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Chinese Journal of Natural Medicines 2019, **17**(4): 0303–0307
Chinese Journal of Natural Medicines

New octadecanoid derivatives from the seeds of *Ipomoea nil*

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Available online 20 Apr., 2019

[ABSTRACT] Four new octadecanoid derivatives (**1**–**4**) including a pair of enantiomers (**1**/**2**), along with 12 known analogues (**5**–**16**), were isolated from the seeds of *Ipomoea nil*. Their structures were determined by detailed spectroscopic analyses and comparison with reported data of structurally related compounds, with the absolute configurations of **1** and **2** being assigned by an *in situ* dimolybdenum ECD method. Our bioassays revealed that these isolates did not show ABTS radical scavenging activity while **10** and **13** displayed better α-glucosidase inhibitory activity than the positive control acarbose (IC_{50} 167.7 ± 1.55 μmol·L^{-1}), with IC_{50} of 92.73 ± 3.12 and 11.39 ± 2.18 μmol·L^{-1}, respectively.

[KEY WORDS] *Ipomoea nil*; Octadecanoid; Fatty acid; Natural enantiomer; α-Glucosidase inhibition

[CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2019)04-0303-05

Introduction

Ipomoea nil (L.) Roth (formerly known as *Pharbitis nil* (L.) Choisy) is an ornamental plant widely cultivated all over the world, and its seeds, called 'Qian niu zi' in traditional Chinese medicine, have commonly been used as diuretic and insecticide or to eliminate phlegm [1]. According to the color, 'Qian niu zi' is normally divided into two types, namely, 'Hei chou' and 'Bai chou' [2]. Previous investigations into the chemical constituents of *I. nil* seeds have revealed diverse structural types including lignans [3-5], diterpenoids and their glycosides [6-8], lipid glycosides [9-10], simple phenolic constituents [5, 11], and so on [12-14]. Most of these natural products were reported to show cytotoxicity against a variety of human cancer cell lines [3-7, 9, 12]. However, the non-polar lipid constituents of *I. nil* seeds have seldom been reported [15]. In our present work, we carried out an intensive chemical investigation on the EtOAc partition generated from the ethanolic extract of the seeds of *I. nil*, which resulted in the isolation and identification of a pair of new octadecanoid glycerides (**1** and **2**), two new octadecanoid ethyl esters (**3** and **4**), and a series of fatty acid analogues (**5**–**16**). The structures of these compounds were elucidated by comprehensive spectroscopic analyses, and the absolute configurations of **1** and **2** were determined by an *in situ* dimolybdenum ECD method. All the compounds (**1**–**16**) were tested for their ABTS radical scavenging and α-glucosidase inhibitory activities, while only the known **10** and **13** exhibited inhibitory effects toward α-glucosidase with IC_{50} of 92.73 ± 3.12 and 11.39 ± 2.18 μmol·L^{-1}, respectively. Herein, we describe the separation, structural determination and biological evaluations of these lipid molecules.

Results and Discussion

Compounds **1**/**2** were assigned the molecular formula of $C_{21}H_{38}O_5$ by (+)-HR-ESIMS analysis at *m/z* 371.2792 ([M + H]$^+$, calcd. 371.2792). The ^{1}H NMR data (Table 1) revealed the presence of a *cis*-form double bond [δ_H 5.27 (1H, dtt, J = 10.8, 7.2, 1.5 Hz), and 5.37 (1H, dtt, J = 10.8, 7.3, 1.5 Hz)], a glycerol moiety [δ_H 3.58 (1H, dd, J = 11.2, 5.9 Hz), 3.68 (1H, brd, J = 11.2 Hz), 3.92 (1H, m), 4.13 (1H, dd, J = 11.6, 6.2 Hz), 4.17 (1H, dd, J = 11.6, 4.7 Hz)], a methyl [δ_H 0.87 (t, J = 7.0 Hz)], and 13 aliphatic methylenes [δ_H 1.30 (12H, m), 1.54 (2H, m), 1.60 (2H, m), 2.01 (2H, qd, J = 7.3, 1.5 Hz), 2.28 (2H, qd, J = 7.4, 1.4 Hz), 2.33 (2H, t, J = 7.5 Hz), 2.38 (2H, t, J = 7.4 Hz), 2.42 (2H, t, J = 7.4 Hz)]. The ^{13}C NMR data

[Received on] 12-Nov.-2018
[Research funding] This work was supported by the Natural Science Foundation of Shandong Province (No. JQ201721), the Young Taishan Scholars Program (No. tsqn20161037), the Director Foundation of XTIPC, CAS (No. 2015RC015), and Shandong Talents Team Cultivation Plan of University Preponderant Discipline (No. 10027).
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These authors have no conflict of interest to declare.

