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Design, synthesis and biological evaluation of 2-aminoquinazolin-4 (3H)-one derivatives as potential SARS-CoV-2 and MERS-CoV treatments

Jun Young Lee a, Young Sup Shin a, Sangeun Jeon b, Se In Lee a, Soojin Noh a, Jung-Eun Cho a, Min Seong Jang c, Seungtaek Kim b, Jong Hwan Song a, Hyoung Rae Kim a, Chul Min Park a, d, C.a

a Center for Convergent Research of Emerging Virus Infection (CEVI), Korea Research Institute of Chemical Technology, 141 Gajeong-ro, Yuseong-gu, Daejeon 34114, South Korea
b Zoonotic Virus Laboratory, Institut Pasteur Korea, Seongnam-si, Gyeonggi-do 13488, South Korea
c Department of Non-Clinical Studies, Korea Institute of Toxicology, Yuseong-gu, Daejeon 34114, South Korea
d Korea University of Science and Technology, Daejeon 34114, South Korea

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ABSTRACT

Despite the rising threat of fatal coronaviruses, there are no general proven effective antivirals to treat them. 2-Aminoquinazolin-4(3H)-one derivatives were newly designed, synthesized, and investigated to show the inhibitory effects on SARS-CoV-2 and MERS-CoV. Among the synthesized derivatives, 7-chloro-2-((3,5-dichlorophenyl)amino)quinazolin-4(3H)-one (9g) and 2-((3,5-dichlorophenyl)amino)-5-hydroxyquinazolin-4 (3H)-one (11e) showed the most potent anti-SARS-CoV-2 activities (IC50 < 0.25 μM) and anti-MERS-CoV activities (IC50 < 1.1 μM) with no cytotoxicity (CC50 > 25 μM). In addition, both compounds showed acceptable results in metabolic stabilities, hERG binding affinities, CYP inhibitions, and preliminary PK studies.

Coronavirus is a single positive-stranded RNA virus that was discovered in 1960 while looking for a new cold virus that infects the upper respiratory tract.1,2 The clinical significance was relatively low because of its weak symptoms.3 However, with the outbreak of SARS-CoV in 2003, its clinical significance has received new attention.4,5 SARS-CoV spread to 4 countries, with 8,422 confirmed cases and 966 deaths, with its mortality rate of 11%, raising the public health awareness.4,5 In 2012, a new outbreak of MERS-CoV occurred and spread to 27 countries by January 2020, resulting in 2519 confirmed cases and 866 deaths, with a mortality rate of 34%.6 Most recently, COVID-19, which was caused by SARS-CoV-2 outbreak in Wuhan, China, in December 2019, has spread worldwide, causing 40 million confirmed cases and 1 million deaths.7 Eventually, on March 11, 2020, COVID-19 was declared as third pandemic after the 1968 Hong Kong flu and 2009 influenza by the WHO.8-9

For treatments of MERS-CoV, it is generally recommended to use drugs such as interferon, immunomodulatory factor, and antiviral drugs such as ribavirin or lopinavir.10 However, there are reports that interferon and ribavirin may cause side effects such as poor bone marrow function, anemia, and virus mutations.11 In addition, although the monkey model showed a therapeutic effect12, it did not show a great effect in actual clinical trials, requiring the development of a safer and more efficient MERS treatment.13

In the case of COVID-19, Remdesivir, which received urgent approval, as well as Nafamostat, and Hydroxychloroquine, are considered as promising therapeutic candidates.14 However, due to side effects and low clinical effects,15 safer and more effective treatments need to be developed.

