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Identification of 4-anilino-6-aminoquinazoline derivatives as potential MERS-CoV inhibitors

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ABSTRACT

New therapies for treating coronaviruses are urgently needed. A series of 4-anilino-6-aminoquinazoline derivatives were synthesized and evaluated to show high anti-MERS-CoV activities. N^4-(3-Chloro-4-fluorophenyl)-N^6-(3-methoxybenzyl)quinazoline-4,6-diamine (**1**) has been identified in a random screen as a hit compound for inhibiting MERS-CoV infection. Throughout optimization process, compound **20** was found to exhibit high inhibitory effect (IC_{50} = 0.157 μM, SI = 25) with no cytotoxicity and moderate *in vivo* PK properties.

Coronaviruses (CoVs) are a group of positive-sense, single-stranded RNA viruses that cause severe respiratory diseases in a broad range of animal species, including humans.[1–3] In 2003, one of the novel coronaviruses, severe acute respiratory syndrome CoV (SARS-CoV), caused a total of 8,422 cases of SARS with 916 deaths.[4] The other novel coronavirus, Middle East respiratory syndrome CoV (MERS-CoV), has emerged in April 2012 and posed a serious threat to public health. As of 4 April 2020, a total of 2,494 human MERS-CoV infections with 858 deaths had been reported from 27 countries.[5] Although MERS-CoV can cause primary infections from direct contact with animal reservoirs like camels,[6] person-to-person transmission of this virus has mainly occurred in health-care facilities and family clusters.[7–9] Recently outbreak of COVID-19, which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in China and has spread to several other countries.

Drug repositioning for an FDA-approved compound library found that numerous compounds inhibited MERS-CoV infection. However, there are still no approved drugs for coronaviruses.[10] Through high content screening (HCS) platform of Institut Pasteur Korea (IPK) using the Korea Chemical Bank (KCB),[11] we found several novel compounds that can inhibit MERS-CoV infection.[12] We found that N^4-(3-chloro-4-fluorophenyl)-N^6-(3-methoxybenzyl)quinazoline-4,6-diamine **1** was effective for inhibiting MERS-CoV infection. Quinazoline derivatives have previously been reported as potent inhibitors of the protein kinase of epidermal growth factor receptor (EGFR).[13] Most of those compounds were approved as anticancer drugs, including Gefitinib,[14] Lapatinib,[15] Erlotinib,[16] and Afatinib[17]. We thought that quinazoline compounds can exhibit good bioavailability and be easily extended to treatment of MERS-CoV infection. Here we report on the synthesis and biological effects of 4-anilino-6-aminoquinazoline derivatives.

The general synthetic route for 4-aminoquinazoline derivatives is described in Scheme 1. 2-Amino-5-nitrobenzonitrile was reacted with dimethylformamide-dimethyl acetal (DMF-DMA) in toluene to give (*E*)-*N′*-(2-cyano-4-nitrophenyl)-*N*,*N′*-dimethylformimidamide **2**. *N*,*N*-dimethylformamidine **2** was treated with acetic acid and anilines at 120 °C to produce 6-nitroquinazoline **3**.[18] Reduction of the nitro group of **3** was carried out using iron powder and NH_4Cl in isopropyl alcohol and water at 100 °C to afford 6-aminoquinazoline **4**. Aromatic amine **4** was reductive alkylated with aldehydes using $NaBH(OAc)_3$ and trifluoroacetic acid in isopropyl acetate at 100 °C to give *N*-substituted quinazolines **5**.

The antiviral activities of the synthesized compounds in Vero cells were determined by immunofluorescence assay (IFA). Vero cells were infected with a korean clinical MERS-CoV isolates and the inhibitory concentration (IC_{50}) and cytotoxic concentration (CC_{50}) values of the compounds were calculated by nonlinear regression analysis.[11]

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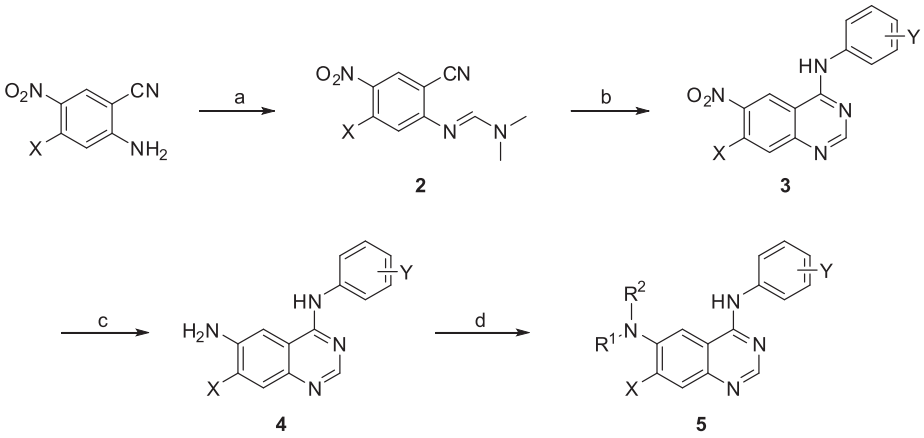


Scheme 1. Synthesis of 6-substituted 4-anilinoquinazoline derivatives. Reagents and conditions: (a) DMF-DMA, toluene, 110 °C, 5 h; (b) $ArNH_2$, AcOH, 120 °C, 7 h; (c) Fe, NH_4Cl, i-PrOH/H_2O (10/1), 110 °C/4h; (d) Aldehyde, $NaBH(OAc)_3$, trifluoroacetic acid, i-PrOAc, 90 °C, 3 h; Acyl chloride, Et_3N, dichloromethane, rt; or carboxylic acid, EDCI, i-Pr_2NEt, DMF, rt.

