Supplemental Information

High-throughput screening of triplex DNA binders from complicated samples by 96-well pate format in conjunction with peak area-fading UHPLC-Orbitrap MS

Hongmei Yang,[a,b] Wenbin Yao,[a] Yihan Wang,[a] Lei Shi,[d] Niusheng Xu,[b] Debin Wan,[c] Rui Su,[a] Wenhui Lian,[a] Changbao Chen,[*a] and Shuying Liu[*a,b]

[a]Changchun University of Chinese Medicine, Changchun 130117, China

[b]Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

[c]Department of Entomology and Comprehensive Cancer Center, University of California, Davis, CA 95616, United States

[d]High Temperature Reactor Holdings Co., Ltd., China Nuclear Engineering Group Co., Beijing 100037, China.

* Corresponding author. E-mail: ccb2021@126.com, syliu@ciac.ac.cn

Keywords: 96-Well plate; Ultra-high-performance liquid chromatography mass spectrometry; Triplex DNA; Binder; Peak area-fading

UHPLC-MS Analysis. The ultra-performance liquid chromatography was performed on an UltiMate 3000 system (Dionex, Sunnyvale, CA, USA). Analytes were separated by a Waters C18 column (100 mm × 2.1 mm i.d., 1.7 μm, Waters, Ireland) at a flow rate of 0.25 mL/min. The gradient solvent system consisted of solvent A (acetonitrile) and solvent B (0.5% acetic acid in water, v/v). For the test mixture separation, the solvent gradient was started with 22% A at injection time and increased linearly to 24% A in 4 min, where it was held for 4 min, further to 30% A over 2 min, followed by a gradient to 90% A in 2 minutes, where it was held for 2 min before it returned to the initial stating condition. In order to separate the extracts of *Rhizoma Coptidis* and *Phellodendron chinense Schneid cortexes*, the solvent A content of the mobile phase was increased from 22% to 24% in 4 min, where it was held for 4 min, and then increased to 30% in the next 2 min before it returned to the initial stating condition.

Mass spectrometric detection was carried out on a Q Exactive Orbitrap mass spectrometer (Thermo, San Jose, CA) equipped with an ESI source in positive ion mode. Nitrogen was supplied as the sheath gas and auxiliary gases at flows of 0.6 and 3 L/min, respectively. Nitrogen was also used as collision gas, and the normalized collision energies were optimized to 45~55%. The capillary temperature was set at 350 °C for all the experiments. The optimized spray voltage and S-lens voltage were 3600 and 55 V, respectively.

Figure legends

Fig. S1 UV absorbance of the used triplex DNA varied as a function of temperature at 260 nm in 20 mM NH_4AC solution (pH 5.5).

Fig. S2 The MS/MS of the standard compounds for the previously known and newly identified triplex binders. (a) columbamine, (b) epiberberine, (c) jatrorrhizine, (d) coptisine, (e) palmatine, (f) berberine, and (g) berberrubine.

Fig. S3 The mass spectra of complexes formed by the triplex DNA with (a) columbamine, (b) palmatine, (c) epiberberine, (d) coptisine, (e) jatrorrhizine, and (f) berberine at the molar ratio of 1:2.

Fig. S1

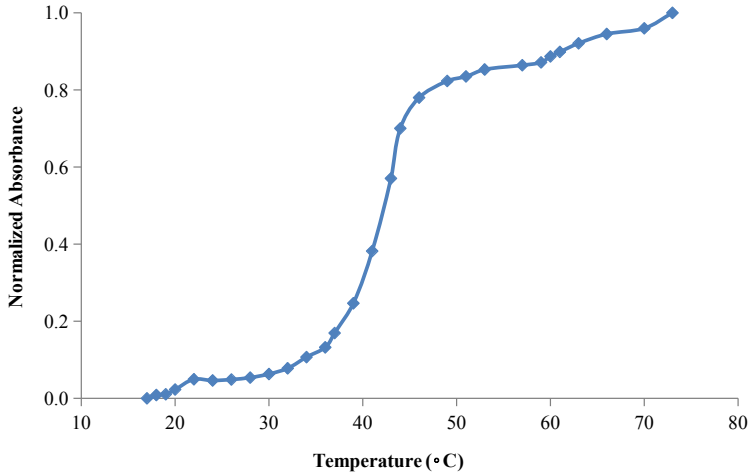
Normalized Absorbance
1.0
0.8
0.6
0.4
0.2
0.0
10
20
30
40
50
60
70
80
Temperature (∘C)

