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Indoleacetic acid derivatives from the seeds of *Ziziphus jujuba* var. *spinosa*

Min Li [a], Yu Wang [b,c], Bun Tsoi [a], Xiao-jie Jin [d], Rong-Rong He [a,e], Xiao-jun Yao [d,f], Yi Dai [a,*], Hiroshi Kurihara [a,e], Xin-Sheng Yao [a,b]

[a] Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, China
[b] College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China
[c] Quality Assurance Center, North China Pharmaceutical Group Semisyntech Co., Ltd., Shijiazhuang 052165, China
[d] Department of Chemistry, Lanzhou University, Lanzhou 730000, China
[e] Anti-stress and Health Research Center, College of Pharmacy, Jinan University, Guangzhou, China
[f] State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Taipa, Macau, China

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ABSTRACT

A pair of diastereoisomers, the *N*-glycosylated derivatives of dioxindole-3-hydroxy-3-acetic acid **1**–**2**, and their conjugates with flavonoids **3**–**8**, was isolated from the seeds of *Ziziphus jujuba* var. *spinosa*. Their structures were elucidated by NMR spectroscopic analyses, and the absolute configurations were determined by circular dichroism method. Compounds **3**–**10** were evaluated for the antioxidant capacity, using the radical absorbance capacity (ORAC) assay.

1. Introduction

Ziziphus jujuba var. *spinosa* spreads widely in China, the fruit of which is a kind of nutritious food. The seeds of this plant are a popular traditional Chinese medicine, Ziziphi Spinosae Semen, used for the treatment of insomnia and palpitation [1]. The previous studies on the chemical constitution of the seeds resulted in the discovery of a series of flavonoids [2–4], saponins [5–7] and alkaloids [8,9], which showed synergetic hypnotic effect with barbiturates by reducing sleep latency and prolonging sleep time [10]. Our further chemical investigation on the crude extract of seeds of *Z. jujuba var. spinosa*, led to the isolation of two new *N*-glycosylated indoleacetic derivatives (**1**, **2**) and a series of their conjugates with flavone C-glycosides (**3**–**8**), as well as spinosin (**9**) and 6‴-feruloylspinosin (**10**) [2] (Fig. 1). Herein we mainly reported the isolation and structure elucidation of the new compounds.

2. Experimental

2.1. General procedures

Optical rotations were determined on a JASCO P-1020 digital polarimeter with a 1 cm cell. UV spectra were recorded on a JASCO V-550 UV/Vis spectrometer. IR spectra were obtained using a JASCO FT/IR-480 plus spectrometer. CD spectra were obtained on a Jasco J-810 spectropolarimeter at room temperature. NMR experiments were performed on Bruker 600 spectrometers. HR-ESI-MS spectra were acquired using a Waters Snapt G2 mass spectrometer. Silica gel (100–200, 200–300 mesh,

* Corresponding author at: Institute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China. Tel.: +86 20 85220785; fax: +86 20 85221559.
E-mail address: daiyi1004@163.com (Y. Dai).

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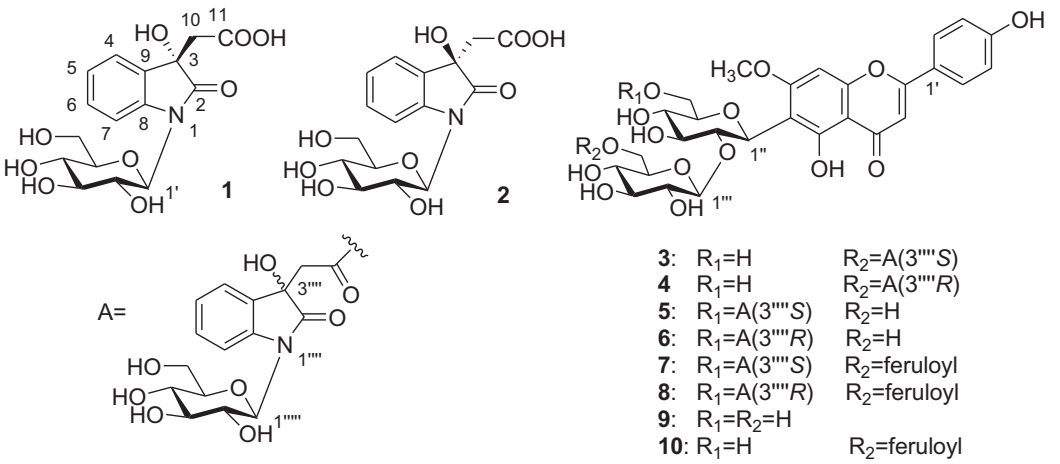

Fig. 1. The structures of compounds **1**–**10**.

Qingdao Marine Chemical Ltd., China), octadecylsilanized (ODS) silica gel (50 μm; YMC Ltd., Japan) and Toyopearl HW40 (TOSOH Ltd., Japan) were used for open column chromatography. The preparative HPLC was performed on Shimadzu LC-6AD series with Cosmosil 5C_{18}-MS-II column (20 × 250 mm, 5 μm).

2.2. Plant material

The seeds of *Z. jujuba* var. *spinosa* were supplied by Shijiazhuang Yiling Pharma Group Co., Ltd. and identified by Dr. Qing-Cun Tian. A voucher specimen (20110810ZSS) had been deposited at the Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou, China.

2.3. Extraction and isolation

The crushed seeds of *Z. jujuba* var. *spinosa* (14.5 kg) were extracted with 70% EtOH (4 × 80 L). After removing the solvent, the residue (1.78 kg) was chromatographed over D101 macroporous resin column eluted with ethanol and water in gradient to give five fractions (Frs. A–E). Fr. C (EtOH/H_2O 1:1 eluate, 106.4 g) was subjected to silica gel column eluted with $CHCl_3$/MeOH in gradient to give 10 fractions (Frs. C1–C10). Fr. C7 ($CHCl_3$/MeOH 8:2 eluate, 13.1 g) was separated by ODS column eluted with MeOH/H_2O in gradient to give 8 subfractions (Frs. C7A–H).

