Supporting Information for Manuscript

Efficient ligand discovery from natural herbs by integrating virtual screen, affinity mass spectrometry and targeted metabolomics

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Content of Supporting Information

Methods for Compound isolation from TCM herbs method

Air-dried, powdered seeds of *Piper Nigrum* (1.0 kg) were extracted with 95% alcohol (3×5 L) at room temperature, each for 24 h. After removal of the organic solvent, the sample was suspended in H_2O and sequentially extracted with petroleum ether and EtOAc. The EtOAc layer was concentrated, subjected to silica gel CC and eluted with petroleum/EtOAc mixtures of increasing polarity (from 10/1 to 5/1) to yield fractions A-H. Fraction F was further separated on a C18 column eluted with MeOH-H_2O (from 3/2 to 1:0) to afford twelve fractions (F1-F12). F6 was subjected to semipreparative HPLC (YMC 5 μm, 250×10 mm, MeOH-H2O 23/2, 2.5 mL/min), HJ-2 (24.6 mg) eluted at 10.8 min, HJ-4 (4.2 mg) eluted at 11.5 min. F8 was separated on the semipreparative HPLC (YMC 5 μm, 250×10 mm, MeOH-H2O 23/2, 2.5 mL/min), HJ-6 (12.5 mg) eluted at 15.3 min. Fraction E was subjected to a C18 column eluted with MeOH-H2O (from 7/3 to 1:0) to afford seven fractions (E1-E7). E5 was subjected to semipreparative HPLC (YMC 5 μm, 250×10 mm, MeCN-H2O 17/3, 2.5 mL/min), HJ-1 (11.4 mg) eluted at 14.9 min. E6 was subjected to semipreparative HPLC (YMC 5 μm, 250×10 mm, MeCN-H2O 17/3, 2.5 mL/min), HJ-3 (3.2 mg) eluted at 15.8 min. HJ-1 to HJ-4 and HJ-6 were identified as pipernonaline, 1-[1- ketone -9 (3,4- methylene two oxy phenyl) -2E, 8E- nonadienyl] piperidine, piperamide-C9:3(2E,4E,8E), piperolein-B, brachyamide B piperamide-C9:2(2E,8E), brachyamide B piperamide-C9:1(8E) by the NMR data which were consistent with that of reported[1-3].

Air-dried, powdered aerial parts of *Piper kadsura* (2.0 kg) were extracted with 95% alcohol (3×10 L) at room temperature, each for 24 h. After removal of the organic solvent, the sample was suspended in H_2O and sequentially extracted with petroleum ether and EtOAc. The EtOAc layer was concentrated, subjected to silica gel CC and eluted with petroleum/actone mixtures of increasing polarity (from 10/1 to 0/1) to yield fractions A-G. Fraction C was subjected to a C18 column eluted with MeOH-H2O (from 3/2 to 1:0) to afford C1-C5. Fraction C3 were further separated on a silica gel column, four fractions afford (C31-C34). Fraction C34 was subjected to semipreparative HPLC (YMC 5μm, 250×10mm, MeCN-H2O 11/9, 2.5 mL/min), HFT-1

(111.3 mg) eluted at 9.8 min. Fraction C32 was subjected to semipreparative HPLC (YMC 5 μm, 250×10 mm, MeCN-H2O 11/9, 2.5 mL/min), HFT-2 (49.9 mg) eluted at 12.5 min. HFT-1 and HFT-2 were identified as (2S,3S,3aR)-3a-allyl-5-methoxy-3-methyl-2-veratryl-2,3,3a,6-tetrahydro-6-oxobenzofuran and (2S,3S,5S)-5-allyl-5-methoxy-3-methyl-2-veratryl-2,3,5,6-tetrahydro-6-oxobenzofuran by the NMR data which were consistent with that of reported[4].

Air-dried, powdered aerial parts of *Salvia miltiorrhiza* (1.0 kg) were extracted with 95% alcohol (3×5 L) at room temperature, each for 24 h. After removal of the organic solvent, the sample was suspended in H2O and extracted with EtOAc. The EtOAc layer was concentrated, subjected to silica gel CC and eluted with chloroform/MeOH mixtures of increasing polarity (from 100/1 to 0/1) to yield fractions A-G. Fraction C were separated by a silica gel column eluted with petroleum/actone mixtures of increasing polarity (from 50/1 to 0/1) to yield DS-1 (6.3 mg). Fraction A were separated by a silica gel column eluted with petroleum/actone mixtures of increasing polarity (from 100/1 to 0/1) to yield DS-3 (5.1 mg). DS-1 and DS-3 were identified as danshenspiroketallactone and △1-dehydromiltirone by the NMR data which were consistent with that of reported[5,6]. NMR spectra for all isolated compounds are shown in Figure S3