Our research for novel antivirals inhibiting these fatal coronaviruses started with a biochemical high content screening (HCS) of a library containing 200,000 compounds from Korea Chemical Bank.16 In the past few years, we reported inhibitors of MERS-CoV such as 2-phenylchrooman-4-one derivatives,17 3-acyl-2-phenylamino-1,4-dihydroquinolin-4-(1H)-one derivatives,18 and 4-aniline-6-aminouquinazoline derivatives19 as well as inhibitors of SARS-CoV-2 such as cyclic sulfonamide derivatives.20

In this study, the core scaffold of 3-acyl-2-phenylamino-1,4-dihydroquinolin-4-(1H)-one of our lead compound (1)18 was changed to 2-amino-quinazolin-4(3H)-one (Fig. 1). Because the aromatic rings substituted with halogen groups or electron withdrawing groups of...
diroquoquinolinolines 1 were important for activity, 2-amino-quinazolin-4(3H)-ones with aromatic rings fixed at the similar positions were designed and evaluated to find compound 2 as a hit (IC₅₀ = 2.6 μM for SARS-CoV-2). 2-Amino-quinazolin-4(3H)-one derivatives have been known to possess high activities as inhibitors of aldose reductase, KᵥATP channel opener, anti-cancer agents, and anti-hyperglycemic agents. Here we report on the synthesis and biological effects of 2-amino-quinazolin-4(3H)-one derivatives.

A series of 2-amino-quinazolin-4(3H)-one analogs were synthesized as shown in Scheme 1. Anthranilic acids 3 were treated with urea by heating 150 °C for 20 h to afford quinazolinediones 4. Dichloroquinazolines 5 were prepared by reacting quinazolinediones 4 with POCl₃ in the presence of trimethylamine. Treatment of dichloroquinazolines 5 with 2 N NaOH led to 2-chloro-4(3H)-quinazolines 6. 2-Chloroquinazolin-4(3H)-ones 6 and anilines 7 with various substituents were heated in DMF at 85 °C for 16 h to obtain 2-amino-quinazolin-4(3H)-ones 8.

The anti-SARS-CoV-2 and anti-MERS-CoV activities of the synthesized compounds were evaluated by using immobilized assay in Vero cells. In this study, Vero cells were stained using antibodies targeting spike protein for MERS-CoV and nucleocapsid protein for SARS-CoV-2, and the infection rate was measured by imaging the infected Vero cells through microscope.

First, we began structure–activity relationship (SAR) studies for anti-SARS-CoV-2 activities by varying anilines at 2 position of quinazololine ring of compound 2 (Table 1). For anilines with one fluoride atom, compound 2b (IC₅₀ = 1.4 μM) bearing aniline substituted with fluoride at meta position showed good effect, whereas compounds with ortho-fluoroaniline (2a) or para-fluoroaniline (2c) showed no inhibitory effect. In the same way, compound with anilines having two fluoride atoms at ortho and para position (2d) had no activity, while compound 2e with 3,5-difluoroaniline (IC₅₀ = 0.24 μM), which has two fluorides at double meta-position, showed a highly increased activity because of the synergic effect of fluoride at meta-position. Changing 2,4-fluoroaniline and 3,5-difluoroaniline to 2,4-chloroaniline (2f) and 3,5-dichloroaniline (2g) showed similar activities (IC₅₀ > 25 and 0.23 μM, respectively). Next, we investigated the substituent effects at meta-position of anilines (9h–9m). Electron-donating groups such as methoxy (9h), hydroxy (9i), and dihydroxy (9j) decreased anti-SARS-CoV-2 activities (IC₅₀ > 11 μM). Electron-withdrawing groups such as cyano (9k) and trifluoromethyl (9l) showed much better effects than those of electron-donating groups (IC₅₀ = 1.7 and 0.68 μM, respectively), while methyl-ester (9m) decreased anti-SARS-CoV-2 activities (IC₅₀ = 8.8 μM). Then, anti-SARS-CoV-2 effects of aliphatic amine substituents such as cyclohexylmethyl amine (9n), isopropyl amine (9o), n-butyral amine (9p), and piperidinyl amine (9q) were evaluated to exhibit decreased potency (IC₅₀ > 14 μM).

