

## Supporting Information

# Efficient discovery of potential inhibitors for SARS-CoV-2 3C-like protease from herbal extracts using a native MS- based affinity-selection method

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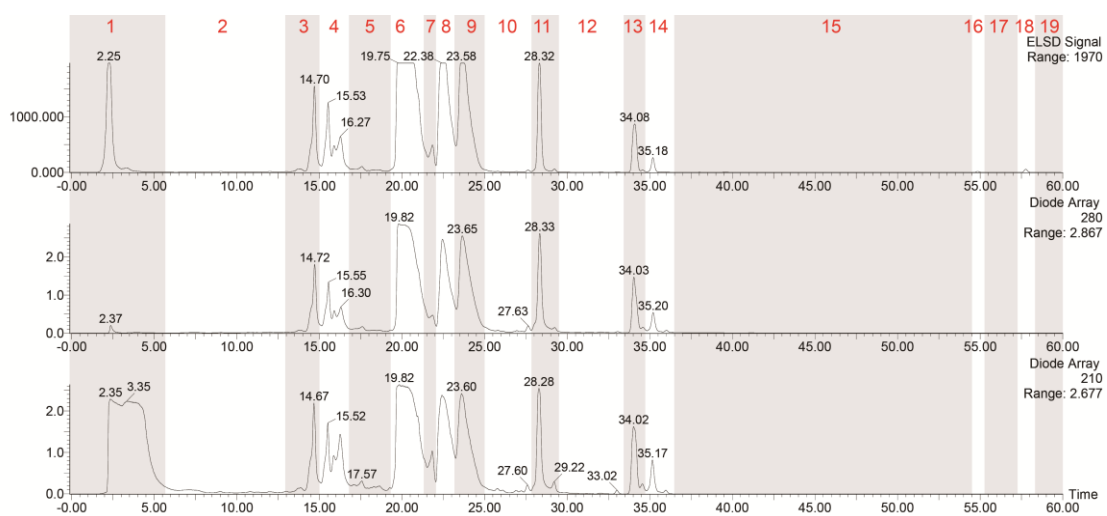


Figure S1. The ELSD and UV response of the crude extracts of *Scutellaria baicalensis*

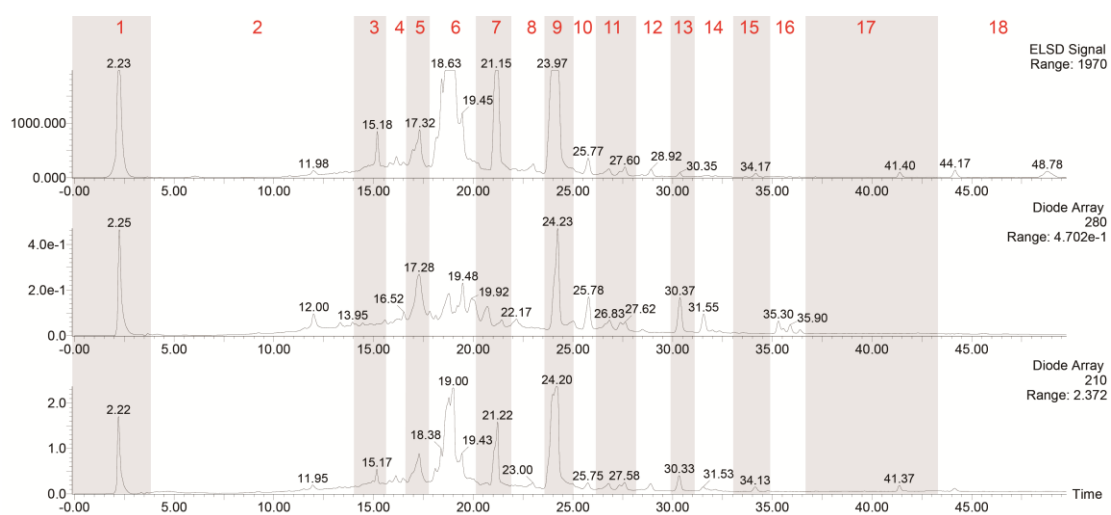


Figure S2. The ELSD and UV response of the crude extracts of *Andrographis paniculata*

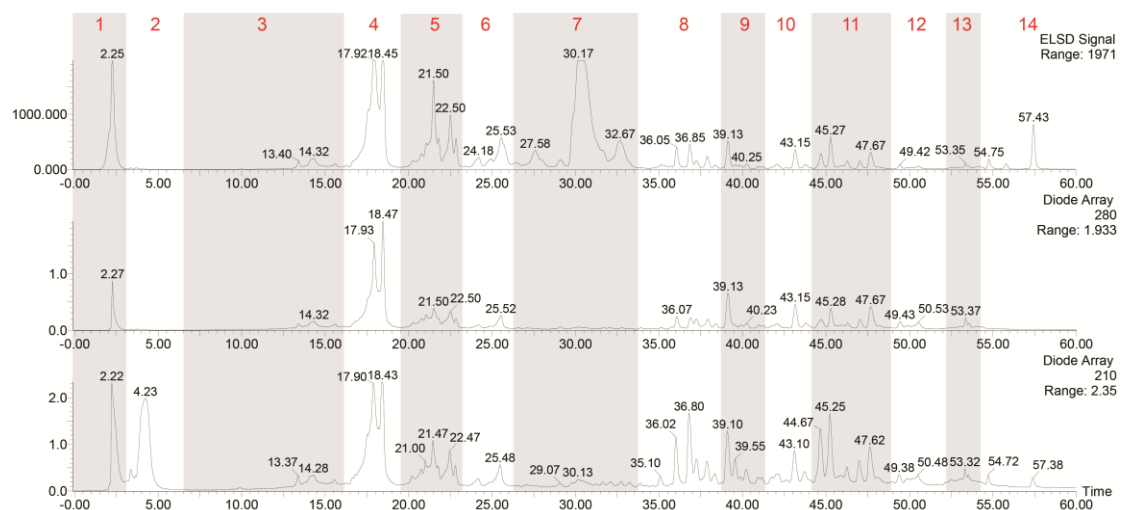


Figure S3. The ELSD and UV response of the crude extracts of *Glycyrrhiza uralensis*

