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Discovery of cyclic sulfonamide derivatives as potent inhibitors of SARS-CoV-2

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ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) continues to spread worldwide, with 25 million confirmed cases and 800 thousand deaths. Effective treatments to target SARS-CoV-2 are urgently needed. In the present study, we have identified a class of cyclic sulfonamide derivatives as novel SARS-CoV-2 inhibitors. Compound **13c** of the synthesized compounds exhibited robust inhibitory activity (IC_{50} = 0.88 μM) against SARS-CoV-2 without cytotoxicity (CC_{50} > 25 μM), with a selectivity index (SI) of 30.7. In addition, compound **13c** exhibited high oral bioavailability (77%) and metabolic stability with good safety profiles in hERG and cytotoxicity studies. The present study identified that cyclic sulfonamide derivatives are a promising new template for the development of anti-SARS-CoV-2 agents.

In December 2019, the novel coronavirus was first reported in Wuhan Province, China.[1] The infection has since spread worldwide, with 25 million confirmed cases and 800 thousand deaths as of 31 August 2020.[2] The new virus, derived from zoonotic transmission, was named by the International Committee on Taxonomy of Viruses (ICTV) as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2).[3] It is a positive-sense single-stranded RNA virus (+ssRNA) that is contagious in humans and other mammals.[3,4] SARS-CoV-2 shares 82% of its genome with SARS-CoV.[5] Although many studies are ongoing, no effective vaccine or treatment for SARS-CoV-2 infection has yet been developed.[6] The U.S. Food and Drug Administration (FDA) approved emergency use of remdesivir, a nucleotide analogue prodrug, in patients hospitalized with severe disease.[7] However, this intravenous antiviral drug did not improve overall survival rates, but it did decrease recovery time in surviving patients.[6] More effective approaches to treatment are urgently needed.

We attempted to find biologically active compounds in the library[8] of the Korea Chemical Bank (KCB) using the Institut Pasteur Korea (IPK) high content screening (HCS) platform. Cyclic sulfonamide compound **1** (Fig. 1) was identified as a hit, and exhibited anti-SARS-CoV-2 activity (IC_{50} = 15.3 μM). Cyclic sulfonamide derivatives are known to have various pharmacological activities such as analgesic[9], anti-inflammatory[10], herbicidal[11], and antidiabetic[12] effects. Here, the present study reported the synthesis and biological effects of cyclic sulfonamide derivatives.

A series of cyclic sulfonamide derivatives were synthesized as shown in Scheme 1. Saccharin was treated with α-bromo ketone and triethylamine to yield the alkylated product **2**. A Gabriel–Colman rearrangement of **2** with sodium ethoxide afforded intermediate **3**, which was reacted with α-chloro amide and α-bromo ketone (or benzyl bromide) under basic conditions using sodium hydride to yield **4** and **5**, respectively. To synthesize a one-carbon homologation compound, **3** was treated with $ClCH_2CH_2CONH$-*p*-CF_3-Ph and sodium hydride. However, elimination of the alkyl chloride substrate yielded an undesired product, CH = CHCONH-*p*-CF_3-Ph. Alternatively, we designed to synthesize α,β-unsaturated amide **8**. Alkenoic acid ester **6** was prepared by reaction of compound **3** and ethyl propiolate with DABCO as a catalyst. Hydrolysis of **6** with lithium hydroxide afforded carboxylic acid **7**. Amide

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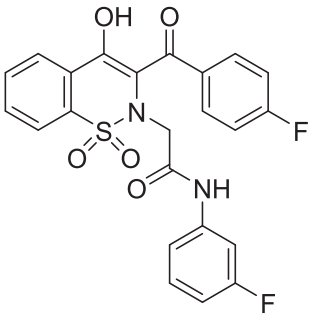


