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.

Bioorg. Med. Chem. Lett. 39 (2021) 127885

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and biological evaluation of 2-aminoquinazolin-4(3*H*)-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,*]

[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*

ARTICLE INFO

Keywords:
Coronavirus
SARS-CoV-2
MERS-CoV
2-aminoquinazolinone
Antiviral

ABSTRACT

Despite the rising threat of fatal coronaviruses, there are no general proven effective antivirals to treat them. 2-Aminoquinazolin-4(3*H*)-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(3*H*)-one (**9g**) and 2-((3,5-dichlorophenyl)amino)-5-hydroxyquinazolin-4 (3*H*)-one (**11e**) showed the most potent anti-SARS-CoV-2 activities ($IC_{50} < 0.25$ μM) and anti-MERS-CoV activities ($IC_{50} < 1.1$ μM) with no cytotoxicity ($CC_{50} > 25$ 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.[3–4] SARS-CoV spread to 4 countries, with 8,422 confirmed cases and 916 deaths, with its mortality rate of 11%, raising the public health awarness.[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 effect[12], 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-phenylchroman-4-one derivatives,[17] 3-acyl-2-phenylamino-1,4-dihydroquinolin-4-(1*H*)-one derivatives,[18] and 4-aniline-6-amino-quinazoline derivatives[19] 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-(1*H*)-one of our lead compound (**1**)[18] was changed to 2-amino-quinazolin-4(3*H*)-one (Fig. 1) . Because the aromatic rings substituted with halogen groups or electron withdrawing groups of

* Corresponding author at: Center for Convergent Research of Emerging Virus Infection (CEVI), Korea Research Institute of Chemical Technology, 141 Gajeong-ro, Yuseong-gu, Daejeon 34114, South Korea.
E-mail address: parkcm@krict.re.kr (C.M. Park).

https://doi.org/10.1016/j.bmcl.2021.127885
Received 15 December 2020; Received in revised form 8 February 2021; Accepted 10 February 2021
Available online 2 March 2021

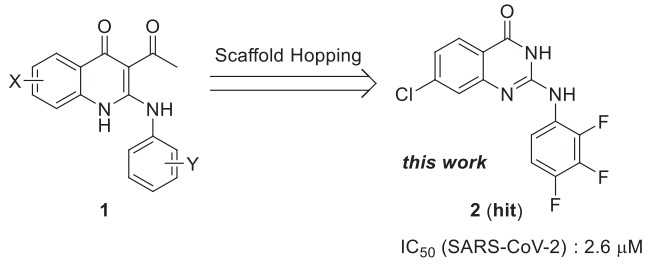


Fig. 1. Our new design of anti-coronavirus Scaffold.

dihydroquinolinones **1** were important for activity, 2-amino-quinazolin-4(3*H*)-ones with aromatic rings fixed at the similar positions were designed and evaluated to find compound **2** as a hit (IC_{50} = 2.6 μM for SARS-CoV-2). 2-Amino-quinazolin-4(3*H*)-one derivatives have been known to possess high activities as inhibitors of aldose reductase,[21a] K_{ATP} channel opener,[21b] anti-cancer agents,[21c] and anti-hyperglycemic agents,[21d] Here we report on the synthesis and biological effects of 2-amino-quinazolin-4(3*H*)-one derivatives.

A series of 2-amino-quinazolin-4(3*H*)-one analogues were synthesized as shown in Scheme 1[22]. 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 $POCl_3$ in the presence of trimethylamine. Treatment of dichloroquinazolines **5** with 2 N NaOH led to 2-chloro-4(3*H*)-quinazolinones **6**. 2-Chloroquinazolin-4(3*H*)-ones **6** and anilines **7** with various substituents were heated in DMF at 85 °C for 16 h to obtain 2-amino-quinazolin-4(3*H*)-ones **8**.