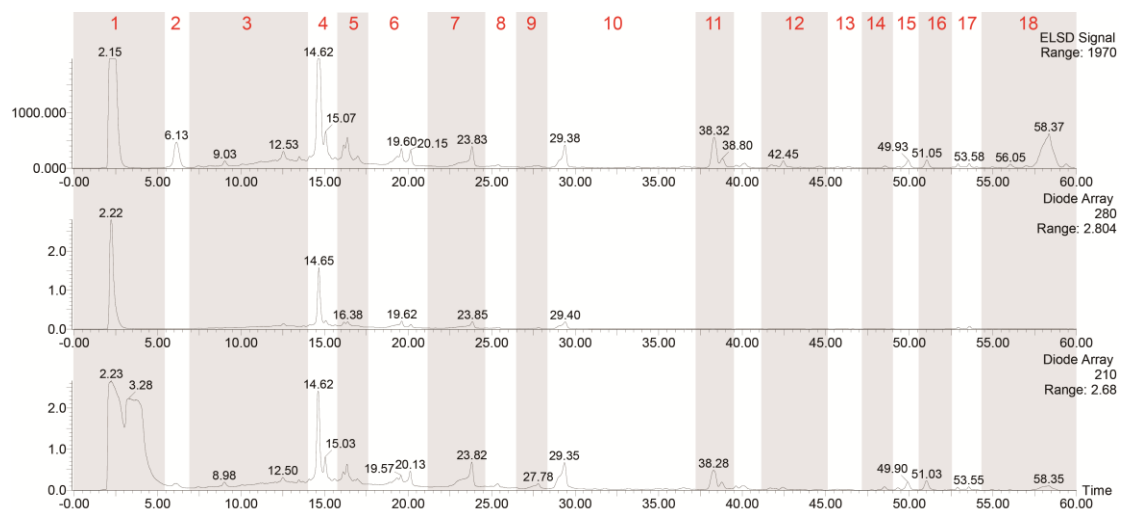


Figure S4. The ELSD and UV response of the crude extracts of *Forsythia suspensa*

