SUPPLEMENTARY MATERIAL

A trace alkaloid, oleraisoindole A from *Portulaca oleracea* L. and its anticholinesterase effect

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Abstract

A new trace alkaloid possessing the lignan structure, named oleraisoindole A, was obtained from the extract of the *Portulaca oleracea* L. The structure of oleraisoindole A was elucidated by 1D and 2D NMR and high resolution electrospray ionization time-of-flight mass spectroscopic methods. The compound presented an anticholinesterase effect with the IC_{50} value of 60.4 μM.

Keywords: *Portulaca oleracea* L.; alkaloids; anticholinesterase effect

Supporting information

Supplementary material relating to this article is available online, alongside, Tables S1-S2, Figure S1-S14 and Experimental section.

Table S1. Full NMR Data of Oleraisoindole A in DMSO-d_6

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Figure S8. HMBC spectrum of Oleraisoindole A in DMSO-d_6

Figure S9. ^{1}H-^{1}H COSY spectrum of Oleraisoindole A in DMSO-d_6

Figure S10. ROESY spectrum of Oleraisoindole A in DMSO-d_6

Figure S11. UHPLC-ESI-Q-TOF/MS of Oleraisoindole A

Table S1. Full NMR Data of Oleraisoindole A in DMSO-d_6

Position	δC	Type	δH,mult (J in Hz)	COSY	HMBC	ROESY
1	166,9/167.4	C				
2		N				
3	166,9/167.4	C				
3a	121.6	C				
4	137.0	C				
4a	131.1	C				
5	110.4	CH	7.12 brs		4, 6, 7, 8a	6'
6	148.7	C				
7	150.0	C				
8	109.5	CH	7.66 brs			9, 7-OMe
8a	130.4	C				
9	121.8	CH	8.20 brs		1, 3a, 4a	8
9a	124.9	C				
7-OMe	55.75	CH_3	3.94 s		7	8
1'	125.6	C				
2'	114.2	CH	6.86 d (1.9)	6'	4, 4', 6'	3'-OMe
3'	147.1	C				
4'	146.5	C				
5'	115.1	CH	6.91 d (7.9)	6'	1', 3'	6'
6'	122.5	CH	6.73 dd (7.9, 1.9)	2', 5'	2', 4', 4	5', 5
3'-OMe	55.69	CH_3	3.74 s		3'	2'
1"	39.0	CH_2	3.66 dd (9.2, 6.9)	2"	1, 3, 2", 1"'	2", 2"'/6"'
2"	32.9	CH_2	2.74 brt (7.6)	1"	1", 1"', 2"'/6"'	1", 2"'/6"'
1"'	128.3	C				
2"'/6"'	129.5	CH	6.95 brd (8.4)	3"'/5"'	2", 2"'/6"', 4"'	1", 2", 3"'/5"'
3"'/5"'	115.2	CH	6.63 brd (8.4)	2"'/6"'	1"', 3"'/5"', 4"'	2"'/6"'
4"'	155.8	C				

Table S2. Comparison of ^{1}H-NMR (600MHz) and ^{13}C-NMR (150MHz) data between Oleraisoindole A and Oleraisoindole in DMSO-d_6

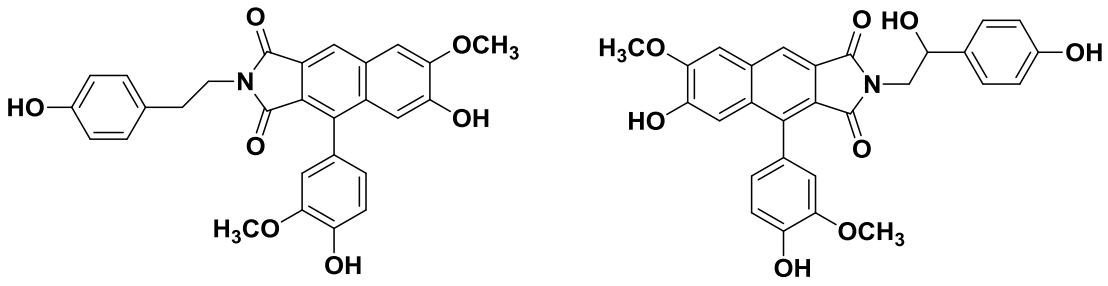
position	type	Oleraisoindole A		Oleraisoindole	
		δC	δH, mult (*J* in Hz)	δC	δH, mult (*J* in Hz)
1	C	166,9/167.4		167.5	
2	N				
3	C	166,9/167.4		166.9/167.1	
3a	C	121.6		121.68/121.74	
4	C	137.0		137.1	
4a	C	131.1		131.4	
5	CH	110.4	7.12, brs	110.4	7.15, s
6	C	148.7		149.8	
7	C	150.0		150.9	
8	CH	109.5	7.66, brs	109.5	7.69, s
8a	C	130.4		130.6	
9	CH	121.8	8.20, brs	121.6	8.22, s
9a	C	124.9		125.20/125.23	
7-OMe	CH_3	55.75	3.94, s	55.68	3.95, s
1'	C	125.6		125.57/125.64	
2'	CH	114.2	6.86, d (1.9)	114.2/114.3	6.83/6.90, d (2.0)
3'	C	147.1		147.07/147.09	
4'	C	146.5		146.5	
5'	CH	115.1	6.91, d (7.9)	115.06/115.09	6.91, d (8.0)
6'	CH	122.5	6.73, dd (7.9, 1.9)	122.6	6.72/6.75, dd (8.0, 2.0)
3'-OMe	CH_3	55.69	3.74, s	55.75	3.74/3.76, s
1"	CH_2	39.0	3.66, dd (6.9, 9.2)	45.3/45.4	a: 3.48/3.49, dd (13.5, 5.0) b: 3.69, brdd (13.5, 8.5)
2"	CH			69.10/69.12	4.78, m
2"	CH_2	32.9	2.74, brt (7.6)		
1"'	C	128.3		133.0	
2"'/6"'	CH	129.5	6.95, brd (8.4)	127.0	7.11/7.12, brd (8.5)
3"'/5"'	CH	115.2	6.63, brd (8.4)	114.81/114.84	6.68/6.69, brd (8.5)
4"'	C	155.8		156.6	