(Table 1) showed signals for a ketone (δ_C 211.3, C-9), an ester carbonyl (δ_C 174.3, C-1), two olefinic (δ_C 127.8, 131.4), 16 methylenes (δ_C 21.8–65.3) and a methyl (δ_C 14.2) carbons. These spectral features were similar to those of (2'*S*)-1-*O*-(9-oxo-10(*E*), 12(*E*)-octadecadienoyl) glycerol isolated from the tuber-barks of *Colocasia antiquorum* var. *esculenta* [16], and the only difference was that compounds **1**/**2** only had one double bond. The positions of the double bond and the ketone were confirmed by examination of 2D NMR data (Fig. 2), with key ^{1}H–^{1}H COSY correlations across H_2-10 to H_2-14 and pivotal HMBC correlations from H_3-18 to C-16, H_2-14 to C-12 and C-16, and H_2-7, H_2-8, H_2-10 and H_2-11 to the C-9 ketone. The glycerol moiety was connected to C-1 to form an ester bond, based on the HMBC correlations from H_2-1′ to C-1 and the downfield chemical shifts for H_2-1′ due to esterification. Thus the planar structures of compounds **1**/**2** were characterized as 1-*O*-(9-oxo-12*Z*-octadecamonoyl) glycerol with only one chiral center. When measuring the optical rotation of **1**/**2**, the near zero value alerted that the enantiomeric purity might need to be checked. Subsequent chiral HPLC analysis revealed a pair of enantiomers in a ratio of *ca.* 3: 2, which led to the further chiral separation of **1** ($[\alpha]_D^{24}$ +3.0) and **2** ($[\alpha]_D^{24}$ −3.3). The absolute configuration **2** was determined to be 2'*R* based on the positive Cotton effect at 311 nm (Δε +6.4) (Fig. 3) in the *in situ* dimolybdenum ECD experiment developed by Snatzke and Frelek [17-18], and that of **1** was thus assigned to be 2'*S*.

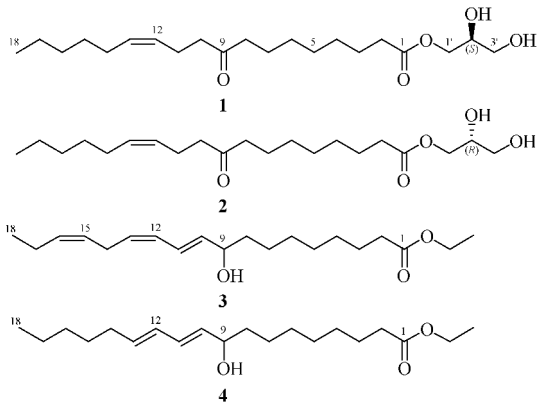

Fig. 1 Chemical structures of 1–4

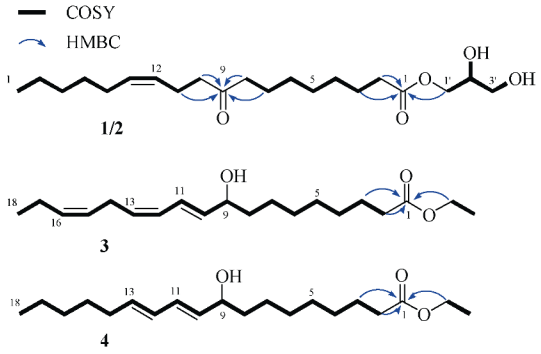

Fig. 2 ^{1}H–^{1}H COSY and selected HMBC correlations for 1/2, 3 and 4

Compound **3**, colorless oil, was assigned the molecular formula of $C_{20}H_{34}O_3$ by (+)-HR-ESIMS analysis at *m/z* 345.2398 ($[M + Na]^+$, calcd. 345.2400). The ^{1}H and ^{13}C NMR data of **3** (Table 1) were highly similar to those of (10*E*, 12*Z*, 15*Z*)-9-hydroxy-10, 12, 15-octadecatrienoic acid methyl ester [19], except that the methoxyl group [δ_H 3.66 (s); δ_C 51.4] in the latter was replaced by an ethoxyl moiety [δ_H 1.25 (3H, t, *J* = 7.2 Hz), 4.12 (2H, q, *J* = 7.2 Hz); δ_C 14.4, 60.3] in **3**. This assignment was further supported by careful inspection of 2D NMR data (Fig. 2). Therefore, the structure of **3** was identified as (10*E*, 12*Z*, 15*Z*)-9-hydroxy-10, 12, 15-octadecatrienoic acid ethyl ester.

