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COMMUNICATION

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Synthesis and biological evaluation of 2-benzylaminoquinazolin-4(3*H*)-one derivatives as a potential treatment for SARS-CoV-2

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Abstract

Despite the continuing global crisis caused by coronavirus disease 2019 (COVID-19), there is still no effective treatment. Therefore, we designed and synthesized a novel series of 2-benzylaminoquinazolin-4(3*H*)-one derivatives and demonstrated that they are effective against SARS-CoV-2. Among the synthesized derivatives, 7-chloro-2-(((4-chlorophenyl)(phenyl)methyl)amino)quinazolin-4(3*H*)-one (Compound **39**) showed highest anti-SARS-CoV-2 activity, with a half-maximal inhibitory concentration value greater than that of remdesivir (IC_{50} = 4.2 μM vs. 7.6 μM, respectively), which gained urgent approval from the U.S. Food and Drug Administration. In addition, Compound **39** showed good results in various assays measuring metabolic stability, human ether a-go-go, Cytochromes P450 (CYPs) inhibition, and plasma protein binding (PPB), and showed better solubility and pharmacokinetics than our previous work.

KEYWORDS
2-benzylaminoquinazolin-4(3*H*)-ones, antiviral activity, coronaviruses, COVID-19

Coronavirus disease 2019 (COVID-19) is a highly contagious and sometimes fatal disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] It was first detected in Wuhan, China, in December 2019 and has since spread worldwide; to date, 180 million confirmed cases and 4 million deaths have been reported as of July 2021.[2] The disease also caused a global economic recession and created a new social phenomenon known as social distancing. Despite the severe situation, no curative therapeutic agents have been developed. During the early days of the pandemic, remdesivir, hydroxychloroquine, lopinavir, and interferon beta-1a were considered to be promising therapeutic agents[3]; however, clinical results showed that their therapeutic effects were insignificant and the drugs had unintended side effects.[4]

Our research unit was studying coronaviruses long before the outbreak of COVID-19; we found that 2-phenylchroman-4-one derivatives,[5] 3-acyl-2-phenylamino-1,4-dihydroquinolin-4-(1*H*)-one derivatives,[6] and 4-anilino-6-amino-quinazoline derivatives[7] are effective MERS-CoV inhibitors. After the COVID-19 outbreak, we also reported that cyclic sulfonamide derivatives[8] and 2-aminoquinazoline-4(3*H*)-ones derivatives[9] are novel SARS-CoV-2 inhibitors.

The most recently reported 2-aminoquinazoline-4(3*H*)-ones derivatives[9] have the advantage of being effective against both MERS-CoV and SARS-CoV-2; with respect to biological activity and cytotoxicity, we found that Compound **1** was the best (Figure 1). However, as we mentioned in our previous paper, our lead Compound **1** had problems regarding solubility and pharmacokinetics; this was due to

