Supporting Information for

Axial Chiral Binaphthoquinone and Perylenequinones from the Stromata of *Hypocrella bambusae* are SARS-CoV-2 Entry Inhibitors

Yu-Ting Li,[†,▽] Chan Yang,[†,▽] Yan Wu,[‡,▽] Jun-Jiang Lv,[§] Xiao Feng,[†] Xiaofei Tian,[^] Zhengzheng Zhou,[‖] Xiaoyan Pan,[*,‡] Shuwen Liu,[*,†] Li-Wen Tian[*,†]

[†]Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, [‖] School of Public Health, Southern Medical University, Guangzhou 510515, People's Republic of China

[‡]State Key Laboratory of Virology, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan 430071, People's Republic of China

[§]School of Pharmaceutical Sciences, Chongqing Medical and Pharmaceutical College, Chongqing 401331, People's Republic of China

[^]School of Biology and Biological Engineering, South China University of Technology, Guangzhou 510006, People's Republic of China

[▽]These authors contribute equally to the manuscript

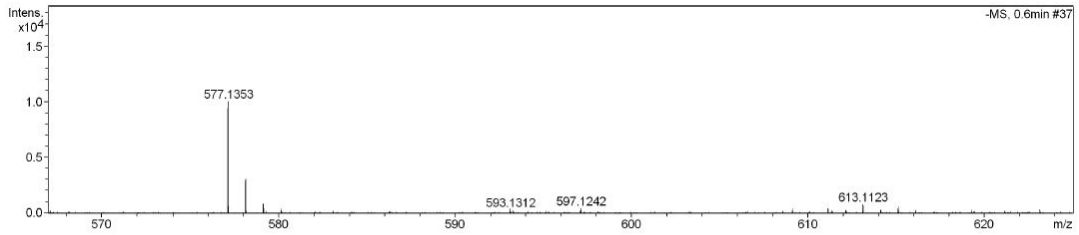
[*]Corresponding authors: Tel.: +86 27 87198352, Email: panxy@wh.iov.cn (X. Y. Pan); Tel.: +86 20 61648538, Email: liusw@smu.edu.cn (S. W. Liu); or Tel.: +86 20 61648594, Email: lwtian@smu.edu.cn (L.W. Tian)

Contents of Supporting Information

Fungal materials collection and identification

Hypocrella was monotypic genus with *H. bambusae* as the typical species. The fungus is parasitic on the culms of *Fargesia* sp. in high chilly alpine area. It mainly distributes in the northwest of Yunnan Province, China, and the southeast of Tibet Autonomous Region, China. The stromata were used by ethnic minorities in Yunnan Province. The stromata were collected at one time on July 2017 from the bamboo forest of Lijiang, Yunnan, China. The stroma was identified based on the dark color, the near spherical shape, and the unique morphological features of the linear ascospores (reference: MycoKeys, 2019, 58, 1-26).

Figure 1S. (-)-HRESIMS spectrum of **1**.



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e⁻ Conf	N-Rule
577.1353	1	C30H25O12	100.00	577.1351	-0.3	-0.2	12.0	18.5	even	ok
613.1123	1	C30H26ClO12	100.00	613.1118	-0.8	-0.5	26.8	17.5	even	ok

Figure 2S. ^{1}H NMR (600 MHz, CDCl3) spectrum of **1**.

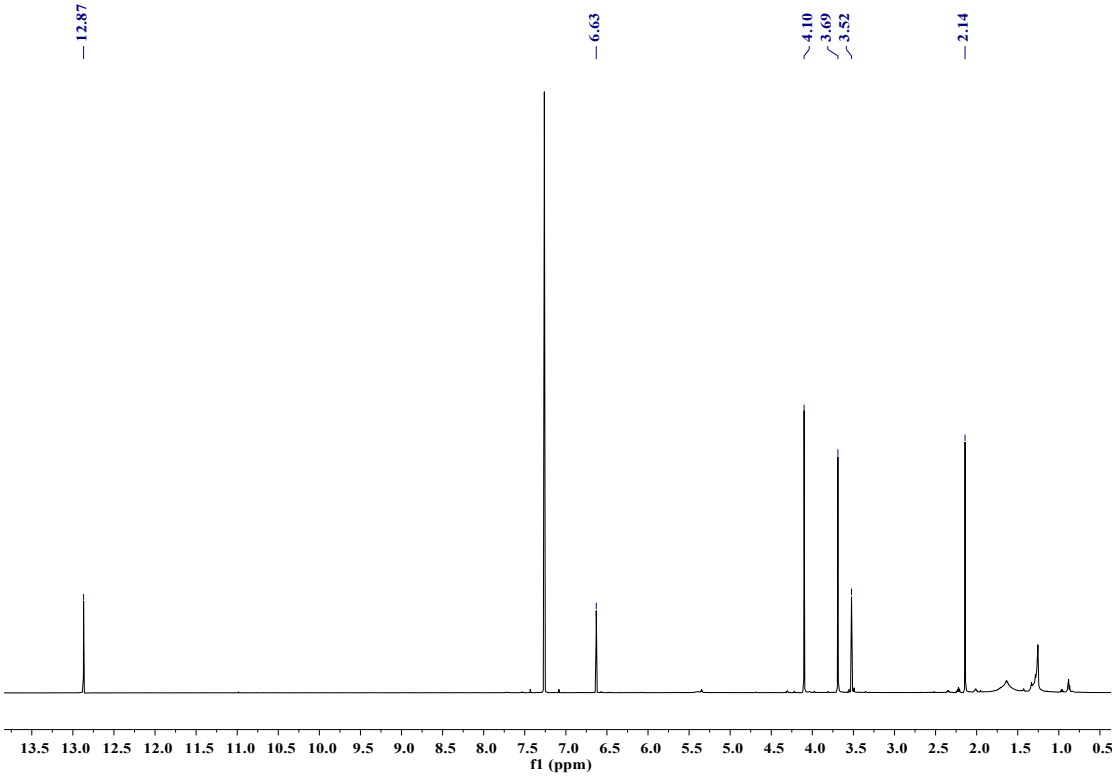


Figure 3S. ^{13}C NMR (150 MHz, $CDCl_3$) of **1**.

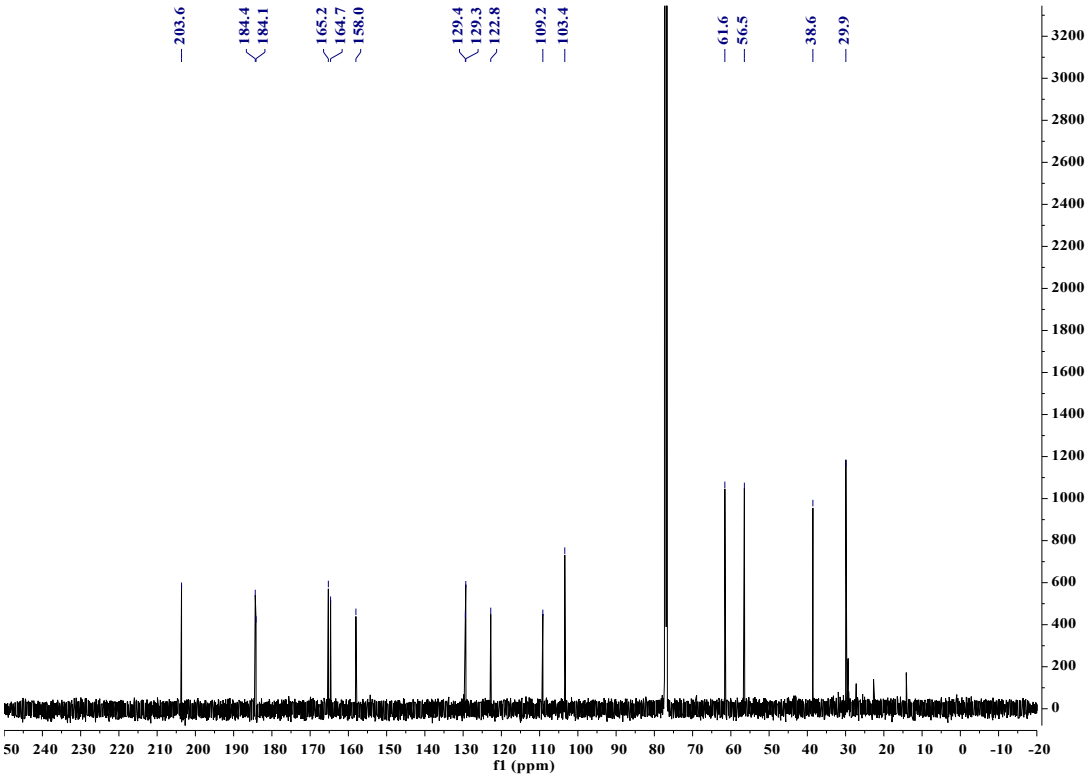


Figure 4S. HSQC spectrum of **1**.

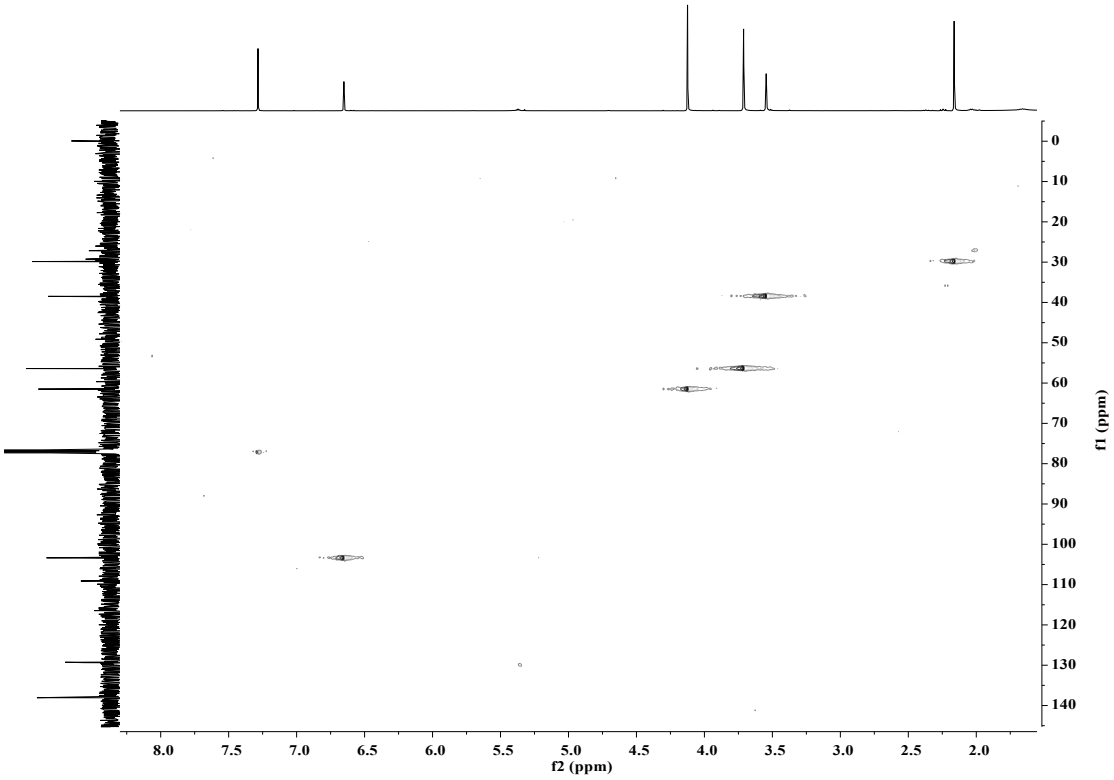


Figure 5S. HMBC spectrum of **1**.