References

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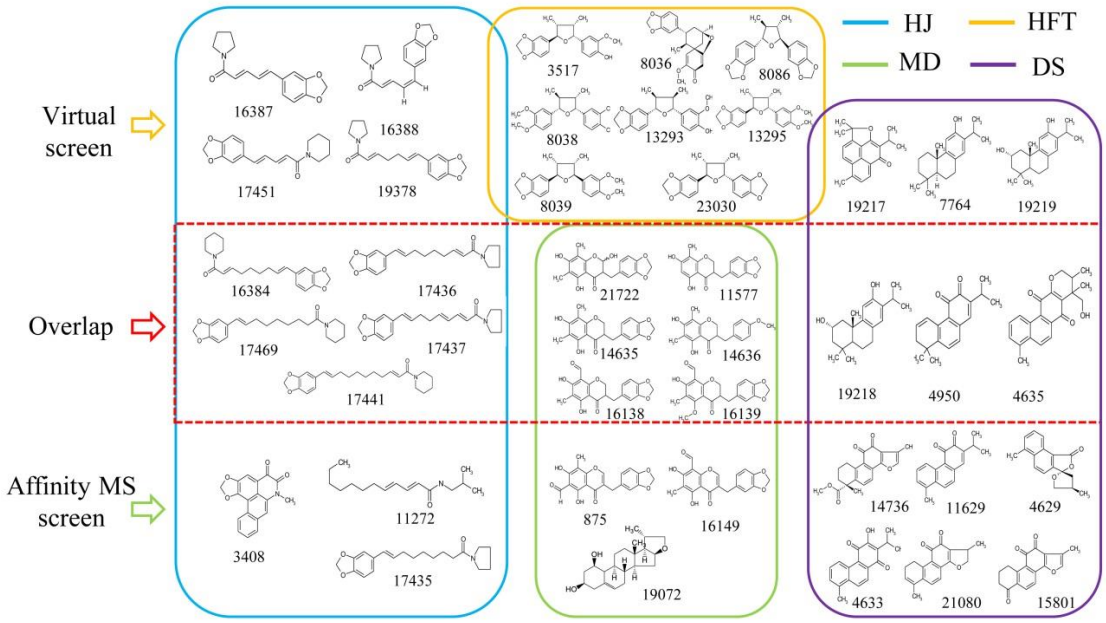

Figure S1. Chemical structures of all initial hits from virtual screen and affinity MS screen. HJ, *Piper nigrum*; MD, *Ophiopogon japonicas*; HFT, *Piper kadsura*; DS, *Salvia miltiorrhiza*;