Fig. S2

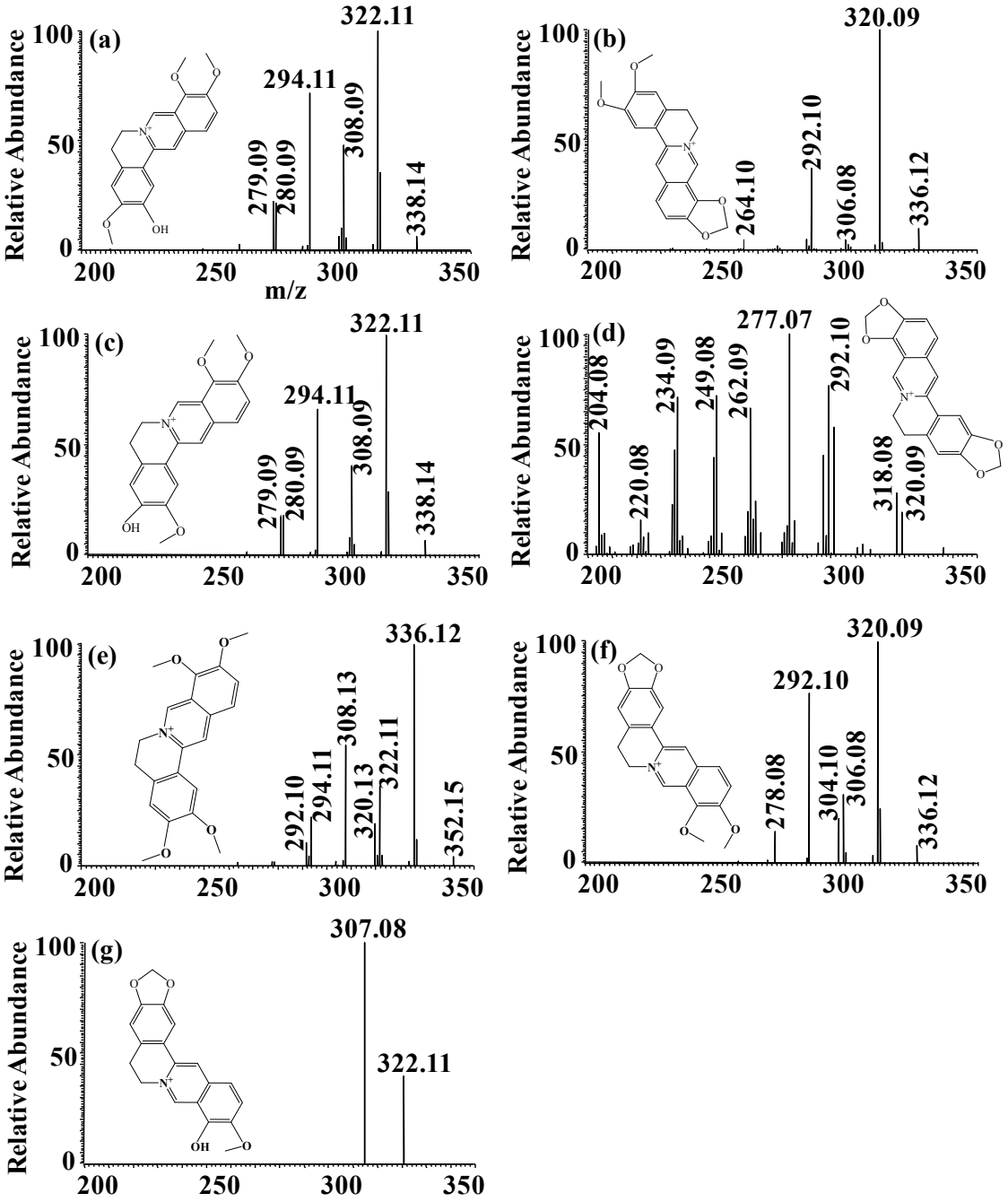
(a)
322.11
294.11
308.09
279.09
280.09
338.14
(b)
320.09
292.10
264.10
306.08
336.12
(c)
322.11
294.11
308.09
279.09
280.09
338.14
(d)
277.07
292.10
204.08
234.09
249.08
262.09
220.08
318.08
320.09
(e)
336.12
308.13
294.11
292.10
320.13
322.11
352.15
(f)
320.09
292.10
278.08
304.10
306.08
336.12
(g)
307.08
322.11
Relative Abundance
m/z