Table S3. IC_{50} (μM) for anticholinesterase effect of Oleraisoindole A and Eserine (*n* = 5)

Compounds and standard inhibitor	AChE IC_{50} (μM)

Oleraisoindole A	60.40± 0.82
Eserine	34.47 ± 0.50

Eserine is the positive control and values are expressed as the means ± SD for *n* = 5.



Oleraisoindole A Oleraisoindole

Figure S1. Comparison of structure between Oleraisoindole A and Oleraisoindole

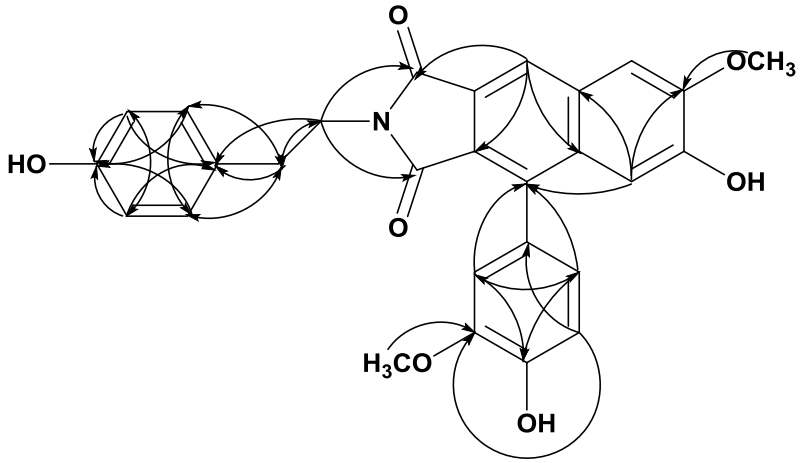


Figure S2. Key HMBC correlations of Oleraisoindole A

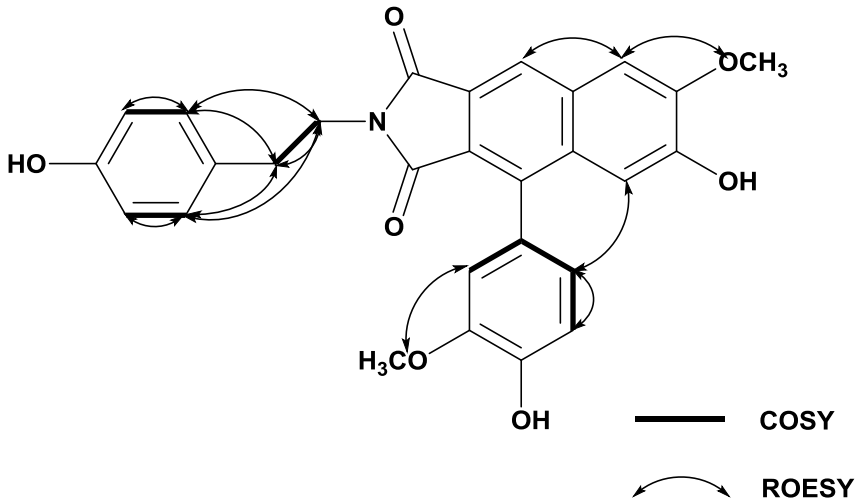


Figure S3. Key ^{1}H-^{1}H COSY and ROESY correlations of Oleraisoindole A

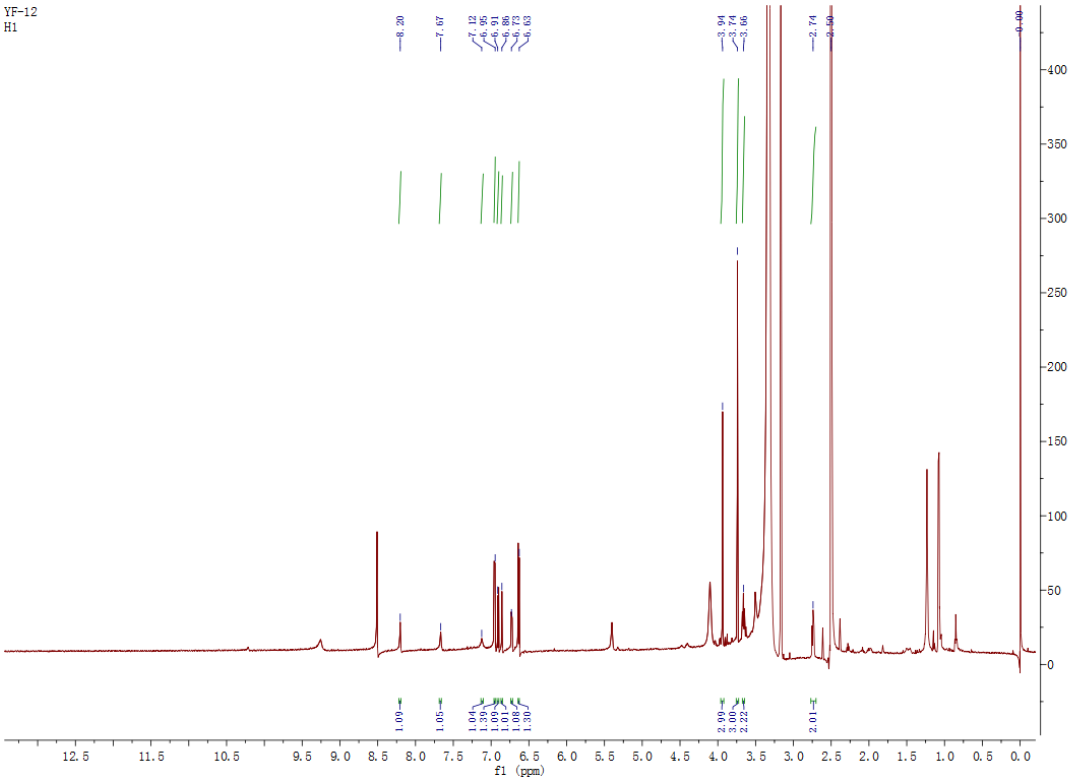


