

# Discovery of Novel Spike Inhibitors against SARS-CoV-2 Infection

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**Supplementary Table S1.**

Ten NCI compounds were verified after virtual screening, each accompanied by its respective identification number and reference code within the article.

<b>Compounds</b>	<b>Code</b>
NSC103846	C1
NSC121365	C2
NSC121969	C3
NSC237531	C4
NSC614438	C5
NSC614440	C6
NSC648665	C7
NSC660825	C8
NSC660826	C9
NSC660827	C10

**Supplementary Table S2.** Pseudovirus-based inhibition assay of the ten purchased compounds (C1-C10). The RLU (%) values were calculated using the following equation:  $RLU (\%) = (A / A_0) \times 100\%$ , where A represents the RLUs of the experimental groups and A<sub>0</sub> represents the RLUs of the DMSO group. The data are presented as means  $\pm$  standard deviation (SD).

Compounds	RLU(% of control)
C1	193.0 $\pm$ 25.6
C2	26.3 $\pm$ 3.2
C3	96.3 $\pm$ 15.0
C4	92.3 $\pm$ 15.5
C5	109.0 $\pm$ 11.3
C6	57.3 $\pm$ 2.9
C7	96.3 $\pm$ 12.6
C8	22.0 $\pm$ 2.0
C9	41.0 $\pm$ 6.2
C10	23.3 $\pm$ 0.6

**Supplementary Table S3.** The relative cell viability percentages for C2, C8, and C10 were determined based on treatments with five concentrations: 100  $\mu$ M, 200  $\mu$ M, 300  $\mu$ M, 400  $\mu$ M, and 500  $\mu$ M. Data were normalized to the viability of the control group treated with DMSO. The results are presented as means  $\pm$  standard deviation (SD).

<b>Compounds</b>	<b>100 <math>\mu</math>M</b>	<b>200 <math>\mu</math>M</b>	<b>300 <math>\mu</math>M</b>	<b>400 <math>\mu</math>M</b>	<b>500 <math>\mu</math>M</b>
C2	123.0 $\pm$ 9.8	117.0 $\pm$ 8.2	96.7 $\pm$ 0.6	93.7 $\pm$ 1.5	91.7 $\pm$ 1.2
C8	130.7 $\pm$ 3.2	123.0 $\pm$ 2.0	105.0 $\pm$ 7.0	94.7 $\pm$ 1.5	91.3 $\pm$ 1.2
C10	130.0 $\pm$ 4.0	111.3 $\pm$ 10.3	101.0 $\pm$ 1.0	91.0 $\pm$ 1.0	91.7 $\pm$ 2.1

**Supplementary Table S4.** The inhibition percentages for C2, C8, and C10 were determined based on treatments with five concentrations: 100  $\mu$ M, 50  $\mu$ M, 25  $\mu$ M, 12.5  $\mu$ M, and 6.25  $\mu$ M. The results are presented as means  $\pm$  standard deviation (SD).

<b>Compounds</b>	<b>100 <math>\mu</math>M</b>	<b>50 <math>\mu</math>M</b>	<b>25 <math>\mu</math>M</b>	<b>12.5 <math>\mu</math>M</b>	<b>6.25 <math>\mu</math>M</b>
C2	86.3 $\pm$ 1.5	78.7 $\pm$ 3.2	73.7 $\pm$ 3.2	65.7 $\pm$ 3.1	28.0 $\pm$ 2.6
C8	92.7 $\pm$ 3.1	83.3 $\pm$ 4.9	78.0 $\pm$ 2.0	75.0 $\pm$ 2.0	38.3 $\pm$ 0.6
C10	93.7 $\pm$ 5.9	81.3 $\pm$ 3.5	76.7 $\pm$ 0.6	72.3 $\pm$ 2.1	35.7 $\pm$ 1.5

**Supplementary Table S5.**

The nine analogs from C2 and C8, each accompanied by their respective identification number and reference code within the article.

<b>Compounds</b>	<b>Code</b>
NSC121360	C2.1
NSC121362	C2.2
NSC630360	C2.3
NSC645888	C2.4
NSC368342	C8.1
NSC660824	C8.2
NSC660828	C8.3
NSC660830	C8.4
NSC660831	C8.5

**Supplementary Table S6.** Pseudovirus-based inhibition assay of the C2 and C8 analogs. The RLU (%) values were calculated using the following equation:  $RLU (\%) = (A / A_0) \times 100\%$ , where A represents the RLUs of the experimental groups and A<sub>0</sub> represents the RLUs of the DMSO group. The data are presented as means  $\pm$  standard deviation (SD).

<b>Compounds</b>	<b>RLU(% of control)</b>
C2.1	33.3 $\pm$ 7.8
C2.2	51.0 $\pm$ 7.0
C8.1	118.3 $\pm$ 7.6
C8.2	2.7 $\pm$ 0.6
C8.3	15.0 $\pm$ 9.5
C8.4	15.3 $\pm$ 7.2
C8.5	29.3 $\pm$ 4.6

**Supplementary Table S7.** The relative cell viability percentages for C8.2 were determined based on treatments with five concentrations: 100  $\mu$ M, 200  $\mu$ M, 300  $\mu$ M, 400  $\mu$ M, and 500  $\mu$ M. Data were normalized to the viability of the control group treated with DMSO. The results are presented as means  $\pm$  standard deviation (SD).