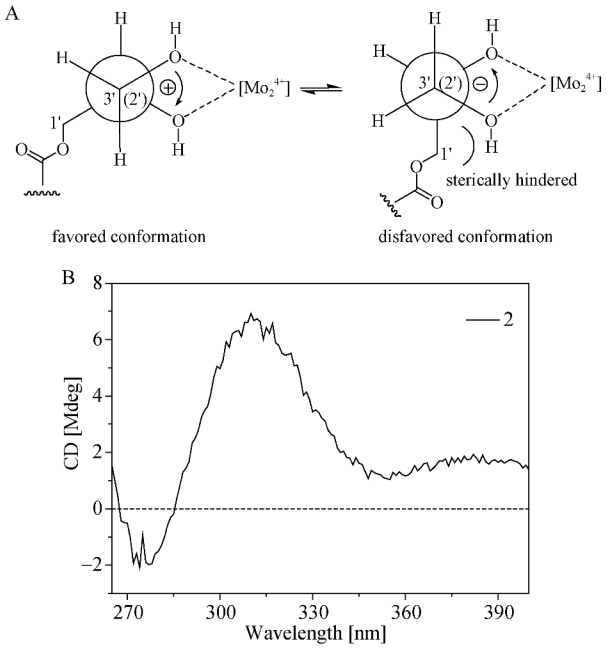

Fig. 3 Conformations and induced ECD spectrum of Mo_2^{4+} complexes of 2

Similarly, compound **4** was assigned the molecular formula of $C_{20}H_{36}O_3$ by (+)-HR-ESIMS analysis at *m/z* 347.2550 ($[M + Na]^+$, calcd 347.2557). The ^{1}H and ^{13}C NMR data of **4** (Table 1) were highly similar to those of (10*E*, 12*E*)-9-hydroxy-10, 12-octadecadienoic acid methyl ester [20], except that the methoxyl group [δ_H 3.67 (s); δ_C 51.3] in the latter was replaced by an ethoxyl moiety [δ_H 1.25 (3H, t, *J* = 7.1 Hz), 4.12 (2H, q, *J* = 7.2 Hz); δ_C 14.4, 60.3] in **4**. Detailed examination of 2D NMR data (Fig. 2) further confirmed this structural assignment. Thus the structure of **4** was determined as (10*E*, 12*E*)-9-hydroxy-10, 12-octadecadienoic acid ethyl ester.

Both **3** and **4** with an ethoxyl group in the structure were possibly artifacts from esterification of their respective free acid or from interesterification of their respective methyl ester, due to use of ethanol as the extracting solvent. The two compounds had only one stereocenter in their structures and showed very small $[\alpha]_D$ values (see Experimental), which also alerted us to check their enantiomeric purities like we did to compounds **1**/**2**. Interestingly, no separable peaks were

