  | 1.9 ± 0.2             | 18.6  |
| 7a    | 9.9 ± 0.8                   | 0.7 ± 0.3             | 13.8  | 2.0 ± 0.2             | 5.1   |
| 7j    | 8.7 ± 0.8                   | 0.6 ± 0.3             | 13.4  | 1.8 ± 0.7             | 4.7   |
| 7k    | 95.3 ± 3.3                  | 9.4 ± 3.6             | 10.2  | 7.7 ± 3.3             | 12.5  |
| 8a    | 9.2 ± 0.1                   | 1.8 ± 0.4             | 5.2   | 1.9 ± 0.4             | 4.9   |
| 8k    | 12.3 ± 1.1                  | 0.2 ± 0.2             | 52.4  | 1.6 ± 0.2             | 7.9   |
| 9a    | 43.5 ± 5.6                  | 3.5 ± 2.7             | 12.4  | 2.2 ± 0.5             | 19.8  |
| 9j    | >100                        | 40.0 ± 20.8           | >2.5  | 22.7 ± 2.8            | >4.4  |
| chloroquine | 33.6 ± 1.3               | 1.3 ± 0.1             | 25.1  | <0.8                  | >40.8 |
| GS-441524 | >100                        | 0.9 ± 0.1             | >112.4 | 1.3 ± 0.07           | >80.0 |

$^c$CC$_{50}$ = 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. $^d$EC$_{50}$ = Effective concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. The values represent the mean ± SD of data derived from duplicate experiments. $^e$SI = Selectivity index calculated as the ratio between CC$_{50}$ and EC$_{50}$.

yielded quinoline 28, which was alkylated with 1-(2-chloroethyl)diethylamine to give target compound 10a.

**Antiviral Activity against SARS-CoV-2**. Compounds 1−14 were tested as inhibitors of SARS-CoV-2 replication, and the data are reported in Table 1. Compounds 9j, 6f,g, 5a, 1f, and 9a showed antiviral activity, with EC$_{50}$ values ranging from 5.9 to 13.0 μM (Figure S1 reports an example of a microscopic picture of GFP-based phenotypic screening), without any sign of cytotoxicity, even at the highest tested concentration of 100 μM. The highest selectivity index (SI) values were observed for compounds that have a couple of methoxy groups on the quinoline ring, such as compounds 9j (6,8-dimethoxy) and 6f,g (5,7-dimethoxy). Other derivatives, such as 8k, 7k, 7a, 8a, 5i, 8j, 10a, and 7j, also showed good antiviral activity (with EC$_{50}$ values from 2.6 to 11.3 μM), but they displayed a more pronounced cytotoxicity for the VeroE6 cells (CC$_{50}$ = 11.1−89.8 μM).

Among the basic side chains in C-4, the piperidine moiety emerged as the most promising to yield antiviral activity, since compounds 7k and 8k were the most potent in the SARS-CoV-2 antiviral assay. On the other hand, the presence of a benzylpiperazine moiety, such as in compounds 1f, 5f, 6f, and 9f, had a variable effect on the antiviral activity, depending on the substitution pattern on the quinoline scaffold, although all of these analogues lacked cytotoxicity. Any other structural variation of hit compound 1a was unfruitful. Indeed, a loss of activity was observed when (i) the ethoxy spacer was replaced by an amide linker (11l); (ii) the p-propoxy group at the 2-phenyl ring was removed (12a); or (iii) the p-propoxynaphenyl ring was replaced by a 5-chlorothienyl (13a) or 2-pyrindyl (14k) moiety.

Overall, the 2-PhQ scaffold emerged as a privileged structure to impart SARS-CoV-2 inhibitory activity. A number of structural features allow us to tune the antiviral activity as well as the cytotoxicity, such as the p-propoxynaphenyl moiety at C-2 of the quinoline scaffold, the methoxy groups on the quinoline nucleus, and the O-alkyl basic side chain at C-4.