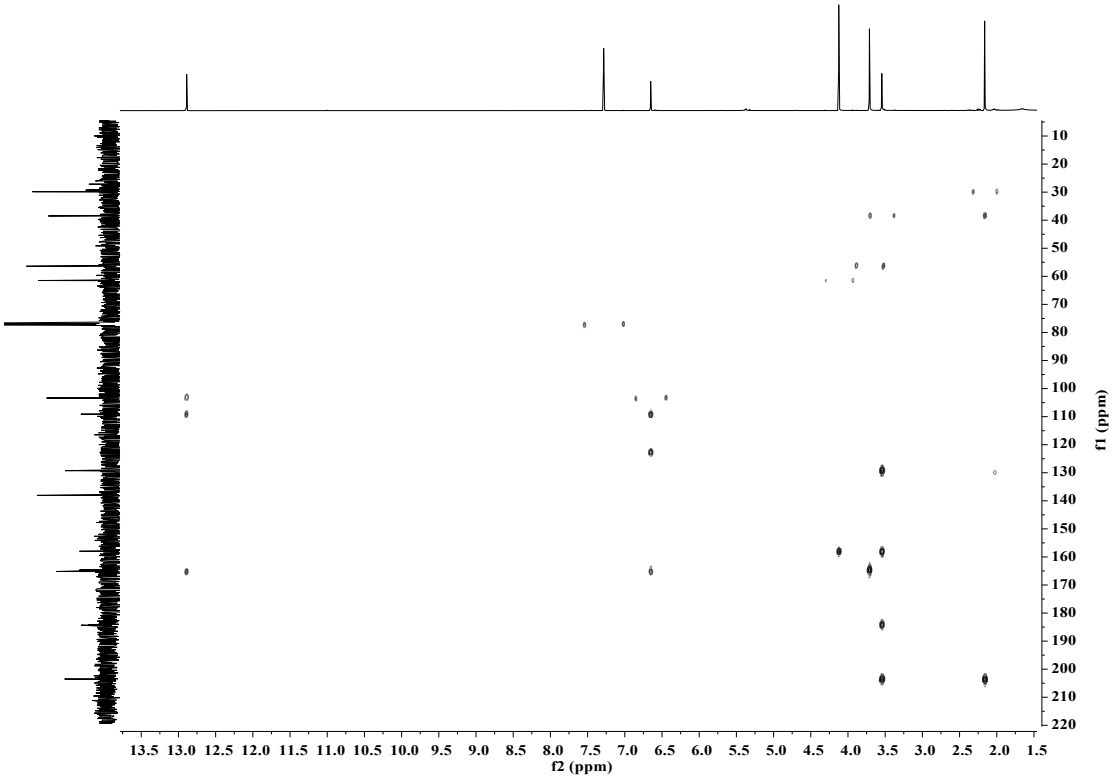
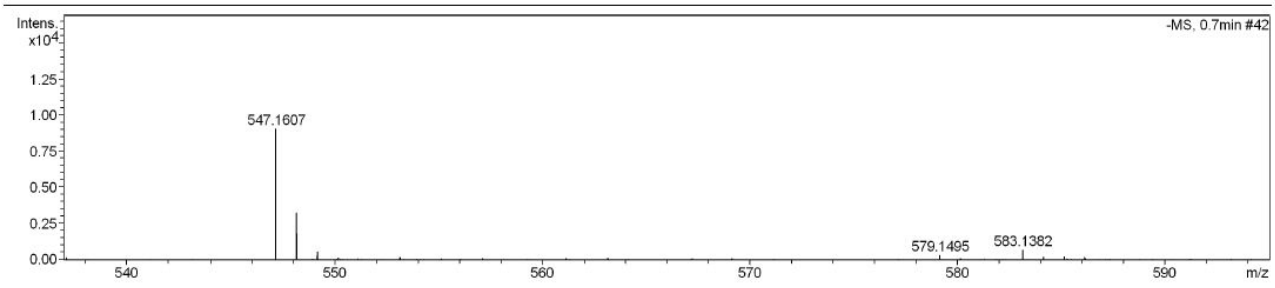


Figure 6S. (-)-HRESIMS spectrum of **2**.



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	err [mDa]	mSigma	rdb	e⁻ Conf	N-Rule
547.1607	1	C30H27O10	100.00	547.1610	0.4	0.2	16.0	17.5	even	ok
583.1382	1	C30H28ClO10	100.00	583.1376	-0.9	-0.5	50.5	16.5	even	ok
1095.3288	1	C60H55O20	100.00	1095.3292	0.4	0.4	15.5	33.5	even	ok
1131.3050	1	C60H56ClO20	100.00	1131.3059	0.8	0.9	25.6	32.5	even	ok

Figure7S. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **2**.

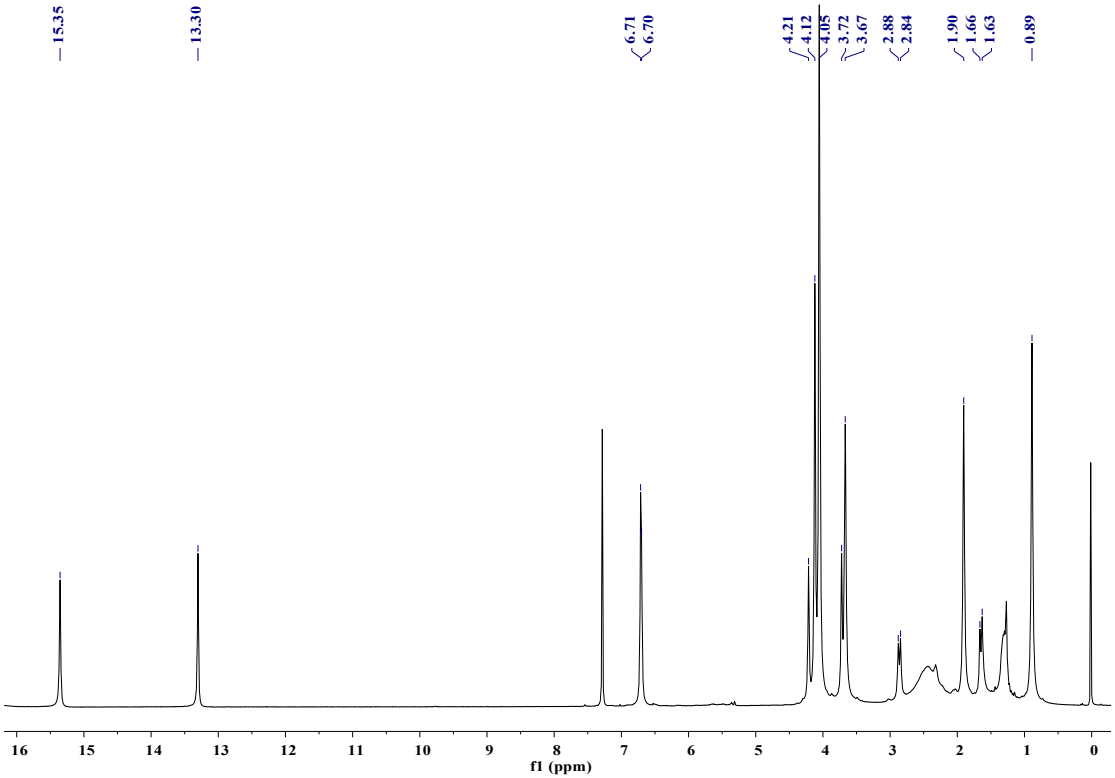


Figure 8S. ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **2**.

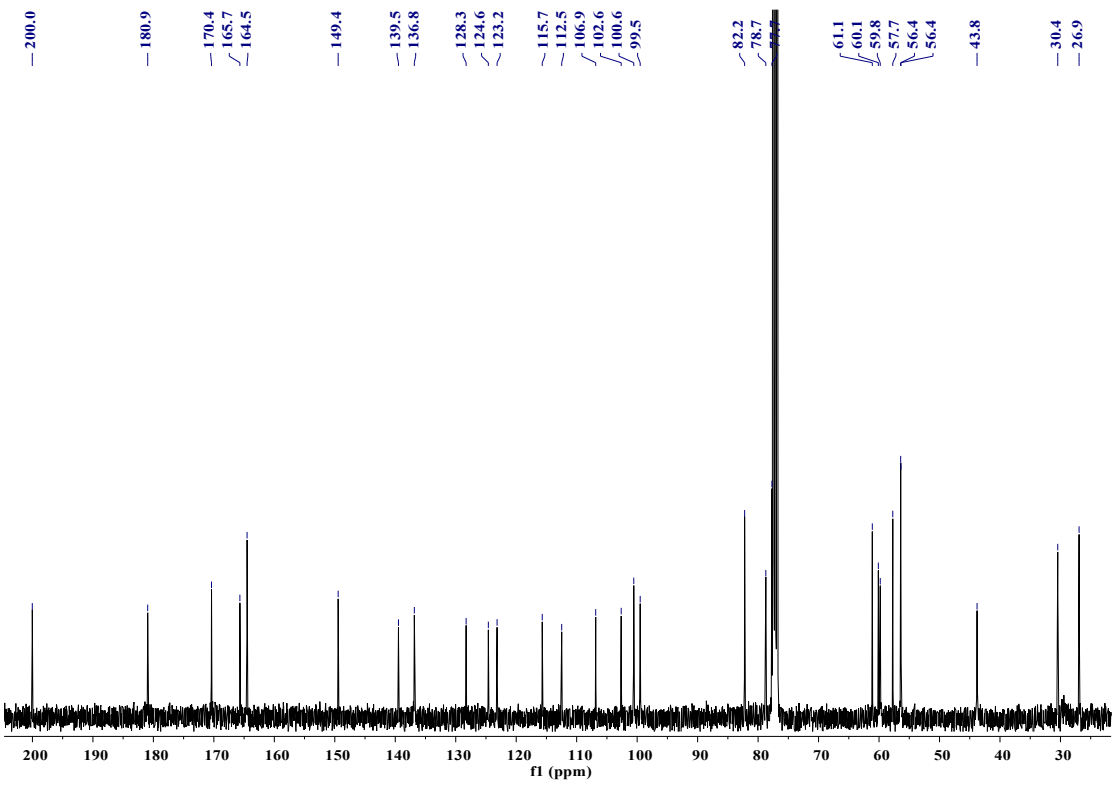


Figure 9S. HSQC spectrum of **2**.

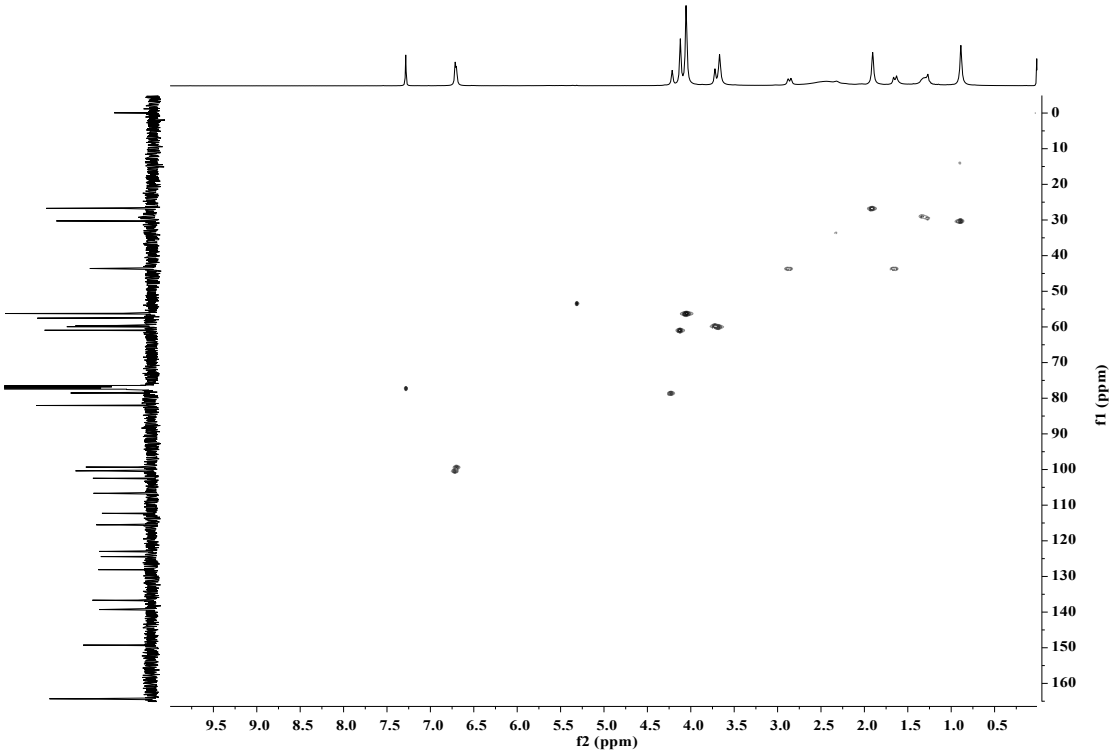


Figure 10S. HMBC spectrum of **2**.