<b>Compounds</b>	<b>100 <math>\mu</math>M</b>	<b>200 <math>\mu</math>M</b>	<b>300 <math>\mu</math>M</b>	<b>400 <math>\mu</math>M</b>	<b>500 <math>\mu</math>M</b>
C8.2	136.0 $\pm$ 11.1	132.7 $\pm$ 8.0	127.7 $\pm$ 8.6	118.0 $\pm$ 7.0	106.3 $\pm$ 8.5



**Supplementary Table S8.** The inhibition percentages for C8.2 were determined based on treatments with five concentrations: 100  $\mu$ M, 50  $\mu$ M, 25  $\mu$ M, 12.5  $\mu$ M, and 6.25  $\mu$ M. The results are presented as means  $\pm$  standard deviation (SD).

<b>Compounds</b>	<b>100 <math>\mu</math>M</b>	<b>50 <math>\mu</math>M</b>	<b>25 <math>\mu</math>M</b>	<b>12.5 <math>\mu</math>M</b>	<b>6.25 <math>\mu</math>M</b>
C8.2	97.3 $\pm$ 0.6	94.0 $\pm$ 1.0	93.0 $\pm$ 4.6	84.0 $\pm$ 2.6	43.7 $\pm$ 1.2

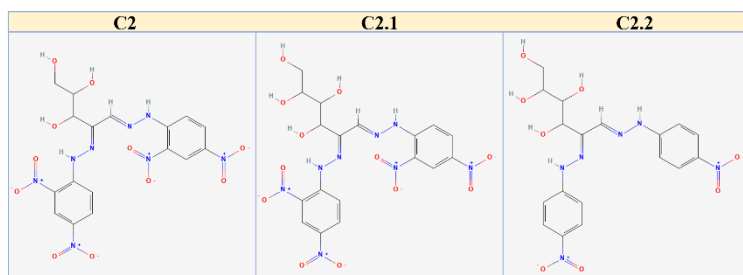
**Supplementary Table S9.** The pseudovirus-based inhibition assay was conducted using the BA.1 strain with C2, C8, C10, and their analogs. The RLU (%) values were calculated using the following equation:  $RLU (\%) = (A / A_0) \times 100\%$ , where A represents the RLUs of the experimental groups and A<sub>0</sub> represents the RLUs of the DMSO group. The data are presented as means  $\pm$  standard deviation (SD).

<b>Compounds</b>	<b>RLU(% of control)</b>
C2	26.0 $\pm$ 3.6
C8	26.7 $\pm$ 1.5
C10	24.7 $\pm$ 1.5
C2.1	38.3 $\pm$ 1.2
C2.2	45.0 $\pm$ 5.6
C8.1	88.3 $\pm$ 11.0
C8.2	14.0 $\pm$ 1.0
C8.3	34.0 $\pm$ 4.6
C8.4	37.3 $\pm$ 1.5
C8.5	51.3 $\pm$ 1.5

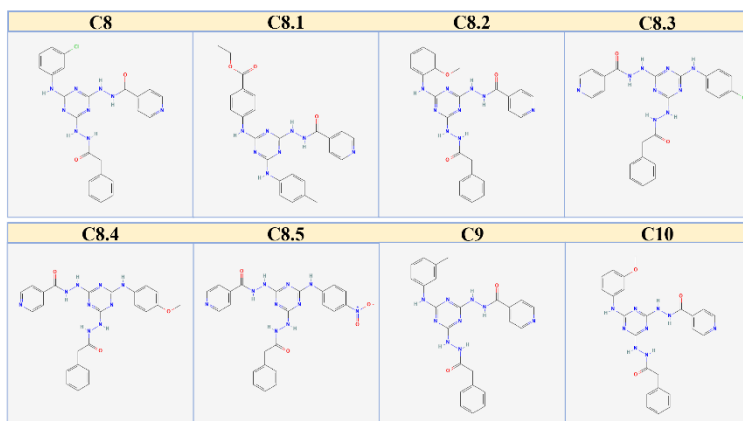
**Supplementary Table S10.** The inhibition percentages for C8.2 on the BA.1 strain were determined based on treatments with five concentrations: 100  $\mu$ M, 50  $\mu$ M, 25  $\mu$ M, 12.5  $\mu$ M, and 6.25  $\mu$ M. The results are presented as means  $\pm$  standard deviation (SD).

<b>Compounds</b>	<b>100 <math>\mu</math>M</b>	<b>50 <math>\mu</math>M</b>	<b>25 <math>\mu</math>M</b>	<b>12.5 <math>\mu</math>M</b>	<b>6.25 <math>\mu</math>M</b>
<b>C8.2</b>	97.3 $\pm$ 0.3	93.9 $\pm$ 1.0	86.0 $\pm$ 1.0	77.4 $\pm$ 1.7	26.2 $\pm$ 0.5

(A)



(B)



**Supplementary Figure S1.** (A) Structures of compound C2 and the C2 analogs. (B) Structures of compounds C2, C9, C10, and the C8 analogs.