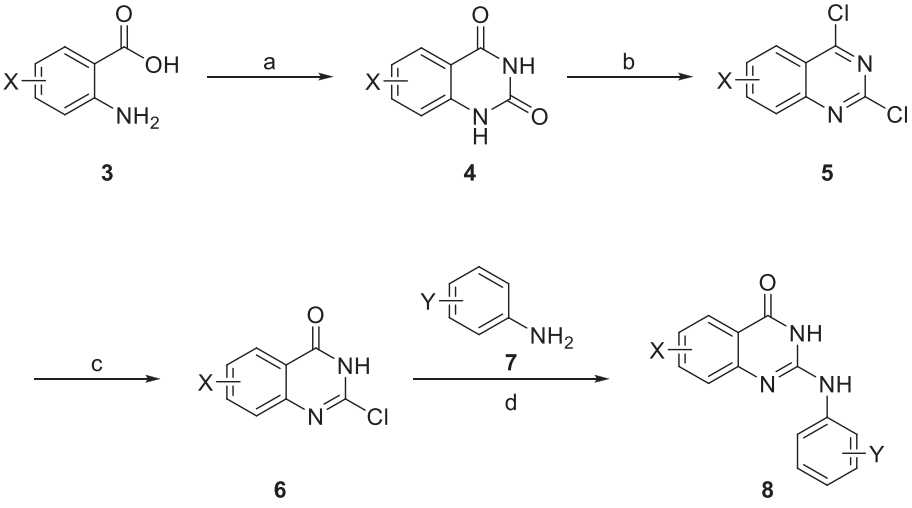
The anti-SARS-CoV-2 and anti-MERS-CoV activities of the synthesized compounds were evaluated by immunofluorescent assay in Vero cells.[16,20] In this study, Vero cells were stained using antibodies targeting spike protein for MERS-CoV and nucleocapsid protein for SARS-CoV-2, and the infection rate was 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 quinazolinone ring of compound **2** (Table 1). For anilines with one fluoride atom, compound **9b** (IC_{50} = 1.4 μM) bearing aniline substituted with fluoride at *meta*-position showed good effect, whereas compounds with *ortho*-fluoroaniline (**9a**) or *para*-fluoroaniline (**9c**) showed no inhibitory effect. In the same way, compound with anilines having two fluoride atoms at ortho and para position (**9d**) had no activity, while compound **9e** with 3,5-difluoroaniline (IC_{50} = 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 (**9f**) and 3,5-dichloroaniline (**9g**) showed similar activities (IC_{50} : >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 (IC_{50} > 11 μM). Electron-withdrawing groups such as cyano (**9k**) and trifluoromethyl (**9l**) showed much better effects than those of electron-donating groups (IC_{50} = 1.7 and 0.68 μM, respectively), while methyl-ester (**9m**) decreased anti-SARS-CoV-2 activities (IC_{50} = 8.8 μM). Then, anti-SARS-CoV-2 effects of aliphatic amine substituents such as cyclohexylmethyl amine (**9n**), isopropyl amine (**9o**), *n*-butyl amine (**9p**), and piperidinyl amine (**9q**) were evaluated to exhibit decreased potency (IC_{50} > 14 μM).

In the next phase of optimization, we evaluated substituent effects of 5 to 8 positions of quinazolinone 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 quinazolinone compound **11k** displayed no anti-SARS-CoV-2 activity.

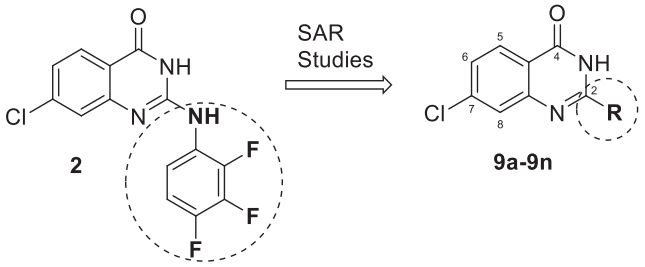
With the compounds having potent activities toward SARS-CoV-2, we tested anti-MERS-CoV activities (Table 3). All compounds except **10b** and **11b** showed good antiviral activities (IC_{50} = 0.39–3.1 μM). It seems that our quinazolinone compounds are potent broad spectrum coronavirus inhibitors. In particular, all the above compounds except **10a** and **11a** displayed no obvious cytotoxicity (CC_{50} > 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) $POCl_3$, TEA, 115 °C, 17 h; (c) 2 N NaOH, rt, 20 h; (d) DMF, 85 °C, 16 h.

Table 1
Lead optimization of 2-anilino groups.