Figure S4. ^{1}H NMR (600 MHz) spectrum of Oleraisoindole A in DMSO-d_6

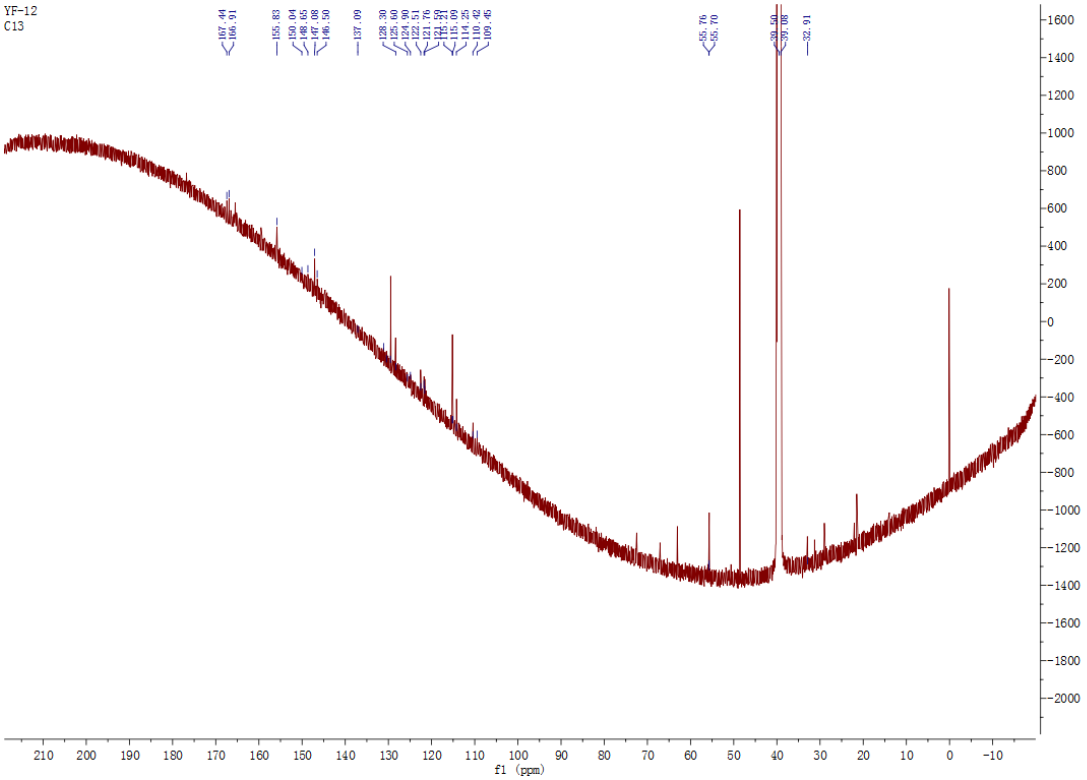


Figure S5. ^{13}C NMR (150 MHz) spectrum of Oleraisoindole A in DMSO-d_6

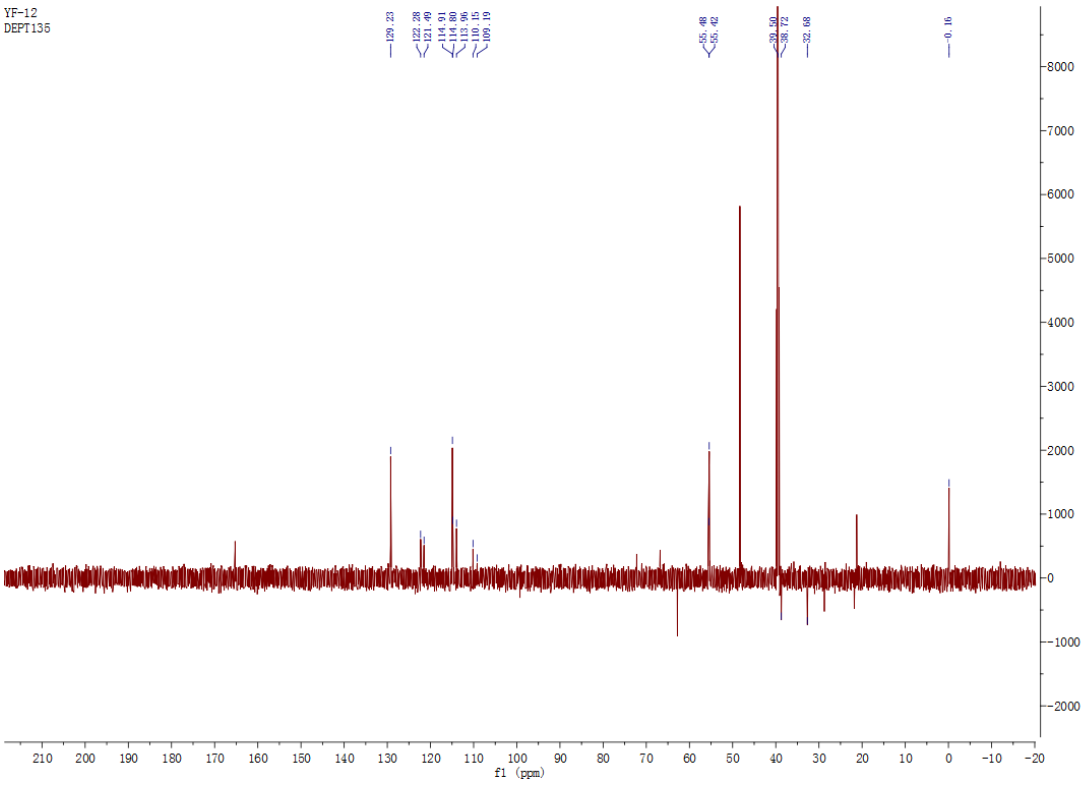


Figure S6. DEPT spectrum of Oleraisoindole A in DMSO-d_6

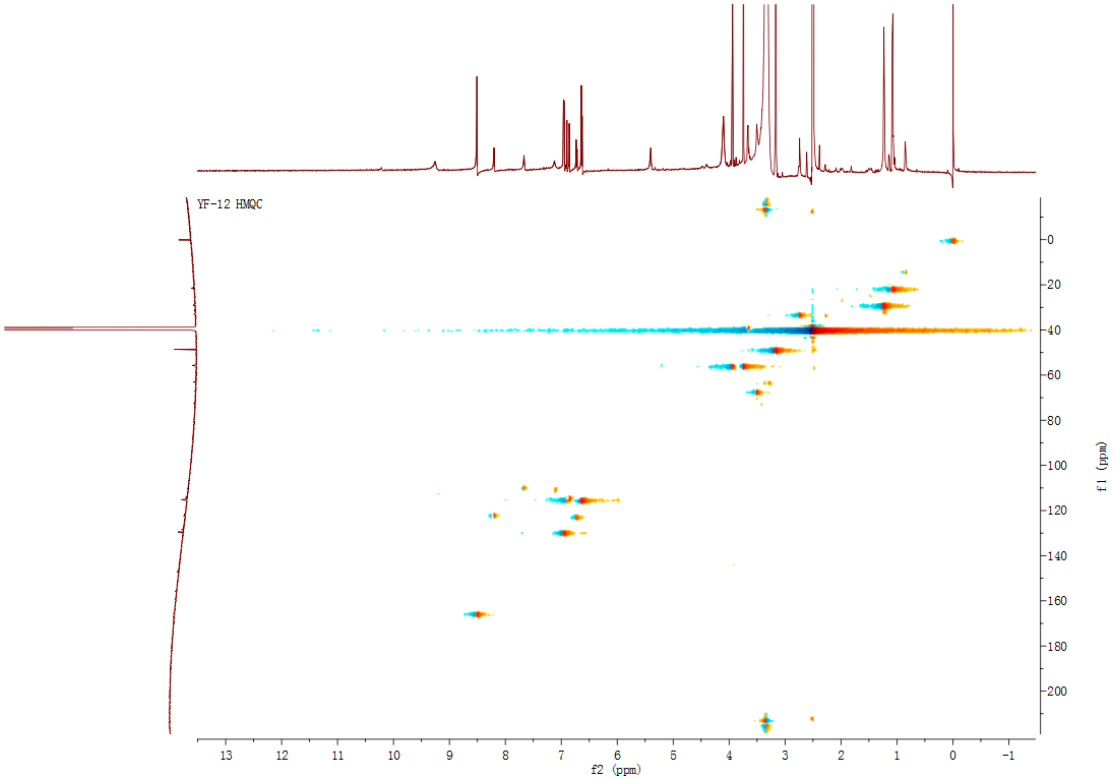
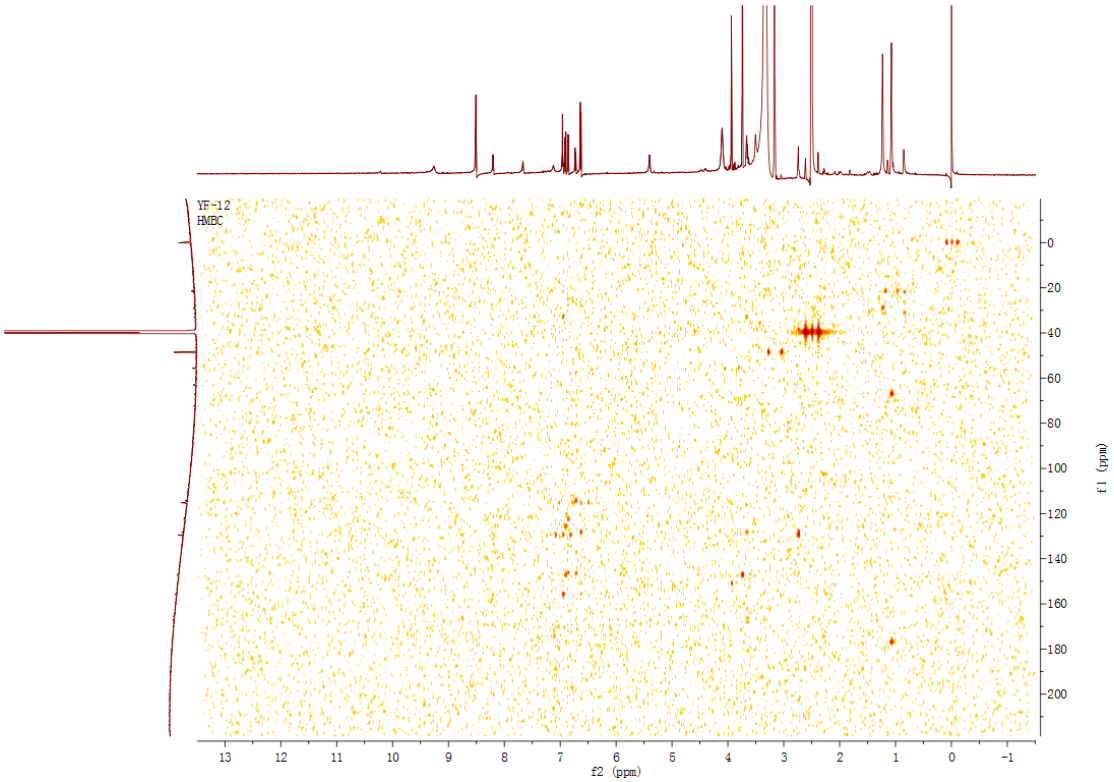