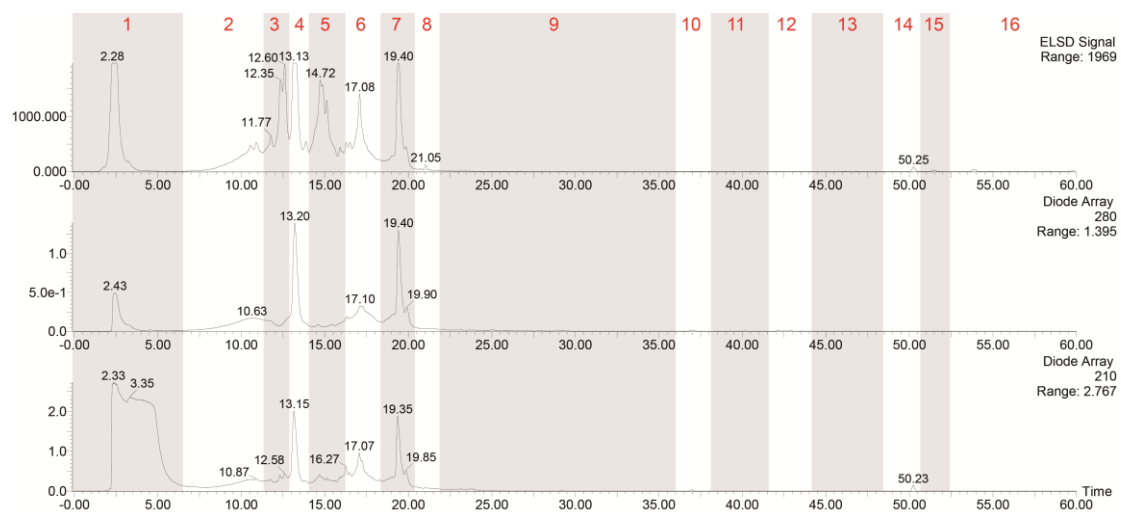


Figure S5. The ELSD and UV response of the crude extracts of *Lonicera japonica*

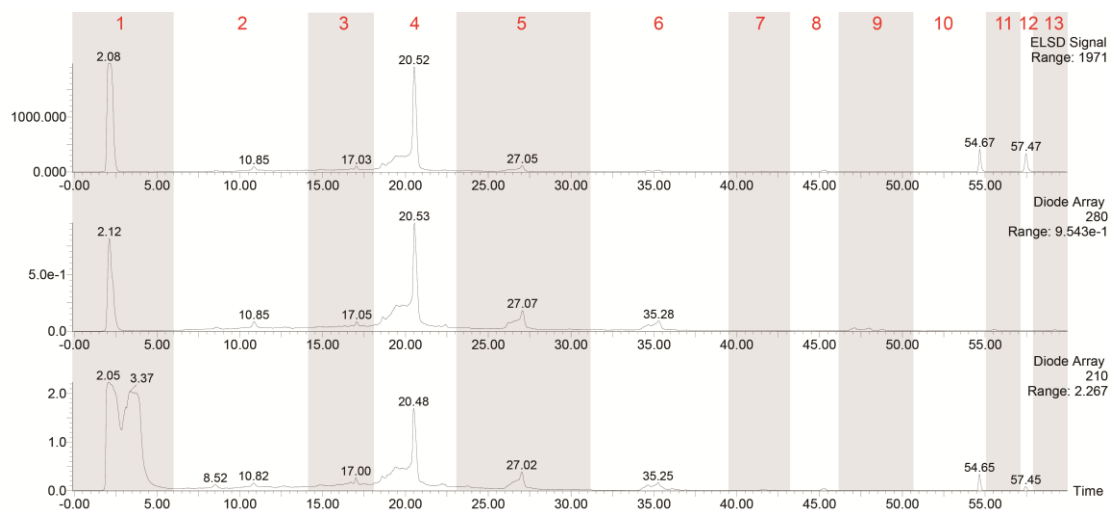


Figure S6. The ELSD and UV response of the crude extracts of *Cirsium japonicum*

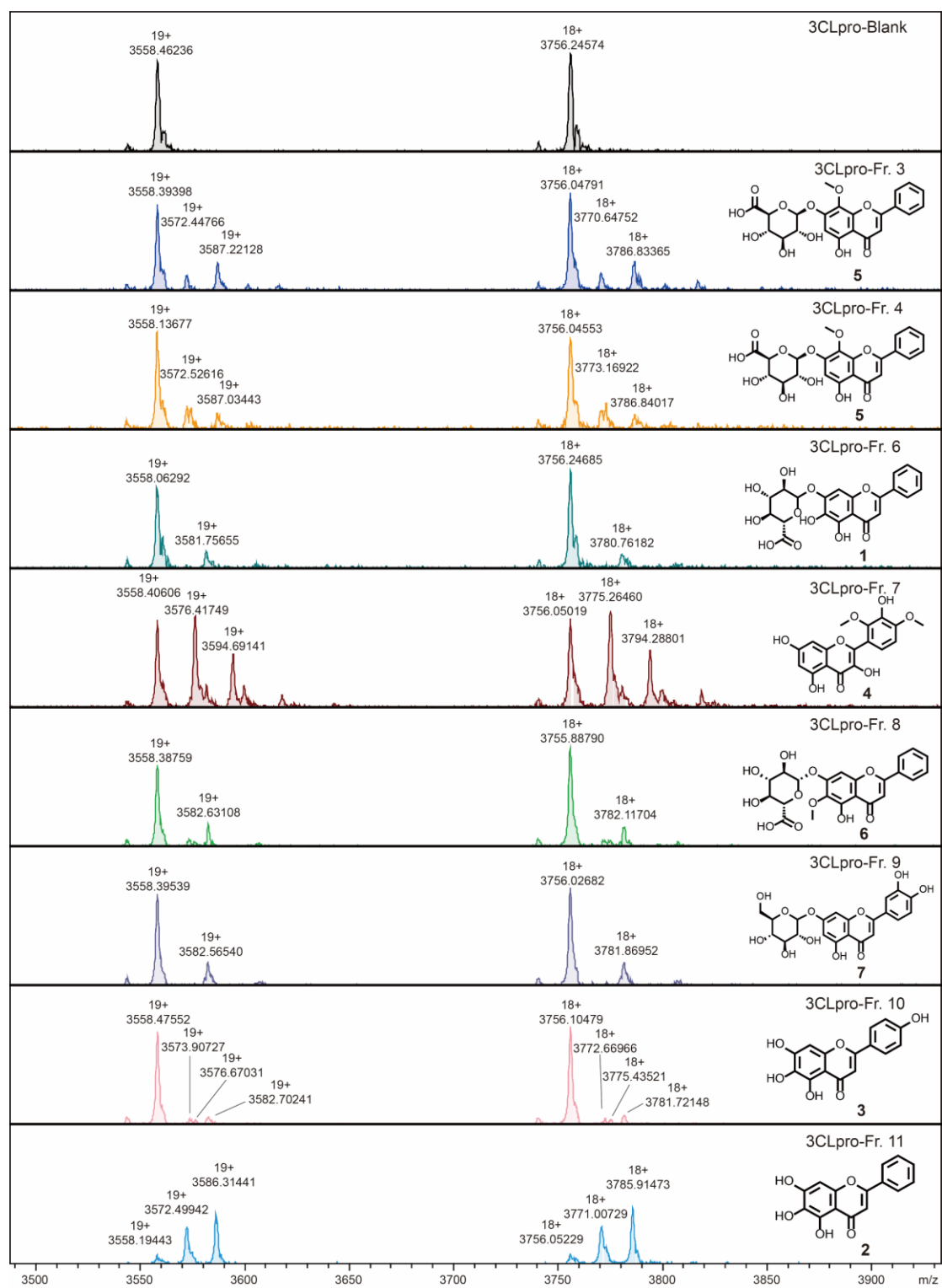


Figure S7. Representative MS spectra of the affinity screening of *Scutellaria baicalensis*

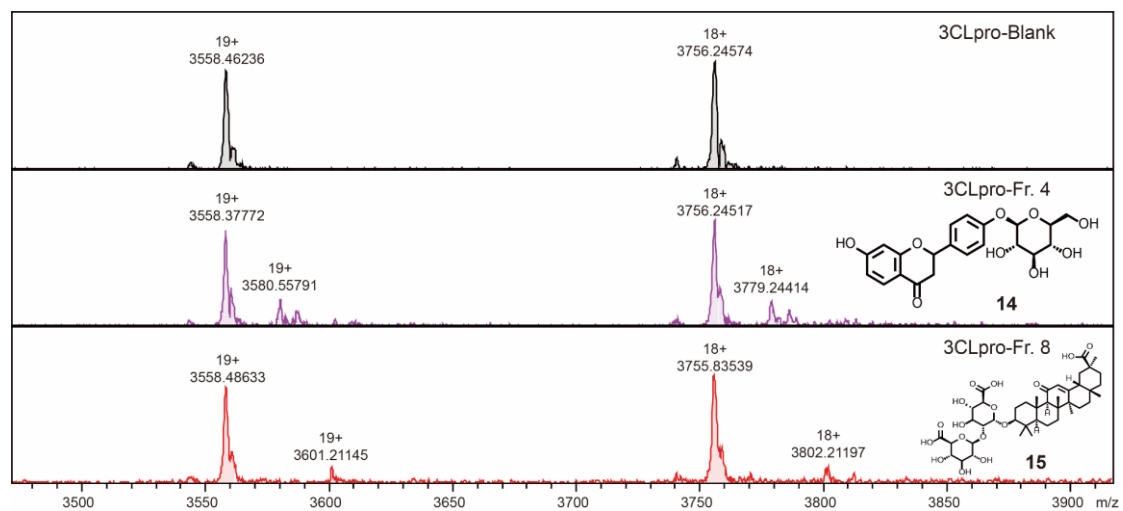


Figure S8. Representative MS spectra of the affinity screening of *Glycyrrhiza uralensis*