Compound	R	SARS-CoV-2		
		IC_{50}[a](μM)	CC_{50}[b](μM)	SI[c]
2	2,3,4-F_3-Ph-NH-	2.6	>25	9.4
9a	2-F-Ph-NH-	>25	>25	1
9b	3-F-Ph-NH-	1.4	>25	18
9c	4-F-Ph-NH-	>25	>25	1
9d	2,4-F_2-Ph-NH-	>25	>25	1
9e	3,5-F_2-Ph-NH-	0.24	18	74
9f	2,4-Cl_2-Ph-NH-	>25	>25	1
9g	3,5-Cl_2-Ph-NH-	0.23	>25	110
9h	3-CH_3O-Ph-NH-	11	>25	2.3
9i	3-HO-Ph-NH-	>25	>25	1
9j	3,5-$(HO)_2$-Ph-NH-	>25	>25	1
9k	3-CN-Ph-NH-	1.7	>25	15
9l	3-CF_3-Ph-NH-	0.68	>25	37
9m	3-CH_3O(O =)C-Ph-NH-	8.8	>25	2.8
9n	Cyclohexylmethyl-NH-	14	>25	1.7
9o	*i*-Pr-NH-	>25	>25	1
9p	*n*-Bu-NH-	>25	>25	1
9q	piperidinyl-N-	>25	>25	1

[a,b] IC_{50} and CC_{50} were derived from the results of at least two dependent experiment in Vero cells infected with SARS-CoV-2
[c] SI(selective index) = CC_{50}/IC_{50} for inhibiting SARS-CoV-2 infection.

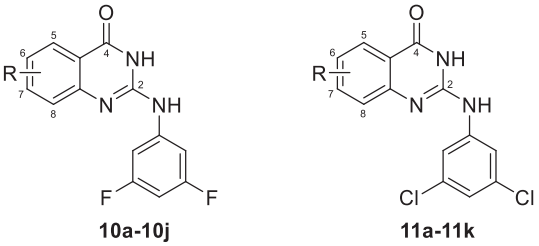
(PPB) and CYP inhibitions (Table 4 and 5). First, the results of microsomal stability show that **9g** is stable in mouse, rat, and human and **11e** is microsomally more unstable than **9g**, which seems to be due to the presence of –OH in the structure. In the cytotoxicity assay, **9g** and **11e** showed a little toxicity in HFL-1 (CC_{50} = 7.5 and 9.6 μM), but did not show toxicity in the rest of the cells (Table 4).

In hERG channel inhibition assay, it was found that both compounds **9g** and **11e** did not interact with hERG channel. In the PPB assay, both compounds **9g** and **11e** showed high binding rates (97–100%). The results of CYP inhibition assay show that **9g** showed little inhibition of the CYP enzyme and **11e** displayed some inhibition (>65% CYP inhibition at 10 μM) in CYP1A2 and CYP3A4.

The preliminary pharmacokinetic properties of **9g** and **11e** were investigated by intravenous (*i.v.*) and oral (*p.o*) routes in rats, with 2 mg/kg and 10 mg/kg, respectively (Table 6). The oral bioavailability of **9g** and **11e** were 15.6% and 7.8%, respectively, partly because the clearance of compound **11e** is higher than that of **9g**. The PK profile of **9g** seemed to be acceptable for the discovery of anti-coronavirus drugs.

In conclusion, we designed and developed 2-aminoquinazolin-4(3*H*)-one derivatives as potent inhibitors against both SARS-CoV-2 and MERS-CoV. Among them, 7-chloro-2-((3,5-dichlorophenyl)amino)quinazolin-4(3*H*)-one (**9g**) and 2-((3,5-dichlorophenyl)amino)-5-hydroxyquinazolin-4(3*H*)-one (**11e**) were considered as new drug candidates because both have high anti-SARS-CoV-2 activities [(**9g**, IC_{50} = 0.23 μM), (**11e,** IC_{50} = 0.15 μM)] and anti-MERS-CoV activities [(**9g**, IC_{50} = 0.93 μM), (**11e**, IC_{50} = 1.02 μM)] with no cytotoxicity (CC_{50} > 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-Aminoquinazolinone derivatives were found to be a promising new scaffold against coronaviruses and further optimizations to increase pharmacokinetic profiles are currently underway.