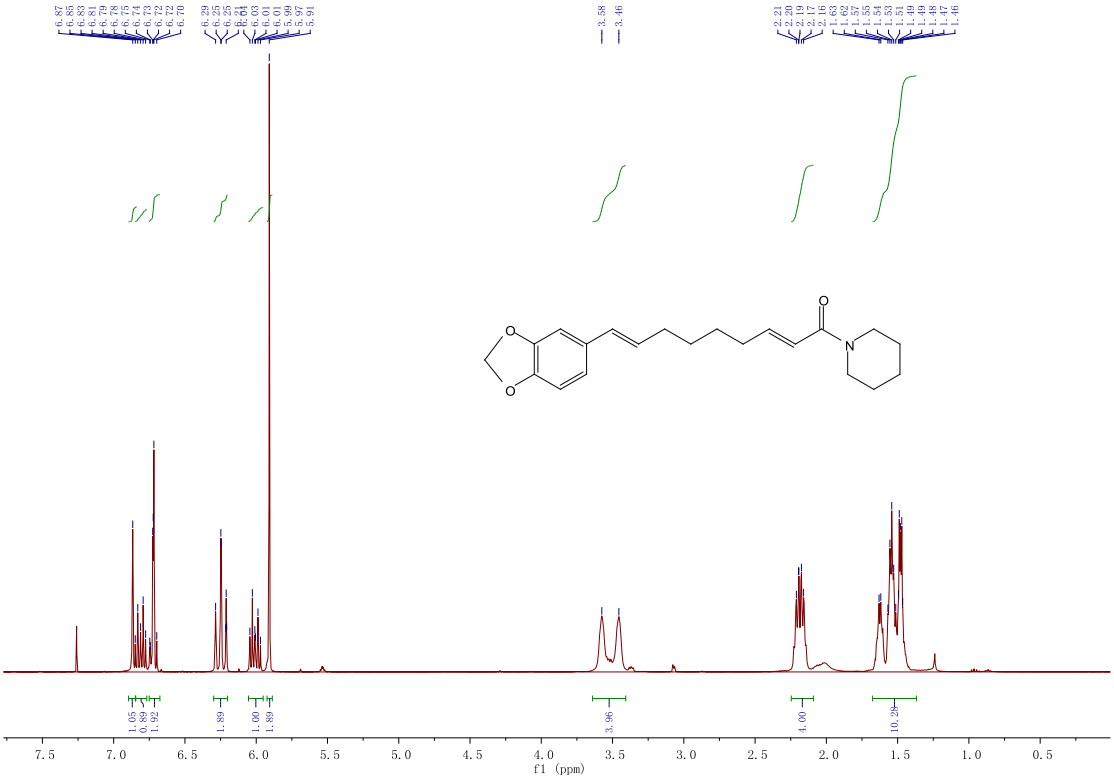

Figure S2-A. ^{1}H NMR (400MHz, $CDCl_3$) data of HJ-1

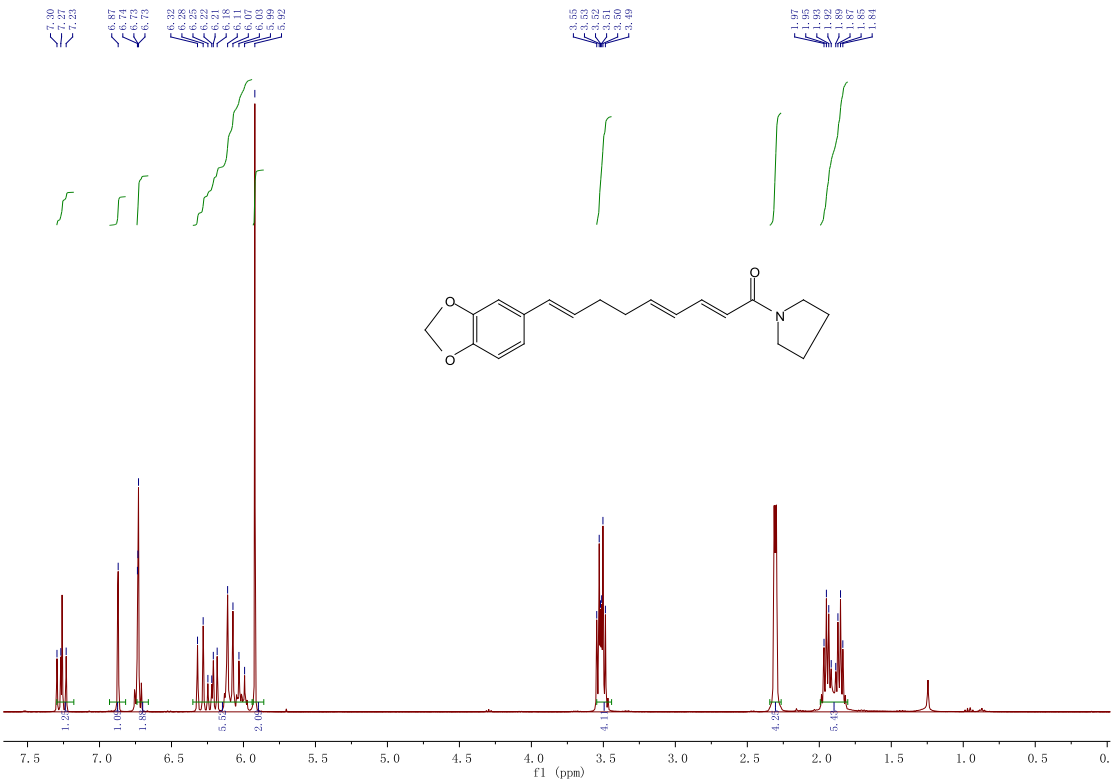

Figure S2-B. ^{1}H NMR (400MHz, $CDCl_3$) data of HJ-2

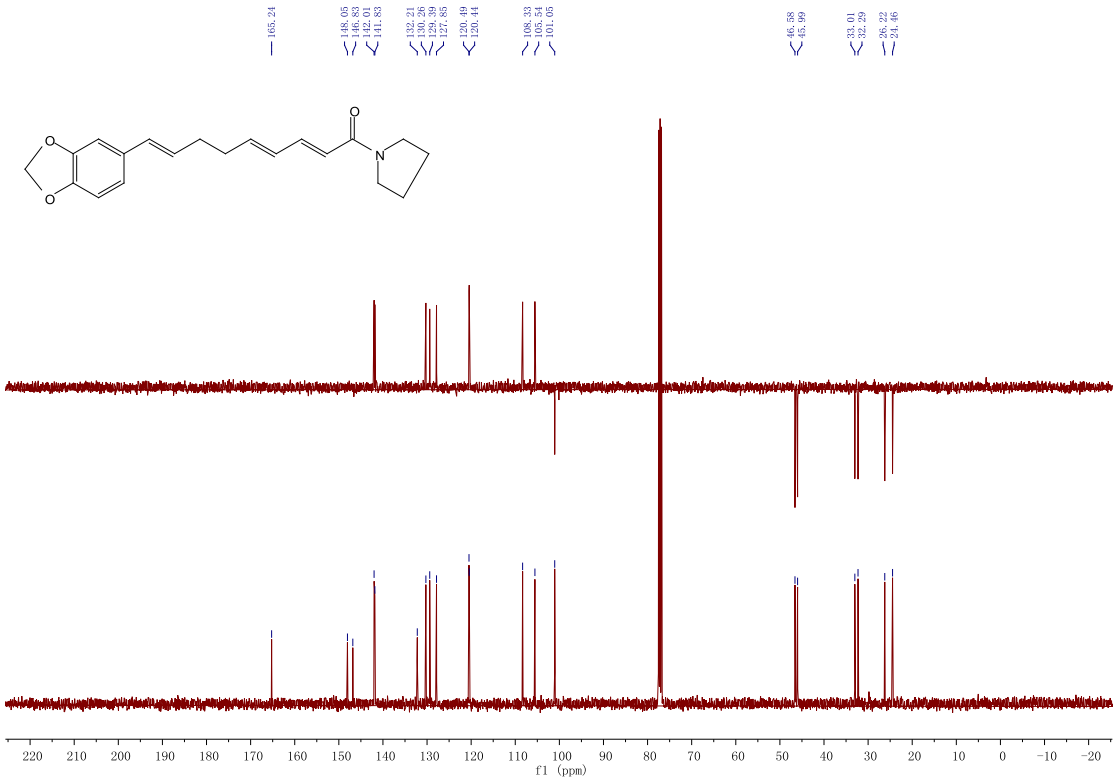