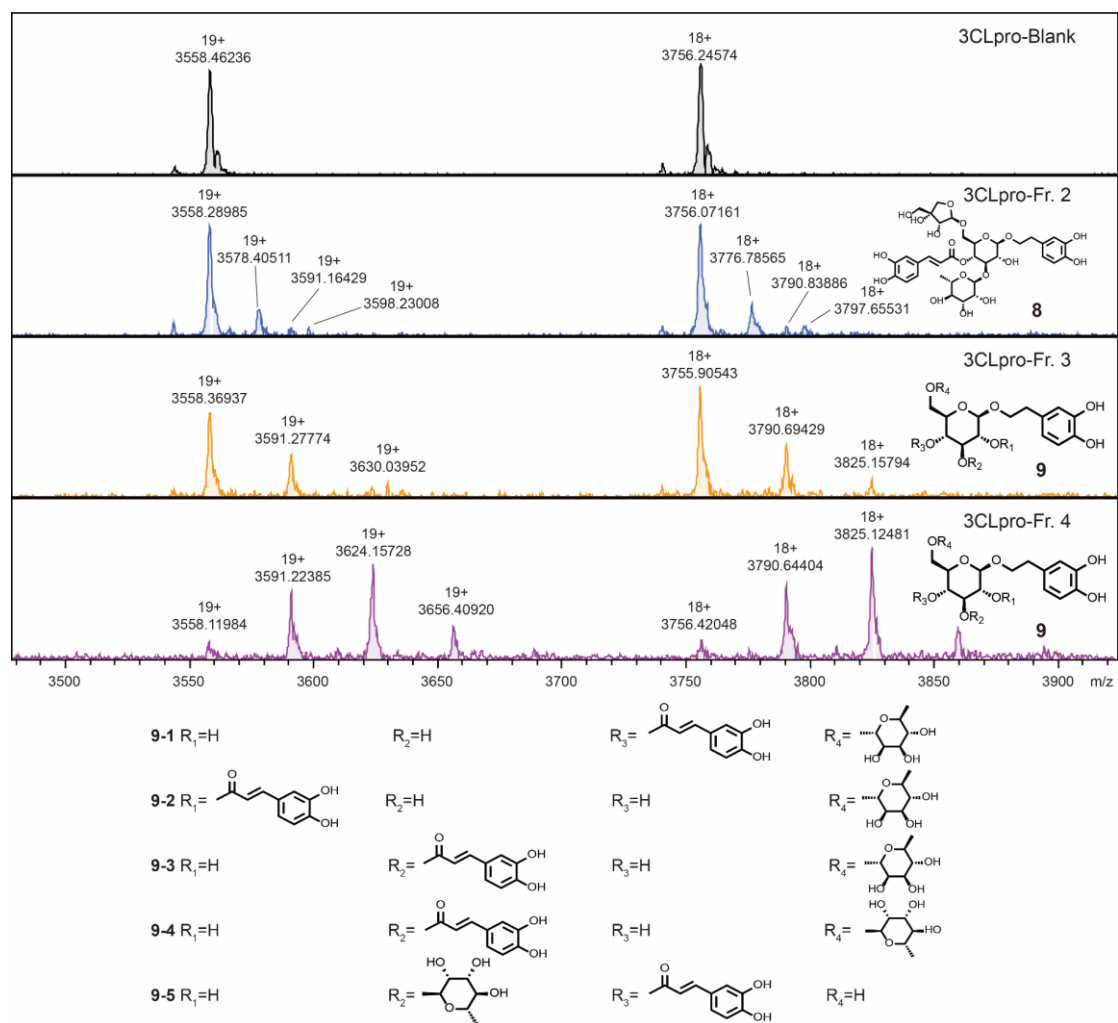


Figure S9. Representative MS spectra of the affinity screening of *Forsythia suspensa*. The compounds in fractions 3 and 4 were isomers or same compound caused by chromatographic peak tail dragging during pre-separation. Their suggested structures are showed at the bottom.