The 2-PhQs with the best profile against SARS-CoV-2 (compounds 1f, 5a,i, 6f,g, 7a,j,k, 8a,k, and 9a,j) were also tested against other HCoVs to evaluate potential broad-spectrum anti-coronavirus activity. An α-coronavirus (HCoV-229E) and another β-coronavirus (HCoV-OC43), which are both causative agents of a common cold, were selected. The antiviral activity, as well as their cytotoxicity, was measured on HEL 299 cells, along with those of CQ and GS-441524, which were used as positive controls (Table 2). Most analogues were
Figure 2. Chloroquine (CQ) treatment (10 μM for 4 h) of VeroE6 cells leads to accumulation of light chain 3-II (LC3-II, red) as visualized by immunofluorescence staining (A, first panel). Treatment with 10 μM of 2-phenylquinolines 9j, 6f, and 6g leads to a similar but less pronounced inhibition of these fusion events. Untreated control cells are shown in the bottom panel; LC3-II localization is shown in red, and nuclei were visualized with DAPI (blue), scale bar 25 μm. High content analysis of the treated cells allows to measure and quantify the increase in cytoplasmic LC3 puncta (B).

**Mode of Action Study.** To date, no quinoline derivatives functionalized with a phenyl ring at C-2 have been reported as possessing anti-CoV activity. However, other quinoline analogues endowed with a plethora of pharmacological activities, including antiviral properties, such as the antimalarial drugs CQ and hydroxychloroquine, display pronounced in vitro activity against SARS-CoV-1, MERS, and SARS-CoV-2. Although their clinical use was revoked for safety issues, more than 90 clinical trials are still in progress to elucidate their therapeutic value. Among the several hypotheses that have been put forward to explain their antiviral mechanism of action, inhibition of autophagy by impairing autophagosome fusion with lysosomes and thereby stalling the autophagic flux received most attention.

Since the 2-PhQs and CQ share the same quinoline scaffold, a selection of 2-PhQs (5g, 6f, 6g, and 9j) was evaluated for autophagy inhibition in VeroE6 cells, and compared to CQ (Figure 2). Confocal microscopy was used in which light chain 3-II (LC3-II, a well-known marker of autophagy) was visualized by immunofluorescence staining, whereas the nuclei were visualized by fluorescent staining with 4',6-diamidino-2-phenylindole (DAPI). Treatment of VeroE6 cells with CQ as positive control led to a 9-fold increase in the intensity of the cytoplasmic LC3 puncta. The tested 2-PhQs demonstrated a less pronounced increased intensity of the cytoplasm of LC3-positive puncta, with compounds 5g and 9j that are devoid of antiviral activity, that showed a minimal effect on the autophagy. Overall, these results show that inhibition of autophagy marginally contributes to the antiviral activity, suggesting that alternative molecular targets might be responsible for the antiviral efficacy of the 2-PhQs.

Recently, many quinoline and quinazoline derivatives have been tested in a cell-based SARS-CoV-2 RdRp reporter system, with a few quinolines being active. This prompted us to investigate the most potent 2-PhQs (compounds 6f, 7k, and 9j) as potential SARS-CoV-2 RdRp inhibitors via a fluorescent-based assay, including non-nucleotide inhibitor F243 as a positive control. All the tested compounds lacked activity against RdRp at the highest tested concentration of 30 μM (Table 3).

The same set of compounds was also evaluated as potential inhibitors of another validated drug target, the NTPase/helicase (nsp13), using the triazole-based compound SSYA10-001, known for its inhibitory activity against helicase of SARS-CoV-1 and MERS-CoV, as reference compound. The SARS-CoV-2 nsp13 helicase uses the energy derived from the hydrolysis of nucleotides to unwind the double-stranded nucleic acids in two single strands along the 5′→3′ direction and possesses two associated activities: RNA unwinding and S′-triphosphatase (NTPase).

While all the compounds were inactive against the unwinding independent ATPase activity, compounds 6g and 7k showed promising activity against the helicase unwinding activity with IC_{50} values of 0.42 and 1.41 μM, respectively. The
Table 3. Inhibitory Activity of Selected 2-PhQs against SARS-CoV-2 Non-structural Proteins

| compd       | RdRp IC₅₀ (μM) | unwinding IC₅₀ (μM) | ATPase IC₅₀ (μM) |
|-------------|----------------|---------------------|-----------------|
| 6f          | >30 (100%)     | >30 (55%)           | >30 (96%)       |
| 6g          | >30 (62%)      | 0.42 ± 0.23         | >30 (90%)       |
| 7k          | >30 (100%)     | 1.41 ± 0.64         | >30 (100%)      |
| 9j          | >30 (100%)     | >30 (57%)           | >30 (100%)      |
| SSYA10-001  | ND             | 0.046 ± 0.015       | >3 (90%)        |
| F243        | 53 ± 3         | ND                  | ND              |