Figure S2-C. ^{13}C NMR (100MHz, $CDCl_3$) data of HJ-2

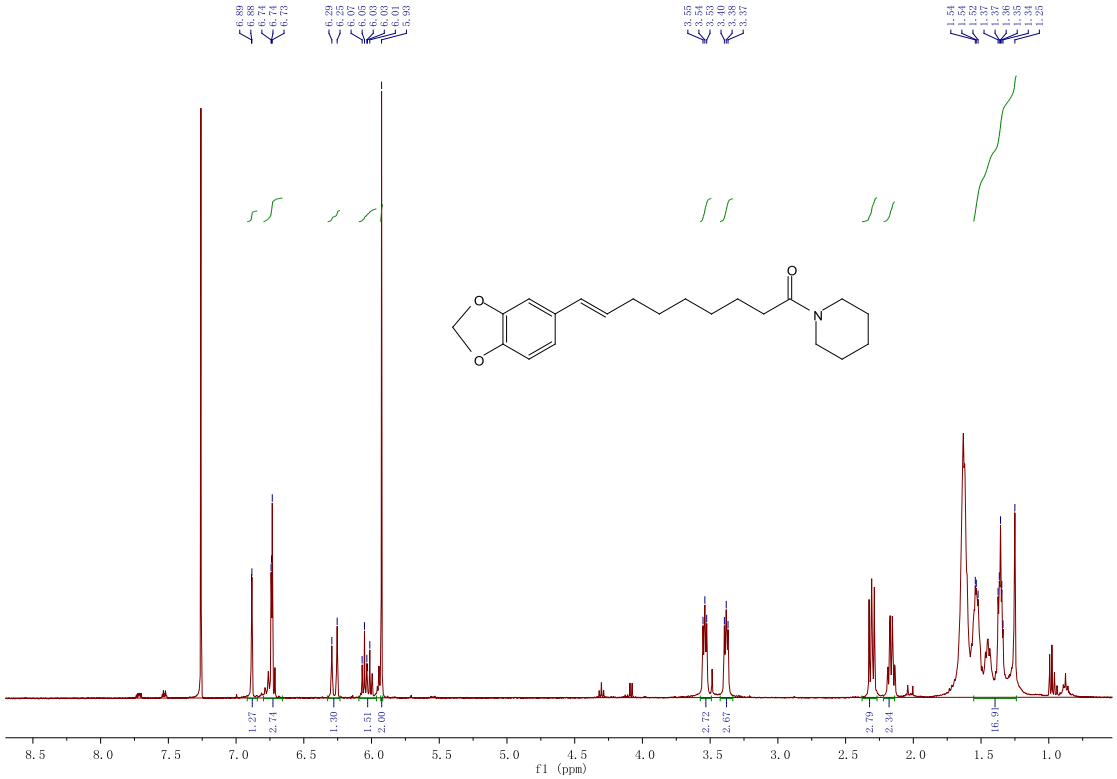

Figure S2-D. ^{1}H NMR (400MHz, $CDCl_3$) data of HJ-3

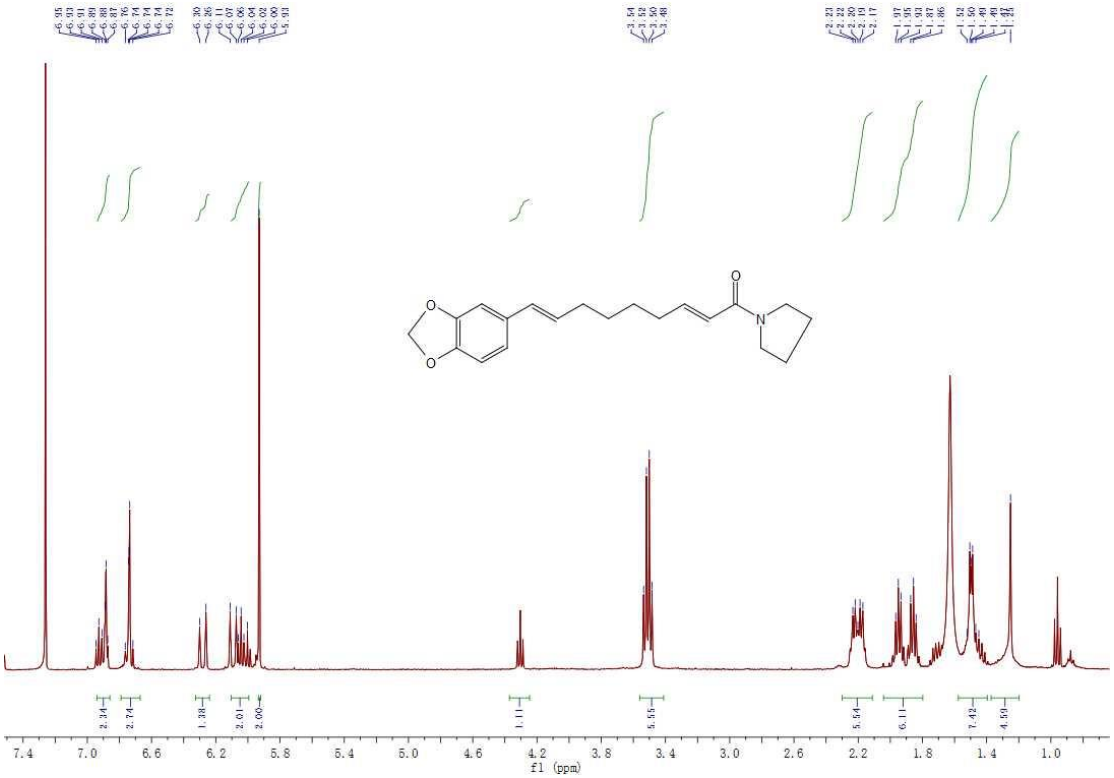