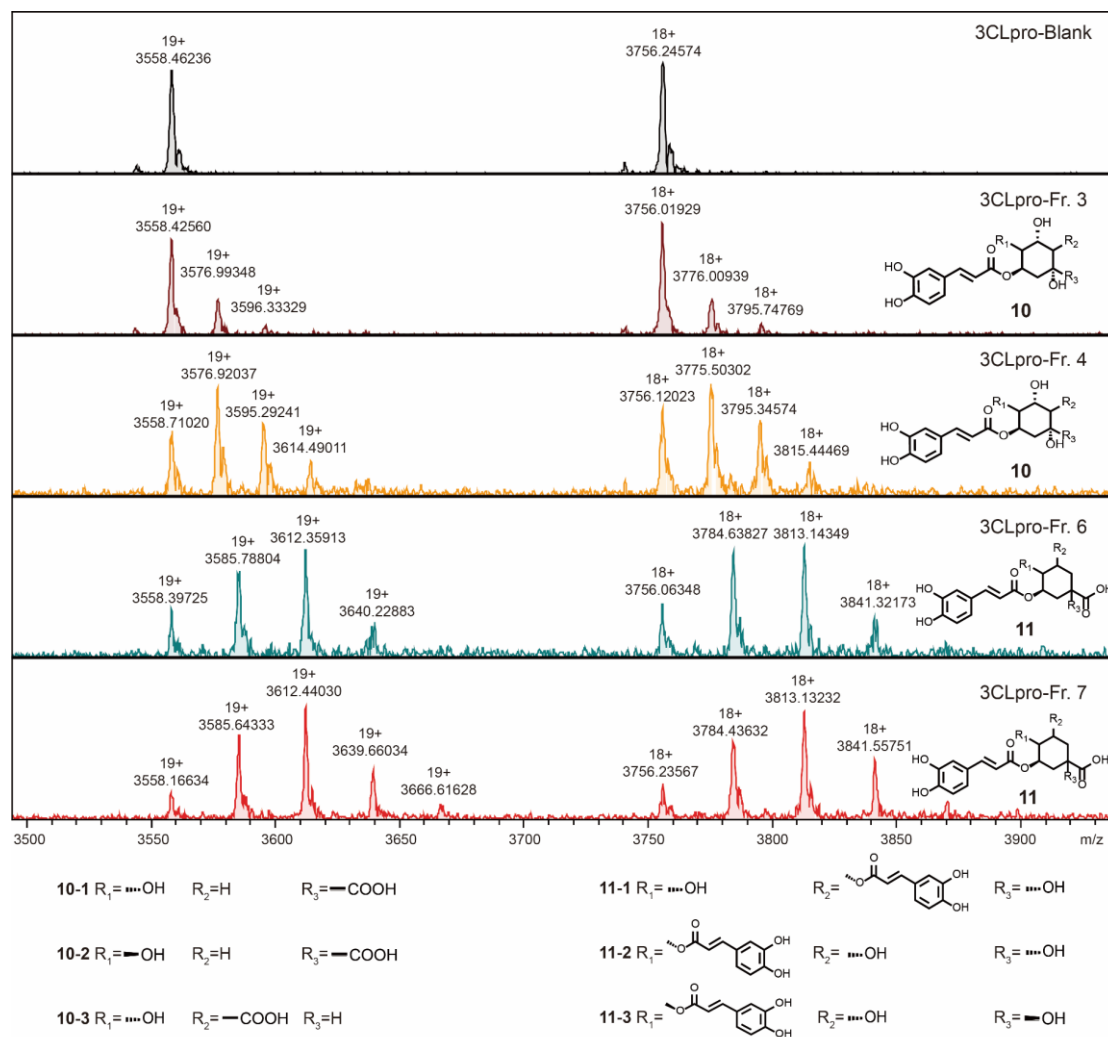


Figure S10. Representative MS spectra of the affinity screening of *Lonicera japonica*. Compound 10 were detected in fractions 3 and 4 and the representative isomers are showed at the bottom. Due to the same reason, the probable isomers of compound 11 which from fractions 6 and 7 are showed at the bottom too.

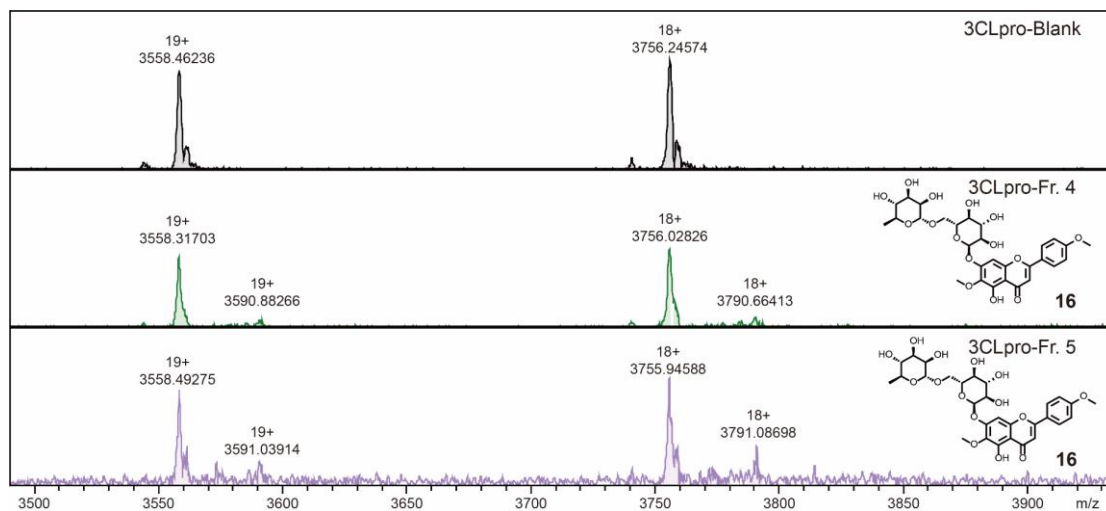


Figure S11. Representative MS spectra of the affinity screening of *Cirsium japonicum*