Table 1 NMR data for 1/2, 3 and 4 in $CDCl_3$

No.	1/2		3		4	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	-	174.3	-	174.1	-	174.1
2	2.33, t (7.5)	34.2	2.28, t (7.5)	34.5	2.28, t (7.5)	34.5
3	1.60, m	24.9	1.60, m	25.1	1.61, m	25.1
4	1.30, m	28.9[a]	1.31, m	29.2[b]	1.31, m	29.2[c]
5	1.30, m	29.1[a]	1.31, m	29.3[b]	1.31, m	29.3[c]
6	1.30, m	29.1[a]	1.31, m	29.5[b]	1.31, m	29.5[c]
7	1.54, m	23.7	1.31, m	25.5	1.31, m	25.5
8	2.38, t (7.4)	42.9	1.50, m	37.4	1.50, m	37.4
9	-	211.3	4.16, m	73.0	4.10, m	73.0
10	2.42, t (7.4)	42.8	5.68, dd (15.2, 6.8)	136.5	5.56, dd (15.3, 7.0)	133.7
11	2.28, qd (7.3, 1.5)	21.8	6.51, dd (15.2, 11.2)	125.7	6.17, dd (15.3, 10.4)	131.2
12	5.27, dtt (10.8, 7.2, 1.5)	127.8	5.98, t (11.2)	127.9	6.02, dd (15.0, 10.4)	129.5
13	5.37, dtt (10.8, 7.3, 1.5)	131.4	5.41, m	130.9	5.70, dt (15.0, 7.0)	135.8
14	2.01, qd (7.3, 1.5)	27.3	2.93, t (7.3)	25.5	2.06, m	32.8
15	1.30, m	29.4	5.31, m	126.6	1.38, m	29.1
16	1.30, m	31.6	5.41, m	132.6	1.31, m	31.6
17	1.30, m	22.7	2.08, m	20.7	1.31, m	22.7
18	0.87, t (7.0)	14.2	0.98, t (7.5)	14.4	0.88, t (7.0)	14.1
OEt	-	-	4.12, q (7.2)	60.3	4.12, q (7.2)	60.3
			1.25, t (7.2)	14.4	1.25, t (7.2)	14.4
1'	4.13, dd (11.6, 6.2)	65.3				
	4.17, dd (11.6, 4.7)					
2'	3.92, m	70.3				
3'	3.68, brd (11.2)	63.5				
	3.58, dd (11.2, 5.9)					
2'-OH	2.96, brs					
3'-OH	2.61, brs					

[a-c] Interchangeable assignments

resolved for both compounds on our three different types of chiral columns (two normal phases and one reversed phase, see Experimental).

The known compounds were identified, on the basis of detailed spectroscopic interpretation, to be 9-octadecenoic acid-2', 3'-dihydroxy propyl ester (**5**) [21], (*Z*)-12-octadecenic-α-glycerol monoester (**6**) [22], glycerol 1-9', 12'-octadecadienoate (**7**) [23], 1-octadecatetraenoyl glycerol (**8**) [24], 2-linoleoylglycerol (**9**) [25], 13-hydroxy-9*Z*, 11*E*-octadecadienoic acid (**10**) [20], 9-oxooctadec-*cis*-12-enoic acid (**11**) [26], linoleic acid (**12**) [27], rabdosia acid A (**13**) [28], oxylipin (**14**) [29], (*Z*)-9, 10, 11-trihydroxy-12-octadecenoic acid (**15**) [30] and (8*R*, 9*R*, 10*S*, 6*Z*)-trihydroxyoctadec-6-enoic acid (**16**) [31] (see Fig. S1, Supporting information).

All the isolates were evaluated in two bioassays, namely, ABTS radical scavenging and *α*-glucosidase inhibitory tests. While none of them showed ABTS radical scavenging activity (at 100μmol·L^{-1}, ascorbic acid as positive control), compounds **10** and **13** displayed inhibitory activity against *α*-glucosidase with IC_{50} of 92.73 ± 3.12 and 11.39 ± 2.18 μmol·L^{-1}, respectively (acarbose as positive control, IC_{50} 167.7 ± 1.55 μmol·L^{-1}).

Experimental

General experimental procedures

Optical rotations were measured on a Rudolph VI polarimeter (Rudolph Research Analytical, Hackettstown, USA) with a 10 cm length cell. NMR experiments were recorded on a Bruker Avance DRX600 spectrometer (Bruker BioSpin AG, Fallanden, Switzerland) and referenced to residual solvent peaks ($CDCl_3$: δ_H 7.26, δ_C 77.16). HR-ESIMS spectra were obtained on an Agilent 6545 Q-TOF mass spectrometer (Agilent Technologies Inc., Waldbronn, Germany). ESIMS analyses were carried out on an Agilent 1260-6460 Triple Quad LC-MS instrument (Agilent Technologies Inc., Waldbronn, Germany). UV spectra were obtained on a Shimadzu UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan) with a 1 cm pathway cell. All normal HPLC separations were performed using an Agilent 1260 series LC instrument (Agilent Technologies Inc., Waldbronn, Germany) coupled with an Agilent SB-C_{18} column (9.4 mm × 250 mm, Agilent Technologies Inc., Santa Clara, USA) unless specified. CHIRALPAK AD-H