Figure S7. HSQC spectrum of Oleraisoindole A in DMSO-d_6



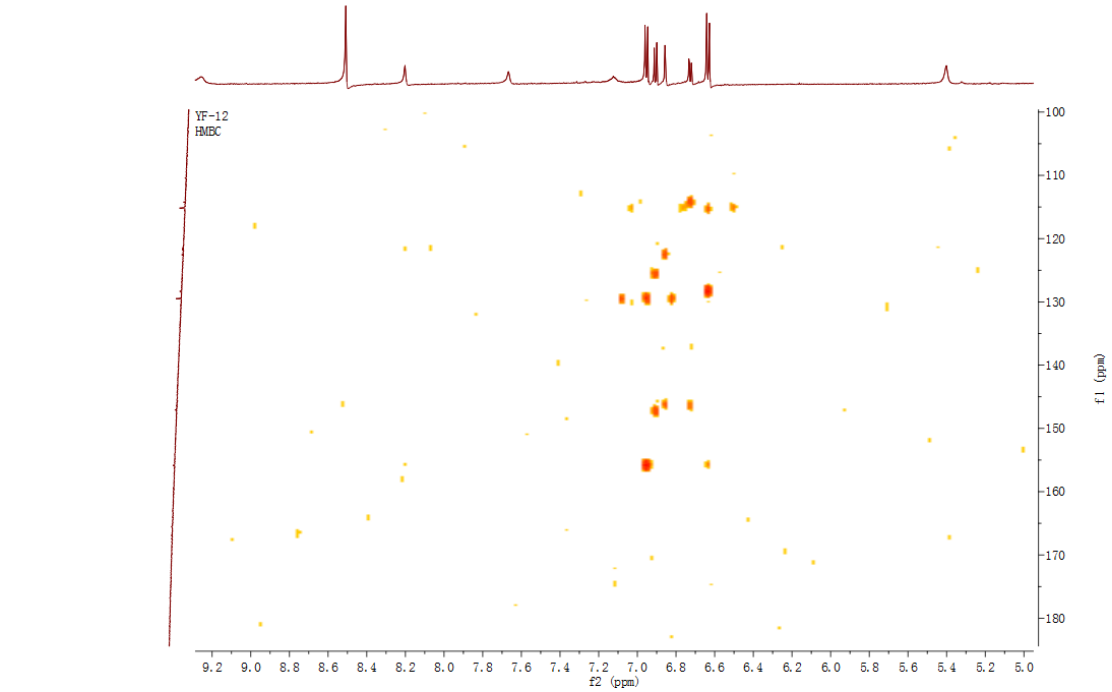


Figure S8. HMBC spectrum of Oleraisoindole A in DMSO-d_6

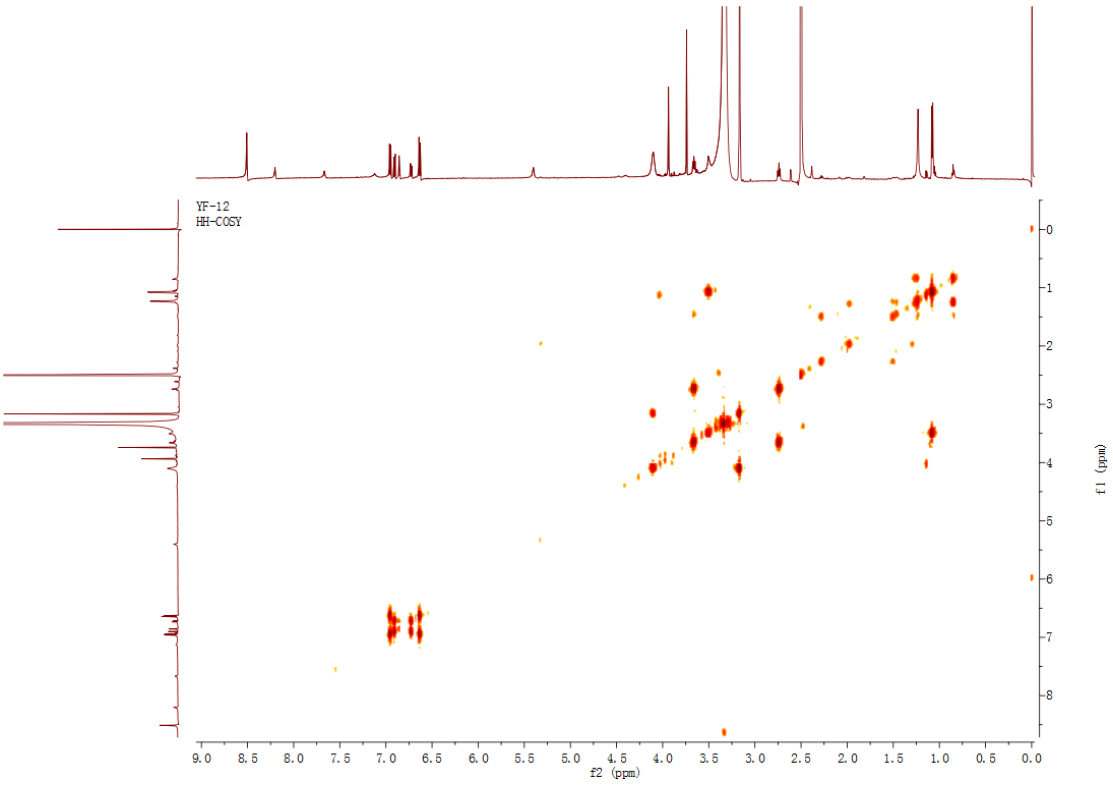


Figure S9. ^{1}H-^{1}H COSY spectrum of Oleraisoindole A in DMSO-d_6

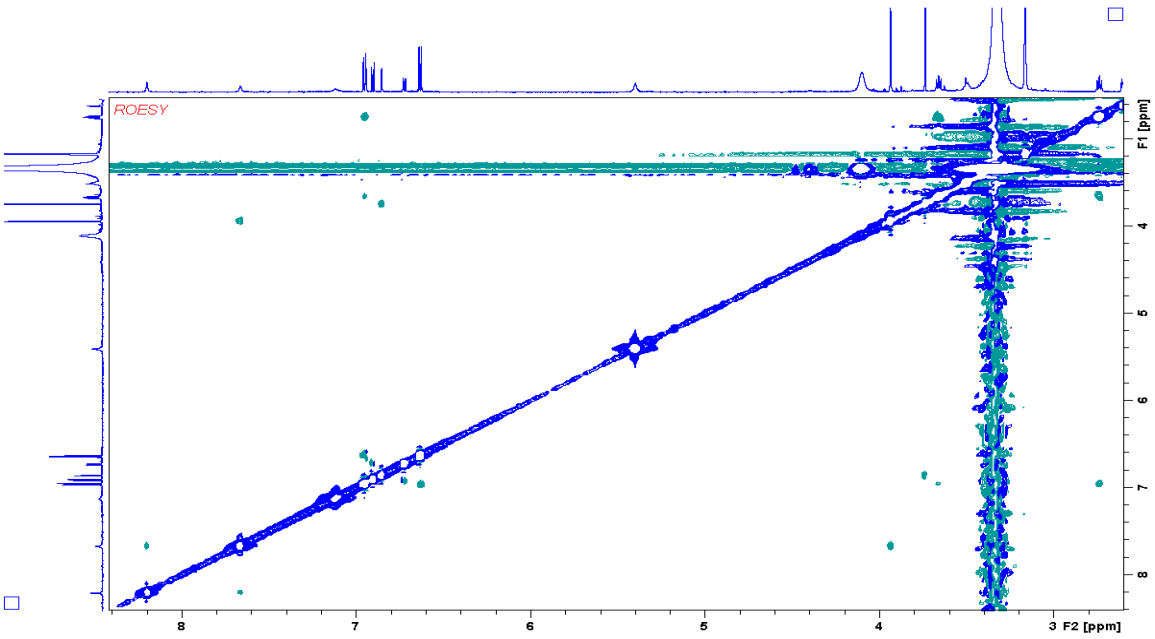