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



Compound	R	SARS-CoV-2		
		IC_{50}[a](μM)	CC_{50}[b](μM)	SI[c]
10a	7-CF_3	0.20	7.6	38
10b	7-NO_2	0.41	>25	61
10c	7-NH_2	>25	>25	1
10d	5-CH_3O	>25	>25	1
10e	5-OH	0.47	>25	54
10f	8-OH	1.6	>25	15
10g	7-OH	>25	>25	1
10h	6,8-$(CH_3)_2$	>25	>25	1
10i	5,8-Cl_2	0.33	>25	76
10j	7,8-Cl_2	0.25	>25	98
11a	7-CF_3	0.21	7.1	34
11b	7-NO_2	0.51	>25	49
11c	7-NH_2	>25	>25	1
11d	5-CH_3O	>25	>25	1
11e	5-OH	0.15	>25	168
11f	8-OH	0.37	>25	65
11g	7-OH	>25	>25	1
11h	6,8-$(CH_3)_2$	>25	>25	1
11i	5,8-Cl_2	0.35	>25	71
11j	7,8-Cl_2	0.24	>25	103
11k	7-Morpholinyl	>25	>25	1

[a,b] IC_{50} and CC_{50} were derived from the results of at least two dependent experiment in Vero cells infected with SARS-CoV-2
[c] SI(selective index) = CC_{50}/IC_{50} for inhibiting SARS-CoV-2 infection.

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

Compound	MERS-CoV			SARS-CoV-2
	IC_{50}[a](μM)	CC_{50}[b](μM)	SI[c]	IC_{50}[d](μM)
9e	0.39	>25	65	0.24
9g	0.93	>25	27	0.23
10a	0.73	>25	35	0.20
11a	0.96	7.3	7.6	0.21
10b	8.5	>25	2.9	0.41
11b	>25	>25	1.0	0.51
10e	1.1	>25	22	0.47
11e	1.02	>25	25	0.15
10f	3.1	>25	8.0	1.64
11f	1.7	>25	14	0.37
10i	0.79	>25	31	0.33
11i	0.62	>25	42	0.35
10j	0.83	>25	30	0.25
11j	0.88	>25	31	0.24

[a,b] IC_{50} and CC_{50} were derived from the results of at least two dependent experiment in Vero cells infected with MERS-CoV
[c] SI(selective index) = CC_{50}/IC_{50} for inhibiting MERS-CoV infection.
[d] IC_{50} were derived from the results of at least two dependent experiment in Vero cells infected with SARS-CoV-2

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.

Table 4
Result of Microsomal stability and cytotoxicity of **9g** and **11e**

Compound	MS[a]			Cytotoxicity (μM)[b]			
	m[a]	r[a]	h[a]	HFL-1	L929	NIH 3 T3	CHO-K1
9g	112	105	99	7.5	31	>100	11
11e	42	54	55	9.6	27	60	28

[a] % of remaining after 30 min
[b] Cell information. HFL-1: human embryonic lung cell line, L929: NCTC clone 929, mouse fibroblast cell line, NIH 3 T3 : mouse embryonic fibroblast cell line, CHO-K1 : Chinese hamster ovary cell line.

Table 5
Result of hERG, PPB, CYP inhibition of **9g** and **11e**

Compound	hERG(10 μM)	PPB (5 μM)		CYP inhibition (10 μM)				
		r	h	1A2	2C9	2C19	2D6	3A4
9g	38	100	100	15	18	<1	20	32
11e	28	97	100	65	34	12	46	65

Table 6
Rat pharmacokinetic study of **9g** and **11e**

Compounds	**9g**		**11e**	
Parameters[a]	I.V., 2 mg/kg	P.O., 10 mg/kg	I.V., 5 mg/kg	P.O., 10 mg/kg
Tmax (h)	NA[b]	2.5	NA	1.0
Cmax (μg/h)	NA	0.9	NA	0.06
$T_{1/2}$ (h)	2.2	5.7	9.2	42
AUC (μg·h/mL)	8.5	6.6	6.5	1.01
CL (L/h/kg)	0.25	NA	0.67	NA
V_{ss}(L/Kg)	1.6	NA	6.8	NA
F_t(%)	NA	15.6	NA	7.8

[a] All results are the mean of experiments using three rats. [b] NA: not applicable

Acknowledgements

The chemical library used in this study was kindly provided by Korea Chemical Bank (http://www.chembank.org/) of Korea Research Institute of Chemical Technology. This research was supported through the National Research Foundation of Korea (NRF) (2020M3E9A1041758) and supported by a grant of National Research Council of Science & Technology (NST) (No. CRC-16-01-KRICT) funded by the ministry of Science and ICT (MSIP).