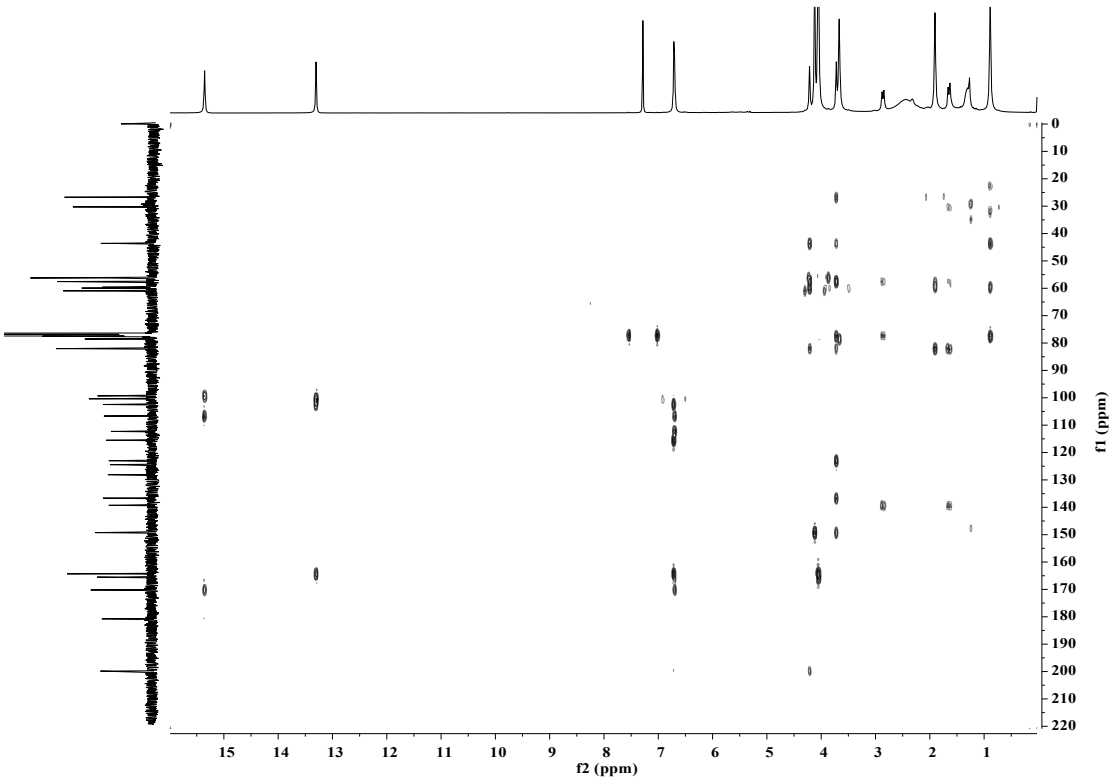


Figure 11S. NOESY spectrum of **2**.

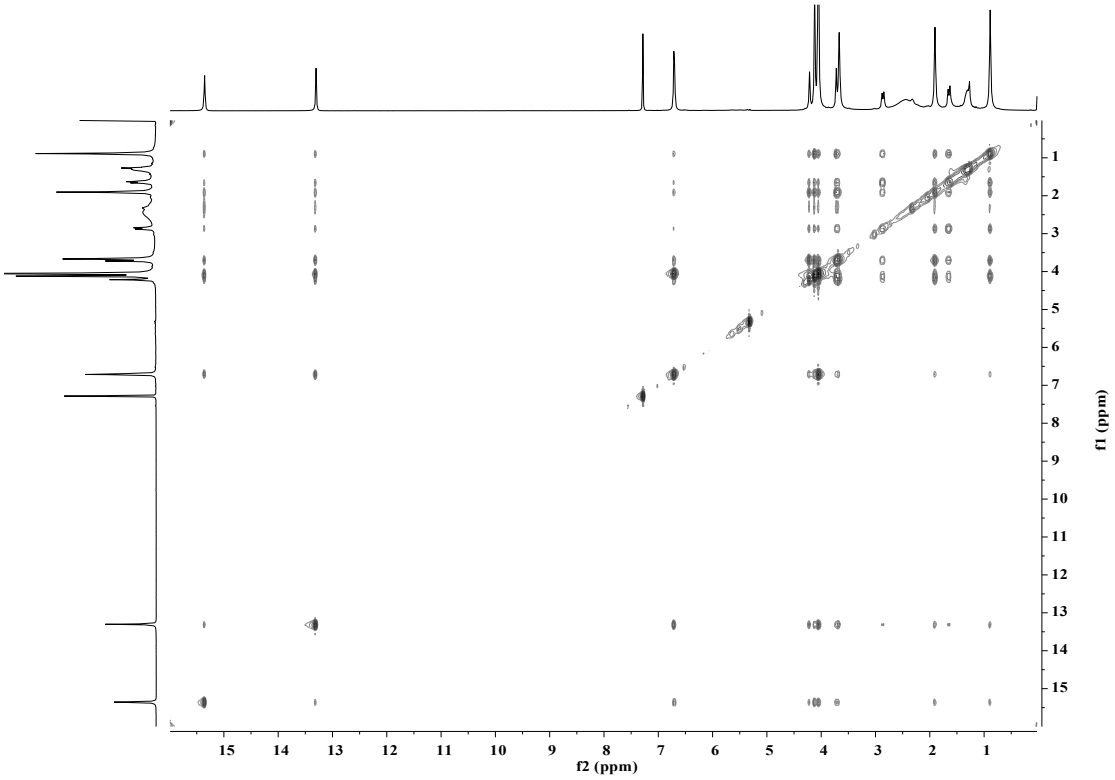


Figure S12. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3**.

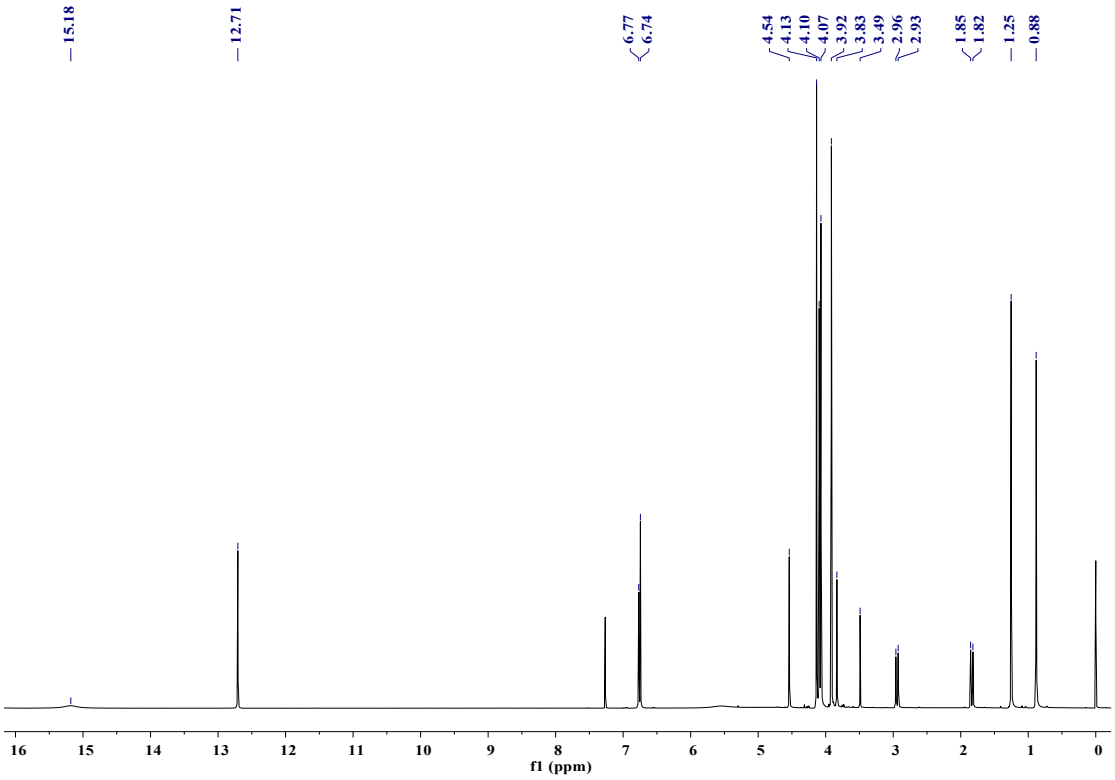


Figure 13S. ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **3**.

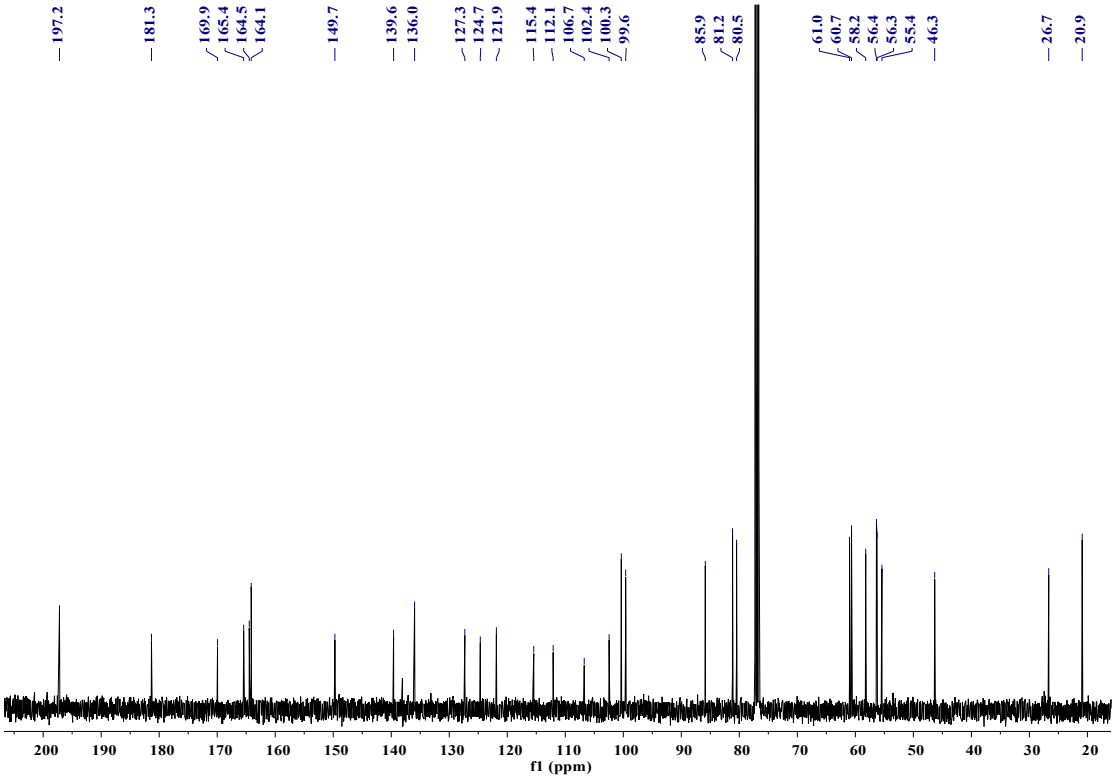


Figure 14S. HSQC spectrum of **3**.