Figure S10. ROESY spectrum of Oleraisoindole A in DMSO-d_6

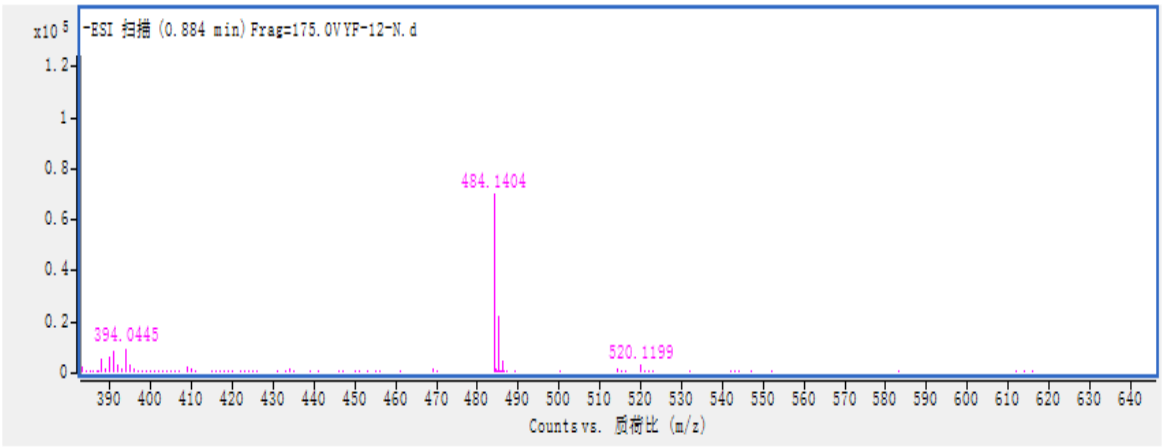


Figure S11. UHPLC-ESI-Q-TOF/MS of Oleraisoindole A

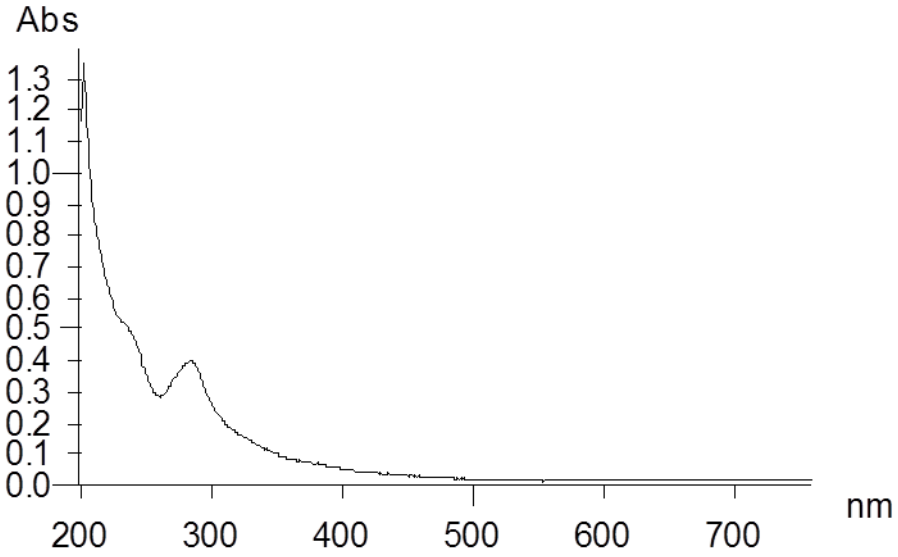
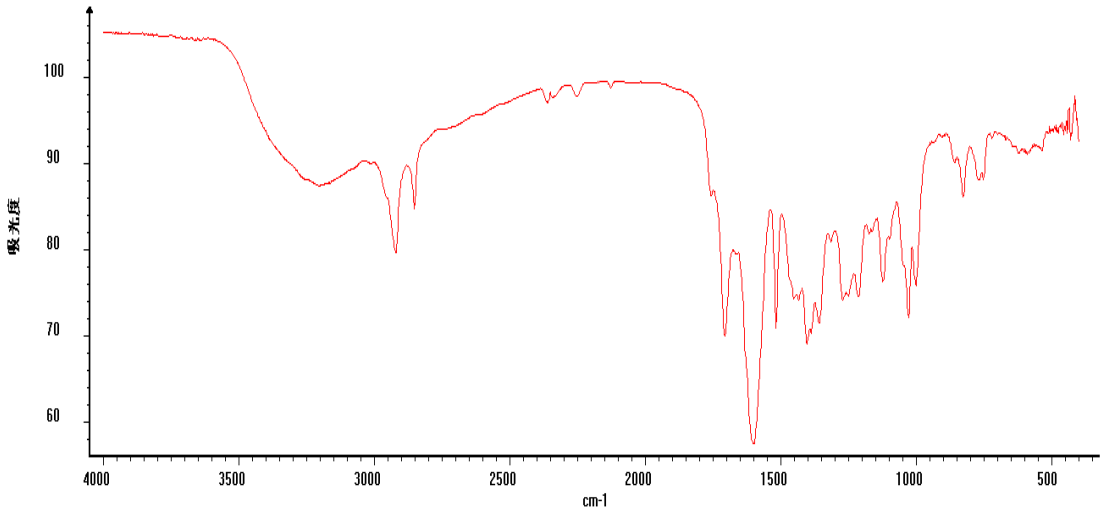


Figure S12. UV spectrum of Oleraisoindole A in methanol