Fig. S3

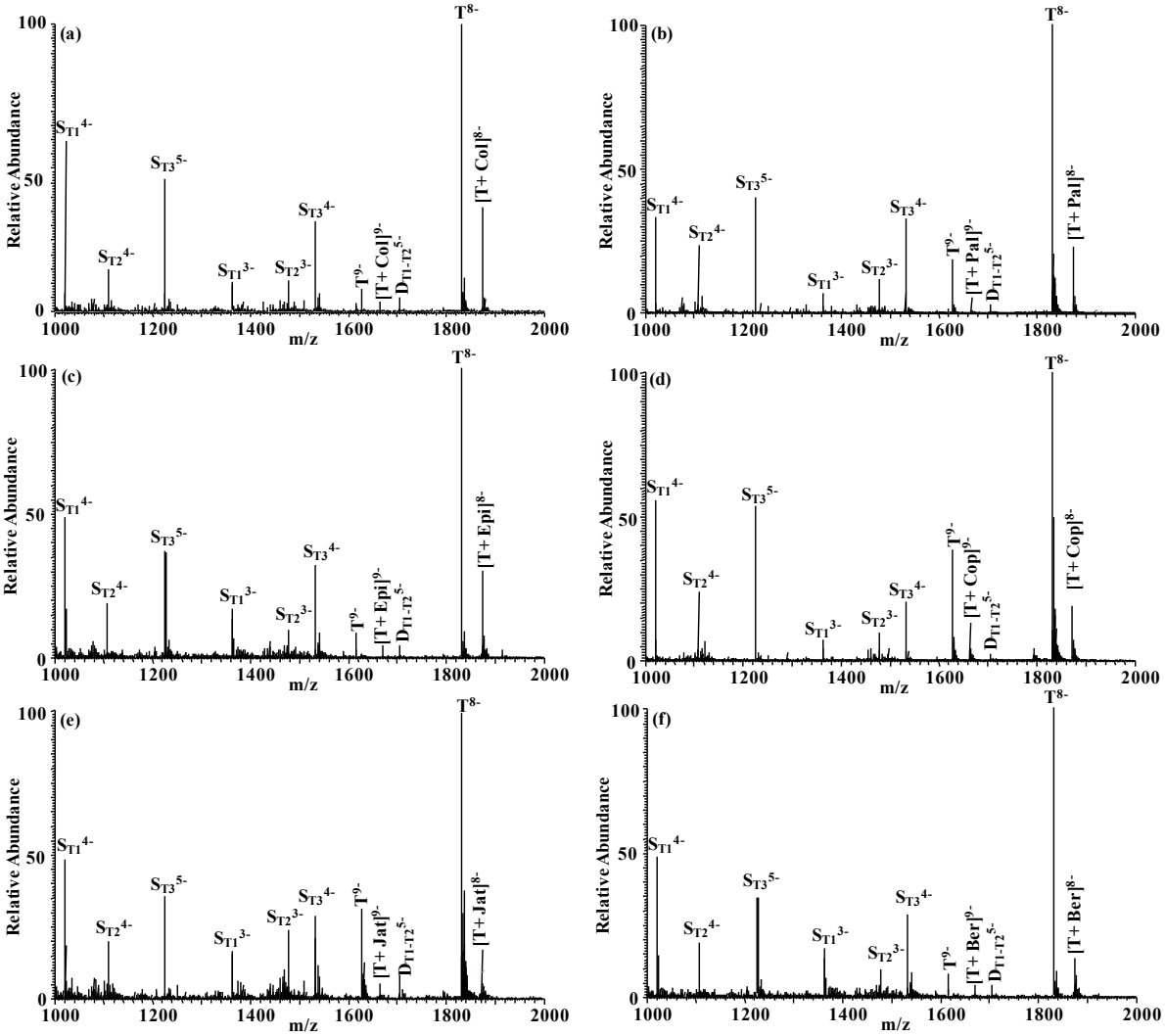
(a)
(b)
(c)
(d)
(e)
(f)
Relative Abundance
m/z
100
50
0
1000
1200
1400
1600
1800
2000
T^{8-}
S_{T1}^{4-}
S_{T2}^{4-}
S_{T3}^{5-}
S_{T1}^{3-}
S_{T2}^{3-}
S_{T3}^{4-}
T^{9-}
$D_{T1\text{-}T2}^{5-}$
[T+ Col]$^{8-}$
[T+ Col]$^{9-}$
[T+ Pal]$^{8-}$
[T+ Pal]$^{9-}$
[T+ Epi]$^{8-}$
[T+ Epi]$^{9-}$
[T+ Cop]$^{8-}$
[T+ Cop]$^{9-}$
[T+ Jat]$^{8-}$
[T+ Jat]$^{9-}$
[T+ Ber]$^{8-}$
[T+ Ber]$^{9-}$

Table S1 Binding degrees of the six compounds to the target triple DNA by 96-well plate format in conjunction with UHPLC-MS.