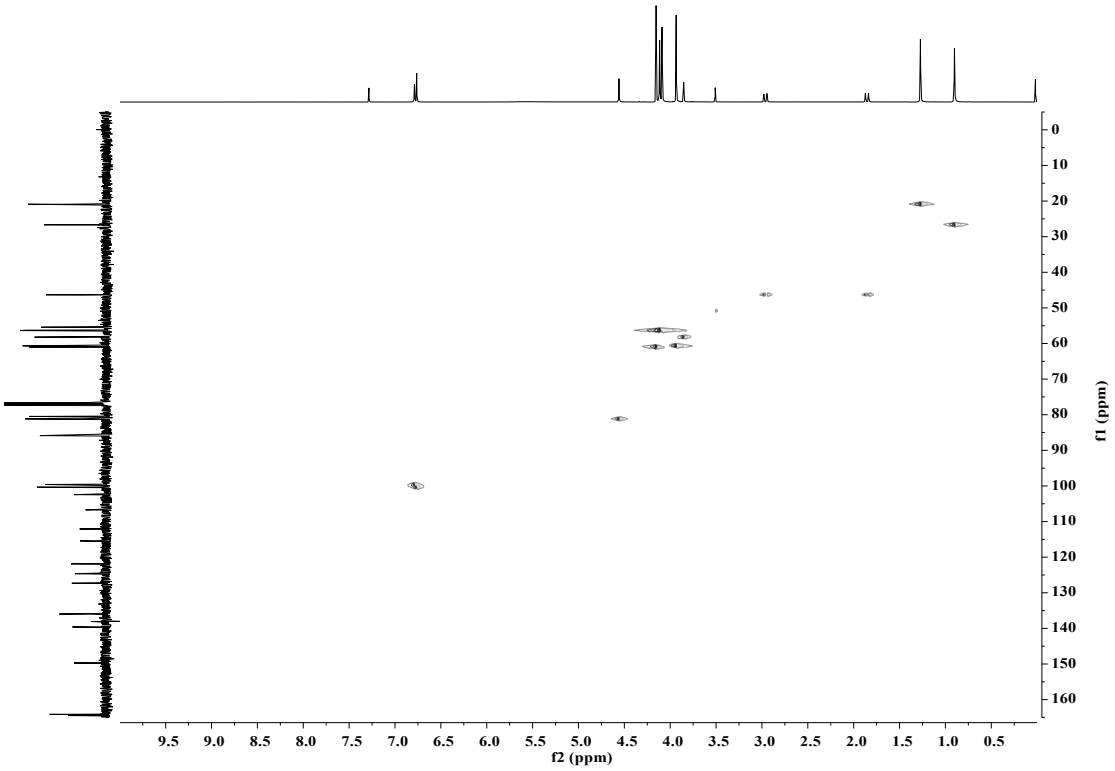


Figure 15S. HMBC spectrum of **3**.

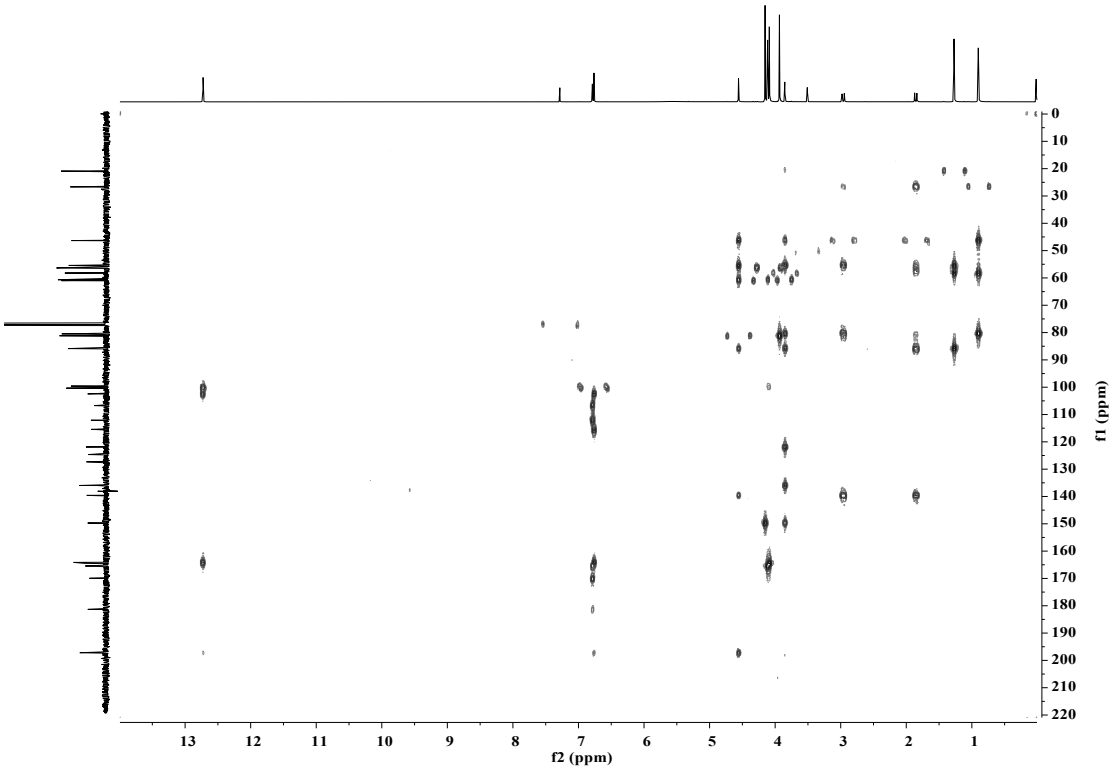


Figure 16S. NOESY spectrum of **3**.

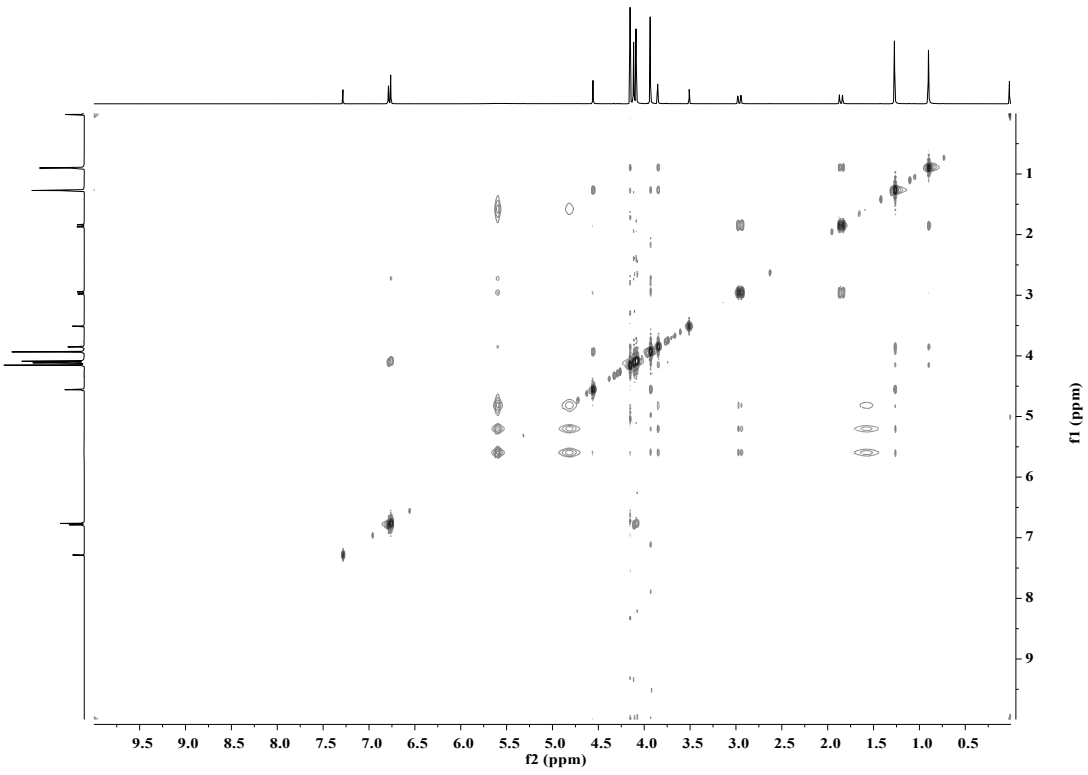


Figure 17S. X-ray crystal structure of **3**.

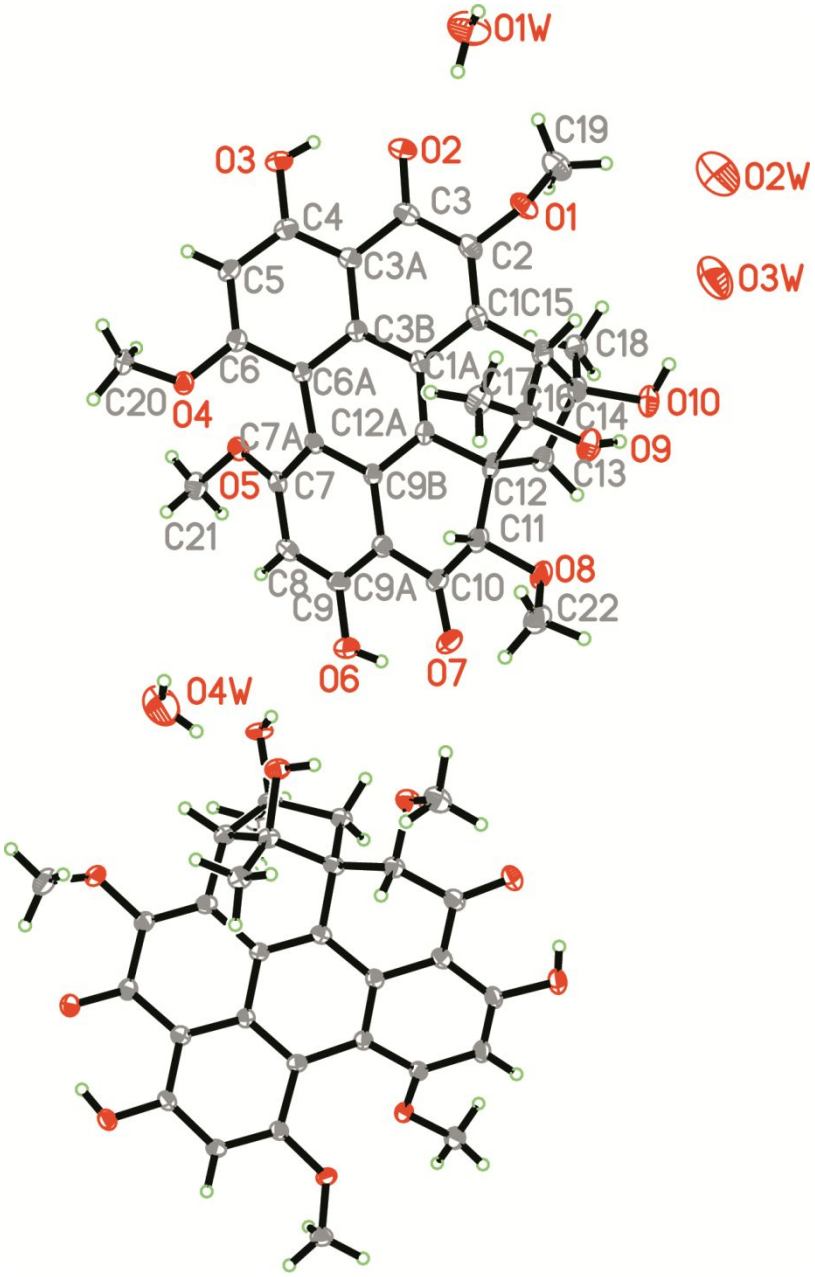


Figure 18S. Experimental CD spectra of **2** and **3**.