1

IC_{50} : 15.3 μM
CC_{50} : > 25 μM
SI : 1.6

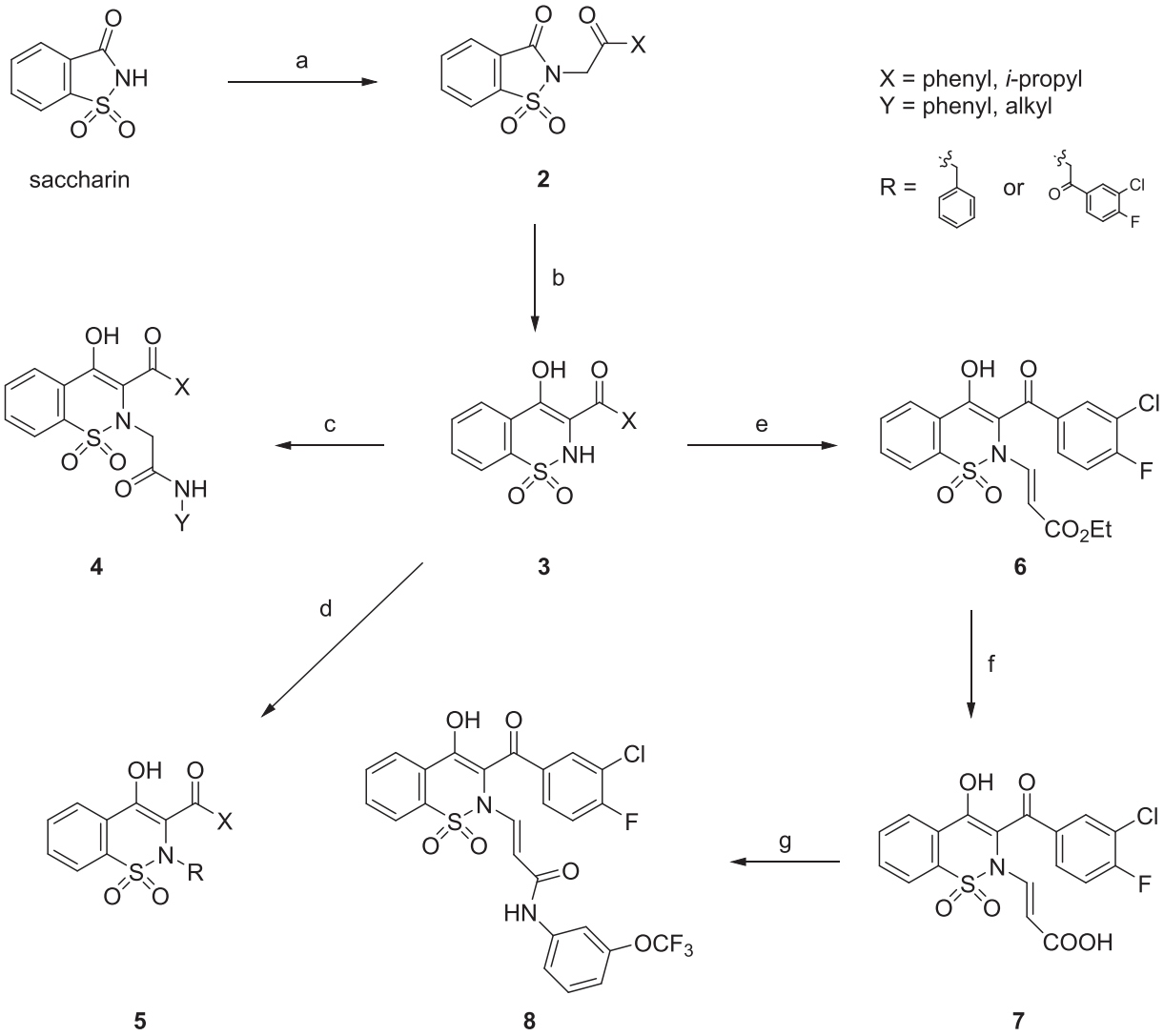
Fig. 1. Anti-SARS-CoV-2 compound **1** identified from the KCB library screen.

coupling of **7** with 3-(trifluoromethoxy)aniline, EDCI, and DMAP yielded amide **8**. To synthesize 7-fluorinated cyclic sulfonamide (Scheme 2), sulfonyl chloride **9** was used as a starting material. Amination of **9** with aqueous ammonium hydroxide yielded sulfonamide **10**. Oxidation of **10** with potassium permanganate afforded compound **11**. Cyclization of **11** with sulfuric acid yielded fluorinated saccharin **12**. Compound **13** was prepared as shown in Scheme 2. **13c** was treated with amine groups to yield *N*-substituted product **14**.