Fr. C7A (MeOH/H_2O 3:7 eluate, 573 mg) was further separated by ODS column eluted with 5% MeOH/H_2O and purified by HPLC with 5% MeOH to afford **1** (12.6 mg) and **2** (7.2 mg). Fr. C7B (MeOH/H_2O 3:7 eluate, 538 mg) was then chromatographed on HW-40 column eluted with 10% MeOH/H_2O and purified by HPLC with 35% MeOH to afford **9** (44.0 mg). Fr. C7C (MeOH/H_2O 35:65 eluate, 3.40 g) was further separated by ODS column eluted with 30% MeOH/H_2O to give 5 subfractions (Frs. C7C1–C7C5). Fr. C7C4 (540 mg) and Fr. C7C5 (536 mg) were then chromatographed respectively on HW-40 column eluted with 10% MeOH/H_2O and purified by HPLC with 18% CH_3CN to afford **3** (41.1 mg), **4** (21.6 mg), **5** (29.7 mg) and **6** (20.6 mg). Fr. C7D (MeOH/H_2O 35:65 eluate, 1.83 g) was further separated by ODS column eluted with MeOH/H_2O in gradient to give 5 subfractions (Frs. C7D1–C7D5). Fr. C7D3 (MeOH/H_2O 3:7 eluate, 1.27 g) and Fr. C7D4 (501 mg) were then chromatographed respectively on HW-40 column eluted with 10% and 30% MeOH/H_2O. Fr. C7D3D (MeOH/H_2O 3:7 eluate, 316 mg) and Fr. C7D4C (MeOH/H_2O 3:7 eluate, 93 mg) were purified by HPLC with 35% MeOH to afford **10** (16.7 mg), **7** (26.5 mg) and **8** (22.2 mg).

3*S*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetic acid (**1**)

Colorless solid; $[\alpha]_D^{27}$ −32.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.37), 252 (3.69) nm; IR (KBr) ν_{max}: 3368, 2886, 1720, 1615, 1375, 1076, 753 cm^{-1}; CD (MeOH) λ_{max} (Δε) 212 (−26.4), 238 (+19.4), 262 (−8.5) nm; ^1H NMR and ^{13}C NMR, see Table 1. HR-ESI-MS *m/z* 392.0966 $[M + Na]^+$ (calcd. for $C_{16}H_{19}NO_9Na$, 392.0958).

Table 1
NMR spectroscopic data for compounds **1** and **2** (^1H 600 MHz, ^{13}C NMR 150 MHz, CD_3OD, δ in ppm).

Position	**1**		**2**	
	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)
2	179.3		179.3	
3	74.3		74.8	
4	125.0	7.44 (d, 7.8)	125.1	7.44 (d, 7.8)
5	124.5	7.11 (t, 7.8)	124.4	7.10 (t, 7.8)
6	130.8	7.31 (t, 7.8)	130.8	7.31 (t, 7.8)
7	113.7	7.27 (d, 7.8)	113.0	7.23 (d, 7.8)
8	142.3		142.8	
9	131.7		131.8	
10	43.7	3.12 (br. s)	43.7	3.01 (br. s)
11	173.9		173.9	
1′	84.2	5.40 (d, 9.0)	83.9	5.31 (d, 9.0)
2′	71.0	4.08 (t, 9.0)	70.6	4.16 (t, 9.0)
3′	78.5	3.56 (m)	78.9	3.53 (m)
4′	71.5	3.50 (m)	71.6	3.48 (m)
5′	81.2	3.50 (m)	81.2	3.48 (m)
6′	62.9	3.92 (d, 12.0)	63.0	3.90 (d, 12.0)
		3.75 (dd, 12.0, 4.2)		3.73 (dd, 12.0, 4.2)

3*R*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetic acid (**2**)

Colorless solid; $[\alpha]_D^{27}$ +0.8 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.21), 252 (3.54) nm; IR (KBr) ν_{max}: 3395, 2882, 1722, 1619, 1376, 1076, 758 cm^{-1}; CD (MeOH) λ_{max} (Δε) 212 (+43.8), 238 (−31.9); ^1H NMR and ^{13}C NMR, see Table 1. HR-ESI-MS *m/z* 392.0963 $[M + Na]^+$ (calcd. for $C_{16}H_{19}NO_9Na$, 392.0958).

6‴-*O*-(3*S*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetyl)spinosin (**3**)

Yellow solid; $[\alpha]_D^{27}$ −71.2 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 211 (4.63), 268 (4.26), 336 (4.33) nm; IR (KBr) ν_{max}: 3398, 2887, 1730, 1608, 1352, 1077, 581 cm^{-1}; CD (MeOH) λ_{max} (Δε) 213 (−11.3), 238 (+8.7), 262 (−6.3) nm; ^1H NMR and ^{13}C NMR, see Tables 2 and 3. HR-ESI-MS *m/z* 960.2764 $[M + H]^+$ (calcd. for $C_{44}H_{50}NO_{23}$, 960.2774).

6‴-*O*-(3*R*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetyl)spinosin (**4**)

Yellow solid; $[\alpha]_D^{27}$ −66.8 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.62), 268 (4.28), 335 (4.33) nm; IR (KBr) ν_{max}:

Table 2
^{13}C NMR spectroscopic data for compounds **3**–**8** (150 MHz, DMSO, δ in ppm).