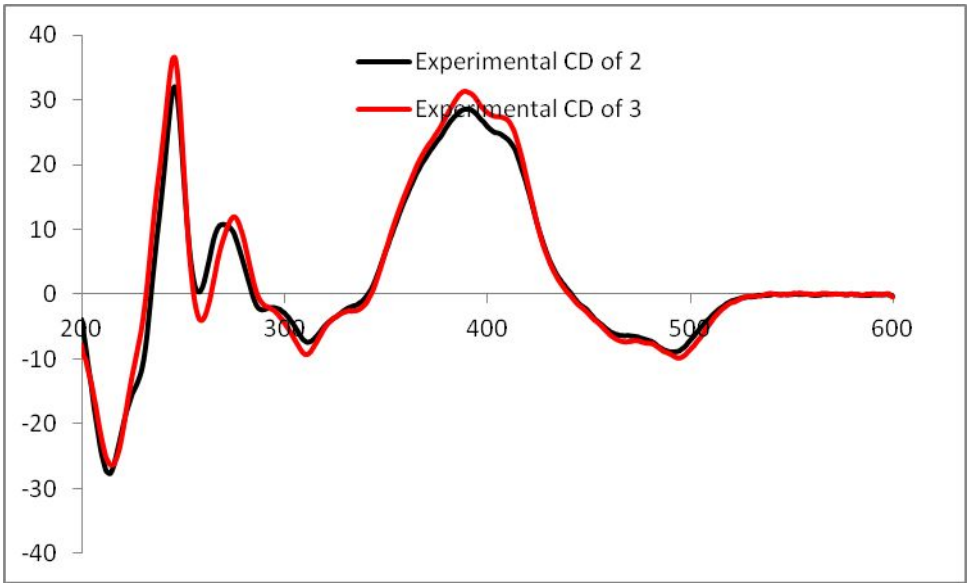


Figure 19S. Experimental CD spectra of **4** and **5**.

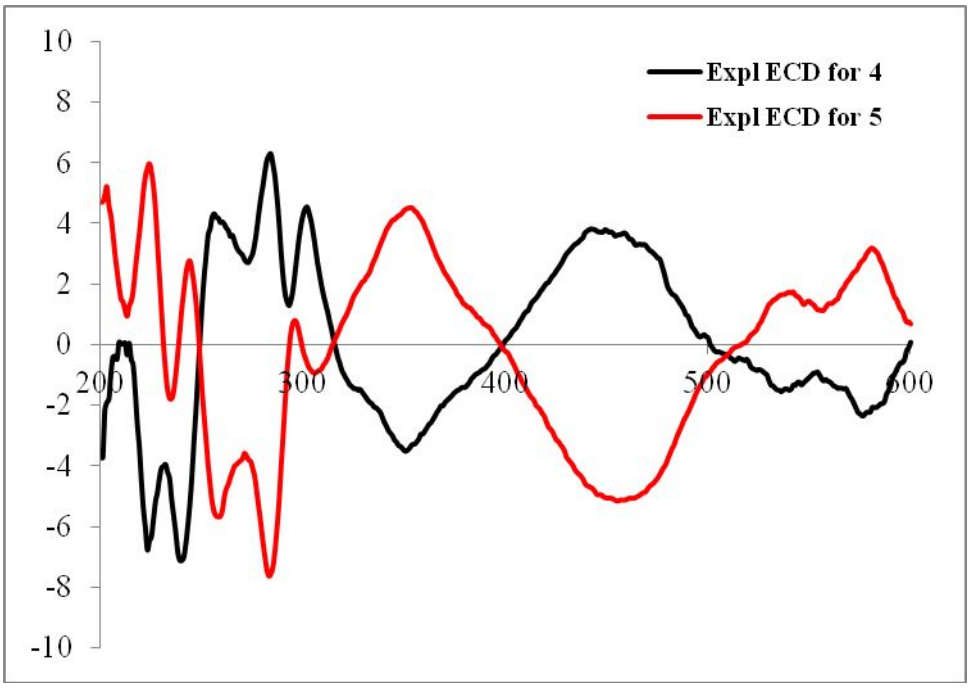


Figure 20S. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **4a**.

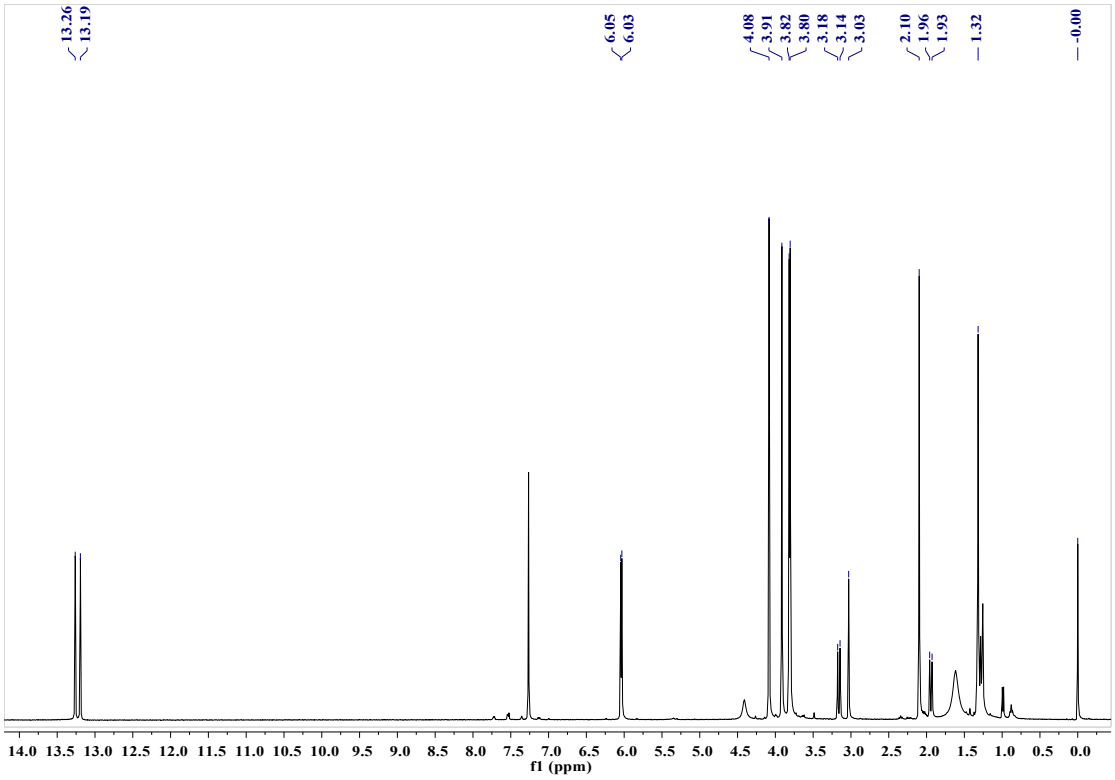


Figure 21S. ^{13}C NMR (100 MHz, $CDCl_3$) spectra of **4a**.

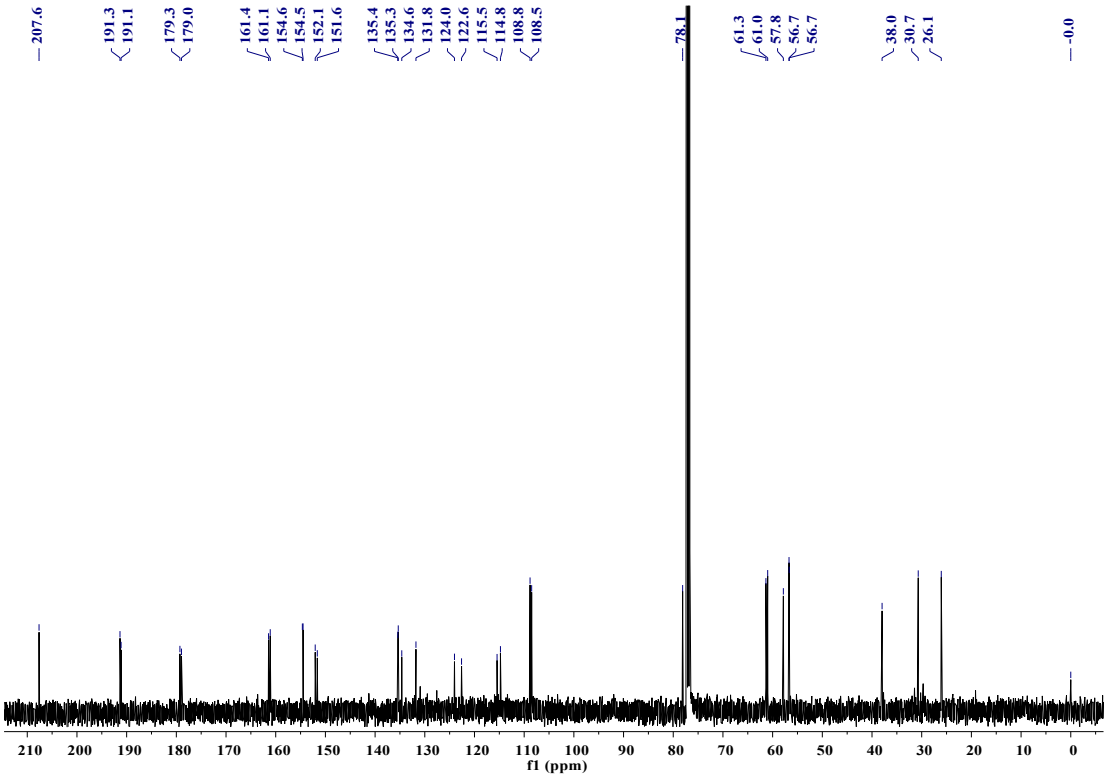
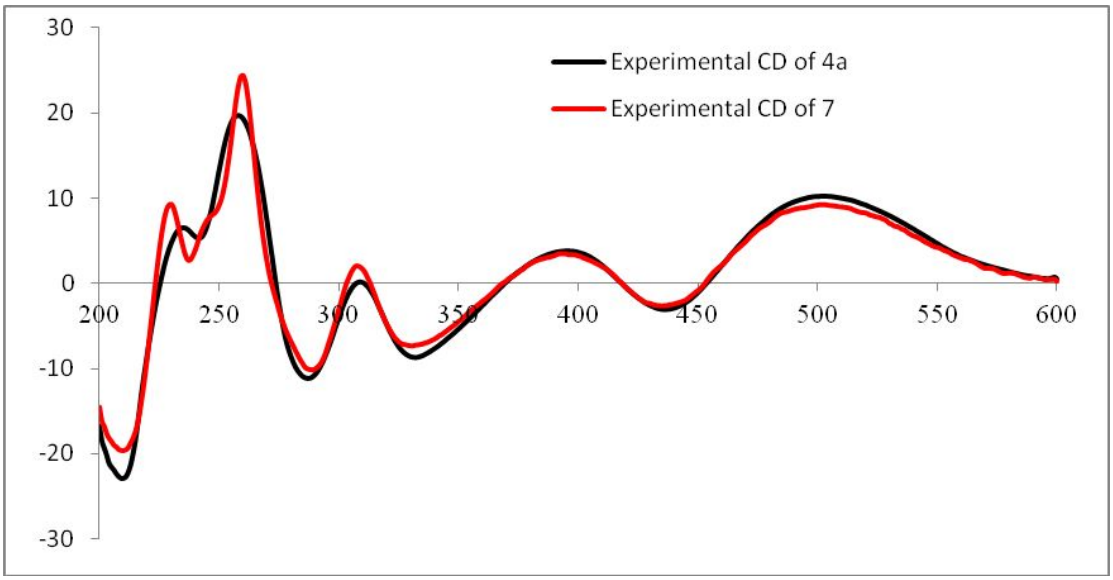


Figure 22S. Experimental CD spectra and optical rotation values of **4a** and **7**.