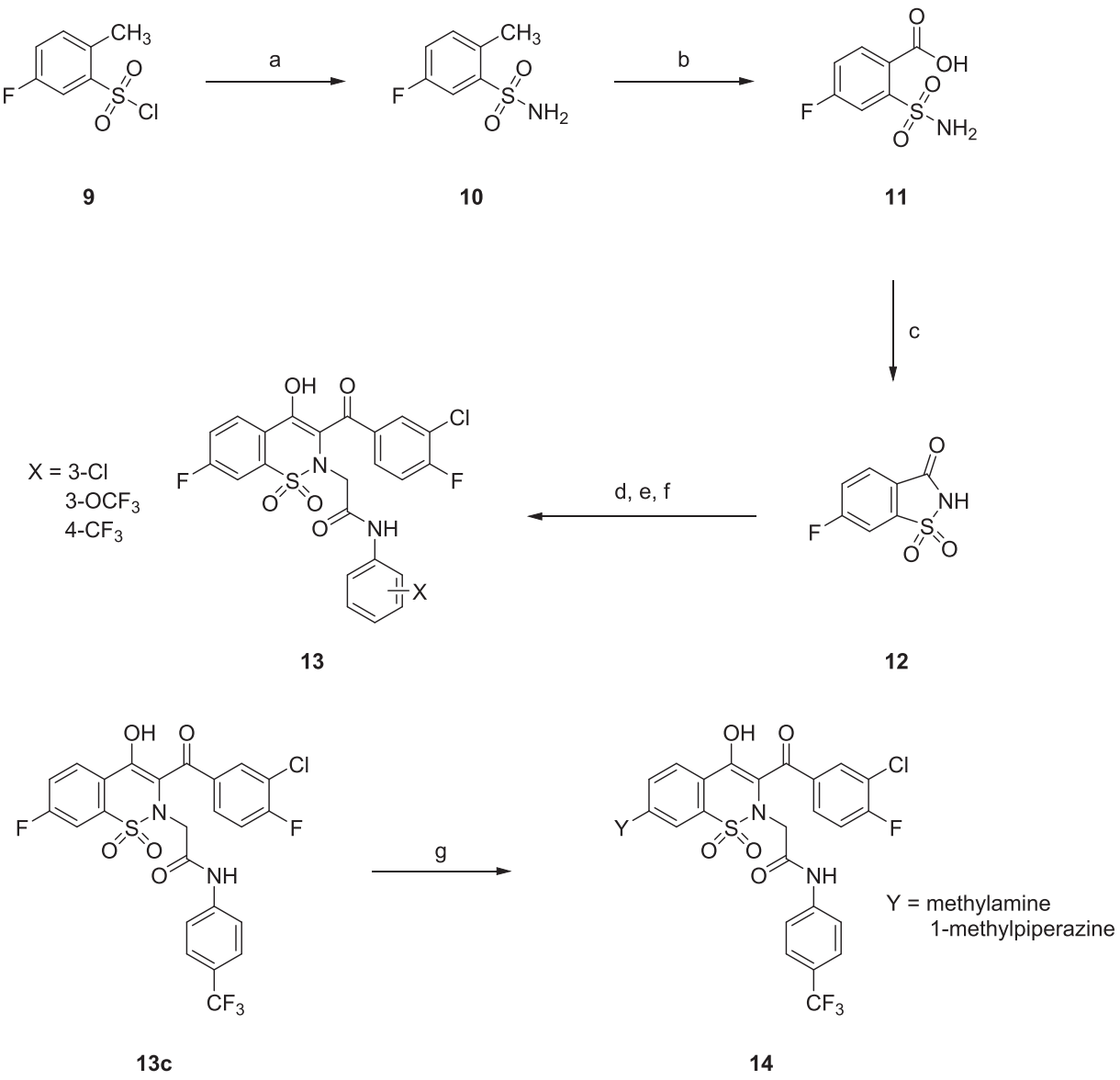
Biological activities of the synthesized cyclic sulfonamide derivatives were evaluated in Vero cells to test both anti-SARS-CoV-2 activity and cytotoxicity by cellular phenotypic screening method[13] as shown in Tables 1 and 2. Chloroquine and remdesivir were used as reference compounds.

We began structure activity relationship (SAR) studies of **1** with varying substituents of the phenyl group at the 2 position, having fixed with a 4-fluoro-substituted benzoyl group at the 3 position (Table 1). Unsubstituent (**4a**) and 2-chloro (**4b**) compounds showed no inhibitory effect. 3-Trifluoromethoxy (**4c**) and 4-trifluoromethyl (**4d**) at the 2 position improved anti-SARS-CoV-2 activities (IC_{50} = 8.90 and 5.30 μM, respectively). **4c** and **4d** exhibited better activity than compound **1** and similar activity to remdesivir and chloroquine (IC_{50} = 7.01 and 8.00 μM, respectively).