Position	3	4	5	6	7	8
2	164.2/164.1	164.2/164.0	163.8/163.8	163.9/163.7	164.3/164.1	164.3/164.1
3	103.2/103.1	103.0/102.9	103.1/103.0	102.7/102.6	102.9/102.7	102.7/102.5
4	182.3/181.9	182.1/181.8	182.3/182.0	182.1/181.8	182.2/181.8	182.2/181.7
5	160.5/159.7	160.5/159.6	160.6/159.7	160.5/159.6	160.8/159.5	160.8/159.5
6	108.7/108.6	108.7	108.2	108.3	108.3/108.3	108.4/108.3
7	165.1/163.8	165.0/163.7	165.0/163.9	164.9/164.0	165.1/163.6	165.1/163.5
8	90.8/90.4	90.6/90.1	90.9/90.4	90.8/90.3	90.8/90.0	90.7/89.9
9	157.1/157.0	157.0/156.8	157.2/157.0	157.1/156.9	157.1/156.9	157.0/156.9
10	104.3/104.1	104.3/104.0	104.5/104.2	104.4/104.1	104.5/104.0	104.4/104.0
1′	121.3/121.2	120.7	121.0/121.0	120.3/120.2	120.7/120.6	120.3/120.3
2′ 6′	128.8/128.8	128.7/128.6	128.6/128.5	128.4	128.6/128.5	128.5/128.4
3′ 5′	116.0/116.0	116.1	116.0	116.1	116.0/116.0	116.1/116.0
4′	161.3/161.2	161.8	161.4	162.2/162.2	162.0	162.0
OCH_3	56.4/56.2	56.4/56.2	56.6/56.2	56.5/56.1	56.5/56.2	56.4/56.2
1″	71.0/70.7	71.1/70.7	71.2/70.7	71.0/70.6	71.1/70.7	71.0/70.6
2″	81.4/81.0	81.1/79.9	80.7/80.4	80.7/80.2	81.7/80.0	81.5/79.9
3″	78.8/78.4	78.8/78.3	78.2/78.1	78.1/77.9	78.4/78.2	78.3/78.1
4″	70.4/70.4	70.4/70.4	70.2/70.1	70.0/70.0	70.1/70.0	69.8
5″	81.9/81.7	81.9/81.6	77.9/77.9	77.8/77.7	78.1/77.9	78.0/77.7
6″	61.5/61.5	61.5/61.5	64.7/64.6	64.3/64.1	64.8/64.7	64.3/64.1
1‴	105.5/105.1	104.9/104.6	105.4/105.2	105.2/105.1	105.7/105.2	105.6/105.1
2‴	74.6/74.4	74.5/74.3	74.7/74.5	74.6/74.5	74.5/74.4	74.4/74.3
3‴	76.1/75.9	76.1/75.9	76.4/76.3	76.3/76.2	76.3/76.2	76.3/76.2
4‴	69.1/68.7	69.0/68.9	69.5/69.2	69.6/69.3	68.9/68.6	68.9/68.6
5‴	73.6/73.3	73.5/73.5	76.7/76.4	76.5/76.3	73.4/73.2	73.4/73.2
6‴	63.6/62.5	63.1/62.7	60.6/60.1	60.7/60.2	62.4/62.1	62.4/62.0
2′′′′	176.7/176.5	175.8/175.7	176.5	175.7	176.5	175.8
3′′′′	72.7	72.3/72.3	72.6/72.6	72.3	72.7	72.3
4′′′′	125.9/125.1	124.1/123.9	125.0/125.0	123.8	125.1/125.1	123.8
5′′′′	122.4/122.4	122.2/122.2	122.2	122.0/121.9	122.2	122.1/122.0
6′′′′	128.9/128.8	129.1	128.9	129.0	129.0	129.0
7′′′′	112.0/111.8	111.9/111.8	112.0/112.0	111.7/111.7	112.0	111.8
8′′′′	140.1/140.1	141.0	140.3	141.1	140.3	141.2
9′′′′	130.1/130.1	130.1/130.1	130.1	130.0/130.0	130.2	130.1/130.1
10′′′′	41.9/41.7	40.7/40.7	42.0/41.9	40.9	42.0/42.0	40.8
11′′′′	168.9/168.8	168.4/168.3	169.3	168.5	169.3	168.6
1′′′′′	82.0/82.0	82.2/82.1	81.9	82.0	82.0	82.0
2′′′′′	68.7	68.5	68.7	68.5	68.7	68.5
3′′′′′	77.1	77.0/76.9	77.0/77.0	77.0	77.0	77.0
4′′′′′	69.9/69.9	69.6	69.8	69.6	69.8	69.6
5′′′′′	80.0/80.0	79.6/79.6	79.9	79.5	80.0	79.6/79.6
6′′′′′	61.1	60.7	61.1	60.7	61.1	60.7
1′′′′′′					125.4/125.2	125.3/125.1
2′′′′′′					110.8/110.7	110.8/110.7
3′′′′′′					148.0/147.9	148.0/147.9
4′′′′′′					149.5/149.5	149.7/149.6
5′′′′′′					115.5/115.4	115.4/115.4
6′′′′′′					123.2/123.1	123.2/123.0
7′′′′′′					144.8/144.8	144.8/144.7
8′′′′′′					113.9/113.5	113.9/113.5
9′′′′′′					166.4/166.3	166.3/166.2
OCH_3					55.7/55.6	55.6/55.6

Table 3
^{1}H NMR spectroscopic data for compounds **3–8** (600 MHz, DMSO, δ in ppm, J in Hz).