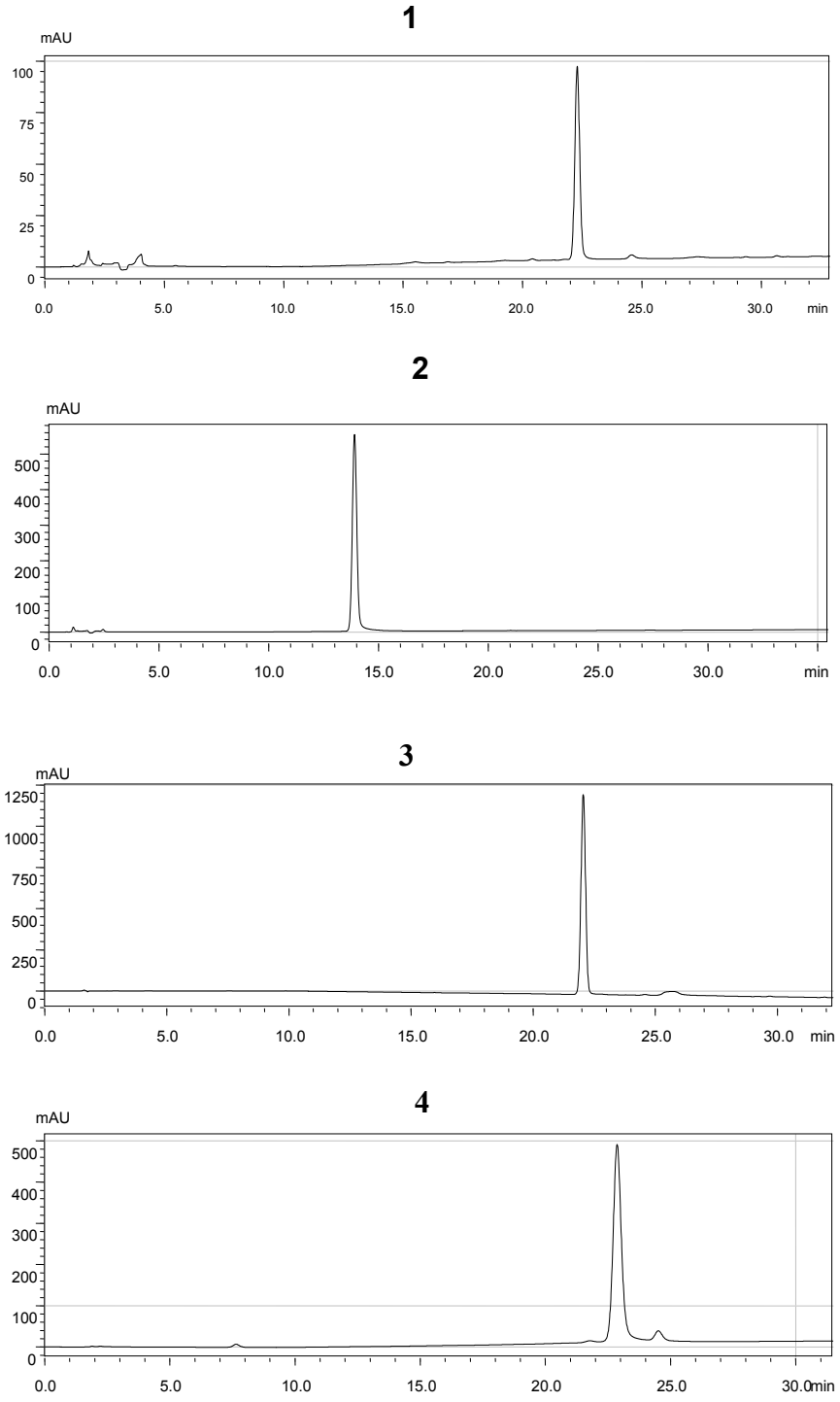
Optical rotation values

4a: [α]25 D +2420 (*c* 0.1 MeOH);

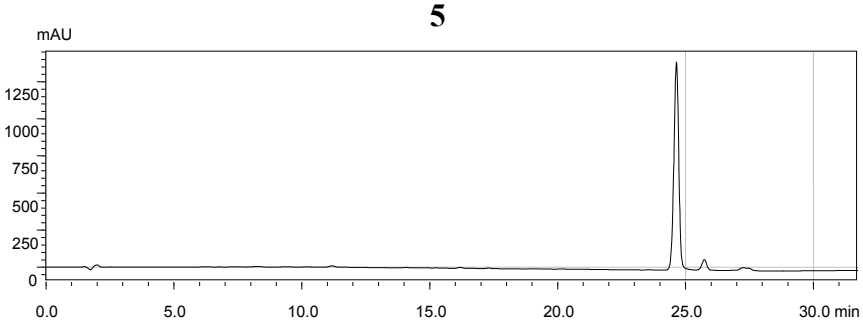
7: [α]25 D +2617 (*c* 0.1 MeOH);

7: [α]20 D +2465.9 (*c* 0.71 acetone) from reference (J. Antibiot. 2004, 59(6), 351-354).

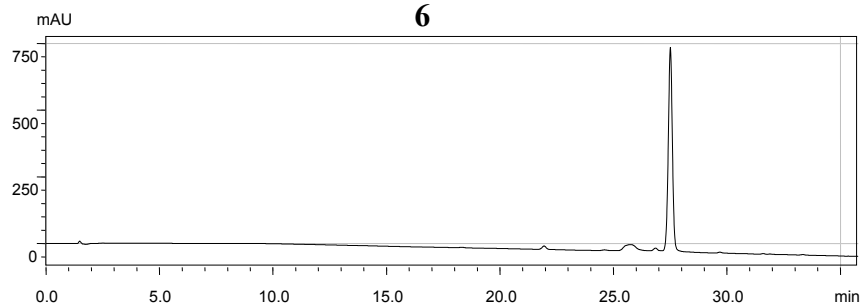
Figure 23S. HPLC purity chromatograms of compounds **1-7** (PDA detection 220 nm). All data were acquired via an HPLC system with RP C18 column (Reprosil, 5 μm, 250 × 4.6 mm) and a CH_3CN-H_2O gradient that increased linearly from 40%-100% CH_3CN over 30 min.



5


mAU
1250
1000
750
500
250
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0 min

6


mAU
750
500
250
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
min

7

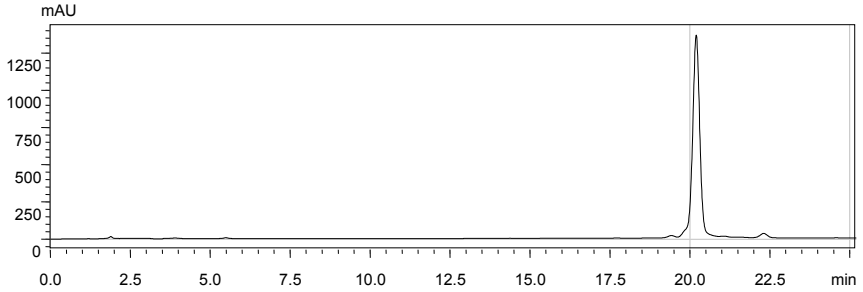

mAU
1250
1000
750
500
250
0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
min

Figure 24S. Cytotoxicity of compounds **1-7** against 293T-ACE2 cell.