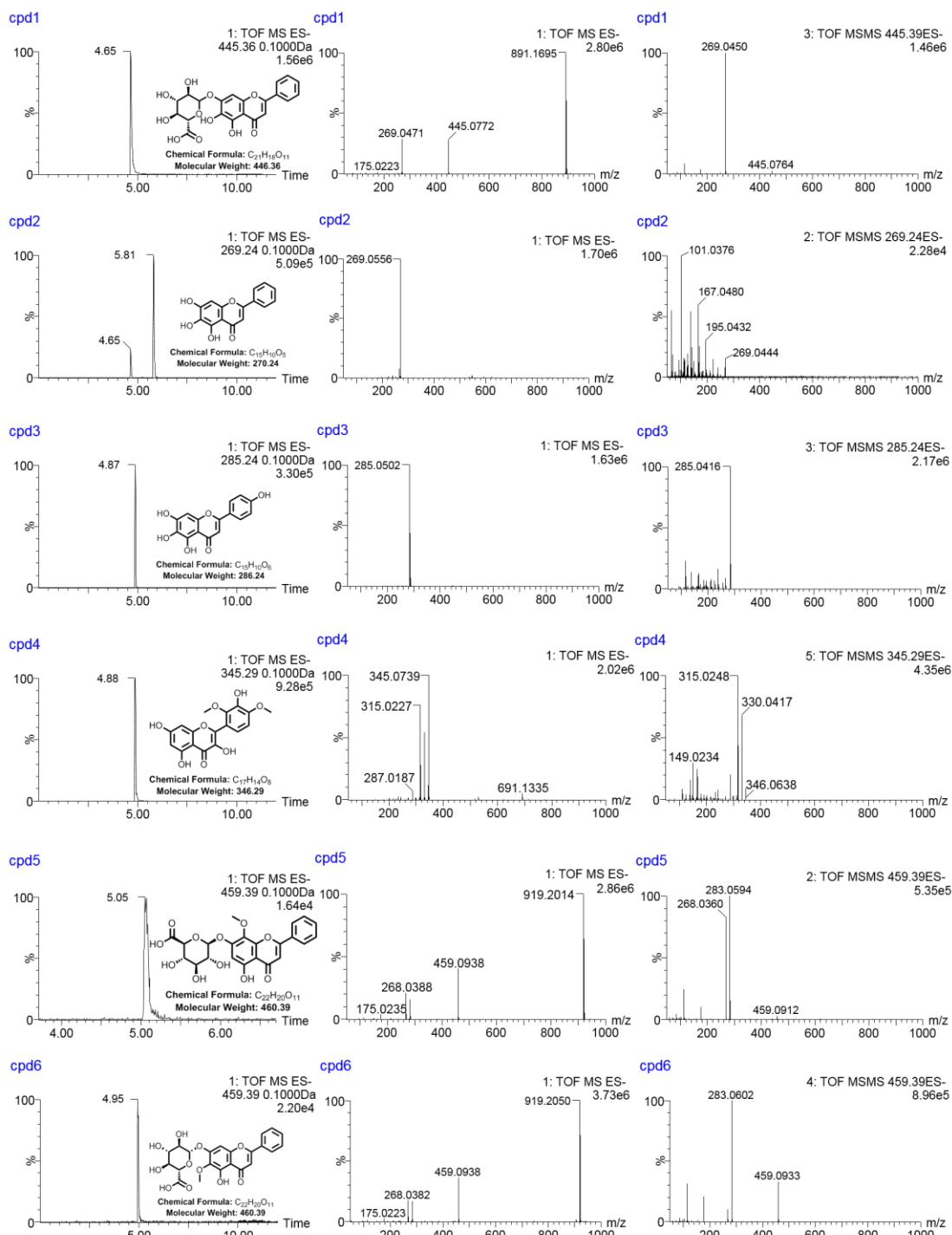


Figure S12. The chromatograms and mass spectra of the hit compounds

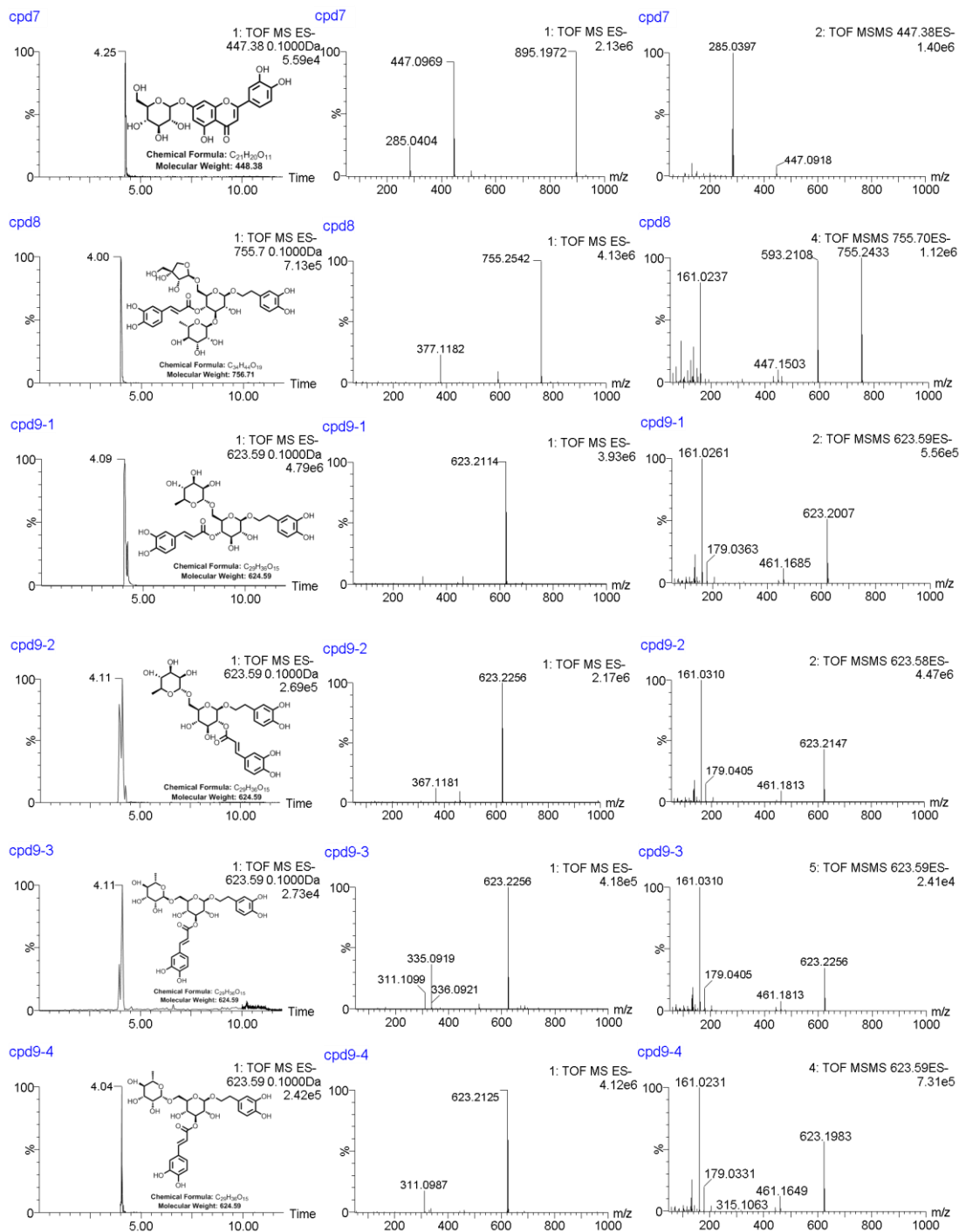


Figure S12. (continued)