Appendix A. Supplementary data

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

References

1 Kendall EJC, Bynoe ML, Tyrrell DA. *J Br Med J*. 1962;2:82.
2 Lim YX, Ng YL, Tam JP, et al. *Diseases*. 2016;4:26.
3 Khan JS, McIntosh K. *Pediatr Infect Dis J*. 2005;24:S223.
4 Woo PC, Lau SK, Huang Y, et al. *Exp Biol Med*. 2009;234:1117.
5 World Health Organization (WHO). Severe Acute Respiratory Syndroms (SARS) Overview. https://www.who.int/health-topics/severe-acute-respiratory-syndrome#tab=tab_1 [online]..
6 World Health Organization (WHO). Middle East respiratory syndrome coronavirus (MERS-CoV) situation update, January 2020 http://www.emro.who.int/pandemic-epidemic-diseases/mers-cov/mers-situation-update-january-2020.html [online].
7 World Health Organization (WHO). Coronavirus Disease (COVID-19) Dashboard, Global situation. https://covid19.who.int/ [online].
8 World Health Organication (WHO) Director-General's opening remarks at the media briefing on COVID-19, 11 March 2020 https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19—11-march-2020 [online].
9 Stawicki SP, Jeanmonod R, Miller AC, et al. *J Global Infect Dis*. 2020;12:47.
10 Omrani AS, Saad MM, Baig K, et al. *Lancet Infect Dis*. 2014;14:1090.
11 (a) Alvarez D, Dieterich DT, Brau N, et al. *J Viral Hepat*. 2006;13:683.(b) Contreas AM, Hiasa Y, He W, et al. *J Virol*. 2002;76:8505.
12 Falzarano D, Wit E, Rasmussen AL, et al. *Nat Med*. 2018;10:721.
13 Arabi YM, Shalhoub S, Mandourah Y, et al. *Clin Infect Dis*. 2020;70:1837.
14 (a) Li G, Clercq ED. *Nat Rev Drug Discov*. 2020;19:149.(b) Wang M, Cao R, Zhang L, et al. *Cell Res*. 2020;30:269.(c) Gil C, Ginex T, Maestro I, et al. *J Med Chem*. 2020;63:12359.
15 (a) Beigel JH, Tomashek KM, Dodd LE, et al. *N Engl J Med*. 2020;383:1813.(b) Pan H, Peto R, Karim QA, et al. *MedRxiv*. 2020. https://doi.org/10.1101/2020.10.15.20209817.
16 Cruz DJ, Bonotto RM, Gomes RG, et al. *PLoS Negl Trop Dis*. 2013;7, e2471.
17 Yoon JH, Lee J, Lee JY, et al. *Bull Korean Chem Soc*. 2019;40:906.
18 Yoon JH, Lee JY, Lee J, et al. *Bioorg Med Chem Lett*. 2019;29, 126727.
19 Lee JY, Shin YS, Lee J, et al. *Bioorg Med Chem Lett*. 2020;30, 127472.
20 Shin YS, Lee JY, Noh S, et al. *Bioorg Med Chem Lett*. 2021;31, 127667.
21 (a) DeRuiter J, Brubaker AN, Millen J, et al. *J Med Chem*. 1986;29:627.(b) Somers F, Ouedraogo R, Antoine M-H, et al. *J Med Chem*. 2001;44:2575.(c) Srivastava V, Sinha D, Tiwari AK, et al. *Cancer Biother Radio*. 2010;25:559.(d) Ram Farhanullah VJ, Tripathi BK, et al. *Bioorg Med Chem Lett*. 2003;11:2439.
22 (a) Prashad M, Har D, Hu B, et al. *Org Process Res Dev*. 2004;8:330.(b) Paul K, Sharma A, Luxami V. *Bioorg Med Chem Lett*. 2014;24:624.