Journal Pre-proofs

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Discovery of Cyclic Sulfonamide derivatives as Potent Inhibitors of SARS-CoV-2

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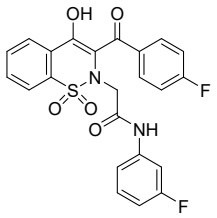
Abstract: Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) continues to spread worldwide, with 25 million confirmed cases and 800 thousand deaths. Effective treatments to target SARS-CoV-2 are urgently needed. In the present study, we have identified a class of cyclic sulfonamide derivatives 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.



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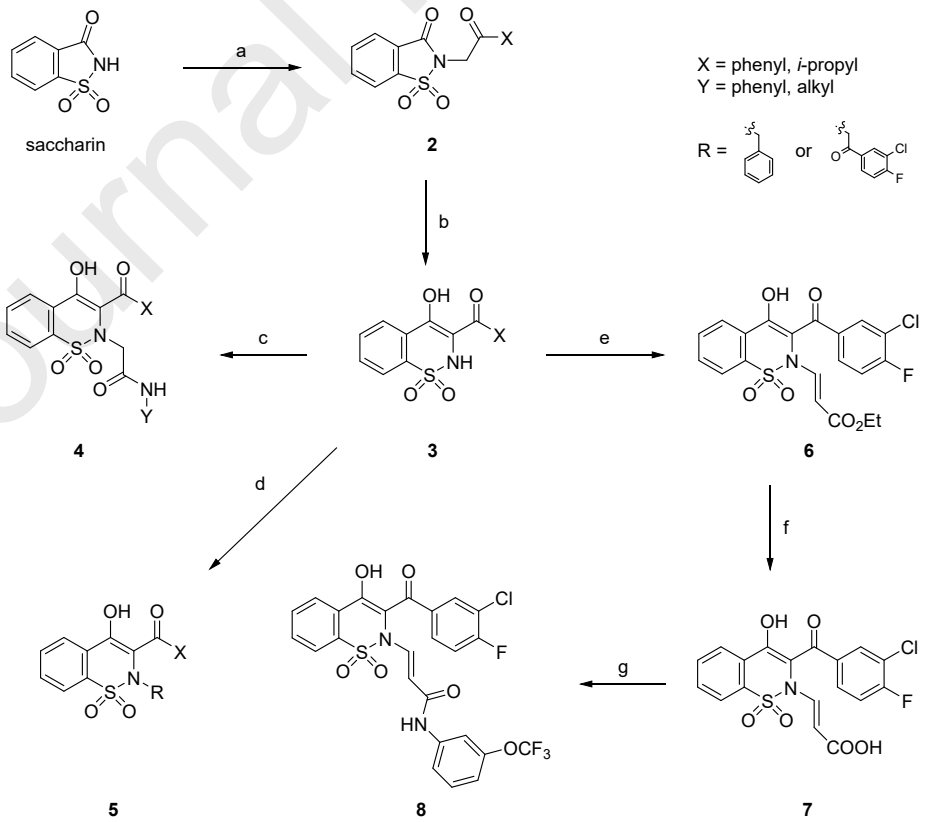
IC_{50} : 15.3 μM
CC_{50} : > 25 μM
SI : 1.6