Position	3	4	5	6	7	8
2						
3	6.81/ 6.80 (s)	6.79/ 6.76 (s)	6.86/ 6.85 (s)	6.85/ 6.84 (s)	6.71/ 6.51 (s)	6.72/ 6.52 (s)
4						
5						
6						
7						
8	6.80/ 6.70 (s)	6.74/ 6.72 (s)	6.81/ 6.78 (s)	6.82/ 6.79 (s)	6.69/ 6.66 (s)	6.70/ 6.68 (s)
9						
10						
1′						
2′ 6′	7.98/ 7.98 (d, 9.0)	7.94/ 7.89 (d, 9.0)	7.98 (d, 9.0)	7.97/ 7.97 (d, 9.0)	7.81/ 7.80 (d, 9.0)	7.81/ 7.80 (d, 9.0)
3′ 5′	6.95/ 6.94 (d, 9.0)	6.93/ 6.92 (d, 9.0)	6.95 (d, 9.0)	6.92 (d, 9.0)	6.88/ 6.83 (d, 9.0)	6.86/ 6.82 (d, 9.0)
4′						
OCH_3	3.84/ 3.67 (s)	3.84/ 3.67 (s)	3.89/ 3.88 (s)	3.91 (s)	3.88/ 3.84 (s)	3.91/ 3.87 (s)
1″	4.70/ 4.68 (d, 9.6)	4.69/ 4.67 (d, 9.6)	4.72/ 4.71 (d, 9.6)	4.68/ 4.67 (d, 9.6)	4.71/ 4.71 (d, 9.6)	4.68/ 4.66 (d, 9.6)
2″	4.49/ 4.28 (t, 9.6)	4.44/ 4.26 (t, 9.6)	4.47/ 4.29 (t, 9.6)	4.44/ 4.26 (t, 9.6)	4.47/ 4.24 (t, 9.6)	4.44/ 4.21 (t, 9.6)
3″	3.45 (m)	3.43 (m)	3.45 (m)	3.41 (m)	3.47 (m)	3.42 (m)
4″	3.16 (m)	3.15 (m)	3.19 (m)	3.15 (m)	3.20 (t, 9.6)	3.15 (m)
5″	3.17 (m)	3.16 (m)	3.42 (m)	3.26 (m)	3.41 (m)	3.25 (m)
6″	3.69 (m) 3.37 (m)	3.70 (m) 3.38 (m)	4.40 (m) 3.87 (m)	4.21 (m) 3.78 (m)	4.41 (m) 3.86 (m)	4.21 (m) 3.78 (m)
1‴	4.25/ 4.21 (d, 7.8)	4.26/ 4.14 (d, 7.8)	4.17/ 4.15 (d, 7.8)	4.15/ 4.13 (d, 7.8)	4.27 (d, 7.8)	4.26/ 4.25 (d, 7.2)
2‴	2.85 (m)	2.84 (m)	2.85 (m)	2.84 (t, 8.4)	2.91 (m)	2.91/ 2.88 (m)
3‴	3.06 (m)	3.04/ 2.99 (t, 9.0)	3.06 (m)	3.04 (m)	3.10 (m)	3.10 (m)
4‴	2.87 (m)	2.86 (m)	2.98 (m)	2.98/ 2.95 (m)	3.08/ 3.04 (m)	3.06 (m)
5‴	3.02/ 2.83 (m)	2.95/ 2.68 (m)	2..75/ 2.56 (m)	2..74/ 2.55 (m)	3.12/ 2.95 (m)	3.11/ 2.93 (m)
6‴	3.79/ 3.65 (m) 3.70/ 3.53 (m)	3.72 (m) 3.52 (m)	3.18 (m) 2.96 (m)	3.18 (m) 2.97 (m)	3.96/ 3.87 (m) 3.79/ 3.62 (m)	3.96/ 3.87 (m) 3.79/ 3.62 (m)
2′′′′						
3′′′′						
4′′′′	7.29 (d, 7.8)	7.34/ 7.32 (d, 7.8)	7.47 (d, 7.2)	7.39 (d, 7.2)	7.46 (d, 7.8)	7.38 (d, 7.2)
5′′′′	7.01/ 6.94 (t, 7.8)	6.99/ 6.95 (t, 7.8)	6.92/ 6.91 (t, 7.2)	6.98/ 6.93 (t, 7.2)	6.91 (t, 7.8)	6.97/ 6.92 (t, 7.2)
6′′′′	7.26/ 7.24 (t, 7.8)	7.25/ 7.18 (t, 7.8)	7.23 (t, 7.2)	7.23/ 7.22 (t, 7.2)	7.22 (o)	7.22 (o)
7′′′′	7.14/ 7.12 (d, 7.8)	7.14/ 7.11 (d, 7.8)	7.13 (d, 7.2)	7.13/ 7.12 (d, 7.2)	7.12 (d, 7.8)	7.12 (d, 7.2)
8′′′′						
9′′′′						
10′′′′	2.83/ 2.81 (o) 2.66/ 2.59 (d, 14.4)	2.96/ 2.95 (o) 2.88/ 2.86 (o)	2.94 (o) 2.81 (d, 15.0)	3.07 (o) 2.95 (o)	2.93 (o) 2.81/ 2.80 (d, 15.0)	3.06 (o) 2.95 (o)
11′′′′						
1′′′′′	5.15/ 5.15 (d, 9.0)	5.15/ 5.13 (d, 9.0)	5.14 (d, 9.0)	5.13/ 5.13 (d, 9.0)	5.14 (d, 9.6)	5.13/ 5.12 (d, 9.0)
2′′′′′	3.80 (m)	3.78 (m)	3.80 (m)	3.78/ 3.78 (m)	3.79 (m)	3.78 (m)
3′′′′′	3.32 (m)	3.31 (m)	3.30 (m)	3.31 (m)	3.30 (m)	3.30 (m)
4′′′′′	3.28 (m)	3.30 (m)	3.26 (m)	3.30 (m)	3.25 (m)	3.30 (m)
5′′′′′	3.32 (m)	3.31 (m)	3.32 (m)	3.31 (m)	3.31 (m)	3.30 (m)
6′′′′′	3.73 (m) 3.49 (m)	3.72 (m) 3.52 (m)	3.73 (d, 11.4) 3.48 (m)	3.72 (m) 3.52 (m)	3.73 (m) 3.47 (m)	3.72 (m) 3.52 (m)
1′′′′′′						
2′′′′′′					7.18/ 7.04 (d, 1.2)	7.18/ 7.05 (d, 1.2)
3′′′′′′						
4′′′′′′						
5′′′′′′					6.78/ 6.74 (d, 7.8)	6.78/ 6.74 (d, 7.8)
6′′′′′′					6.93/ 6.79 (dd, 7.8, 1.2)	6.94/ 6.80 (dd, 7.8, 1.2)
7′′′′′′					7.22/ 7.07 (d, 15.6)	7.23/ 7.09 (d, 15.6)
8′′′′′′					6.24/ 6.15 (d, 15.6)	6.24/ 6.15 (d, 15.6)
9′′′′′′						
OCH_3					3.81/ 3.80 (s)	3.82/ 3.81 (s)