“Compound concentration required to inhibit the SARS-CoV-2 RdRp-associated activity by 50%.”
“Compound concentration required to inhibit the SARS-CoV-2 nsp13 helicase-associated activity by 50%.”
“Compound concentration required to inhibit the SARS-CoV-2 nsp13 ATPase-associated activity by 50%.”
“Percentage of control measured in the presence of the highest concentration of tested compound.

other analogues showed only moderate nsp13 inhibition, reducing the enzymatic activity by 45% at 30 μM (Table 3).

Thus, the 2-PhQ scaffold seems to have emerged as suitable to inhibit the nsp13 helicase activity, with various potency depending on the substitution patterns around the quinoline ring.

Based on the results achieved on helicase, to better elucidate the role of the dimethoxy tetrahydroisoquinoline of the most potent 6g, a few analogs were designed by removing one or both of the methoxy groups, as in compounds 6m and 6n, or by reducing the size of the bicycle, giving the dihydroisoindole derivative 6o. All these structure modifications gave compounds devoid of any activity on SARS-CoV-2 replication (Table 1), confirming 6g as the right combination to impart both antiviral and anti-helicase activity.

In conclusion, screening a proprietary compound library as potential inhibitors of SARS-CoV-2 replication led to the identification of the 2-PhQ 1a, displaying an EC₅₀ value of 6 μM and a low SI of 3. The subsequent testing of close analogues, along with the synthesis of properly designed derivatives, confirmed the 2-PhQ scaffold as being very suitable to impart anti-SARS-CoV-2 activity. The correct functionalization of the quinoline core with two methoxy groups coupled with a C-2 p-proxopyphenyl ring and a suitable basic ethoxy side chain at C-4 yielded derivatives showing EC₅₀ values ranging from 2.6 to 13 μM and lacking cytotoxicity (CC₅₀ > 100 μM). Most analogues were also active against other HCoVs (such as OCA43 and 229E) with low μM activity (0.2–9.4 μM), thus emerging as pan-CoV inhibitors with the potential to hit also future HCoV outbreaks.

Preliminary studies on the mechanism of action revealed that 2-PhQs were weak inhibitors of the autophagy pathway and were inactive as RdRp inhibitors; on the other hand, some analogues inhibited the helicase unwinding activity of nsp13 with low μM potency. Nsp13 helicase is a very promising target, being highly conserved, with a 99.8% sequence identity shared between SARS-CoV-2 and SARS-CoV-1, suggesting that drugs targeting nsp13 will be active against emerging HCoVs outbreaks. For compounds 6g and 7k, the helicase inhibition appears to mainly contribute to the antiviral activity, with bis-dimethoxyquinoline derivative 6g emerging as the most promising.

The synthesis of a few analogues of 6g, compounds 6m–o, gave clear insights on the essential role of the 6,7-dimethoxy-tetrahydroisoquinoline moiety in conferring antiviral activity through the helicase unwinding inhibition. The co-crystallographic experiments, in progress for 6g, will establish the importance of this group for nsp13 inhibition and will guide the design of improved 2-PhQ derivatives.

ASSOCIATED CONTENT

# Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00123.

Table S1, overview of tested compounds; Figure S1, antiviral activity of compound 6g in the SARS-CoV-2/ VeroE6-eGFP assay; experimental details and synthetic procedures; ¹H NMR, ¹³C NMR, and HPLC for the final compounds; biological experimental procedures (PDF)

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Funding
This work was supported by FISR project F75F21000880001 (O.T.). The SARS-CoV2 screening was performed using the ‘Caps-It’ research infrastructure (project ZW13-02) that was financially supported by the Hercules Foundation (Research Foundation Flanders) and Rega Foundation, KU Leuven.

Notes
The authors declare no competing financial interest.

ABBREVIATIONS
SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SARS-CoV-1, Severe Acute Respiratory Syndrome Coronavirus 1; MERS-CoV, Middle East Respiratory Syndrome; HCoV, human coronavirus; 2-PhQ, 2-phenylquinoline; CQ, chloroquine; LC3-II, light chain 3-II; RdRp, RNA-dependent RNA polymerase; SÅR, structure–activity relationship; TLC, thin-layer chromatography

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