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

Young Sup Shin, Jun Young Lee, Soojin Noh, Yoonna Kwak, Sangeun Jeon, Sunoh Kwon, Young-hee Jin, Min Seong Jang, Seungtaek Kim, Jong Hwan Song, Hyoun Rae Kim, Chul Min Park

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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 (IC50 = 0.88 μM) against SARS-CoV-2 without cytotoxicity (CC50 > 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.8 More effective approaches to treatment are urgently needed.

We attempted to find biologically active compounds in the library 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 (IC50 = 15.3 μM). Cyclic sulfonamide derivatives are known to have various pharmacological activities such as analgesic9, anti-inflammatory10, herbicidal11, and antidiabetic12 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 CICH2CH2CONH-p-CF3-Ph and sodium hydride. However, elimination of the alkyl chloride substrate yielded an undesired product, CH = CHCONH-p-CF3-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\(^2\) 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).

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

Scheme 1. Synthesis of cyclic sulfonamide derivatives. Reagents and conditions: (a) BrCH\(_2\)COX (X = phenyl groups, i-propyl), Et\(_3\)N, DMF, rt, 9 h (b) 21% NaOEt, EtOH, 60 °C, 0.5 h (c) CICH\(_2\)CONHY (Y = phenyl, alkyl groups), NaH, DMF, rt, 3 h (d) BrCH\(_2\)COPh-3-Cl-4-F or BrCH\(_2\)Ph, NaH, DMF, rt, 3 h (e) ethyl propiolate, DABCO, DCM, 60 °C, 3.5 h (f) LiOH, THF/MeOH/H\(_2\)O, rt, 5 h (g) 3-(trifluoromethoxy)aniline, EDCI, DMAP, DCM, rt, 9 h.
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$_3$O-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-O CF$_3$ 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.

| Entry | Cpd | R<sup>1</sup> | R<sup>2</sup> | IC<sub>50</sub> (μM) | CC<sub>50</sub> (μM) | SI |
|-------|-----|------------|------------|-----------------|-----------------|----|
| 1     | 1   | 4-F-Ph     | 3-F-Ph     | 15.3            | >25             | 1.0|
| 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<sub>3</sub>O-Ph | 8.90 | >25 | 2.7|
| 5     | 4d  | 4-F-Ph     | 4-CF<sub>3</sub>-Ph | 5.30 | >25 | 4.7|
| 6     | 4e  | Ph         | 3-CF<sub>3</sub>O-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<sub>3</sub>O-Ph | 10.10 | >25 | 2.3|
| 10    | 4i  | 3-CN-Ph    | 3-CF<sub>3</sub>O-Ph | 14.30 | >25 | 1.6|
| 11    | 4j  | 3-Cl-Ph    | 3-CF<sub>3</sub>O-Ph | 11.90 | >25 | 2.1|
| 12    | 4k  | 3-Cl-4-F-Ph| 3-CF<sub>3</sub>O-Ph | 9.20 | >25 | 2.8|
| 13    | 4l  | 3-Cl-4-F-Ph| 3-CF<sub>3</sub>O-Ph | 8.50 | >25 | 2.9|
| 14    | 4m  | 4-CN-Ph    | 3-CF<sub>3</sub>O-Ph | 14.30 | >25 | 1.6|
| 15    | 4n  | 4-CN-Ph    | 3-CF<sub>3</sub>O-Ph | 11.60 | >25 | 2.0|
| 16    | 4o  | i-propyl   | 3-CF<sub>3</sub>O-Ph | 10.80 | >25 | 1.9|
| 17    | 4p  | 3-F-Ph     | 4-CF<sub>3</sub>-Ph | 7.00 | >25 | 3.2|
| 18    | 4q  | 3-CN-Ph    | 4-CF<sub>3</sub>-Ph | 10.70 | >25 | 1.5|
| 19    | 4r  | 3-CN-Ph    | 4-CF<sub>3</sub>-Ph | 4.10 | >25 | 5.8|
| 20    | 4s  | 4-CN-Ph    | 4-CF<sub>3</sub>-Ph | 2.50 | >25 | 11.1|
| 21    | 4t  | 4-CN-Ph    | 4-CF<sub>3</sub>-Ph | 4.00 | >25 | 6.0|
| 22    | 4u  | 4-CN-Ph    | 4-CF<sub>3</sub>-Ph | 9.30 | >25 | 1.4|
| 23    | 4v  | 4-CN-Ph    | 4-CF<sub>3</sub>-Ph | 8.60 | >25 | 2.5|
| 24    | 4w  | i-propyl   | 4-CF<sub>3</sub>-Ph | 7.30 | >25 | 3.0|
| 25    | 5a  | Ph         | PhCH<sub>2</sub> | >25             | >25             | 1.0|
| 26    | 5b  | 3-CN-Ph    | 3-Cl-4-F-PhCOCH<sub>2</sub> | >25 | >25 | 1.0|
| 27    | 5c  | 4-CN-Ph    | 3-Cl-4-F-PhCOCH<sub>2</sub> | >25 | >25 | 1.0|
| 28    |      | chloroquine |            | 8.00             | >25             | 3.1|
| 29    |      | remdesivir |            | 7.01             | >25             | 3.6|

<sup>a</sup> IC<sub>50</sub> and CC<sub>50</sub> were derived from the results of at least two independent experiments in Vero cells.
<sup>b</sup> SI (selectivity index) = CC<sub>50</sub>/IC<sub>50</sub> 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<sub>50</sub> (μM) | CC<sub>50</sub> (μM) | SI |
|-------|-----|---|---|-----------------|-----------------|----|
| 1     | 4a  | H | -CH<sub>2</sub>CONH-4-CF<sub>3</sub>-Ph | 2.50 | >25 | 11.1|
| 2     | 7   | H | -CH=CHOH | >25 | >25 | 1.0|
| 3     | 8   | H | -CH=CHONH-3-CF<sub>3</sub>O-Ph | 6.60 | >25 | 3.6|
| 4     | 13a | F | -CH<sub>2</sub>CONH-3-Cl-Ph | 2.20 | >25 | 12.1|
| 5     | 13b | F | -CH<sub>2</sub>CONH-3-CF<sub>3</sub>O-Ph | 3.10 | >25 | 8.9|
| 6     | 13c | F | -CH<sub>2</sub>CONH-4-CF<sub>3</sub>-Ph | 0.88 | >25 | 30.7|
| 7     | 14a | NHMe | -CH<sub>2</sub>CONH-4-CF<sub>3</sub>-Ph | 13.80 | >25 | 1.3|
| 8     | 14b | 1-methyl-piperazine | -CH<sub>2</sub>CONH-4-CF<sub>3</sub>-Ph | 14.00 | >25 | 1.6|

<sup>a</sup> IC<sub>50</sub> and CC<sub>50</sub> were derived from the results of at least two independent experiments in Vero cells.
<sup>b</sup> SI (selectivity index) = CC<sub>50</sub>/IC<sub>50</sub> for inhibiting SARS-CoV-2 infection.
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.

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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.

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