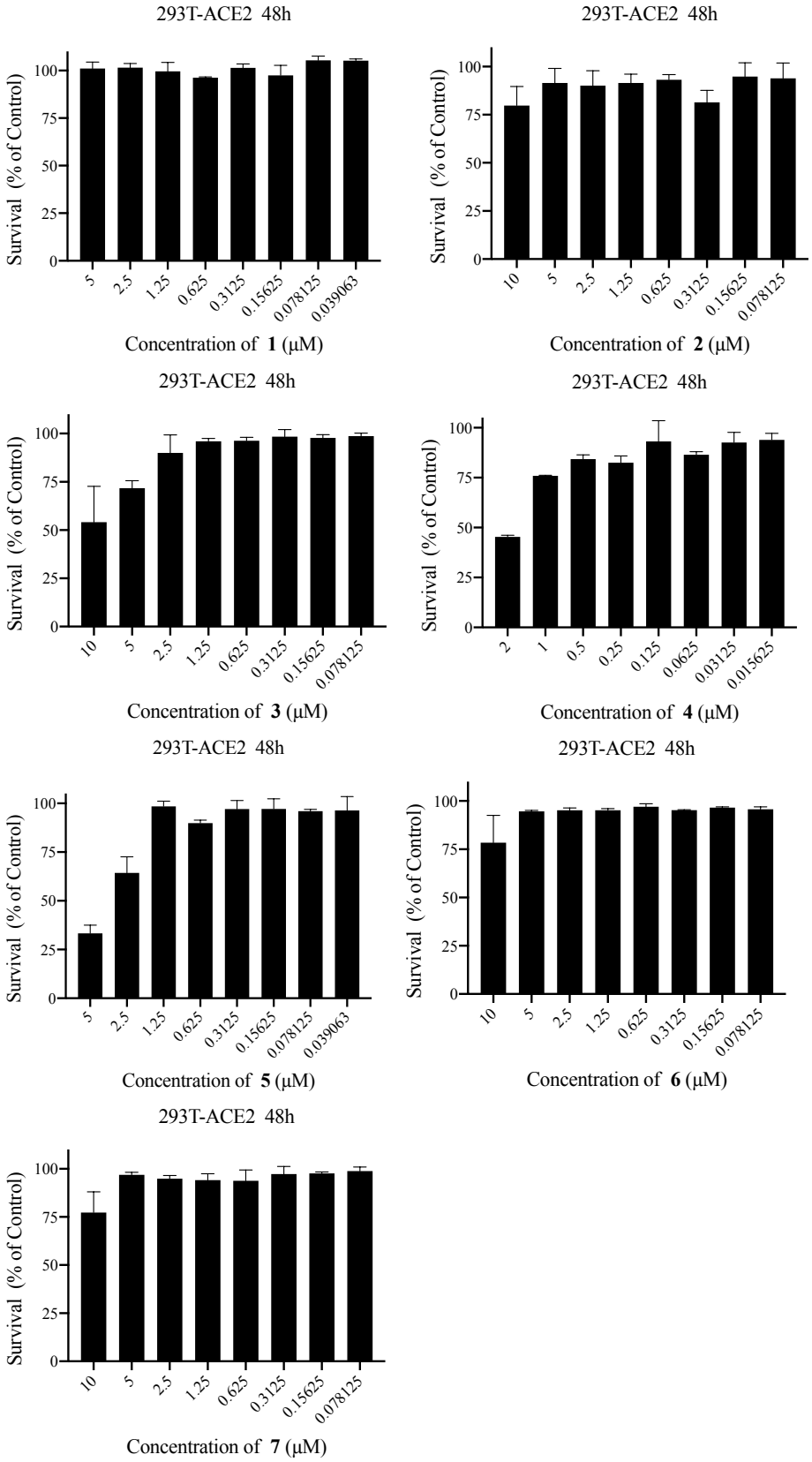
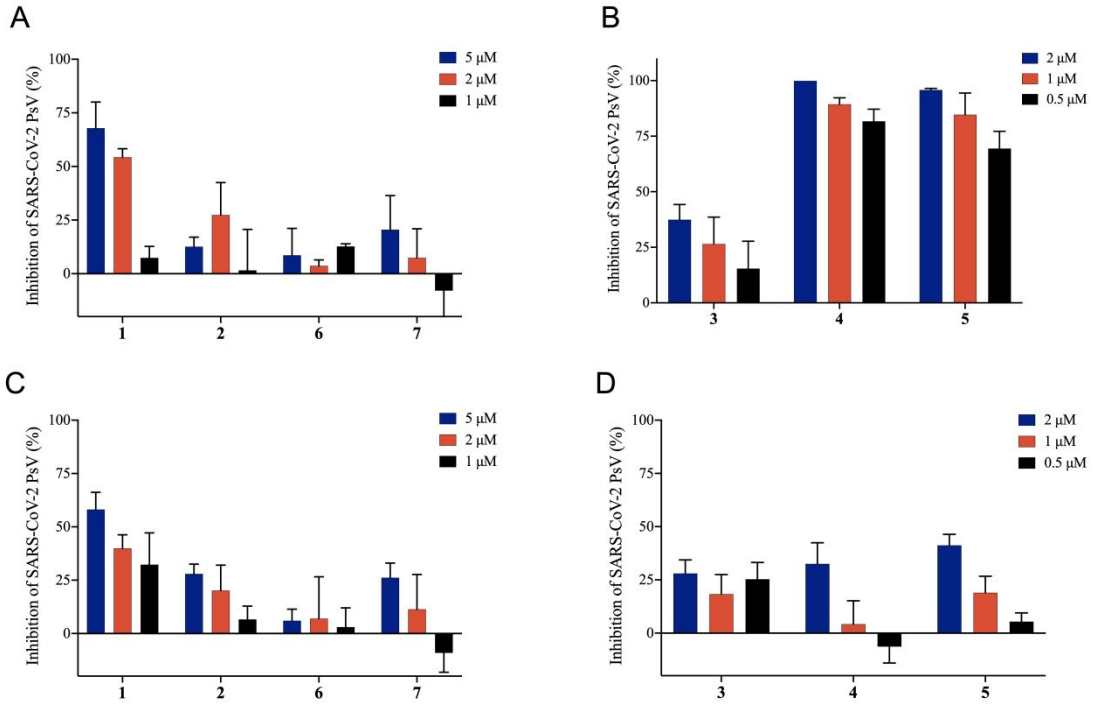
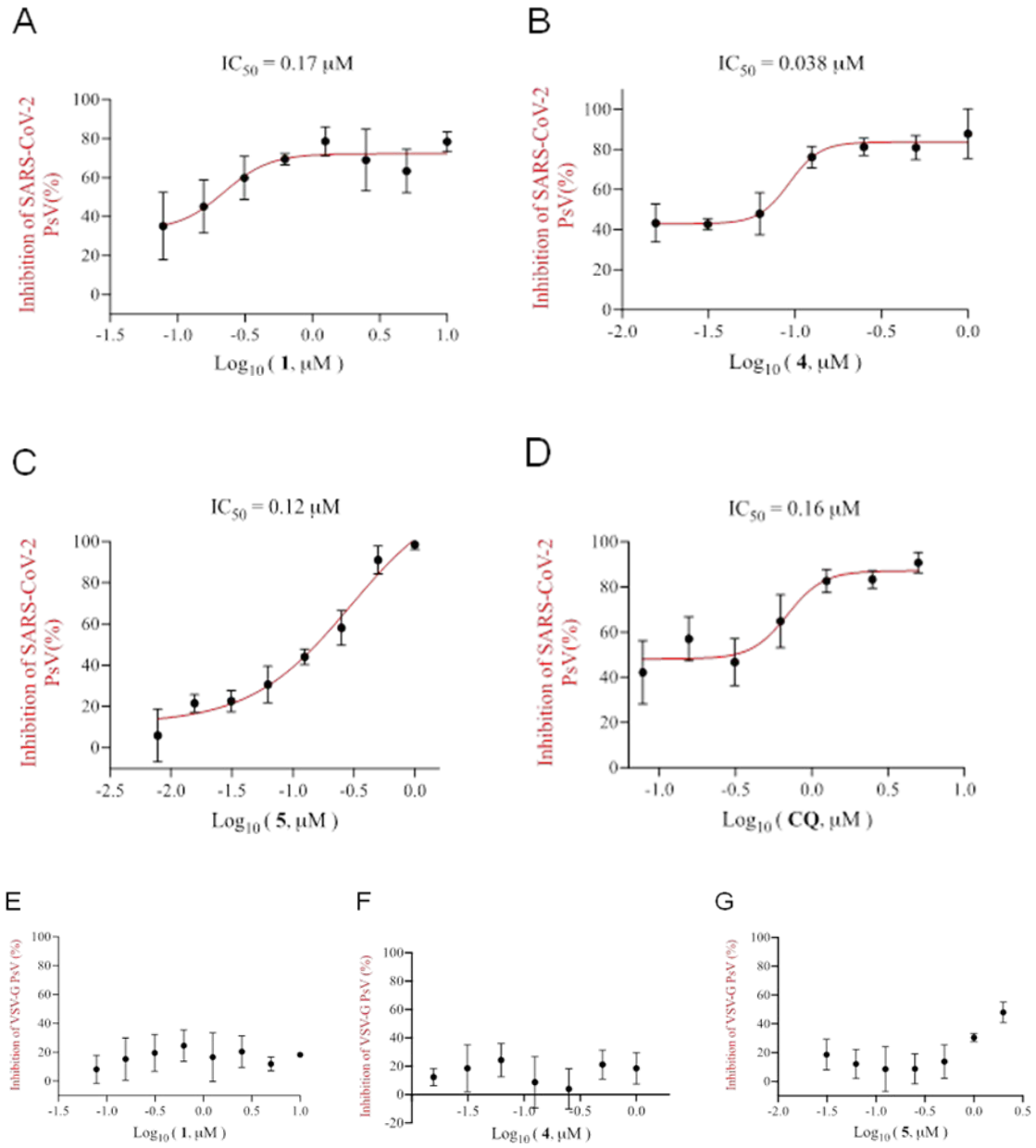


Figure 25S. Preliminary anti-SARS-CoV-2 activity evaluation for compounds **1**-**7**.



The inhibitory activities of compounds **1**-**7** against SARS-CoV-2 PsV infection in the ongoing-infection model (A and B). The inhibitory activities of compounds **1**-**7** against SARS-CoV-2 PsV infection in the pre-infecrion model (C and D). All experiments were done in triplicates and repeated three times, data are expressed as means ± SD.

Figure 26S. The inhibitory activities of **1**, **4**, **5** and chloroquine against SARS-CoV-2 PsV infection.



Compounds **1** (A), **4** (B), **5** (C) and Chloroquine (D) inhibited SARS-CoV-2 PsV infection in 293T-ACE2 cells in 48 hours. Compounds **1** (E), **4** (F), and **5** (G) showed no inhibitory activity against VSV-G PsV in 293T/ACE2 cells in 48 hours. Data were presented as mean ± SD of triplicate samples from a representative experiment.

Figure 27S. The cytotoxicity of compounds **1**, **4**, and **5** against Vero-E6 cell after 24 hours.

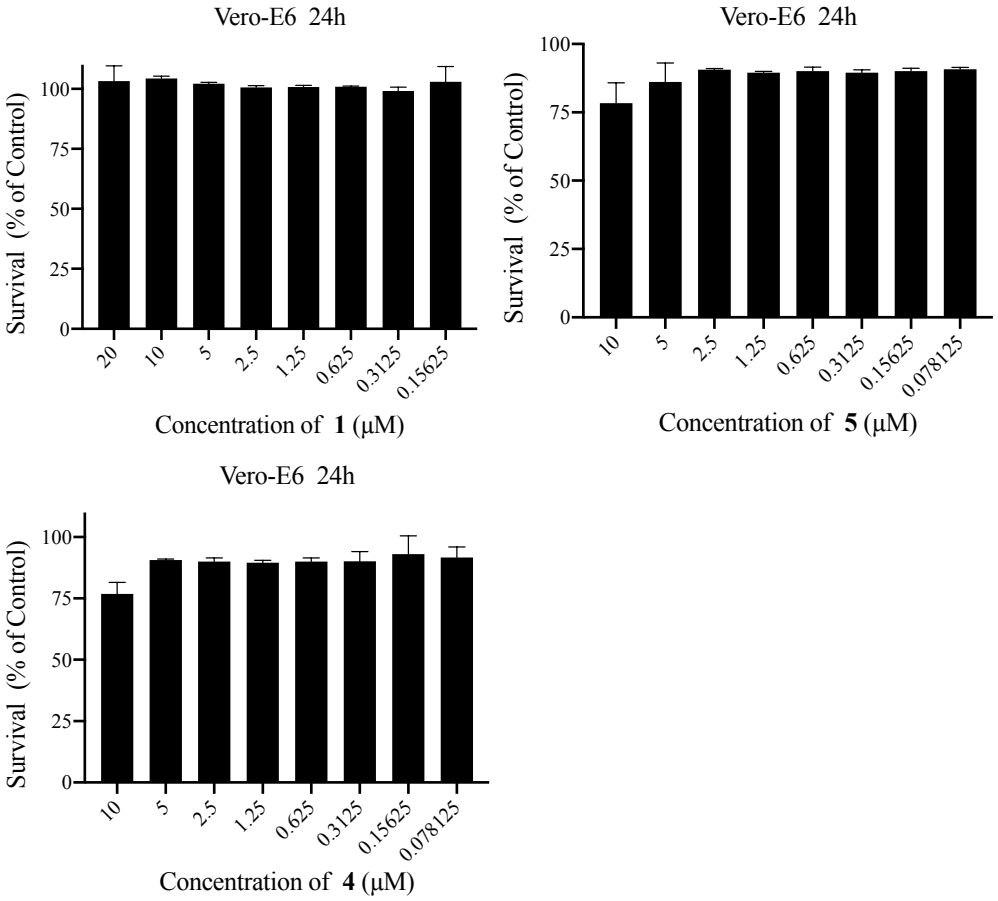
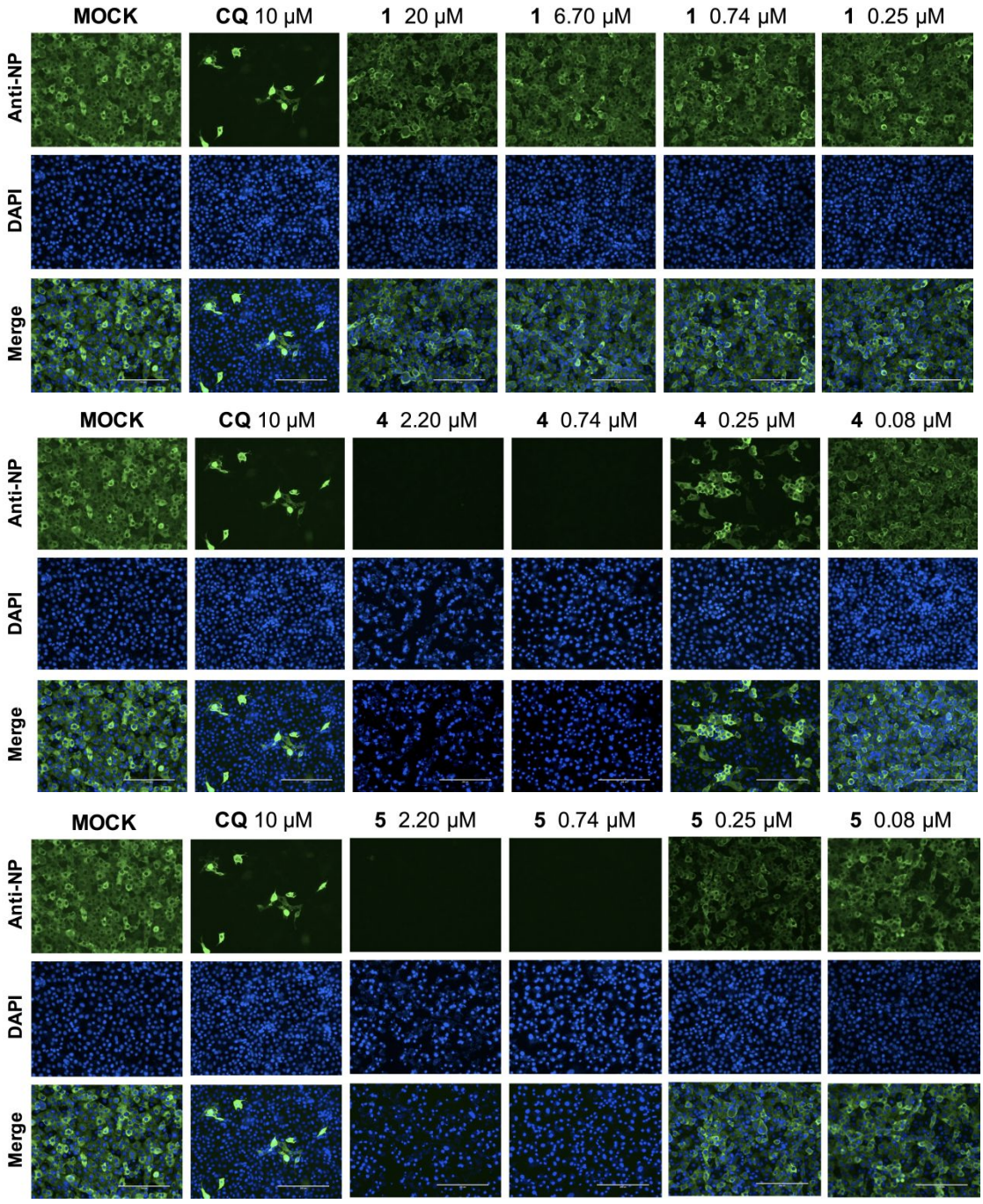