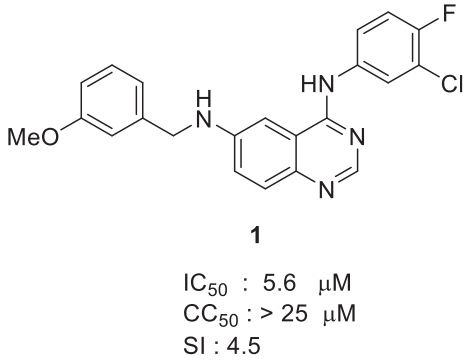
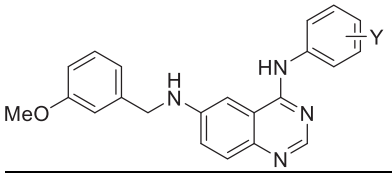


Fig. 1. Hit against MERS-CoV from a KCB library screen.

Molecules with higher selective index (SI) were considered as active molecules (Fig 1).

Our preliminary structure activity relationship (SAR) studies surrounding **1** were carried out by introducing halide groups on phenyl ring of 4-anilino group (Table 1). 4-Bromo substituent (**6**) maintained the potency. 4-Fluoro (**7**), 3-chloro (**8**), 4-cyano (**9**), and 3-acetyl (**10**) reduced potency compared to **1**. Interestingly, 4-trifluoromethyl (**11**) can slightly improve the potency. In the next phase optimization, we observed the effects of the substituents of 6 position of quinazoline ring, having fixed with 3-chloro-4-fluoro and 4-trifluoromethyl substituents on phenyl ring at 4 position (Table 2).

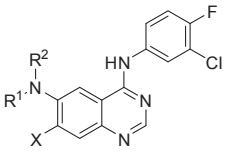
Table 1
Antiviral effect and toxicity of 4-anilino groups of hit compound **1.**

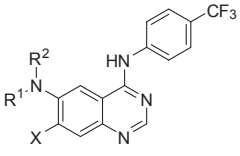


Compound	Y	IC_{50}(μM)[a]	CC_{50}(μM)[b]	SI[c]
1	3-Cl,4-F	5.6	> 25	4.5
6	4-Br	7.4	> 25	3.4
7	4-F	22.7	> 25	1.1
8	3-Cl	> 25	23.0	1.0
9	4-CN	> 25	> 25	1.0
10	3-$COCH_3$	> 25	> 25	1.0
11	4-CF_3	1.8	> 25	1.0

[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.

Table 2
Structure-Activity Relation of Derivatives of **1** and **11.**


12-31


32-36

Compound	X	R_1	R_2	IC_{50}(μM)[a]	CC_{50}(μM)[b]	SI[c]
1	H	3-CH_3O-$PhCH_2$	H	5.6	> 25	4.5
12	H	2-CH_3O-$PhCH_2$	H	3.8	> 25	7
13	H	2-OH-$PhCH_2$	H	3.6	> 25	7
14	H	3,4-F_2-$PhCH_2$	H	4.6	23.0	5
15	H	4-F-$PhCH_2$	H	4.6	14.4	3
16	H	2-NO_2-$PhCH_2$	H	3.3	> 25	8
17	H	3-NO_2-$PhCH_2$	H	6.1	> 25	4
18	H	4-NO_2-$PhCH_2$	H	2.7	> 25	8
19	H	2-CN-$PhCH_2$	H	> 25	> 25	1
20	H	3-CN-$PhCH_2$	H	0.157 ± 0.002	3.59 ± 1.1	25
21	H	4-CN-$PhCH_2$	H	4.8	> 25	5
22	H	3-H_2NCO-$PhCH_2$	H	> 25	> 25	1
23	H	3-CF_3-$PhCH_2$	H	2.3	9.2	4
24	H	*n*-butyl	*n*-butyl	> 25	> 25	1
25	H	cyclohexyl	H	15	> 25	1
26	H	CH_3CO	H	> 25	> 25	1
27	H	3-CN-PhCO	H	> 25	> 25	1
28	H	3-CN-*trans*-cynnamoyl	H	> 25	> 25	1
29	OMe	3-CN-$PhCH_2$	H	5.8	13.2	2
30	OH	3-CN-$PhCH_2$	H	> 25	> 25	1
11	H	3-CH_3O-$PhCH_2$	H	1.8	> 25	1
31	H	2-CN-$PhCH_2$	H	> 25	> 25	1
32	H	3-CN-$PhCH_2$	H	3.6	> 25	7
33	H	4-CN-$PhCH_2$	H	4.1	> 25	6
34	H	3-NO_2-$PhCH_2$	H	3.7	> 25	7
35	H	4-NO_2-$PhCH_2$	H	3.3	> 25	8

[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.