(continued on next page)

Table 3 *(continued)*

3398, 2887, 1732, 1608, 1351, 1075, 581 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 209 (+13.5), 239 (−12.2), 277 (−3.7) nm; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 3. HR-ESI-MS m/z 960.2774 $[M + H]^+$ (calcd. for $C_{44}H_{50}NO_{23}$, 960.2774).

6″-*O*-(3*S*-1-*N*-*β*-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetyl)spinosin (**5**)

Yellow solid; $[\alpha]_D^{27}$ −92.4 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.61), 268 (4.27), 335 (4.35) nm; IR (KBr) ν_{max}: 3411, 2882, 1732, 1608, 1351, 1076, 581 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 205 (−4.4), 238 (+16.9), 262 (−8.1) nm; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 3. HR-ESI-MS m/z 960.2761 $[M + H]^+$ (calcd. for $C_{44}H_{50}NO_{23}$, 960.2774).

6″-*O*-(3*R*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetyl)spinosin (**6**)

Yellow solid; $[\alpha]_D^{27}$ −42.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.70), 268 (4.38), 335 (4.46) nm; IR (KBr) ν_{max}: 3398, 2882, 1733, 1608, 1351, 1075, 581 cm^{-1}; CD (MeOH) λ_{max} (Δε) 213 (+12.0), 240 (−13.4), 263 (+1.4) nm; ^1H NMR and ^{13}C NMR, see Tables 2 and 3. HR-ESI-MS *m/z* 960.2777 $[M + H]^+$ (calcd. for $C_{44}H_{50}NO_{23}$, 960.2774).

6″-*O*-(3*S*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetyl)-6‴-feruloylspinosin (**7**)

Yellow solid; $[\alpha]_D^{27}$ −59.2 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.76), 283 (4.45), 330 (4.61) nm; IR (KBr) ν_{max}: 3412, 2882, 1721, 1607, 1354, 1077, 572 cm^{-1}; CD (MeOH) λ_{max} (Δε) 208 (−16.4), 240 (+10.9), 262 (−6.2), 308 (+9.9), 347 (−11.1) nm; ^1H NMR and ^{13}C NMR, see Tables 2 and 3. HR-ESI-MS *m/z* 1136.3250 $[M + H]^+$ (calcd. for $C_{54}H_{58}NO_{26}$, 1136.3247).

6″-*O*-(3*R*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetyl)-6‴-feruloylspinosin (**8**)

Yellow solid; $[\alpha]_D^{27}$ −28.0 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.68), 283 (4.35), 330 (4.52) nm; IR (KBr) ν_{max}: 3410, 2882, 1721, 1607, 1354, 1076, 581 cm^{-1}; CD (MeOH) λ_{max} (Δε) 215 (+9.9), 234 (−8.4), 262 (+6.2), 308 (+9.8), 346 (−11.8) nm; ^1H NMR and ^{13}C NMR, see Tables 2 and 3. HR-ESI-MS *m/z* 1136.3229 $[M + H]^+$ (calcd. for $C_{54}H_{58}NO_{26}$, 1136.3247).

2.4. Acid hydrolysis and sugar identification

The compounds **1**–**6** (each 1 mg) were respectively hydrolyzed by 2 M HCl (2 mL) at 90 °C for 2 h. The reaction mixture was concentrated to dryness. After extraction with CH_3Cl–H_2O, the H_2O layer was dried and reacted with L-cysteine methyl ester hydrochloride (1 mg) and *o*-tolyl isothiocyanate (5 μL) in pyridine (1 mL) at 60 °C, as Tanaka et al. already reported [11]. The mixture was analyzed by HPLC [Cosmosil $5C_{18}$-MS-II, 250 × 4.6 mm; mobile phase, 25% CH_3CN (0.1% CH_3COOH); UV 250 nm]. Compared with the derivatives of standard sugars, D-glucose were detected from derivatives of **1**–**6**.

2.5. Mild alkaline hydrolysis

Solutions of compounds **5** and **6** (each 10 mg) were respectively hydrolyzed by 0.05 mol/L NH_4OH (10 ml) at room temperature for 1.5 h [4]. The reaction mixture was neutralized with formic acid, was and then extracted with EtOAc. The H_2O layer was further purified by HPLC to afford **5a** (2.5 mg) and **6a** (2.2 mg), respectively [YMC–Pack ODS-A, 250 × 10 mm; mobile phase, 5% CH_3OH (0.1% HCOOH); UV 254 nm].

2.6. Quantum chemical ECD calculation method

In theoretical calculations, the geometry of the molecules was optimized with Gaussian 09 package at B3LYP/6-31G(d) computational level. The minimum nature of the structure was confirmed by frequency calculations at the same computational level. Then ECD calculations were carried out in the methanol solvent medium using time-dependent density functional theory (TDDFT) with B3LYP functional and DGDZVP basis set.

2.7. ORAC assay

Automated ORAC assay was carried out on a GENios luciferase-based microplate reader (TECAN, Switzerland) with an excitation/emission filter pair of 485/527 nm as previously described [12]. Fluorescein was used as a fluorescence probe, and the reaction was initiated with the addition of AAPH. Trolox was used as a standard. The final results were calculated on the basis of the difference in the area under the fluorescence decay curve between the AAPH control and each sample. Samples were prepared in stock solutions of 5 μM, 2.5 μM and 1.25 μM respectively. Each sample at a scheduled concentration was done in quadruplicate. Data are expressed as micromoles of Trolox equivalents (TE) per microliter of sample (μmol TE/mL or U/mL).