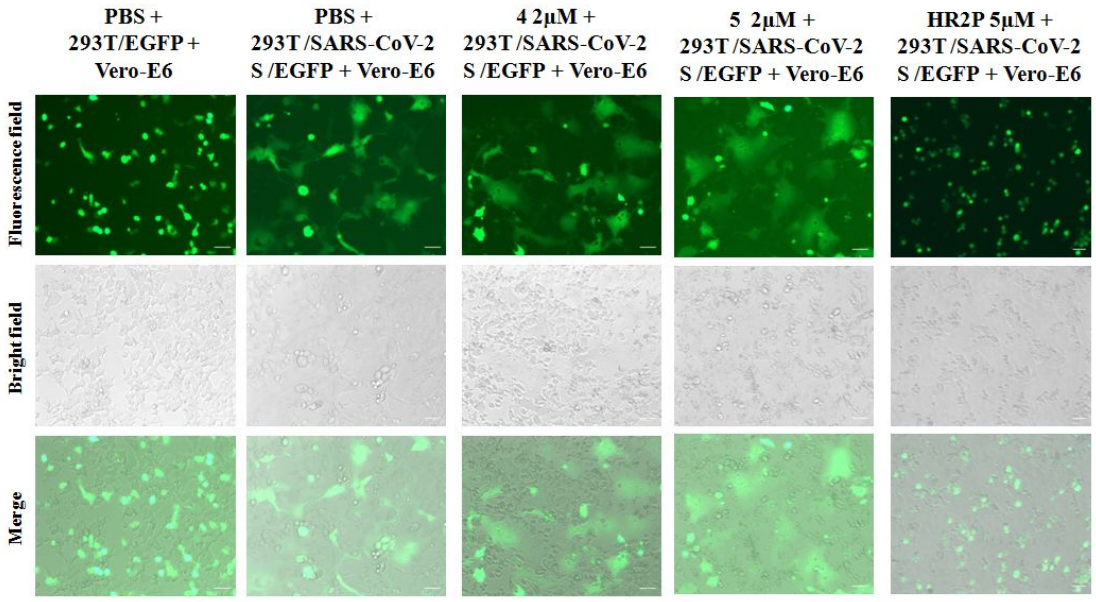
Figure 28S. Immunofluorescence images of SARS-CoV-2 N protein expressions.



Green: SARS-CoV-2 N protein, blue: DAPI. CQ was used as a positive drug control.

scale bars = 200 μm.

Figure 29S. Cell-cell fusion assay results.



Schematic representation of SARS-CoV-2 S protein mediated cell-cell fusion after treatment with 2 μM of compounds **4** and **5** for 24 hours. Representative results were shown from one field selected randomly each sample with scale bars of 100 μm. Peptide HR2P was used as a positive control.

Figure 30S. Molecular docking of **4** and **5** with receptor-binding domain

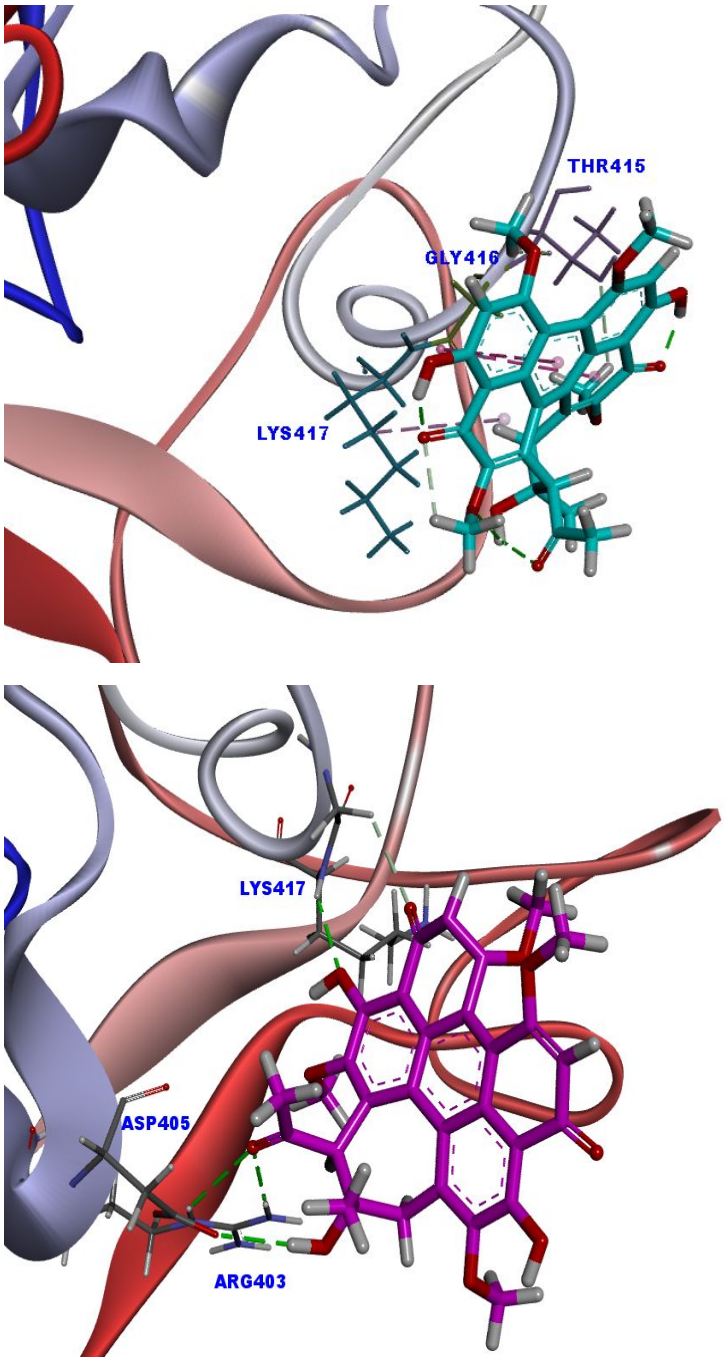
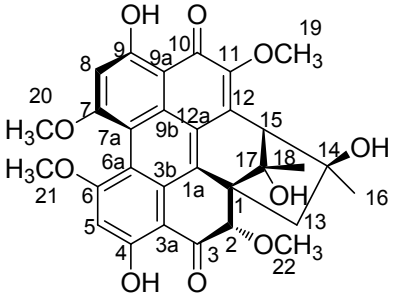


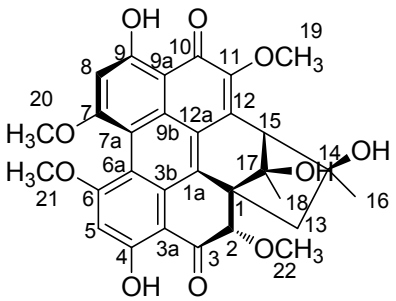
Table 1S NMR spectroscopic data of **2**, **3**, and (*M*)-hypomycin A in $CDCl_3$

	2		**3**		(*M*)-hypomycin[a]	
No.	δ_C, type	δ_H (*J*, Hz)	δ_C, type	δ_H (*J*, Hz)	δ_C, type	δ_H (*J*, Hz)
1	57.7, C		55.4, C		55.6, C	
1a	139.5,C		139.6, C		139.8, C	
2	78.7, CH	4.21, s	81.2, CH	4.56, s	81.3, CH	4.54, s
3	200.0, C		197.2, C		197.4, C	
3a	102.6, C		102.5, C		102.6, C	
3b	128.3, C		127.3, C		127.5, C	
4	164.5, C		164.2, C		164.3, C	
5	100.6, CH	6.71, s	100.3, CH	6.76, s	100.5, CH	6.74, s
6	164.5, C		164.5, C		164.6, C	
6a	115.7, C		115.4, C		115.6, C	
7	165.7, C		165.4, C		165.6, C	
7a	112.5, C		112.1, C		112.3, C	
8	99.5, CH	6.70, s	99.6, CH	6.79, s	99.8, CH	6.77, s
9	170.4, C		169.9, C		170.1, C	
9a	106.9, C		106.7, C		106.9, C	
9b	124.6, C		124.7, C		124.8, C	
10	180.9, C		181.3, C		181.5, C	
11	149.4, C		149.7, C		149.9, C	
12	136.8, C		136.0, C		136.2, C	
12a	123.2, C		121.9, C		122.0, C	
13	43.8, CH_2	2.86, d (13.6)	46.3, CH_2	2.97, d (13.4)	46.5, CH_2	1.84, d (13.6)
		1.65, d (13.6)		1.86, d (13.4)		2.94, d (13.6)
14	77.7, C		80.5, C		80.7, C	
15	59.8, CH	3.72, s	58.2, CH	3.85, s	58.4, CH	3.83, s
16	26.9, CH_3	0.89, s	26.7, CH_3	0.90, s	26.9, CH_3	0.88, s
17	82.2, C		85.9, C		86.1, C	
18	30.5, CH_3	1.90, s	20.9, CH_3	1.27, s	21.1, CH_3	1.25, s
19	61.1, CH_3	4.12, s	61.0, CH_3	4.15, s	61.2, CH_3	4.13, s
20	56.4, CH_3	4.05, s	56.3, CH_3	4.12, s	56.5, CH_3	4.09, s
21	56.4, CH_3	4.05, s	56.4, CH_3	4.10, s	56.6, CH_3	4.07, s
22	60.1, CH_3	3.67, s	60.7, CH_3	3.93, s	60.9, CH_3	3.91, s
OH-4		13.3, s		12.7, s		12.71, s
OH-9		15.4, s		15.4, s		15.19, s

[a] Reported NMR data in the literature [J. Nat. Prod. 2020, 83(8), 2490-2500].



(*P*, 1*R*, 2*S*, 14*S*, 15*R*, 17*R*)-**2**



(*P*, 1*R*, 2*S*, 14*S*, 15*R*, 17*S*)-**3**