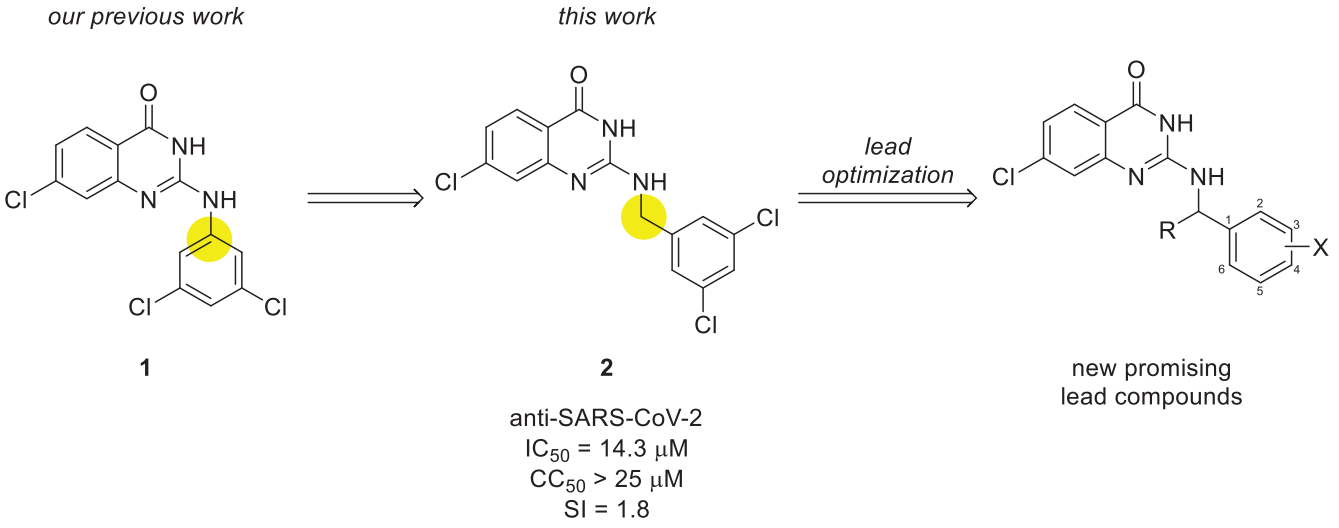
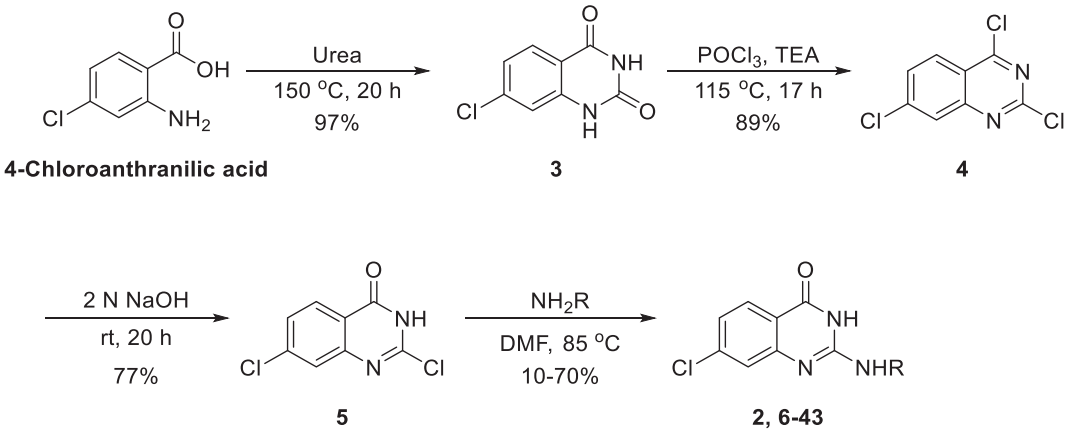


FIGURE 1 Design of the novel anti-coronavirus scaffolds



SCHEME 1 Synthesis of 2-benzylaminoquinazolin-4(3*H*)-ones derivatives

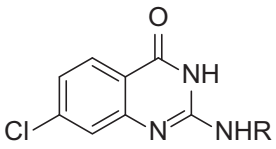
its planar structure. In addition, recent studies warned that aniline-based drugs may cause side effects such as hepatotoxicity, blood dyscrasias, and cutaneous adverse drug reaction (ADR).[10] Indeed, we can confirm that aniline-based drugs such as amodiaquine, bromfenace, flutamide, and lumiracoxib have been withdrawn.[10] Although our lead Compound **1** showed no cytotoxicity against Vero cells, it has potential toxicity due to its aniline-based structure.

Therefore, we synthesized Compound **2** by substituting 3,5-dichlorobenzyl amine at position 2 of the quinazolinone structure and then evaluated its biological activity. However, the anti-SARS-CoV-2 activity was weaker (IC_{50} = 14.3 μM) than that of Compound **1**; therefore, we synthesized derivatives of Compound **2** to improve the activity by substituting various types of benzyl amine including α-substituted benzyl amines (Figure 1).