Further optimizations of the 2 position were conducted with an unsubstituted benzoyl group (**4e**) at the 3-position, as **4e** and **4c** had similar anti-SARS-CoV-2 effects (IC_{50} = 11.5 and 8.9 μM, respectively).



Scheme 1. Synthesis of cyclic sulfonamide derivatives. Reagents and conditions: (a) $BrCH_2COX$ (X = phenyl groups, *i*-propyl), Et_3N, DMF, rt, 9 h (b) 21% NaOEt, EtOH, 60 °C, 0.5 h (c) $ClCH_2CONHY$ (Y = phenyl, alkyl groups), NaH, DMF, rt, 3 h (d) $BrCH_2COPh$-3-Cl-4-F or $BrCH_2Ph$, NaH, DMF, rt, 3 h (e) ethyl propiolate, DABCO, DCM, 60 °C, 3.5 h (f) LiOH, THF/MeOH/H_2O, rt, 5 h (g) 3-(trifluoromethoxy)aniline, EDCI, DMAP, DCM, rt, 9 h.



Scheme 2. Synthesis of cyclic 7-substituted sulfonamide derivatives. Reagents and conditions: (a) aq. NH_4OH, 100 °C, 1 h (b) $KMnO_4$, 5% aq. NaOH, 120 °C, 5 h (c) sulfuric acid, rt, 1.5 h (d) $BrCH_2COPh$-3-Cl-4-F, Et_3N, DMF, rt, 9 h (e) 21% NaOEt, EtOH, 60 °C, 0.5 h (f) $ClCH_2CONHPhX$ (X = 3-Cl, 3-OCF_3, 4-CF_3), NaH, DMF, rt, 3 h (g) methylamine or 1-methylpiperazine, K_2CO_3, DMSO, 80 °C, 9 h.

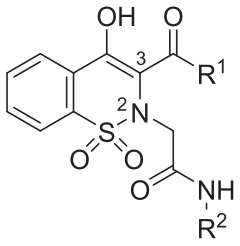
Aliphatic amide derivatives (**4f** and **4 g**) were detrimental for anti-SARS-CoV-2 activities. Benzyl (**5a**) and phenylacetyl groups (**5b** and **5c**) at the 2 position had no anti-SARS-CoV-2 activities.

Subsequently, substituents at the 3 position (**4 h**–**4o**) were optimized in the compound containing 3-CF_3O-phenyl acetamide (**4c**) at the 2 position. 3-Fluoro (**4 h**) and 3-chloro (**4j**) showed no significant difference in antiviral activity (IC_{50} = 10.10 and 11.90 μM, respectively). The activity of an electron donating group, 4-methoxy compound **4n,** also did not improve anti-SARS-CoV-2 activity (IC_{50} = 11.60 μM). Compound **4o**, substituted with an isopropyl, alkyl group instead of phenyl, had similar activity (IC_{50} = 10.80 μM). 3-Cyano (**4i**) and 4-cyano (**4m**) substituents decreased activity (IC_{50} = 14.30 μM), compared with **4c**. 3-Chloro-4-fluoro (**4k**) and 4-chloro (**4l**) exhibited marginally improved antiviral activities (IC_{50} = 9.20 and 8.50, respectively). Next, substituent effects at the 3 position with 4-CF_3-aryl at the 2 position were investigated (**4p**–**4w**). Compounds with 4-CF_3 at the 2 position were generally more active than compounds with 3-OCF_3 at the 2 position. 3-Fluoro (**4p**), 3-cyano (**4q**), 4-cyano (**4u**), 4-methoxy (**4v**), and isopropyl (**4w**) compounds, maintaining 4-CF_3 at the 2 position, also displayed moderate antiviral activities (IC_{50} = 7.00–10.70 μM). 3-Chloro (**4r**), 3-chloro-4-fluoro (**4s**), and 4-chloro (**4t**) had good antiviral activities (IC_{50} = 4.10, 2.50, and 4.00 μM, respectively). Compound **4s** was identified as a potent inhibitor of SARS-CoV-2.