and OD-H (both 4.6 mm × 250 mm) columns (Daicel Corporation, Tokyo, Japan) and Chiral MZ (2) RH 5u (4.6 mm × 250 mm) column (Phenomenex, Washington D.C., USA), were used for chiral HPLC analysis and separation. Column chromatography (CC) was performed on D101-macroporous absorption resin (Sinopharm Chemical Reagent Co., Ltd., Shanghai), reversed phase C18 silica gel (Merck KGaA, Darmstadt, Germany), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and silica gel (300–400 mesh; Qingdao Marine Chemical Co. Ltd., Qingdao, China). All solvents used for CC were of analytical grade (Tianjin Fuyu Fine Chemical Co. Ltd., Tianjin, China) and solvents used for HPLC were of HPLC grade (Oceanpak Alexative Chemical Ltd., Goteborg, Sweden). Pre-coated silica gel GF254 plates (Qingdao Marine Chemical Co. Ltd., Qingdao, China) were used for TLC monitoring.

Plant materials

The seeds of *Ipomoea nil* (L.) Roth were bought in Kunming 'Juhuayuan' herbal market and were authenticated by Prof. ZHOU Jie from University of Jinan. A voucher specimen has been deposited at School of Biological Science and Technology, University of Jinan (Accession number: npmc-024).

Extraction and isolation

The air-dried powder of the seeds of *I. nil* (30 kg) was extracted with 95% EtOH at room temperature three times to afford a crude extract (2.8 kg). The extract was then suspended in 2.0 L water and partitioned with EtOAc (2.0 L × 3). The EtOAc soluble extract (445 g) was subjected to CC over D101-macroporous absorption resin, eluted with EtOH–H_2O (30%, 50%, 80% and 90%), to afford four fractions (A, B, C and D). Fraction C (74 g) was subjected to silica gel CC, eluted with petroleum ether–ethyl acetate (50 : 1 to 1 : 2, *V*/*V*), to produce 40 subfractions (C1–C40). Fraction C12 was separated by silica gel CC (petroleum ether–chloroform, 5 : 1 to 1 : 2, *V*/*V*) to produce three subfractions (C12-1–C12-3), and then C12-1 was first fractionated by Sephadex LH-20 CC (MeOH) and further purified by HPLC (3.0 mL·min^{-1}, 80% MeCN-H_2O) to afford **12** (1.6 mg, t_R = 15.0 min). Fraction C14 was separated by silica gel CC, eluted with petroleum ether–chloroform (5 : 1 to 1 : 2, *V*/*V*), to produce two subfractions (C14-1 and C14-2), and then C14-2 was first fractionated by Sephadex LH-20 CC (MeOH) and further separated by HPLC (3.0 mL·min^{-1}, 72% MeCN–H_2O, t_R = 11.6, 12.5 and 15.0 min, respectively) to afford **3** (2.8 mg), **10** (5.4 mg) and **4** (2.8 mg). Fraction C20 was separated by silica gel CC, eluted with petroleum ether–acetone (10 : 1 to 1 : 2, *V*/*V*), to produce three subfractions (C20-1–C20-3), and then C20-2 was purified by HPLC (3.0 mL·min^{-1}, 50% MeCN–H_2O) to afford **5** (5.6 mg, t_R = 11.5 min). Fraction C28 was subjected to Sephadex LH-20 CC (MeOH) to give one major subfraction which was further purified by HPLC (3.0 mL·min^{-1}, 67% MeCN–H_2O) to furnish **13** (15.0 mg, t_R = 11.6 min). Fraction C33 was separated by silica gel CC, eluted with petroleum ether-chloroform (10 : 1 to 1 : 2, *V*/*V*), to produce three subfractions (C33-1–C33-3), and then C33-2 was first fractionated by Sephadex LH-20 CC (MeOH) and further purified by HPLC (3.0 mL·min^{-1}, 55% MeCN-H_2O, t_R = 7.0, 9.0 and 9.5 min, respectively) to afford **14** (1.2 mg), **15** (3.1 mg) and **16** (2.4 mg). Fraction C37 was first subjected to Sephadex LH-20 CC (MeOH) to give two major subfractions (C37-1 and C37-2) and C37-1 was then purified by HPLC (3.0 mL·min^{-1}, 50%–95% MeCN–H_2O in 20 min, t_R = 5.0, 7.0, 8.5, 16.0, 18.5 and 21.5 min, respectively) to afford **1**/**2** (22.5 mg), **8** (2.5 mg), **11** (1.6 mg), **9** (15.2 mg), **7** (163.8 mg) and **6** (33.3 mg). Compounds **1** (1.7 mg, t_R = 11.0 min) and **2** (1.1 mg, t_R = 12.0 min) were then separated from each other on an OD-H chiral column (1.0 mL·min^{-1}, 10% isopropanol in *n*-hexane).