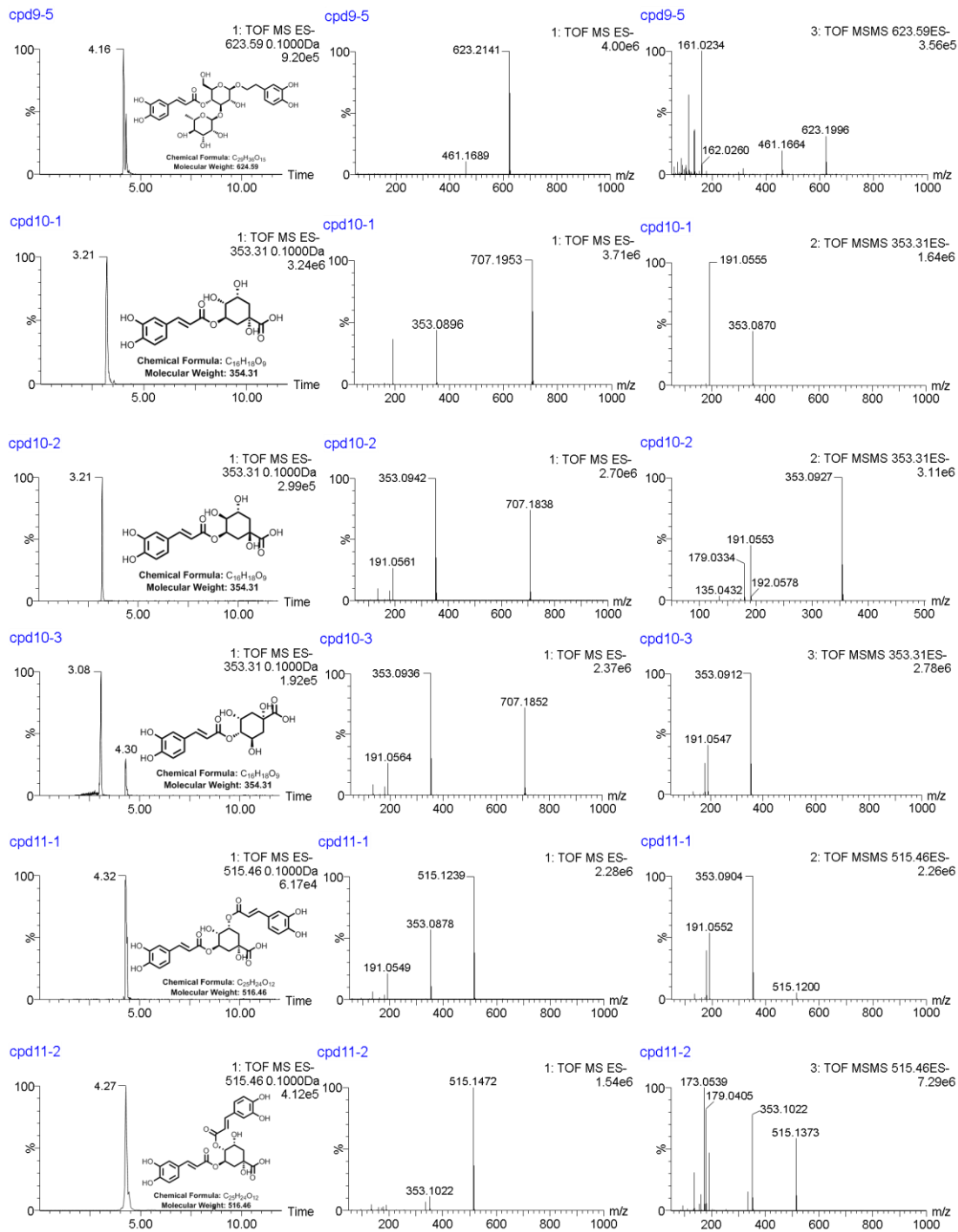


Figure S12. (continued)

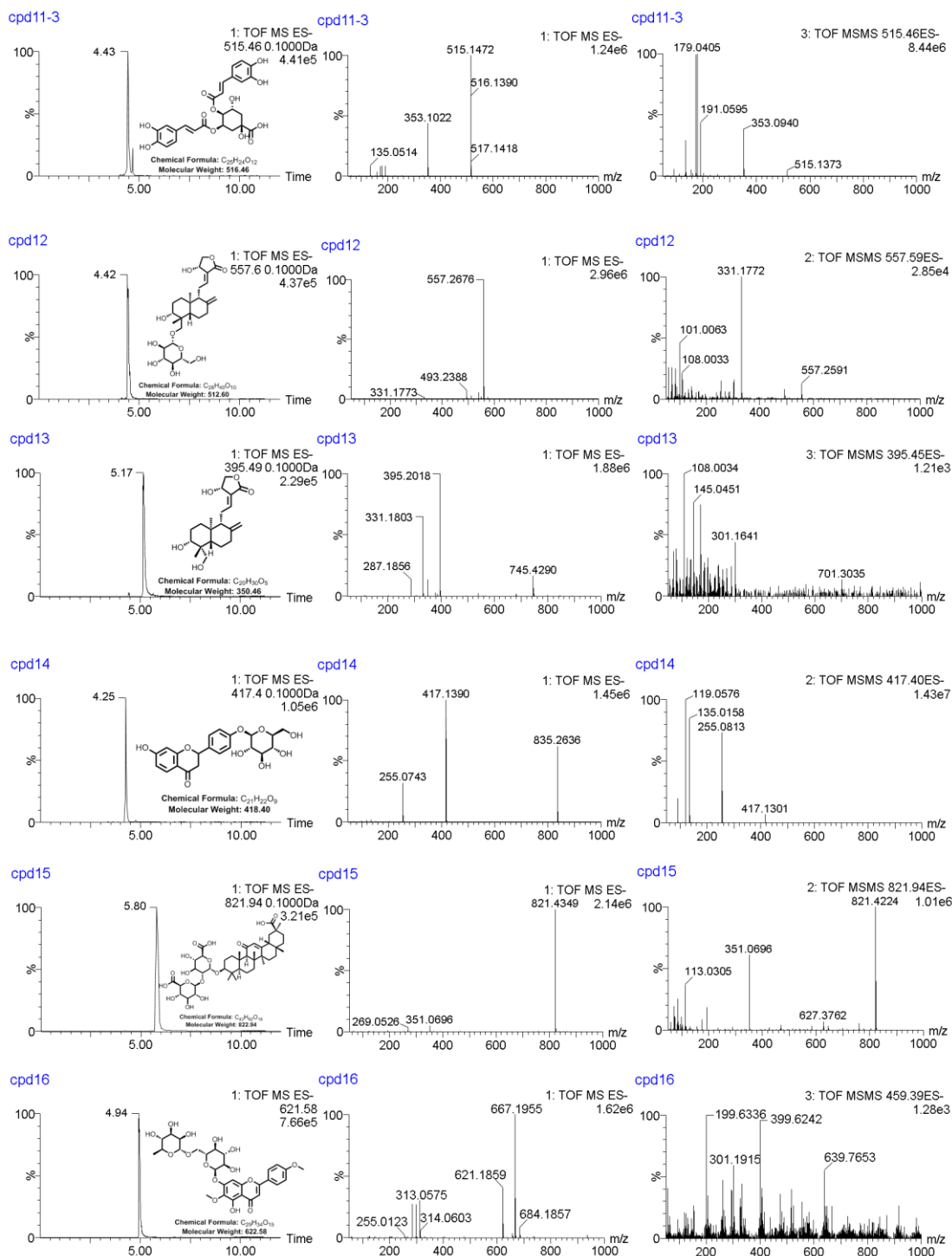


Figure S12. (continued)