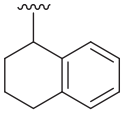
The 2-benzylaminoquinazoline derivatives were synthesized as reported previously (Appendix S1).[9,11] Commercially available 4-chloroanthranilic acid was used as the starting material. First, 4-chloroanthranilic acid was added to urea and heated to 150 °C to obtain 7-chloroquinazoline-2,4(1*H*,3*H*)-dione (Compound **3**). The next step was chlorination of Compound **3**. To achieve this, $POCl_3$ and triethylamine were added to Compound **3** and refluxed for 17 h to obtain 2,4,7-trichloroquinazoline (Compound **4**). Then, oxidation of the 4-position of the chloride was performed by dissolving Compound **4** in 2 N NaOH, followed by stirring of the suspension at room temperature (r.t) for 20 h to produce 2,7-dichloroquinazolin-4(3*H*)-one (Compound **5**). 2-Benzylaminoquinazoline derivatives were obtained by heating various types of benzyl amine with Compound **5** in N,N-Dimethylformamide at 85 °C; the chemical yield was 10%–70% (Scheme 1).

Biological evaluation of the efficacy of the synthesized derivatives against SARS-CoV-2 was conducted in BSL-3 facilities. Viral infectivity was determined in Vero cells and expression of the SARS-CoV-2 nucleocapsid protein was examined in an immunofluorescence assay (IFA).[12] The selectivity index (SI) was calculated by dividing the

TABLE 1 Lead optimization of 2-benzylamino groups



2, 6-43

Compounds	R	Efficacy against SARS-CoV-2		
		IC_{50}	CC_{50}	SI[a]
2	3,5—Cl_2—$PhCH_2$—	14.3	>25	>1.7
6	2—F—$PhCH_2$—	>25	>25	1
7	3—F—$PhCH_2$—	>25	>25	1
8	4—F—$PhCH_2$—	>25	>25	1
9	2,4—F_2—$PhCH_2$—	>25	>25	1
10	3,4-F_2—$PhCH_2$—	14.5	>25	>1.7
11	3,5—F_2—$PhCH_2$—	14.0	>25	>1.7
12	2—Cl—$PhCH_2$—	13.1	>25	>1.9
13	3—Cl—$PhCH_2$—	13.9	>25	>1.7
14	4—Cl—$PhCH_2$—	13.5	>25	>1.8
15	2,4—Cl_2—$PhCH_2$—	5.3	>25	>4.7
16	3,4—Cl_2—$PhCH_2$—	10.5	>25	>2.3
17	2—Br—$PhCH_2$—	11.6	>25	>2.1
18	3—Br—$PhCH_2$—	>25	>25	1
19	4—Br—$PhCH_2$—	14.6	>25	>1.7
20	2—NO_2—$PhCH_2$—	>25	>25	1
21	3—NO_2—$PhCH_2$—	14.6	>25	>1.7
22	4—NO_2—$PhCH_2$—	9.6	>25	>2.6
23	2—CN—$PhCH_2$—	>25	>25	1
24	3—CN—$PhCH_2$—	>25	>25	1
25	4—CN—$PhCH_2$—	>25	>25	1
26	2—CF_3—$PhCH_2$—	10.4	>25	>2.4
27	3—CF_3—$PhCH_2$—	14.0	>25	>1.7
28	4—CF_3—$PhCH_2$—	5.8	>25	>4.3
29	2—CH_3—$PhCH_2$—	14.1	>25	>1.7
30	3—CH_3—$PhCH_2$—	>25	>25	1
31	4—CH_3—$PhCH_2$—	>25	>25	1
32	2—NH_2—$PhCH_2$—	>25	>25	1
33	3—NH_2—$PhCH_2$—	>25	>25	1
34	4—NH_2—$PhCH_2$—	>25	>25	1
35	2—OCH_3—$PhCH_2$—	17.2	>25	>1.4
36	4—OCH_3—$PhCH_2$—	>25	>25	1
37	4—OH—$PhCH_2$—	17.3	>25	>1.4
38	3—OH—4—OMe—$PhCH_2$—	>25	>25	1
39	(4—Cl—Ph)PhCH—	4.2	14.3	3.4
40	(4—Cl—Ph)CH_3CH—	4.3	21.4	5.0
41	Ph_2CH—	4.5	13.1	2.9
42	(Ph)CH_3CH—	13.3	>25	>1.8
43		7.3	>25	>3.4

(Continues)

TABLE 1 (Continued)

Compounds	R	Efficacy against SARS-CoV-2		
		IC_{50}	CC_{50}	SI[a]
remdesivir		7.6	>25	>3.2
chloroquine		9.4	>25	>2.6
lopinavir		16.6	>25	>1.5

Note: The IC_{50} and CC_{50} were derived from the results of at least two dependent experiments in Vero cells.
[a]Selective index (SI) = CC_{50}/IC_{50}.

