

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

Li-Te Tai ¹, Cheng-Yun Yeh ², Yu-Jen Chang ³, Ju-Fang Liu ⁴, Kai-Cheng Hsu ⁵,
Ju-Chien Cheng ^{6,*} and Chih-Hao Lu ^{7,8,9,*}

¹ Industrial Development Graduate Program of College of Biological Science and Technology, National Yang Ming Chiao Tung University, Hsinchu 300193, Taiwan; duncan19950626@gmail.com

² Graduate Institute of Biomedical Sciences, China Medical University, Taichung 404333, Taiwan; simplefeeling8211@gmail.com

³ The Ph.D. Program of Biotechnology and Biomedical Industry, China Medical University, Taichung 404333, Taiwan; uzang199431465@gmail.com

⁴ School of Oral Hygiene, College of Oral Medicine, Taipei Medical University, Taipei 110301, Taiwan; jufangliu@tmu.edu.tw

⁵ Graduate Institute of Cancer Biology and Drug Discovery, Taipei Medical University, Taipei 110301, Taiwan; piki@tmu.edu.tw

⁶ Department of Medical Laboratory Science and Biotechnology, China Medical University, Taichung 404333, Taiwan

⁷ Institute of Bioinformatics and Systems Biology, National Yang Ming Chiao Tung University, Hsinchu 300193, Taiwan

⁸ Department of Biological Science and Technology, National Yang Ming Chiao Tung University, Hsinchu 300193, Taiwan

⁹ Center for Intelligent Drug Systems and Smart Bio-Devices (IDS2B), National Yang Ming Chiao Tung University, Hsinchu 300193, Taiwan

* Correspondence: jcheng@mail.cmu.edu.tw (J.-C.C.); chlu@nycu.edu.tw (C.-H.L.)

Supplementary Table S1.

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

Compounds	Code
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 A₀ 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 μ M, 200 μ M, 300 μ M, 400 μ M, and 500 μ M. Data were normalized to the viability of the control group treated with DMSO. The results are presented as means \pm standard deviation (SD).

Compounds	100 μM	200 μM	300 μM	400 μM	500 μM
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 μM , 50 μM , 25 μM , 12.5 μM , and 6.25 μM . The results are presented as means \pm standard deviation (SD).

Compounds	100 μM	50 μM	25 μM	12.5 μM	6.25 μM
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.

Compounds	Code
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 A₀ represents the RLUs of the DMSO group. The data are presented as means \pm standard deviation (SD).

Compounds	RLU(% of control)
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 μ M, 200 μ M, 300 μ M, 400 μ M, and 500 μ M. Data were normalized to the viability of the control group treated with DMSO. The results are presented as means \pm standard deviation (SD).

Compounds	100 μM	200 μM	300 μM	400 μM	500 μM
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 μM , 50 μM , 25 μM , 12.5 μM , and 6.25 μM . The results are presented as means \pm standard deviation (SD).

Compounds	100 μM	50 μM	25 μM	12.5 μM	6.25 μM
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

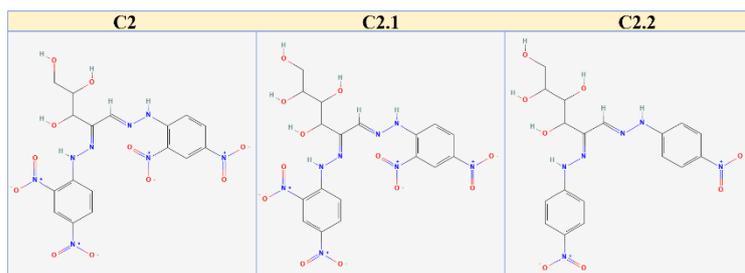
Supplementary Table S9. The pseudovirus-based inhibition assay was conducted using the BA.1 strain with C2, C8, C10, and their analogs. The RLU (%) values were calculated using the following equation: $RLU (\%) = (A / A_0) \times 100\%$, where A represents the RLUs of the experimental groups and A0 represents the RLUs of the DMSO group. The data are presented as means \pm standard deviation (SD).

Compounds	RLU(% of control)
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

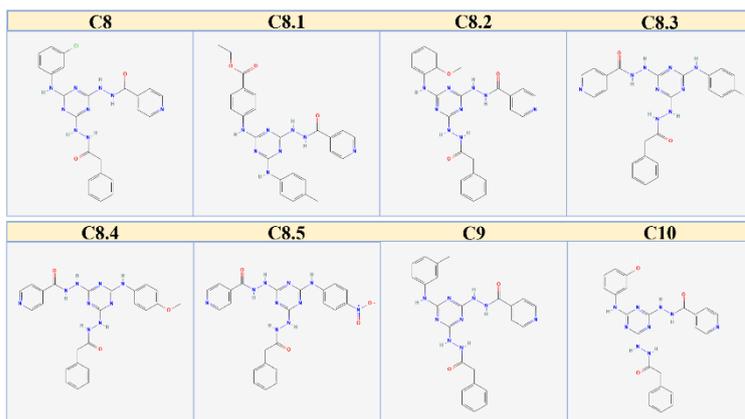
Supplementary Table S10. The inhibition percentages for C8.2 on the BA.1 strain were determined based on treatments with five concentrations: 100 μM , 50 μM , 25 μM , 12.5 μM , and 6.25 μM . The results are presented as means \pm standard deviation (SD).

Compounds	100 μM	50 μM	25 μM	12.5 μM	6.25 μM
C8.2	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.