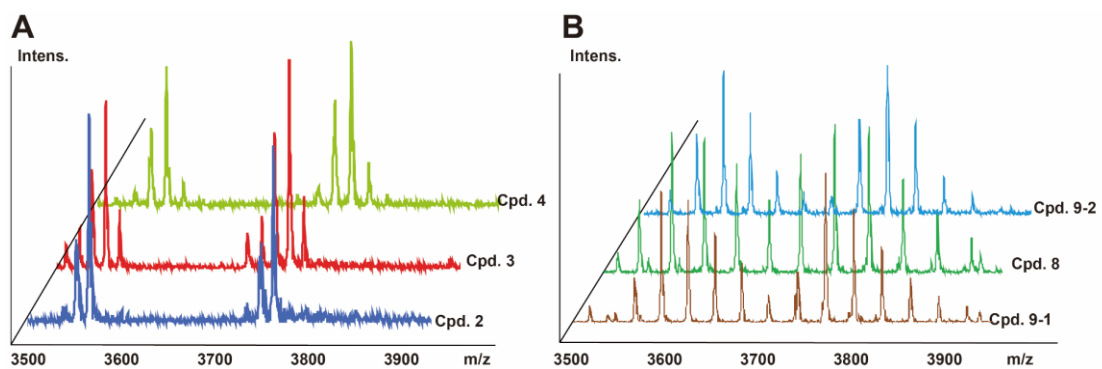


Figure S13. MS spectra of 3CLpro incubated with the representative compounds at the same concentration. (A) Representative MS spectra of 3CLpro incubated with compound 2, 3 and 4 at the concentration of 12.5  $\mu\text{M}$ . (B) Representative MS spectra of 3CLpro incubated with compound 8 or 9 at the concentration of 12.5  $\mu\text{M}$ .

Table S1. The weights of the 96 fractions after lyophilization

<i>Scutellaria baicalensis</i>			<i>Andrographis paniculata</i>		
Fraction ID	RT window (min)	Weight (mg)	Fraction ID	RT window (min)	Weight (mg)
1	0.0-5.6	3.1	1	0.0-3.8	19.5
2	5.6-13.0	5.2	2	3.8-14.0	12.1
3	13.0-15.0	10.1	3	14.0-15.7	5.6
4	15.0-16.8	8.1	4	15.7-16.1	4.1
5	16.8-19.4	3.0	5	16.1-17.8	4.0
6	19.4-21.3	65.5	6	17.8-20.1	33.1
7	21.3-22.0	4.5	7	20.1-21.9	13.8
8	22.0-23.2	16.1	8	21.9-23.6	5.2
9	23.2-25.0	20.1	9	23.6-25.1	21.6
10	25.0-27.8	3.1	10	25.1-26.2	2.5
11	27.8-29.5	5.2	11	26.2-28.2	1.9
12	29.5-33.4	0.6	12	28.2-29.9	1.2
13	33.4-34.7	4.3	13	29.9-31.2	0.4
14	34.7-36.5	1.7	14	31.2-33.1	0.5
15	36.5-54.5	1.5	15	33.1-34.8	1.1
16	54.5-55.4	0.6	16	34.8-36.7	0.4
17	55.4-57.3	0.7	17	36.7-43.7	2.1
18	57.3-58.4	0.1	18	43.7-60.0	5.2
19	58.4-60.0	0.3			

<i>Glycyrrhiza uralensis</i>			<i>Forsythia suspensa</i>		
Fraction ID	RT window (min)	Weight (mg)	Fraction ID	RT window (min)	Weight (mg)
1	0.0-3.2	14.8	1	0.0-5.4	71.3
2	3.2-6.6	1.5	2	5.4-6.9	15.4
3	6.6-16.2	11.2	3	6.9-13.9	19.3
4	16.2-19.6	28.7	4	13.9-15.7	18.0
5	19.6-23.3	20.6	5	15.7-17.1	12.3
6	23.3-26.3	9.5	6	17.1-21.2	11.7
7	26.3-29.5	8.9	7	21.2-24.6	7.3
8	29.5-33.7	37.4	8	24.6-26.5	3.5
9	33.7-38.8	10.2	9	26.5-28.4	2.0
10	38.8-41.4	4.3	10	28.4-30.5	3.9
11	41.4-44.1	58.1	11	30.5-37.2	6.0
12	44.1-49.0	8.1	12	37.2-39.5	4.5
13	49.0-52.2	2.5	13	39.5-41.2	1.1
14	52.2-54.4	3.8	14	41.2-45.2	4.2
15	54.4-60.0	3.0	15	45.2-47.1	1.6
			16	47.1-49.1	2.0
			17	49.1-50.6	0.6



18	50.6-52.7	1.3
19	52.7-54.4	3.8
20	54.4-60.0	8.8

<i>Lonicera japonica</i>			<i>Cirsium japonicum</i>		
Fraction ID	RT window (min)	Weight (mg)	Fraction ID	RT window (min)	Weight (mg)
1	0.0-6.5	46.3	1	0.0-6.1	13.1
2	6.5-11.3	20.2	2	6.1-14.2	8.3
3	11.3-12.8	23.6	3	14.2-18.1	8.4
4	12.8-14.0	21.0	4	18.1-23.1	16.4
5	14.0-16.2	29.5	5	23.1-31.2	6.0
6	16.2-18.3	17.9	6	31.2-39.5	4.3
7	18.3-20.4	13.2	7	39.5-43.4	1.5
8	20.4-21.8	1.3	8	43.4-46.2	0.6
9	21.8-36.0	5.1	9	46.2-50.7	2.1
10	36.0-38.1	0.9	10	50.7-55.1	2.4
11	38.1-41.5	0.4	11	55.1-57.2	0.3
12	41.5-44.2	0.4	12	57.2-57.9	2.5
13	44.2-48.5	0.4	13	57.9-60.0	1.1
14	48.5-50.7	1.0			
15	50.7-53.0	0.2			
16	53.0-60.0	0.9			