TABLE 2 Performance of **39** in the hERG, cytotoxicity, kinetic solubility, PPB, CYP inhibition, and microsomal stability assays

		Compound 39
hERG[a]		16.8
Cytotoxicity[b]	Vero	11.2
	HFL-1	7.8
	L929	7.4
	NIH 3 T3	7.4
	CHO-K1	38.6
Solubility (kinetics)[c]		228.4 ± 2.1 μM (90.5 ± 0.8 μg/ml)
PPB[d]	Rat	99.9
	Man	99.6
CYP inhibition[e]	1A2	<1
	2C9	26.7
	2C19	13.0
	2D6	<1
	3A4	<1
Microsomal stability[f]	Mouse	>99
	Rat	>99
	Dog	>99
	Monkey	>99
	Human	>99

[a]hERG patch clamp assay: inhibition at 10 μM (%).
[b]IC_{50} (μM) against human embryonic lung cells (HFL-1), L929 (NCTC clone 929; mouse fibroblast cells), NIH 3 T3 (mouse embryonic fibroblast cells), and CHO-K1 (Chinese hamster ovary cells).
[c]DMSO stock solution (5% in water).
[d]Plasma protein-binding rate (%) at 5 μM.
[e]% CYP inhibition in human liver microsomes (tested at 10 μM in a cocktail substrate assay).
[f]Liver microsomal phase I stability (%remaining after 30 min).

TABLE 3 Pharmacokinetic study of **39** in rats

Parameters	Compound 39	
	I.V., 5 mg/kg	P.O., 10 mg/kg
T_{max} (h)	NA	6
C_{max} (μg/ml)	NA	0.24
$T_{1/2}$ (h)	6.0	11.4
AUC_{last} (μg·h/ml)	9.0	3.1
AUC_{∞} (μg·h/ml)	10.0	4.4
CL (L/H/kg)	0.5	NA
V_{ss} (L/kg)	4.7	NA
F_t (%)	NA	17.2

Abbreviation: NA, not applicable.

cytotoxic concentration (CC_{50}) by the inhibitory concentration (IC_{50}). Therefore, compounds with a high SI are suitable therapeutic agents because they selectively inhibit the virus without causing cytotoxicity.

The structure–activity relationship (SAR) study focused on substituting various benzyl amines at position 2 of the quinazolinone in Compound **5**. The first step was optimization. To examine this, we substituted halogenated benzyl amines at position 2. We synthesized Compounds **6** (ortho-), **7** (meta-), and **8** (para-) by substituting a monofluorobenzyl amine moiety at these sites. However, these compounds had no activity (IC_{50} > 25 μM). Next, we synthesized Compounds **9**, **10**, and **11** by substituting difluorobenzyl amine; again, anti-SARS-CoV-2 activity was poor (IC_{50} = 14–25 μM).

Compounds **12** (ortho-), **13** (meta-), and **14** (para-) were derived by substituting monochlorobenzyl; again the antiviral effects were insignificant (IC_{50} = 13 μM). However, dichlorobenzylamine-substituted derivatives **15** and **16** (along with **2)** showed good anti-SARS-CoV-2 activity (IC_{50} = 5–10 μM). Among them, the 2,4-dichlorobenzylamine-substituted Compound **15** (IC_{50} = 5.3 μM) showed better activity than remdesivir (IC_{50} = 7.8 μM). Compounds **17** (ortho-), **18** (meta-), and **19** (para-), which were substituted with a monobromobenzyl amine, showed low efficacy against SARS-CoV-2 (IC_{50} = 11–25 μM) (Table 1).