In the next phase of optimization, we evaluated substituent effects of 5 to 8 positions of quinazolinoine ring, having fixed with 3,5-difluoroaniline or 3,5-dichloroaniline at 2 position (Table 2). Compounds with electron-withdrawing groups, such as 7-trifluoromethyl (10a and 11a) and 7-nitro (10b and 11b), showed high binding affinities (0.20–0.51 μM). In the case of compounds with electron-donating groups, variations of inhibitory effects were shown. Compounds with 5-hydroxy (10e and 11e), 8-hydroxy (10f and 11f), 5,8-dichloro (10i and 11i), and 7,8-dichloro (10j and 11j) showed high binding affinities (0.15–1.6 μM). 7-Amino (10c and 11c), 5-methoxy (10d and 11d), 7-hydroxy (10g and 11g), and 6,8-dimethyl (10h and 11h) derivatives showed no inhibitory effects. The 7-N-substituted quinazolinoine compound 11k displayed no anti-SARS-CoV-2 activity.

With the compounds having potent activities toward SARS-CoV-2, we tested anti-MERS-CoV activities (Table 3). All compounds except 10b and 11b showed good antiviral activities (IC₅₀ = 0.39–3.1 μM). It seems that our quinazolinoine compounds are potent broad spectrum coronavirus inhibitors. In particular, all the above compounds except 10a and 11a displayed no obvious cytotoxicity (CC₅₀ > 25 μM).

We selected the compounds for the purpose of further pharmacological investigations on SARS-CoV-2. In the case of electron-withdrawing substituents in the aromatic ring of quinazolinone, compound 9g with chloro substituent at position 7 was selected because it has the highest SI (110). And we chose compound 11e with electron-donating substituent (hydroxyl) at 5 position because it is the most active on SARS-CoV-2 and has the highest SI (168). Compound 9g and 11e were further evaluated for their microsomal stabilities, cytotoxicities, human ether a-go-go (hERG) bindings, plasma protein bindings.

**Scheme 1.** Synthesis of 2-aminoquinazolin-4(3H)-ones derivatives. Reagents and conditions: (a) Urea, 150 °C, 20 h; (b) POCl₃, TEA, 115 °C, 17 h; (c) 2 N NaOH, rt, 20 h; (d) DMF, 85 °C, 16 h.
μ-presence of somal stability show that -PPB) and CYP inhibitions (Table 4 and 5). First, the results of micro-Lead optimization of 2-anilino groups.

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In conclusion, we designed and developed 2-aminoquinazolin-4(3H)- ones Derivatives as potent inhibitors against both SARS-CoV-2 and MERS-CoV. Among them, 7-chloro-2-((3,5-dichlorophenyl)amino)quinazinazol-4(3H)-one (9g) and 2-((3,5-dichlorophenyl)amino)-5-hydroxyquinazinazol-4(3H)-one (11e) were considered as new drug candidates because both have high anti-SARS-CoV-2 activities ([9g, IC50 = 0.23 μM], [11e, IC50 = 0.15 μM]) and anti-MERS-CoV activities ([9g, IC50 = 0.93 μM], [11e, IC50 = 1.02 μM]) with no cytotoxicity (CC50 > 25 μM).

Our two lead compounds also showed good microsomal stabilities, relatively low cytotoxicities, low hERG binding affinities and CYP inhibitions. The PK profile of 9g seemed to be acceptable for the discovery of antivirals. 2-Aminoquinazinazolone derivatives were found to be a promising new scaffold against coronaviruses and further optimizations to increase pharmacokinetic profiles are currently underway.

Table 1
Lead optimization of 2-anilino groups.

Table 2
SAR studies of 2-anilinoquinazolin-4(3H)-one derivatives

Table 3
Anti-MERS-CoV activity of 2-Aminoquinazoloin-4(3H)-ones Derivatives

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.

a,b IC50 and CC50 were derived from the results of at least two dependent experiment in Vero cells infected with SARS-CoV-2

a SI(selective index) = CC50/IC50 for inhibiting SARS-CoV-2 infection.

b IC50 and CC50 were derived from the results of at least two dependent experiment in Vero cells infected with MERS-CoV

c SI(selective index) = CC50/IC50 for inhibiting MERS-CoV infection.

ab IC50 and CC50 were derived from the results of at least two dependent experiment in Vero cells infected with SARS-CoV-2.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127885.

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