First, substituent effects at 6 position of **1** having 3-chloro-4-fluoro aniline at 4 position were evaluated. Changing 3-methoxybenzyl amine to 2-methoxybenzyl amine group **12** showed similar activity

Table 3
Result of hERG, Microsomal stability, cytotoxicity of **20.**

Compound	hERG (μM)	MS[a]		Cytotoxicity (μM)[b]				
		m[a]	h[a]	Vero[c]	HFL-1	L929	NIH 3T3	CHO-K1
20	> 50	60.7	56.4	> 100	> 100	> 100	> 100	> 100

[a] % original compound remained after 30 min incubation.
[b] Cell information. Vero: African green monkey kidney cell line, HFL-1: human embryonic lung cell line, L929: NCTC clone 929, mouse fibroblast cell line, NIH 3T3: mouse embryonic fibroblast cell line, CHO-K1: Chinese hamster ovary cell line.
[c] Vero cell was not infected with MERS-CoV.

Table 4
In vivo pharmacokinetic profiles in rat of **20.**

Parameters*	I.V., 2 mg/kg	P.O., 5 mg/kg
Tmax (h)	NA**	2.0
Cmax (μg/h)	NA	0.2
$T_{1/2}$ (h)	5.9	5.5
AUC (μg·h/mL)	1.11	0.57
CL (L/h/kg)	1.73	NA
V_{ss} (L/Kg)	6.3	NA
F_t (%)	NA	21

* All results are the mean of experiments using three rats.
** NA: not applicable.

(IC_{50} = 3.8 μM). Introducing 2-hydroxybenzyl amine at 6 position of quinazoline ring **13** was tolerated (IC_{50} = 3.6 μM). 3,4-Difluoro (**14**) and 4-fluoro (**15**) compounds gave similar activities (IC_{50} = 4.6 μM). We next explored the effects of electron-withdrawing groups. Compounds with nitro groups (**16** to **18**) showed similar inhibitory effects (IC_{50} = 3.3, 6.1 and 2.7 μM, respectively). Compound with 2-cyanobenzyl amine at 6 position (**19**) showed no inhibitory effect, whereas 4-cyanoebnzyl amine (**21**) functionality was tolerated (IC_{50} = 4.8 μM). Gratifyingly, 3-cyanobenzyl amine analogue **20** resulted in significant higher activity (IC_{50} = 0.157 μM). We tested other electron-withdrawing groups at meta position of benzyl amine substituent. 3-Amidobenzyl amine analogue **22** was detrimental for activity but trifluoromethyl substituent at 3 position **23** retained the inhibitory effect (IC_{50} = 2.3 μM). Then benzyl amine substituents at 6 position were changed to aliphatic amines and amides (**24**–**28**). Aliphatic amine substituents, such as di-*n*-butyl (**24**) and cyclohexyl (**25**), showed little inhibitory effects and amide groups (**26**–**28**) were detrimental. We introduced several substituents at 7 position of **20**. Methoxy at 7 position gave a little decreased activity (**29**, IC_{50} = 5.8 μM) but hydroxy (**30**) showed no inhibitory effects.

The antiviral effects of derivatives at 6 position of **11** with 4-trifluoromethyl aniline group at 4 position were also examined. Most of substituents were well tolerated. 3-Cyanobenzyl (**32**), 4-cyanobenzyl (**33**), 3-nitrobenzyl (**34**) and 4-nitrobenzyl (**35**) showed similar activities (IC_{50} = 3.3 to 4.1 μM) to **11**. However, 2-cyanobenzyl (**31**) substituent exhibited decreased potency.

Considering activity and cytotoxicity, **20** was thought to be the best compounds for anti-MERS drug and evaluated for its hERG, metabolic stability, cytotoxicity (Table 3). Our optimized lead compound **20** was found to show no hERG binding and have good microsomal stability in both mouse and human. Compound **20** showed no cytotoxicity toward Vero (not infected with MERS-CoV) as well as toward HFL-1, L929 NIH 3 T3 and CHO-K1 cell lines, which shows that potential interaction between compound **20** and viruses might affect the cell viability. The pharmacokinetic parameters of **20** were evaluated in rats by intravenous (i.v.) and oral (p.o.) routes at 2 and 5 mg/kg, respectively (Table 4). **20** showed reasonable oral bioavailability (21%).

In conclusion, we have synthesized a series of 4-anilino-6-aminoquinazoline derivatives and most of the compounds showed anti-MERS-CoV activity in Vero cell. The best compound among the derivatives was **20**, which had the form of quinazoline with 3-Chloro-4-fluoroaniline at 4 position and 3-cyanobenzyl amine at 6 position. Compound **20** showed high anti-MERS-CoV activity (IC_{50} = 0.157 μM, SI = 25) with no cytotoxicity and moderate *in vivo* PK property. Further studies on additional SAR and pharmacological investigation of these compounds are currently underway.

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.

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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.2020.127472.

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