Figure S2-E. ^{1}H NMR (400MHz, $CDCl_3$) data of HJ-4

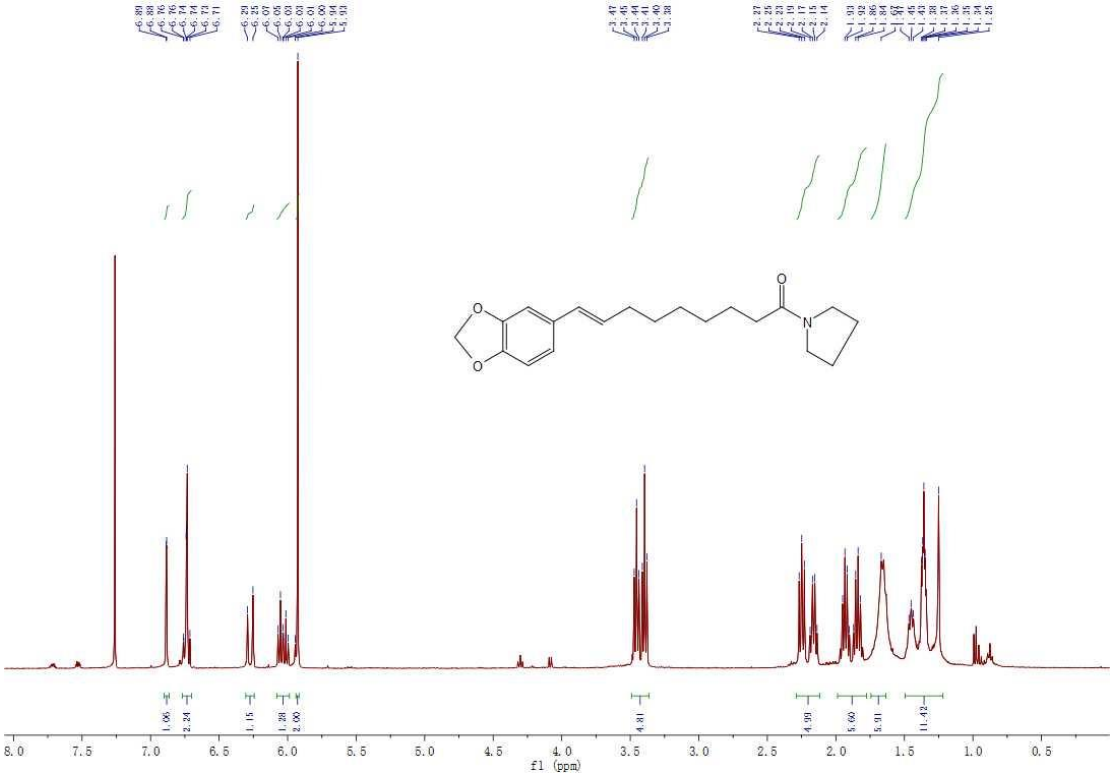

Figure S2-F. ^{1}H NMR (400MHz, $CDCl_3$) data of HJ-6

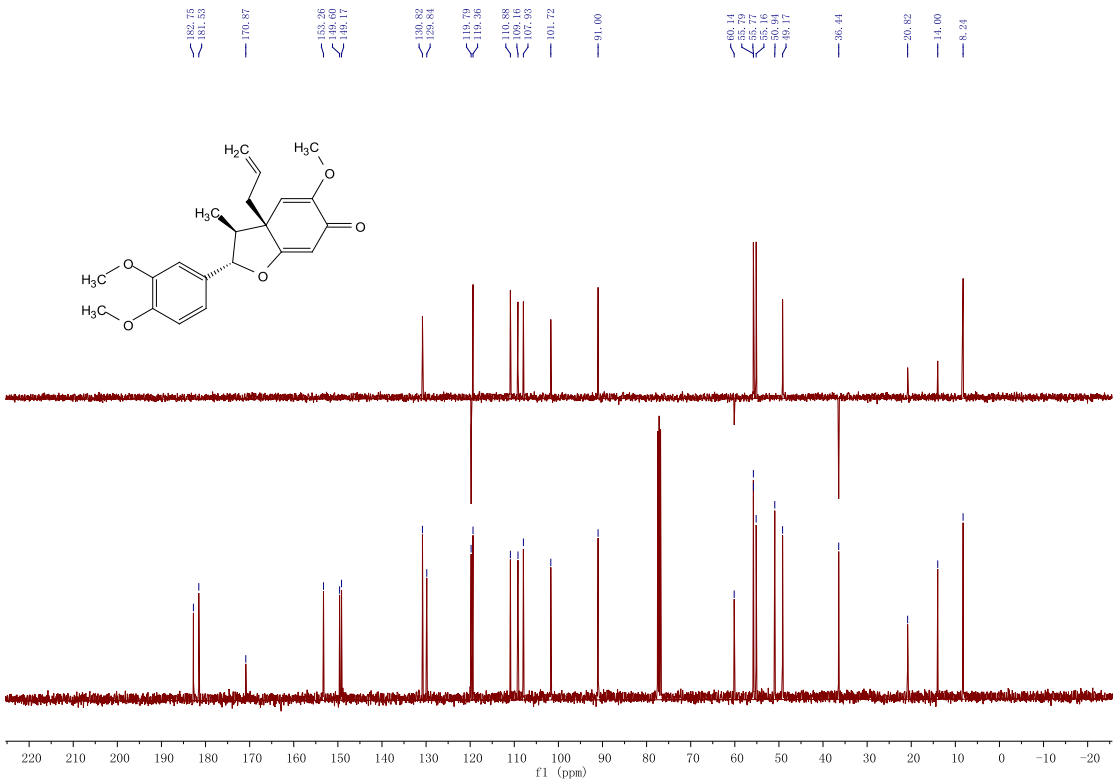

Figure S2-G. ^{13}C NMR (100MHz, $CDCl_3$) data of HFT-1