We conducted further modifications to increase activity (Table 2). Carboxylic acid **7** exhibited no antiviral effect. Substitution of α,β-unsaturated amide **8** for acetamide slightly decreased antiviral activity (IC_{50} = 6.60 μM). Interestingly, 7-fluorinated cyclic sulfonamide (**13a-c**) improved antiviral activity (0.88–3.10 μM). Compound **13c** showed the most potent inhibitory activity against SARS-CoV-2 (IC_{50} = 0.88 μM) without cytotoxicity, having a selectivity index of 30.7. The 7-*N*-substituted products **14a** and **14b** had decreased antiviral activity (IC_{50} = 13.80 and 14.00 μM, respectively), compared with the 7-fluorinated compounds (**13a-c**).

Compound **13c**, found to be a potential anti-SARS-CoV-2 agent, was evaluated for its metabolic stability, human ether a-go-go (hERG) binding, cytotoxicity, and *in vivo* PK profile (Table 3). **13c** exhibited good microsomal stability in human and dog, low binding with hERG, and no cytotoxicity toward Vero, HFL-1, L929, NIH 3T3, and CHO-K1

Table 1
Anti-SARS-CoV-2 activity and cytotoxicity of cyclic sulfonamide derivatives.



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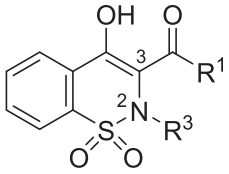

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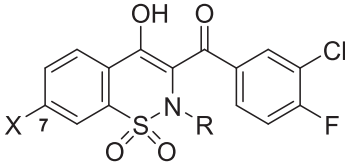
Entry	Cpd	R^1	R^2	R^3	IC_{50}^a (μM)	CC_{50}^b (μM)	SI
1	**1**	4-F-Ph	3-F-Ph	–	15.3	>25	1.6
2	**4a**	4-F-Ph	Ph	–	>25	>25	1.0
3	**4b**	4-F-Ph	2-Cl-Ph	–	>25	>25	1.0
4	**4c**	4-F-Ph	3-CF_3O-Ph	–	8.90	>25	2.7
5	**4d**	4-F-Ph	4-CF_3-Ph	–	5.30	>25	4.7
6	**4e**	Ph	3-CF_3O-Ph	–	11.50	>25	2.1
7	**4f**	Ph	ethyl	–	>25	>25	1.0
8	**4g**	Ph	cyclohexyl	–	>25	>25	1.0
9	**4h**	3-F-Ph	3-CF_3O-Ph	–	10.10	>25	2.3
10	**4i**	3-CN-Ph	3-CF_3O-Ph	–	14.30	>25	1.6
11	**4j**	3-Cl-Ph	3-CF_3O-Ph	–	11.90	>25	2.1
12	**4k**	3-Cl-4-F-Ph	3-CF_3O-Ph	–	9.20	>25	2.8
13	**4l**	4-Cl-Ph	3-CF_3O-Ph	–	8.50	>25	2.9
14	**4m**	4-CN-Ph	3-CF_3O-Ph	–	14.30	>25	1.6
15	**4n**	4-OMe-Ph	3-CF_3O-Ph	–	11.60	>25	2.0
16	**4o**	*i*-propyl	3-CF_3O-Ph	–	10.80	>25	1.9
17	**4p**	3-F-Ph	4-CF_3-Ph	–	7.00	>25	3.2
18	**4q**	3-CN-Ph	4-CF_3-Ph	–	10.70	>25	1.5
19	**4r**	3-Cl-Ph	4-CF_3-Ph	–	4.10	>25	5.8
20	**4s**	3-Cl-4-F-Ph	4-CF_3-Ph	–	2.50	>25	11.1
21	**4t**	4-Cl-Ph	4-CF_3-Ph	–	4.00	>25	6.0
22	**4u**	4-CN-Ph	4-CF_3-Ph	–	9.30	>25	1.4
23	**4v**	4-OMe-Ph	4-CF_3-Ph	–	8.60	>25	2.5
24	**4w**	*i*-propyl	4-CF_3-Ph	–	7.30	>25	3.0
25	**5a**	Ph	–	$PhCH_2$	>25	>25	1.0
26	**5b**	3-CN-Ph	–	3-Cl-4-F-$PhCOCH_2$	>25	>25	1.0
27	**5c**	4-CN-Ph	–	3-Cl-4-F-$PhCOCH_2$	>25	>25	1.0
28	chloroquine				8.00	>25	3.1
29	remdesivir				7.01	>25	3.6

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

Table 2
Anti-SARS-CoV-2 activity and cytotoxicity of further modified cyclic sulfonamide derivatives.