Compounds	Binding degree (%, ±SD, n=3)
vitexin	16.27 ±1.74
EB	11.24 ± 0.91
palmatine	22.67 ± 1.85
berberine	15.91± 1.68
coralyne	78.95 ± 1.36
formononetin	1.06 ± 0.72

The concentration of each compound was calculated according to the corresponding standard calibration curve described as follows: A = 274554C + 119566 (R^2 = 0.9995) for vitexin, A = 1237260C + 4389110 (R^2 = 0.9991) for EB, A = 1736820C + 2818590 (R^2 =0.9994) for palmatine, A = 71899830C + 6855060 (R^2 = 0.9998) for berberine, A = 1919850C + 6208250 (R^2 = 0.9998) for coralyne, A = 224152C + 219680 (R^2 = 0.9993) for formononetin. Here, C denotes the concentration of ligands, and A signifies the corresponding peak area.

Table S2 The observed and calculated *m/z* values of fragment ions for each of the identified putative binders.

identified putative	Molecular formula	Observed *m/z*	Calculated	Δppm
columbamine	$C_{19}H_{16}NO_4$	322.1064	322.1079	5
	$C_{18}H_{14}NO_4$	308.0915	308.0923	3
	$C_{18}H_{16}NO_3$	294.1126	294.1130	1
	$C_{17}H_{14}NO_3$	280.0979	280.0974	2
	$C_{17}H_{13}NO_3$	279.0884	279.0895	4
epiberberine	$C_{19}H_{14}NO_4$	320.0918	320.0923	2
	$C_{18}H_{12}NO_4$	306.0753	306.0766	4
	$C_{18}H_{14}NO_3$	292.0967	292.0974	2
	$C_{17}H_{14}NO_2$	264.1014	264.1024	4
jatrorrhizine	$C_{19}H_{16}O_4N$	322.1075	322.1079	1
	$C_{18}H_{14}O_4N$	308.0912	308.0923	4
	$C_{18}H_{16}O_3N$	294.1125	294.1130	2
	$C_{17}H_{14}O_3N$	280.0963	280.0974	4
	$C_{17}H_{13}O_3N$	279.0911	279.0895	6
coptisine	$C_{19}H_{12}NO_4$	318.0771	318.0766	2
	$C_{18}H_{14}NO_3$	292.0953	292.0974	7
	$C_{17}H_{11}NO_3$	277.0735	277.0739	1
	$C_{17}H_{12}NO_2$	262.0863	262.0868	2
	$C_{16}H_{11}NO_2$	249.0801	249.0790	4
	$C_{16}H_{12}NO$	234.0914	234.0919	2
	$C_{15}H_{10}NO$	220.0775	220.0762	6
	$C_{15}H_{10}N$	204.0822	204.0813	4
palmatine	$C_{20}H_{18}NO_4$	336.1243	336.1236	2
	$C_{19}H_{16}NO_4$	322.1051	322.1079	9
	$C_{20}H_{18}NO_3$	320.1293	320.1287	2
	$C_{19}H_{18}NO_3$	308.1299	308.1287	4
	$C_{18}H_{16}O_3$	294.1148	294.1130	6
	$C_{18}H_{14}O_3$	292.0989	292.0974	5
berberine	$C_{19}H_{14}NO4$	320.0919	320.0923	2
	$C_{18}H_{12}NO_4$	306.0761	306.0766	2
	$C_{19}H_{14}NO_3$	304.0969	304.0974	2
	$C_{18}H_{14}NO_3$	292.0969	292.0974	2
	$C_{17}H_{12}NO_3$	278.0812	278.0817	2
berberrubine	$C_{18}H_{13}NO_4$	307.0859	307.0844	5

Table S3 Binding degrees of the six components in the extract of Rhizoma Coptidis by direct infusion ESI-MS method.

Compounds	Binding degree (%, ±SD, n=3)
columbamine	26.12±1.83
palmatine	17.20 ± 1.51
epiberberine	17.70 ± 1.76
coptisine	11.41± 1.28
jatrorrhizine	11.80 ± 1.29
berberine	7.95 ± 0.66