The major bands of the FT-IR spectrum and their corresponding functional groups included bands of amide group (3425 and 1515 cm^{-1}) and carbonyl group (1706 cm^{-1})

Figure S13. IR (KBr) spectrum of Oleraisoindole A

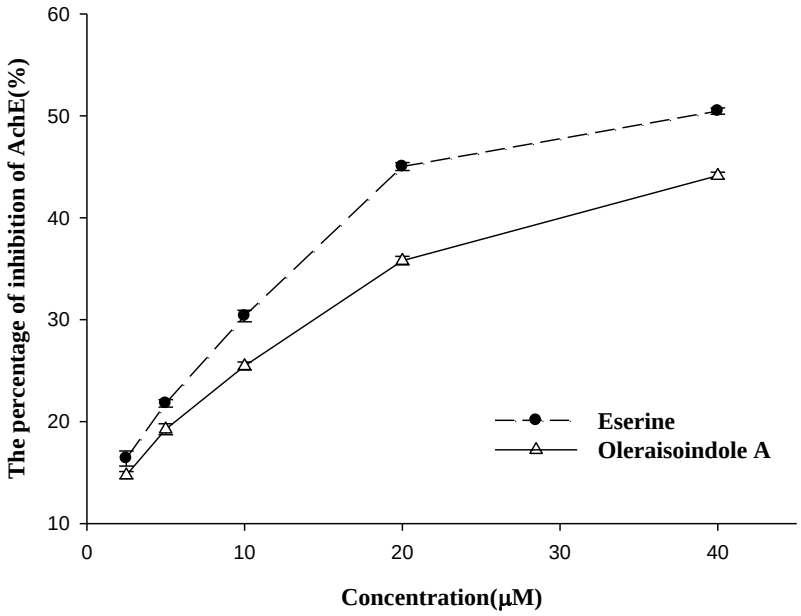


Figure S14. Anticholinesterase effect of Oleraisoindole A and Eserine (*n*=5).