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

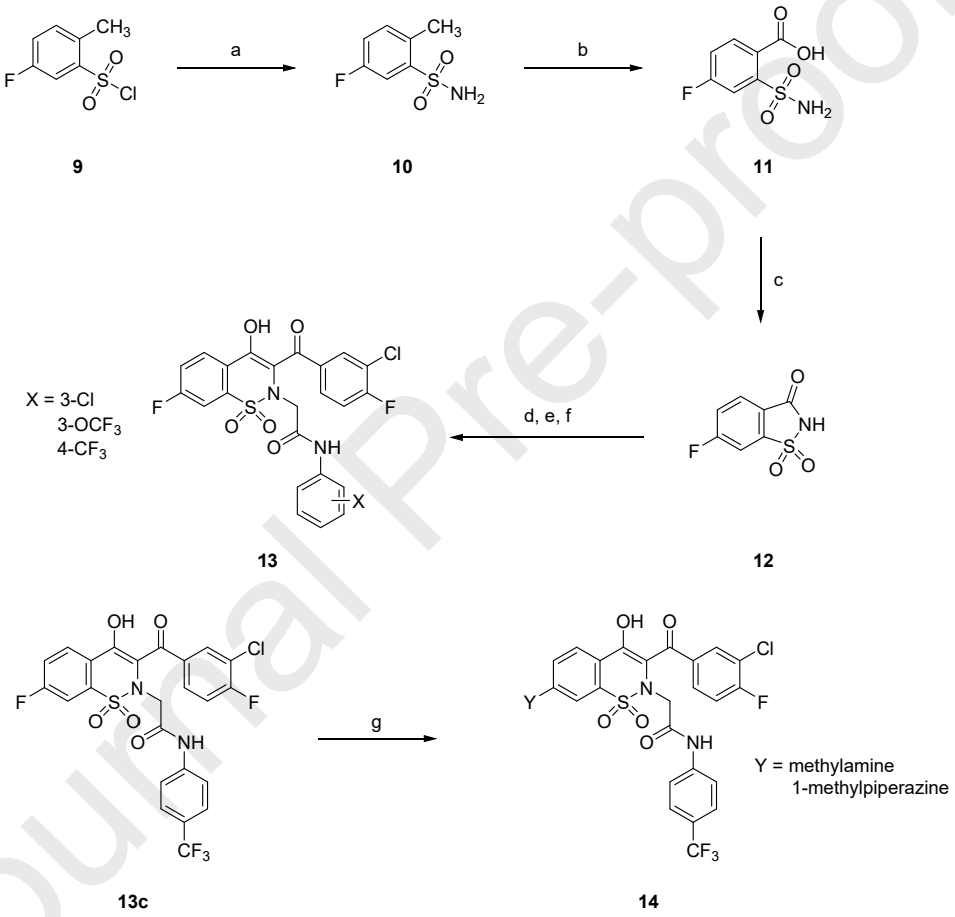
A series of cyclic sulfonamide derivatives were synthesized as shown in Scheme 1. Saccharin was treated with α-bromo ketone and triethylamine to yield the alkylated product **2**. A Gabriel–Colman rearrangement of **2** with sodium ethoxide afforded intermediate **3**, which was reacted

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 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 Table 1 and 2. Chloroquine and remdesivir were used as reference compounds.



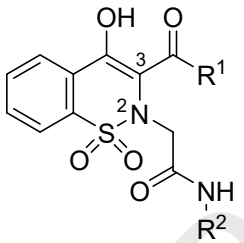
Scheme 1. Synthesis of cyclic sulfonamide derivatives. Reagents and conditions: (a) $BrCH_2COX$ (X = phenyl groups, *i*-propyl), Et_3N, DMF, rt, 9 h (b) 21% NaOEt, EtOH, 60 °C, 0.5 h (c) $ClCH_2CONHY$ (Y = phenyl, alkyl groups), NaH, DMF, rt, 3 h (d) $BrCH_2COPh$-3-Cl-4-F or $BrCH_2Ph$, NaH, DMF, rt, 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.

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).

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



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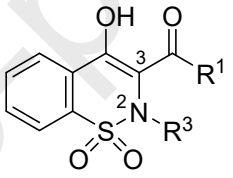

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Entry	Cpd	R^1	R^2	R^3	IC_{50}[a] (μM)	CC_{50}[b] (μM)	SI
1	**1**	4-F-Ph	3-F-Ph	-	15.3	>25	1.6
2	**4a**	4-F-Ph	Ph	-	>25	>25	1.0
3	**4b**	4-F-Ph	2-Cl-Ph	-	>25	>25	1.0
4	**4c**	4-F-Ph	3-CF_3O-Ph	-	8.90	>25	2.7
5	**4d**	4-F-Ph	4-CF_3-Ph	-	5.30	>25	4.7
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

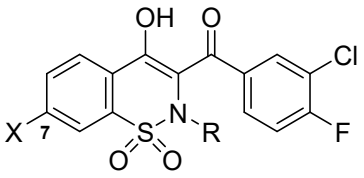
[a,b] IC_{50} and CC_{50} were derived from the results of at least two independent experiments in Vero cells.

[c] SI (selectivity index) = CC_{50}/IC_{50} for inhibiting SARS-CoV-2 infection.

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). Aliphatic amide derivatives (**4f** and **4g**) 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 (**4h–4o**) were optimized in the compound containing 3-CF_3O-phenyl acetamide (**4c**) at the 2 position. 3-Fluoro (**4h**) 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.