3. Results and discussion

The crude extract of the seeds of *Z. jujuba* var. *spinosa* was subjected to macroporous resin, silica gel and HW-40 column, followed by repeated RP-HPLC to afford eight new compounds **1**–**8**.

Compounds **1** and **2** were isolated as colorless solids. The HRESIMS of **1** showed the molecular ion peak at *m/z* 392.0966 $[M + Na]^+$, corresponding to the molecular formula $C_{16}H_{19}NO_9$, with eight degrees of unsaturation. The ^1H NMR spectrum of **1** revealed the presence of 1,2-disubstituted benzene ring, as well as one sugar moiety with the anomeric proton at δ 5.40 (1H, d, $J = 9.0$ Hz). In the ^{13}C NMR spectrum, only 15 carbon signals were observed, including one carbonyl carbon (δ 179.3), one quaternary carbon (δ 74.3) and one methylene (δ 43.7), as well as signals for benzene ring and sugar moiety. Acid hydrolysis of **1** yielded D-glucose, which was detected by HPLC analysis [11]. The large coupling constant (9.0 Hz) of the anomeric proton indicated a β configuration. The complete assignments of the benzene and glycosidic signals were achieved by 2D-NMR experiments. The planar structure was established according to the HMBC correlations from H-1′ to C-2/C-8, from H-4 to C-3, and from H-10 to C-2/C-3/C-9/C-11 (Fig. 2). The molecular formula of compound **2** was also determined as $C_{16}H_{19}NO_9$ by HRESIMS at *m/z* 392.0963 $[M + Na]^+$. The NMR data of **2** was similar to those of **1** (Table 1). Comprehensive analysis of 2D-NMR spectra established the planar structure of **2**, which was the same as **1**.

Compounds **3**–**8** were all obtained as yellow amorphous powders. The molecular formula of **3** was established to be $C_{44}H_{49}NO_{23}$ by HRESIMS at *m/z* 960.2764 $[M + H]^+$, with 21 degrees of unsaturation. The UV absorption bands at 211, 268, and 336 nm were recorded, which were the characteristic absorptions of a flavone skeleton. The ^1H NMR and ^{13}C NMR spectra showed a doubling of many of the signals, which were in a nearly 1:1 ratio. This phenomenon has been reported previously in flavone C-glycosides from Ziziphi Spinosae Semen, such as spinosin (**9**) and 6‴-feruloylspinosin (**10**). It was deemed that there were two stable conformers produced by rotational barrier 7-OCH_3 in flavone-6-C-glycoside at low

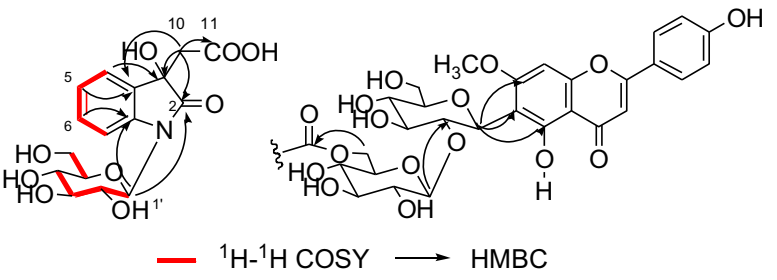

Fig. 2. Key ^{1}H-^{1}HCOSY and HMBC correlations of **1**, key HMBC correlations of spinosin moiety.

temperature [2,13]. In the following structure elucidation, we adopted the pair of signals to be assigned to one carbon/proton.

By comparing with ^{13}C NMR data of spinosin (**9**), 28 carbon signals could be easily attributed to the skeleton of a flavone C-glycosides. The HMBC correlations established the gross structure of spinosin moiety (Fig. 2), allowed the assignment of the ^{1}H NMR and ^{13}C NMR spectral data for spinosin (Tables 2 and 3). The remaining signals were consistent with the partial molecular formula $C_{16}H_{18}NO_8$ with 8 degrees of unsaturation. In the ^{1}H NMR spectrum, four aromatic protons at δ 7.29 (1H, d, J = 7.8 Hz), 7.26/7.24 (1H, t, J = 7.8 Hz), 7.14/7.12 (1H, d, J = 7.8 Hz), and 7.01/6.94 (1H, t, J = 7.8 Hz) implied the presence of 1,2-disubstituted benzene ring. Moreover, the anomeric proton at δ 5.15/5.15 (1H, d, J = 9.0Hz) and upfield shifted carbon at δ 82.0 indicated an *N*-glycosidic unit. Acid hydrolysis of **3** yielded D-glucose, which was detected by HPLC analysis of their derivative products. The large coupling constant (9.0 Hz) of the anomeric proton indicated a β-anomeric configuration. The ^{13}C NMR