Entry	Cpd	X	R	IC_{50}^a (μM)	CC_{50}^b (μM)	SI
1	**4s**	H	–CH_2CONH-4-CF_3-Ph	2.50	>25	11.1
2	**7**	H	–CH═CHCOOH	>25	>25	1.0
3	**8**	H	–CH═CHCONH-3-CF_3O-Ph	6.60	>25	3.6
4	**13a**	F	–CH_2CONH-3-Cl-Ph	2.20	>25	12.1
5	**13b**	F	–CH_2CONH-3-CF_3O-Ph	3.10	>25	8.9
6	**13c**	F	–CH_2CONH-4-CF_3-Ph	0.88	>25	30.7
7	**14a**	NHMe	–CH_2CONH-4-CF_3-Ph	13.80	>25	1.3
8	**14b**	1-methyl-piperazine	–CH_2CONH-4-CF_3-Ph	14.00	>25	1.6

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

Table 3
hERG, microsomal stability (MS), cytotoxicity, and PK profile of **13c**.

Compound	hERG inhibition %at 10 μM	MS[a]	Cytotoxicity (μM)[b]	PK[c] in rats
13c	<1%	93% (human) 61% (monkey)	Vero: 42.1 HFL-1: 44.2 L929: 31.4 NIH 3 T3: 68.0 CHO-K1: 10.6	C_{max} = 14.33 μg/mL $T_{1/2}$ = 18.5 h CL = 0.04 l/h/kg F = 77%

[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 3 T3: mouse embryonic fibroblast cell line, CHO-K1: Chinese hamster ovary cell line.
[c] Rats (n = 3) were dosed at IV 5 mg/kg and PO 10 mg/kg.

cell lines. Moreover, an *in vivo* PK study of **13c** identified good bioavailability of 77% in rats by intravenous (IV) and oral (PO) routes at 5 and 10 mg/kg, respectively.

In conclusion, we identified a novel class of cyclic sulfonamide derivatives as SARS-CoV-2 inhibitors using SAR optimization, viral inhibitory assays, cytotoxicity assays, and PK studies. Compound **13c** is a potent SARS-CoV-2 inhibitor (IC_{50} = 0.88 μM), has no cytotoxicity, and has a selectivity index of 30.7. Further evaluation of compound **13c** was conducted to determine the PK profile of cyclic sulfonamide. Compound **13c** showed good oral bioavailability of 77%, metabolic stability, low binding with hERG, and no cytotoxicity. This study identified that cyclic sulfonamide derivatives are a promising new template for the development of SARS-CoV-2 inhibitors.

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.

Acknowledgements

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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.2020.127667.

References

1 Zhou P, Yang X-L, Wang X-G, et al. *Nature*. 2020;579:270.
2 World Health Organization (WHO). Weekly Epidemiological Update, 31 August 2020; https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200831-weekly-epi-update-3.pdf?sfvrsn=d7032a2a_4 [online].
3 Yang P, Wang X. *Cell Mol Immunol*. 2020;17:555.
4 Akhtar MJ. *Bioorg Chem*. 2020;101, 104027.
5 Chan JF, Kok K-H, Zhu Z, et al. *Emerg Microbes Infect*. 2020;9:221.
6 Baden LR, Rubin EJ. *N Engl J Med*. 2020;382:1851.
7 U.S. Food & Drug Administration, Coronavirus (COVID-19) Update: FDA Issues Emergency Use Authorization for Potential COVID-19 Treatment, FDA News Release, May 1, 2020; https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment [online].
8 Lee JY, Shin YS, Lee J, et al. *Bioorg Med Chem Lett*. 2020;30, 127472.
9 Ukrainets IV, Petrushova LA, Sidorenko LV, et al. *Sci Pharm*. 2016;84:497.
10 Gannarapu MR, Vasamsetti SB, Punna N, et al. *Eur J Med Chem*. 2014;75:143.
11 Lei K, Hua X-W, Tao Y-Y, et al. *Bioorg Med Chem*. 2016;24:92.
12 Kim SH, Ramu R, Kwon SW, et al. *Bioorg Med Chem Lett*. 2010;20:1065.
13 Jeon S, Ko M, Lee J, et al. *Antimicrob Agents Chemother*. 2020;64, e00819.