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.

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 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.

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 3T3: 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 3T3: 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.

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.

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)

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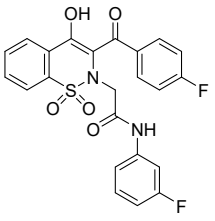

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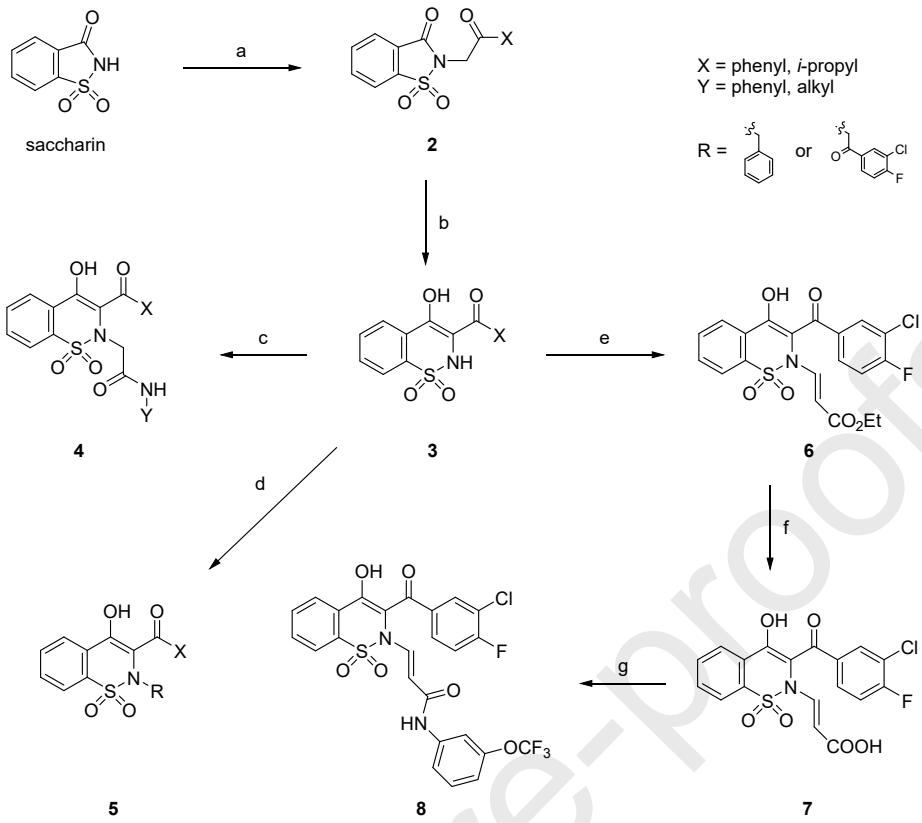
Figure 1. Anti-SARS-CoV-2 compound **1** identified from the KCB library screen.