Experimental section

General experimental procedures

The data of UV spectrum and IR spectrum were recorded by a HITACHI U-3010 spectrophotometer (Hitachi Ltd, Tokyo, Japan) and an IR200 spectrophotometer (Thermo Electron Corporation, Waltham, MA), respectively. NMR spectra in DMSO-d_6 were obtained from an AVANCE 600 MHz instrument (Bruker Corporation, Switzerland). Relative molecular weights were obtained using a 6520 quadrupole time-of-flight mass spectrometer (Agilent, Palo Alto, CA). Purity was checked on a Nexera X2 UHPLC LC-30A system (Shimadzu, Kyoto, Japan) by using a Kromasil C18 column (150 mm x 4.6 mm, 5 μm, Dalian Johnsson Separation Science and

Technology Corporation). In the separation process, column chromatography (CC) includes silica gel (100-220 and 200-300 mesh, Qingdao Ocean Chemical Co., Ltd., Qingdao, China), polyamide resin (80-100 mesh, Luqiao Sijia, Taizhou, Zhejiang) Biochemical Plastics), China) and ODS (20-40μm, GE Healthcare, Marlborough, MA). Silica gel GF_{254} (Qingdao Ocean Chemical Co., Qingdao, China) was used to prepare TLC.

Plant materials and chemicals

The whole herbs of *P. oleracea* were collected in Shijiazhuang (Hebei, China) in June 2017, and identified by Prof. Xixiang Ying. The voucher specimen (No. 20171001) was displayed at Liaonig University of Traditional Chinese Medicine. Methanol, acetonitrile and formic acid are all of HPLC grade provided by Damao Chemical Reagent Plant (Tianjin, China, purity ≥ 99.9%). Acetylthiocholine iodide (ATCI) (purity≥99%) and acetylcholinesterase (AChE) (vitality≥200 units/mg protein) were purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Eserine (purity≥98%) was purchased from Shanghai Hanxiang Biotechnology Co., Ltd. (Shanghai, China). 5,5-dithiobis-2-nitrobenzoic acid (DTNB) (purity≥99%) was purchased from Shanghai Jinshui Biotechnology Co., Ltd. (Shanghai, China). Na_2HPO_4 and NaH_2PO_4 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) (purity≥99.0%). All other reagents were of analytical grade purchased from Jinfeng Chemical Factory (Tianjin, China), and the water was WAHAHA purified water (Shenyang, China).

Isolation and identification

A sample (250 kg) of the air-dried aerial parts *P. oleracea* was treated twice with 10 volumes of water for 2 hours each time to afford a crude extract (21 kg). The extract was concentrated and subjected to a 100-200 mesh silica-gel column chromatography with a 2 volumes of ethyl acetate 3 times as the isocratic elution, affording the fraction evaporated (500 g). Then, the fraction eluted with water, 30%, 50%, 70% and pure ethanol was chromatographed respectively on a 80-100 mesh polyamide resin column (120 × 8 cm, approximately, 2.5 kg) to obtain five fractions (4 L each). The fraction of 50% extracting was condensed under the reflux to obtain the extract of 58 g, which was subjected to a 200-300 mesh silica-gel column (120 × 8 cm, approximately, 200 g) for further purification with ethyl acetate, ethyl acetate and methanol (5:1, 2:1, 1:2, 1:4, v/v) as the gradient eluant, obtaining five fractions (Frs. 1-5, 500 mL each). The fractions (38 g), which turns orange and cyan when exposed to Dragendorff and ferric chloride reagent, were applied to an ODS column chromatography (20-40 um, 150 g, Ultimate XB-C18, φ3x70 cm) and eluted with methanol (40%, 50%, 60%, 70%, respectively). At medium pressure, five fractions (A1-A5, 100 mL each) were obtained. A3 (0.46 g) was changed to cyan when exposed to ferric chloride reagent, and then purified on a Sephadex LH-20 column (100 g, φ 2 × 150 cm) using methanol solvent to give fractions B1-B5, and then B5 was prepared by with ultra-high-performance liquid chromatography (UHPLC), using Acetonitrile-0.1% formic acid as the mobile phase, with flow rate of 1.0 mL/min, and obtained oleraisoindole A (1 mg, purity of > 99% with UHPLC, t_R 5.269 min, Acetonitrile-0.1% formic acid, 43:57, v/v).

Acetylcholinesterase inhibition assay

Anticholinesterase effect was determined according to the modified Ellman method (Ellman et al. 1961). The sample solutions in methanol was diluted into five different concentrations and the AChE solution in buffer of PBS (pH 8.0, containing 0.1 mol/L Na_2HPO_4 and NaH_2PO_4) were mixed in wells a 96-well microplate. Then incubated for 10 minutes at 37°C and added 5,5-dithiobis-2-nitrobenzoic acid (DTNB) to the buffer. Finally, acetylthiocholine iodide (ATCI) was added and the absorbance was monitored spectrophotometrically at 405 nm. The same concentration of eserine was used as a positive control. Instead of samples, methanol was included in the blank control. Each sample was measured in parallel five times and the percentage of inhibition of AChE was calculated using the following formula (Šinko et al. 2007):

Anticholinesterase effect (%) = $[(A_{blank} - A_{sample})/A_{blank}] \times 100\%$

where, A_{sample} is the absorbance of the test compound, and A_{blank} is the absorbance of the blank control. The anticholinesterase effect was evaluated by the value of IC_{50}, and values are expressed as the means ± SD for $n = 5$.

References

Ellman GL, Courtney KD, Jr AV and Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7:88-90.

Šinko G, Čalić M, Bosak A and Kovarik Z. 2007. Limitation of the ellman method: cholinesterase activity measurement in the presence of oximes. Anal Biochem. 370:223-227.