Identification of new compounds

*Compounds **1/2***

Colorless oil; $[\alpha]_D^{24}$ +3.0 (*c* 0.17, MeOH) for **1**, –3.3 (*c* 0.11, MeOH) for **2**; (+)-ESIMS *m*/*z* 371.2 $[M + H]^+$; (+)-HR-ESIMS *m*/*z* 371.2792 $[M + H]^+$ (Calcd. for $C_{21}H_{39}O_5$, 371.2792); ^1H and ^{13}C NMR data ($CDCl_3$) see Table 1.

*Compound **3***

Colorless oil; $[\alpha]_D^{24}$ –1.3 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε): 204 (3.57), 217 (3.50) nm; (+)-ESIMS *m*/*z* 345.2 $[M + Na]^+$; (+)-HR-ESIMS *m*/*z* 345.2398 $[M + Na]^+$ (Calcd. for $C_{20}H_{34}O_3Na$, 345.2400); ^1H and ^{13}C NMR ($CDCl_3$) data see Table 1.

*Compound **4***

Colorless oil; $[\alpha]_D^{24}$ +1.4 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε): 224 (3.66) nm; (+)-ESIMS *m*/*z* 347.2 $[M + Na]^+$; (+)-HR-ESIMS *m*/*z* 347.2550 $[M + Na]^+$ (Calcd. for $C_{20}H_{36}O_3Na$, 347.2557); ^1H and ^{13}C NMR data ($CDCl_3$) see Table 1.

α-Glucosidase inhibitory assay

Briefly, 0.2 U of α-glucosidase from *Saccharomyes cerevisiae* (Sigma-Aldrich, St. Louis, MO, USA) was diluted by 0.1 mol·L^{-1} phosphate buffer consisting of Na_2HPO_4 and NaH_2PO_4 (pH 6.8). The assay was conducted in a 200 μL reaction system containing 98 μL of buffer, 25 μL of diluted enzyme solution and 2 μL of DMSO (blank control) or tested samples (dissolved in DMSO). After 20 min of incubation in 96-well plates at 37 °C, 25 μL of 0.4 mmol·L^{-1} PNPG (4-nitrophenyl-*β*-D-glucopyranoside, Aladdin, Shanghai, China) was added as substrate to start the enzymatic reaction. The plate was incubated for an additional 15 min at 37 °C, followed by the addition of 50 μL of 0.2 mol·L^{-1} Na_2CO_3 to stop the reaction. The optical density (OD) was measured at an absorbance wavelength of 405 nm using a Microplate Reader (Tecan, Switzerland). The inhibitory effects of all the isolates were first assessed at the concentration of 100 μmol·L^{-1}, and only compounds showing > 50% inhibition rate were further subjected for IC_{50} measurements. Acarbose (Aladdin, Shanghai, China) was used as a positive control with an IC_{50} of 167.7 ± 1.55 μmol·L^{-1}.

ABTS radical scavenging assay

The ABTS radical scavenging assay was performed as

we reported previously [32]. All the compounds were first assessed at the concentration of 100 μmol·L^{-1}, while none of them exhibited enough activity for further IC_{50} measurement. Ascorbic acid was used as a positive control.

Acknowledgements

We thank Prof. ZHOU Jie for the identification of the plant materials.

Supporting information

Original spectroscopic data including chiral HPLC separation, HR-ESIMS and NMR (^{1}H, ^{13}C, ^{1}H-^{1}H COSY, HSQC and HMBC) spectra for compounds **1**–**4** were provided.

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Cite this article as: SONG Xiu-Qing, ZHANG Jun-Sheng, YU Shu-Juan, YU Jin-Hai, ZHANG Hua. New octadecanoid derivatives from the seeds of *Ipomoea nil* [J]. *Chin J Nat Med*, 2019, **17**(4): 303-307.