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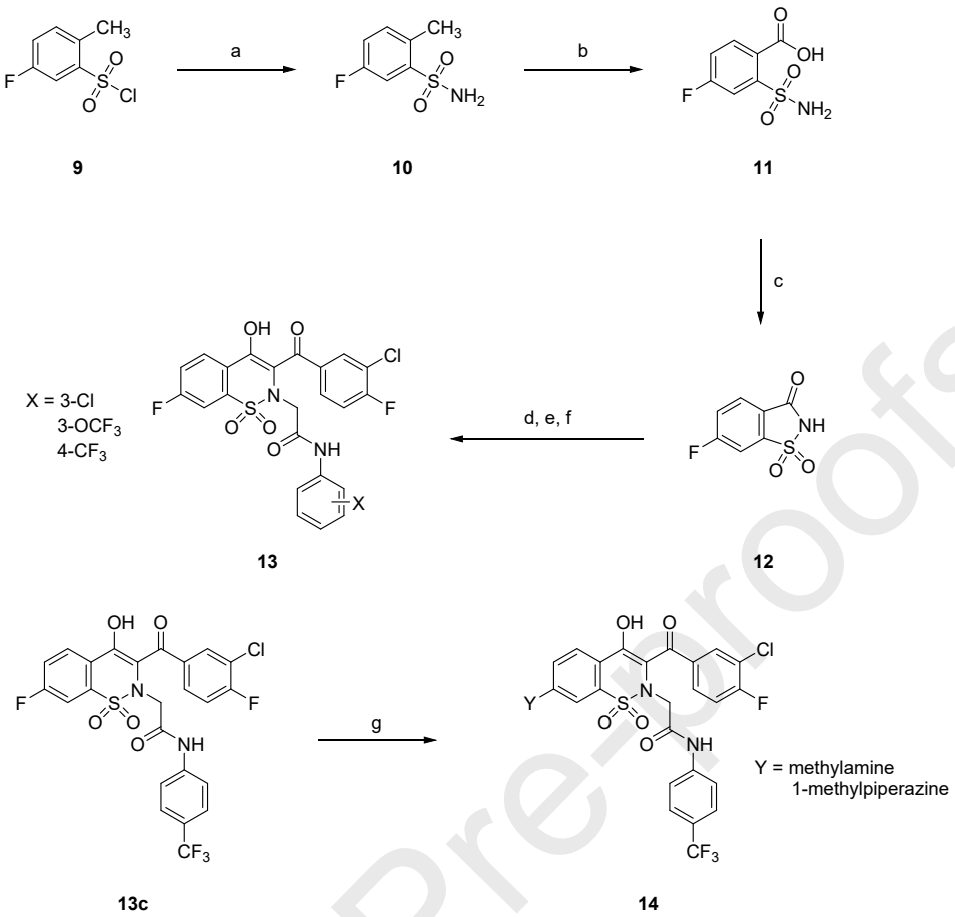
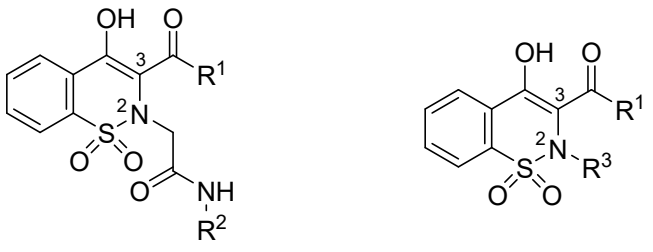


Scheme 2. Synthesis of cyclic 7-substituted sulfonamide derivatives. Reagents and conditions: (a) aq. NH_4OH, 100 °C, 1 h (b) $KMnO_4$, 5% aq. NaOH, 120 °C, 5 h (c) sulfuric acid, rt, 1.5 h (d) $BrCH_2COPh$-3-Cl-4-F, Et_3N, DMF, rt, 9 h (e) 21% NaOEt, EtOH, 60 °C, 0.5 h (f) $ClCH_2CONHPhX$ (X = 3-Cl,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

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



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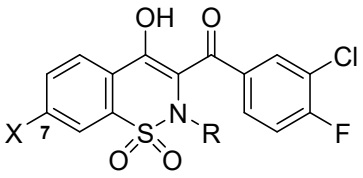
1	**1**	4-F-Ph	3-F-Ph	-	15.3	>25	1.6
2	**4a**	4-F-Ph	Ph	-	>25	>25	1.0
3	**4b**	4-F-Ph	2-Cl-Ph	-	>25	>25	1.0
4	**4c**	4-F-Ph	3-CF_3O-Ph	-	8.90	>25	2.7
5	**4d**	4-F-Ph	4-CF_3-Ph	-	5.30	>25	4.7
6	**4e**	Ph	3-CF_3O-Ph	-	11.50	>25	2.1
7	**4f**	Ph	ethyl	-	>25	>25	1.0
8	**4g**	Ph	cyclohexyl	-	>25	>25	1.0
9	**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.

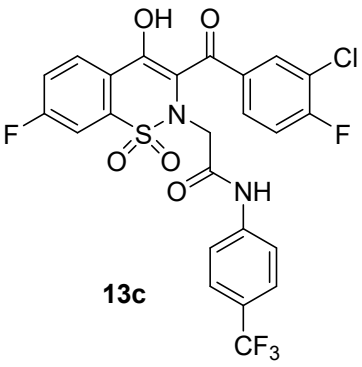
Table 3. hERG, microsomal stability (MS), cytotoxicity, and PK profile of **13c**

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 3T3: 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 3T3: 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.



13c

IC_{50} : 0.88 μM
CC_{50} : > 25 μM
SI : 30.7