The next step of lead compound optimization was involved attachment of a benzyl amine harboring electron withdrawing groups (EWGs; i.e., —NO_2, —CN, or —CF_3) at position 2 of quinazolinone. Among the three nitro-substituted compounds (**21–22**), only Compound **22**, which was substituted at the para position, showed moderate anti-SARS-CoV-2 activity (IC_{50} = 9.6 μM). None of the nitrile-substituted compounds (**23–25**) inhibited SARS-CoV-2 (IC_{50} > 25 μM). Trifluoromethyl-substituted derivatives (**26–28**) were more active than other EWG-substituted derivatives. Among them, **28** had better biological activity than remdesivir (IC_{50} = 5.8 μM vs. 7.6 μM, respectively).

For further optimization, benzyl amines substituted with electron donating groups (EDGs; i.e., —CH_3, —NH_2, —OMe, or —OH) were attached to quinazolinone. Our

previous research[9] suggested that the EDG did not increase anti-SARS-CoV-2 activity. Similarly, in this study, the improvement in activity was insignificant. Of the methyl-substituted derivatives (**29–31**), only **29** showed partial inhibition of SARS-CoV-2 (IC_{50} = 14.1 μM). None of the NH_2-substituted derivatives (**32–34**) had anti-SARS-CoV-2 activity (IC_{50} > 25 μM). Derivatives substituted with —OMe (**35–36**) or —OH (**37**), or both (**38**), appeared to have weak biological activity (IC_{50} = 14–25 μM).

Finally, to test the effects of the hydrophobic moieties in benzylamines, we synthesized **39**–**43** by inserting bulky functional groups such as diphenylmethylamines, α-methylbenzylamine, and 1,2,3,4-tetrahydro-1-naphthylamine. Compounds with diphenylmethylamines (**39**, **41**) showed good efficacy (IC_{50} = 4.2 and 4.5 μM, respectively) regardless of the presence of chlorine. In the case of compounds substituted with α-methylbenzylamines, the presence of chlorine in phenyl group showed good activity (**40**, IC_{50} = 4.3 μM), but unsubstituted phenyl decreased the activity (**42**, IC_{50} = 13.3 μM). Compound substituted with 1,2,3,4-tetrahydro-1-naphthylamine (**43**) showed moderate activity (IC_{50} = 7.3 μM).

Based on the above results, we selected **39** as the new lead compound because it has highest IC_{50} value among all derivatives. We then assessed its potential therapeutic value by conducting various assays. First, the hERG assay confirmed that it was safe with respect to cardiac toxicity; **39** showed 16.8% binding at 10 μM. Next, cytotoxicity assays based on Vero and CHO-K1 cells revealed that **39** had low toxicity, with a CC_{50} value > 10 μM. However, it showed mild toxicity against HFL-1, L929, and NIH 3 T3 cells (CC_{50} < 10 μM) (Table 2).

The kinetic solubility of Compound **39** in a DMSO stock solution (5% in water) was 228.4 ± 2.1 μM (90.5 ± 0.8 μg/ml). In the plasma protein-binding assay, **39** showed 99% binding affinity for both rat and human. The CYP inhibition assay confirmed that none of the CYP isoforms were inhibited significantly. In the microsomal stability test, Compound **39** was stable in all experimental models (mouse, rat, dog, monkey, and human) (Table 3).

The preliminary pharmacokinetic study of Compound **39** was conducted in rats. The compound was administered via the intravenous (i.v.) route at 5 mg/kg and via the per oral (p.o.) route at 10 mg/kg. A comparison of AUC_{last} values revealed that the i.v. route yielded a value of 9.0 μg h/ml and the p.o. route a value of 3.1 μg h/ml, indicating that the drug was better absorbed when given i.v. Compound **39** showed moderate oral bioavailability (17.2%).

CONCLUSION

We synthesized various 2-benzylaminoquinazoline derivatives, some of which showed better activity than the control drugs (remdesivir, chloroquine, and lopinavir). Among them, Compound **39**, which showed the best activity, was selected as our new lead compound. The compound performed satisfactorily in cytotoxicity, microsomal stability, hERG, CYP inhibition, and PPB assays. Also, **39** showed reasonable bioavailability (17.2%) in the rat pharmacokinetic study. Further studies to develop highly effective COVID-19 treatments are on-going.

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