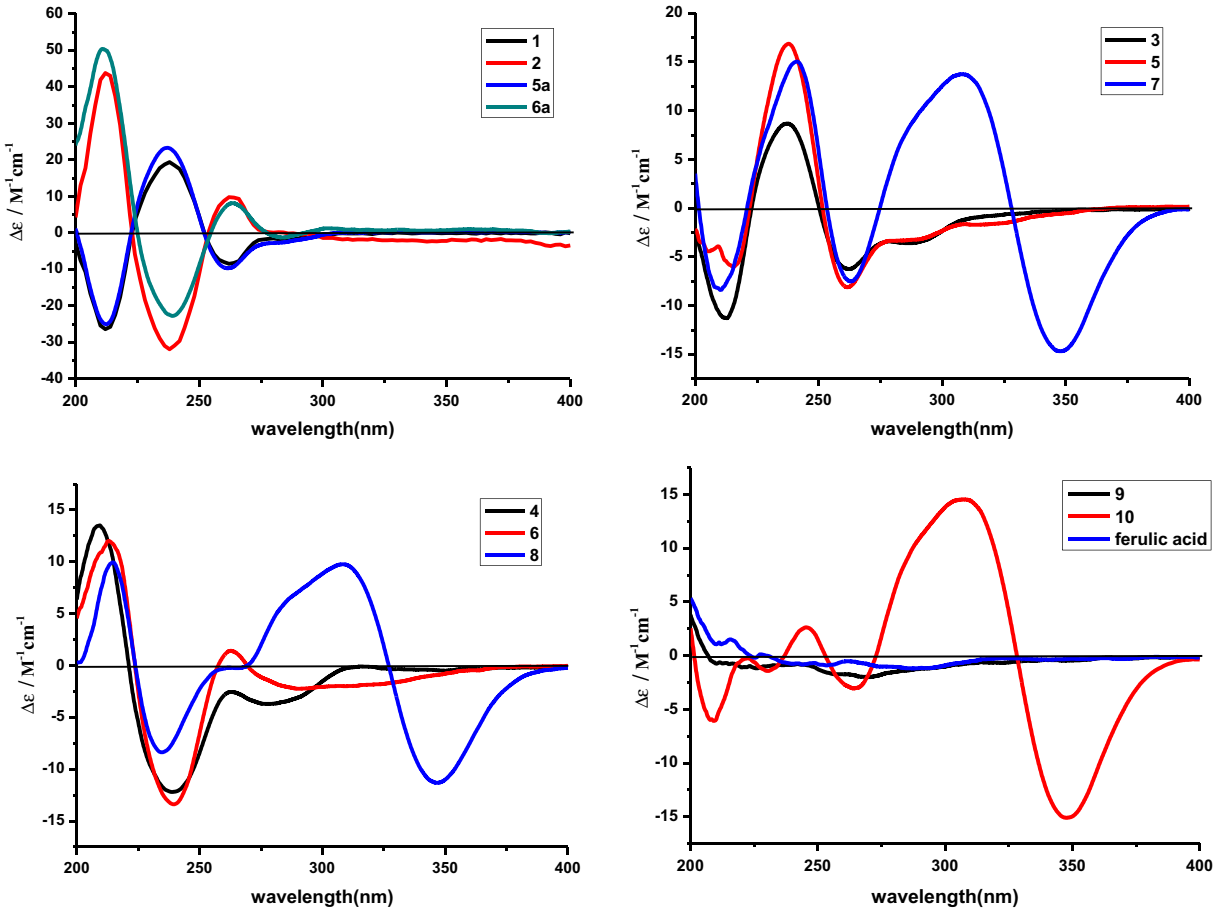

Fig. 3. CD spectra of **1**–**10**, **5a**, **6a** and ferulic acid in MeOH.

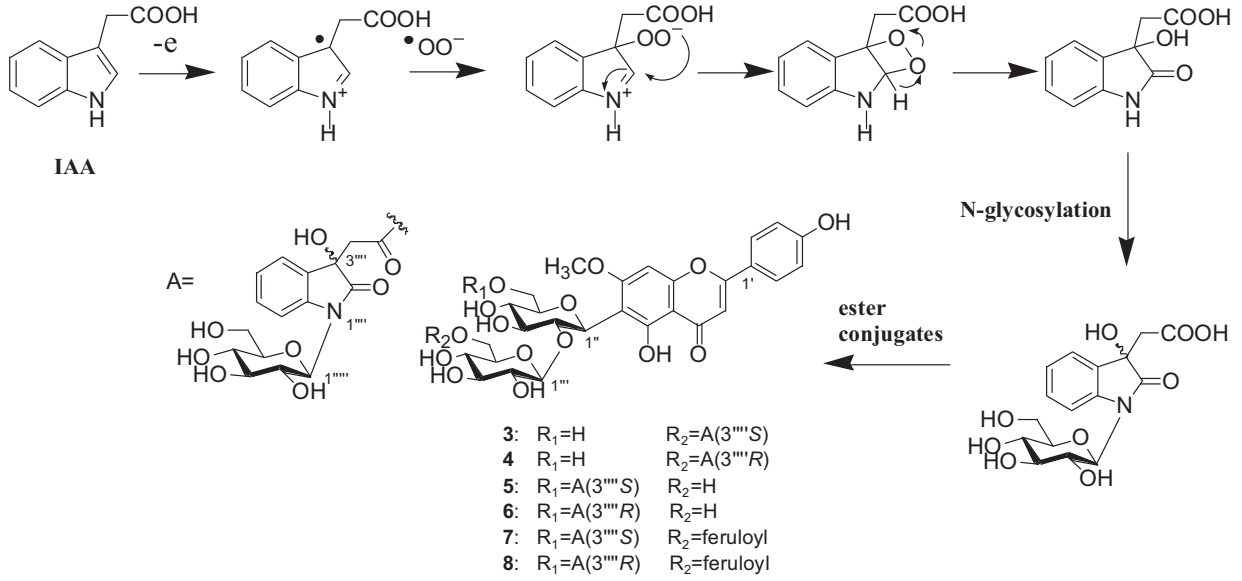

Fig. 4. Proposed biosynthetic pathway for indoleacetic acid derivatives.

spectrum also showed several remaining signals, which were attributed to two carbonyl carbons (δ 176.7/176.5, 168.9/168.8), one quaternary carbon (δ 72.7), and one methylene (δ 41.9/41.7), as well as those signals of benzene ring and *N*-glycosidic unit. The planar structure of this partial moiety was established by HMBC correlations (Fig. 2), which was the same planar as **1**. Compared with ^{13}C NMR data of spinosin (**9**) and **1**, the obviously shifted carbon signals for C-6‴ and C-11′′′′ demonstrated that the indoleacetic acid derivative moiety was attached to C-6‴ of the outer glucose unit of spinosin moiety. It was further confirmed by the HMBC correlation between H-6‴ and C-11′′′′ (Fig. 2). Compound **4** had the same molecular formula $C_{44}H_{49}NO_{23}$ as that of **3**, which was revealed by HRESIMS experiment (*m/z* 960.2774 $[M + H]^+$). The NMR data of **4** was also similar to those of **3**, except for some slightly shifted signals for indoleacetic acid moiety. Comprehensive analysis of 1D- and 2D NMR data constructed the planar structure of **4**.