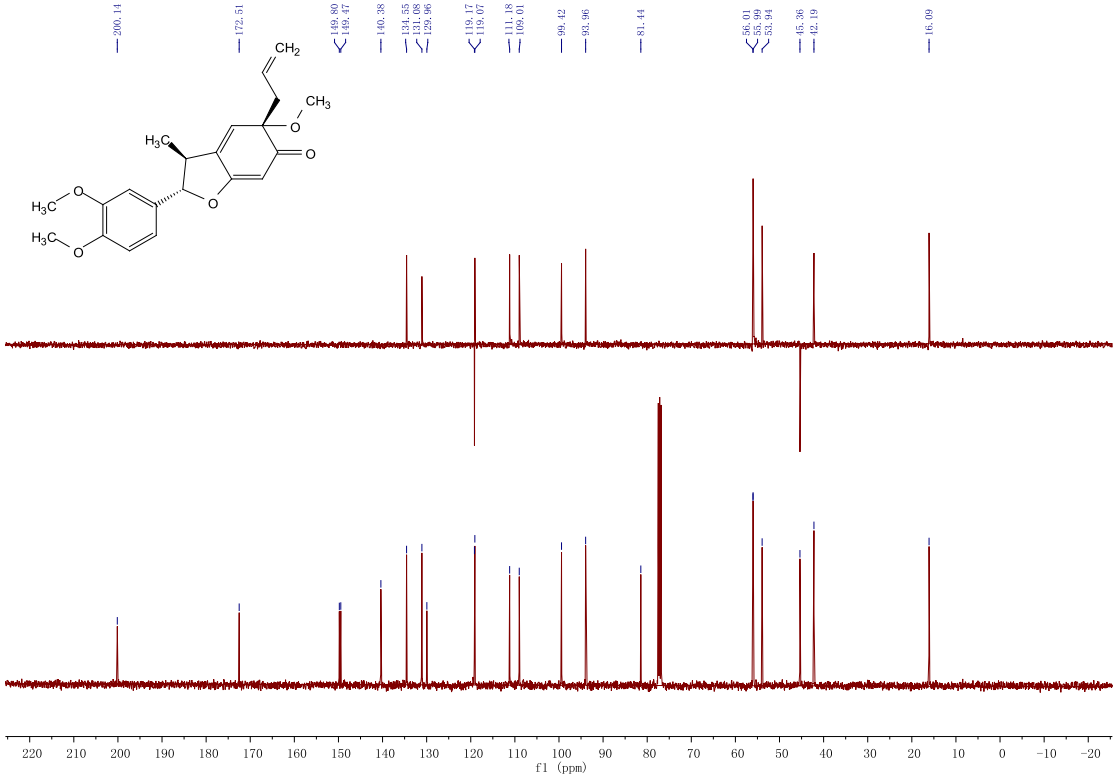

Figure S2-H. ^{13}C NMR (100MHz, $CDCl_3$) data of HFT-2

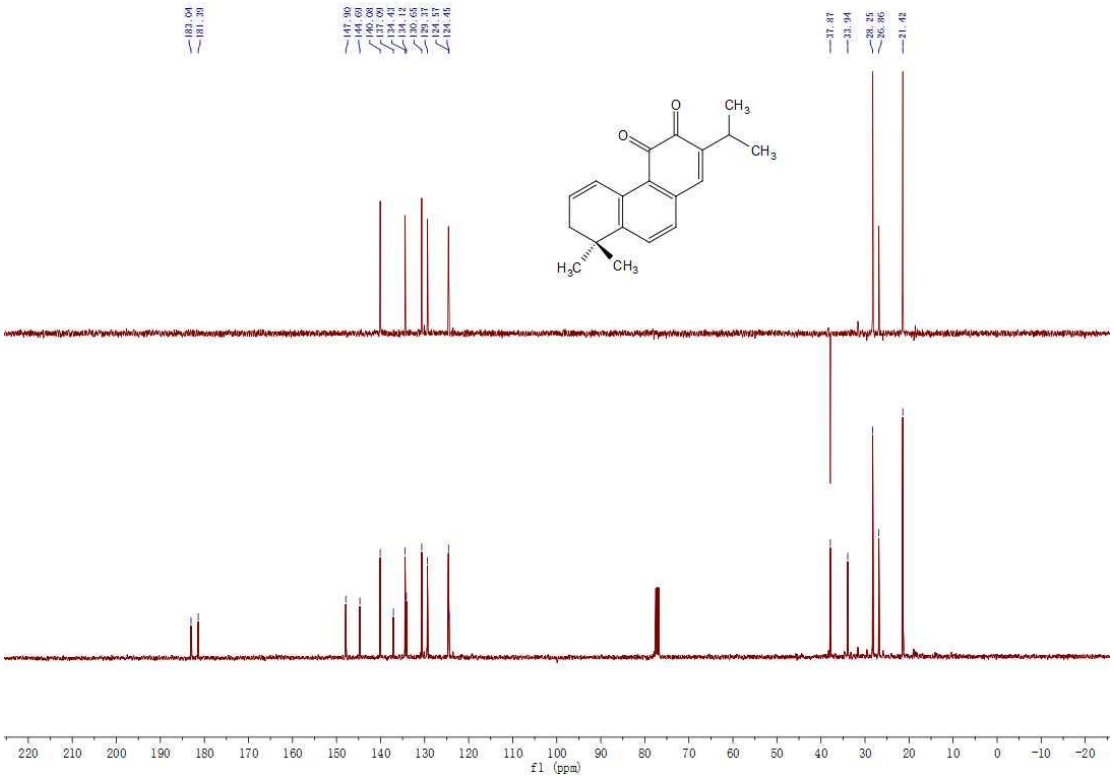

Figure S2-I. ^{13}C NMR (100MHz, $CDCl_3$) data of DS-1

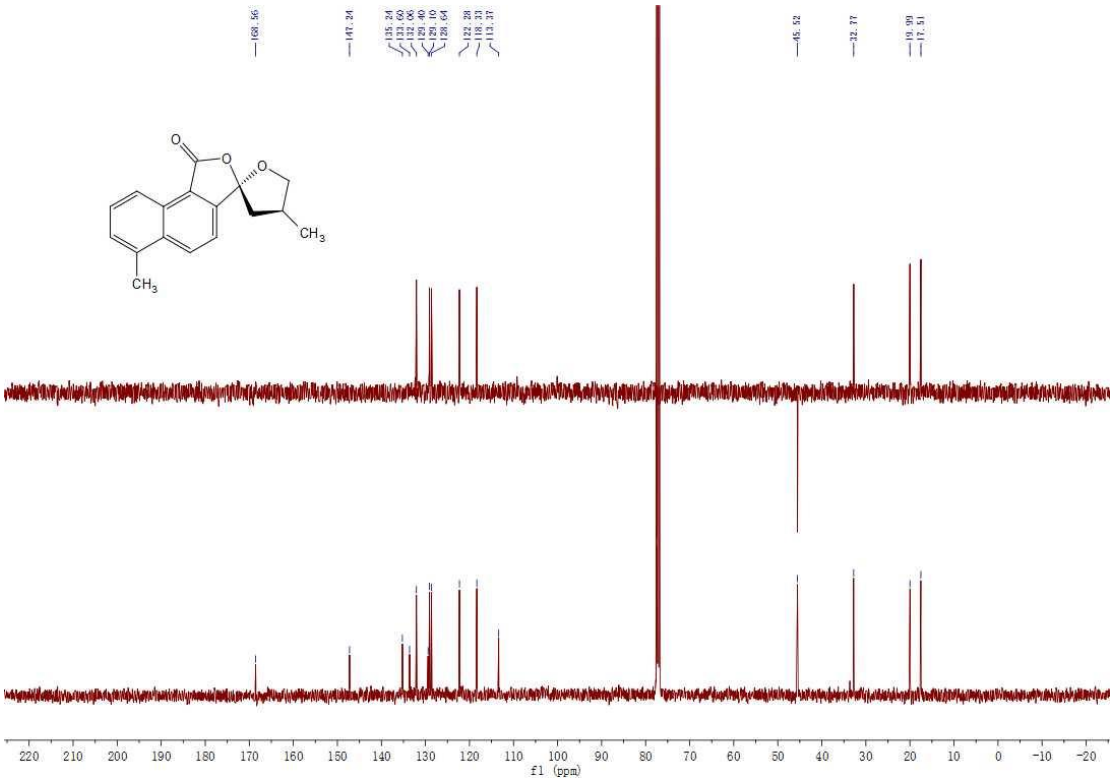

Figure S2-J. ^{13}C NMR (100MHz, $CDCl_3$) data of DS-3

Figure S2 (A-J). NMR spectra for all compounds isolated from TCM herbs

Table S2 Docking scores and BI values for all initial hits from screening of four TCM herbs.