The molecular formulas of compounds **5** and **6** were both established as $C_{44}H_{49}NO_{23}$ by HRESIMS *m/z* 960.2761 $[M + H]^+$ and *m/z* 960.2777 $[M + H]^+$, which were the same as those of **3** and **4**. Their ^1H NMR and ^{13}C NMR spectra were similar to those of **3** and **4**, except for some signals of glycosyl moiety. After the comprehensive analysis of the glycosidic signals by 2D-NMR experiments, the key HMBC correlations of H-6″/C-11′′′′ indicated that the indole derivative moieties of compounds **5** and **6** were both attached to the inner glucose unit of spinosin moieties.

The molecular formulas of compounds **7** and **8** were both established as $C_{54}H_{57}NO_{26}$ on the basis of HRESIMS (*m/z* 1136.3250 $[M + H]^+$ and *m/z* 1136.3229 $[M + H]^+$), which were $C_{10}H_8O_3$ more than those of **5** and **6**. A *trans*-feruloyl moiety was obtained by the ^1H NMR, COSY, HSQC and HMBC spectra (Table 3). The location of the moiety was assigned at C-6‴ of outer glucose unit of spinosin moiety due to the HMBC correlation between H-6‴ and the ester carbonyl carbon C-9′′′′′.

The absolute configuration of compounds **1** and **2**, 3-hydroxyoxindole derivatives, could be determined by CD method [14,15]. The CD spectrum of **1** was recorded, and a positive Cotton effect in the 250–220 nm was observed (Fig. 3), indicating that the absolute configuration of **1** was 3*S*. Conversely, the CD spectrum of **2** exhibited a negative Cotton effect in the 250–220 nm. Thus, the absolute configuration of **2** was determined to be 3*R*. In addition, the electronic circular dichroism (ECD) spectra of **1** and **2** were calculated by quantum mechanical time dependent density functional theory (TDDFT) to further validate the absolute configuration (Fig. S1-9 and S2-9, Supplementary data). Therefore, the structures of **1** and **2** were deduced as 3*S*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetic acid and 3*R*-1-*N*-β-D-glucopyranosyl-2-oxo-3-hydroxy-indole-3-acetic acid, respectively.

In order to determine the absolute configuration of compounds **3**–**8**, the mild alkaline hydrolysis of compounds **5** and **6** were performed, yielding **5a** and **6a**, respectively [4]. The ^1H NMR and CD spectra of **5a** which were identical to those of **1**

Table 4
ORAC values of compounds **3**–**10**.

Compd.	ORAC (1 μmol Trolox equiv./mL)		
	5 μM	2.5 μM	1.25 μM
3	166.6 ± 5	150.9 ± 12	
4	146.6 ± 9	154.3 ± 2	
5	64.5 ± 3	78.1 ± 7	
6	144.2 ± 4	142.0 ± 6	
7		199.9 ± 12	165.6 ± 9
8	132.6 ± 2	136.4 ± 4	
9		265.4 ± 11	298.1 ± 6
10		216.1 ± 5	244.3 ± 15

(Fig. 3; Fig. S5a, Supplementary data), demonstrated that absolute configuration of **5** was 3′′′′*S*. Similarly, compound **6** was established to be 3′′′′*R*.

In addition, the CD spectra of compounds **3**–**8** were also recorded (Fig. 3). It was found that, below 260 nm, the CD curves of compounds **3**, **5** and **7** were in accordance with that of **1**, while those of compounds **4**, **6** and **8** were consistent with that of **2**. It is noted that there was a strong Cotton effect in the regions above 280 nm observed in compounds **7**, **8** and **10**. However, it could not be observed in compound **9** and ferulic acid (Fig. 3). It seemed that this strong Cotton effect was the characteristic CD spectrum of 6‴-feruloylspinosin, without interfering the judgment of the configurations of indoleacetic acid derivatives. Therefore, compounds **3**, **5** and **7** were established to be 3′′′′*S* by the positive Cotton effect of 250–220 nm, while compounds **4**, **6** and **8** were assigned as 3′′′′*R* by the negative Cotton effect.

As compounds **3**–**8** could be regarded as the artifacts from esterification of flavonoid **9**/**10** with **1**/**2**, the ultrasonic extraction of the seeds was analyzed by UPLC-MS. All the new compounds could be detected in the ultrasonic extract, indicating that all of them were natural products (Fig. S9, Supplementary data). Since dioxindole-3-hydroxy-3-acetic acid (DiOxIAA) was regarded as the oxidative product of indoleacetic acid (IAA) [16], compounds **1**–**8** may be the further metabolites by the means of *N*-glycosylation and esterification. The possible catabolic pathway from IAA was proposed (Fig. 4). *N*-glycosylated indoles are rare natural products. Far as we know, it has only been isolated from *Rheum maximowiczii* [17], *Brucea mollis* [15] and *Streptomyces* sp. GW48/1497 [18]. It is the first report of *N*-glycosylated indole conjugates with flavonoids, which enriched the structural types of *N*-glycosylated indole derivatives of natural origin.

In addition, compounds **3**–**10** were evaluated for the antioxidant capacity (Table 4), using the radical absorbance capacity (ORAC) assay [12]. As a result, new compounds **3**–**8** showed significant antioxidant capacities. Among them, compound **7** is the most active one, with ORAC value of 199.9 μmol trolox equivalent (TE)/mL when tested at the concentration of 2.5 μM.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.09.001.

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