Piper nigrum (HJ)

TCMHD ID	Virtual screen (kcal/mol)[a]				Affinity screen
	AutoDock	VINA	Glide SP	Glide XP	BI[b]
16384[c]	-6.51[d]	-6.30	-4.74	-5.56	8.36 ± 0.52
16387	-6.50	-6.20	-5.99	-6.14	1.66 ± 0.29
16388	-5.93	-6.10	-5.74	-5.72	1.59 ± 0.23
17436	-6.21	-6.30	-5.36	-6.68	8.61 ± 0.82
17437	-6.41	-6.40	-6.05	-6.24	5.72 ± 0.26
17451	-6.42	-6.40	-5.13	-5.89	1.91 ± 0.22
17469	-5.99	-6.70	-4.70	-5.66	8.69 ± 0.89
19378	-6.01	-6.10	-5.68	-5.96	ND[e]
3408	-5.49	-6.40	-4.24	-4.21	16.09 ± 2.80
11272	-4.26	-5.30	-1.12	-3.55	8.57 ± 0.96
17435	-5.90	-6.00	-4.57	-6.75	6.70 ± 0.41
17441	-6.16	-7.20	-4.43	-5.90	61.14 ± 14.49

Salvia miltiorrhiza (DS)

TCMHD ID	Virtual screen (kcal/mol)[a]				Affinity screen
	AutoDock	VINA	Glide SP	Glide XP	BI[b]
19217	-6.09	-6.40	-4.87	-4.52	ND
19218	-6.43	-6.60	-4.94	-4.78	1863 ± 2.88
19219	-7.35	-6.50	-5.30	-5.23	ND
4635	-6.36	-6.20	-5.00	-4.69	2.59 ± 0.16
4950	-6.65	-6.40	-4.64	-5.34	6.92 ± 0.27
7764	-6.92	-6.30	-5.51	-5.17	ND
14736	-5.96	-6.40	-4.28	-3.60	9.05 ±1.37
11629	-7.11	-6.30	-3.74	0.02	9.06 ± 0.44
4629	-6.64	-6.90	-4.58	-4.36	10.65 ± 1.41
4633	-6.50	-5.90	-3,92	-1.39	14.79 ± 2.92
21080	-6.36	-6.30	-4.74	-3.76	10.39 ± 1.12
15801	-6.60	-6.30	-4.30	-3.24	8.12 ± 1.34

Ophiopogon japonicas (MD)

TCMHD ID	Virtual screen (kcal/mol)[a]				Affinity screen
	AutoDock	VINA	Glide SP	Glide XP	BI[b]
21722	-5.96	-6.20	-5.07	-5.94	2.47 ± 0.32
11577	-6.31	-6.70	-6.09	-5.07	3.07 ± 0.48
14635	-6.47	-6.80	-5.90	-5.84	3.47 ± 0.29
14636	-6.07	-6.50	-5.61	-4.68	3.53 ± 0.34
16138	-6.22	-6.50	-5.87	-5.78	10.58 ± 1.85
16139	-6.37	-6.40	-5.74	-5.47	10.72 ± 1.21
875	-5.82	-6.00	-6.27	-4.98	13.61 ±1.71
16149	-6.15	-5.90	-5.88	-5.96	10.63 ± 2.73
19072	-6.48	-6.50	-3.39	-2.12	13.4 ± 2.44

Piper kadsura (HFT)

TCMHD ID	Virtual screen (kcal/mol)[a]				Affinity screen
	AutoDock	VINA	Glide SP	Glide XP	BI[b]
3517	-6.72	-6.70	-5.34	-5.61	1.04 ± 0.04
8036	-6.82	-6.60	-4.63	-6.39	1.55 ± 0.15
8038	-6.82	-6.60	-6.01	-6.13	1.33 ± 0.04
8039	-6.78	-6.50	-4.55	-6.18	1.48 ± 0.38
8086	-6.85	-6.90	-5.37	-5.04	1.49 ± 0.06
13293	-6.80	-6.30	-5.13	-6.07	1.22 ± 0.16
13295	-6.37	-6.60	-5.42	-6.12	1.30 ± 0.24
23030	-6.84	-7.00	-5.14	-5.19	1.17 ± 0.17

[a] Virtual screening binding energy of each compound to the hydrophobic pocket of EBOV NP.

[b] BI (binding index) of each compound measured by affinity mass spectrometry

[c] Compounds in bold ID are co-identified by two screening approaches.

[d] Docking scores in blue rank among top 30% by the specific docking tools.

[e] ND indicates the compound was not detected in the herbal extracts by LC-MS analysis.

Table S3 Thermal stability shift of NPs incubated with specific compounds

Name	ΔT$_1$ (℃)[a]	ΔT$_2$ (℃)	ΔT$_3$ (℃)	Ave ΔT (℃)
HJ-1	-4	-4	-4	-4
HJ-2	-1	-2	-2	-2
HJ-3	-3	-3	-3	-3
HJ-4	-6	-6	-6	-6
HJ-6	-4	-4	-4	-4
MD-1	-4	-4	-4	-4
MD-2	0	0	0	0
MD-4	NA[d]	NA	NA	NA
DS-1	-2	-2	-2	-3
DS-3	0	0	0	0
HFT-1	0	0	0	0
HFT-2	0	0	0	0
GC-7[b]	-7	-7	-7	-7
VP35[c]	8	8	8	8

[a] Change of the melting temperature (ΔT) of the NP protein incubated with each compound relative to the free NP protein. Results were from three independent experiments.

[b] GC-7 is a known ligand of EBVO NP which can reduce the protein thermostability.

[c] VP35 peptide is a known ligand of EBVO NP which can enhance the protein thermostability.

[d] NA indicates that no reliable data was acquired from an abnormal melting curve.