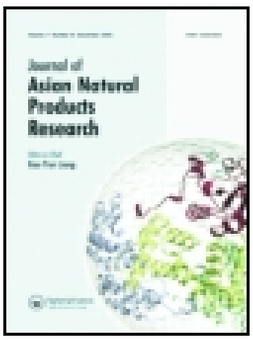

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Indole alkaloid glucosides from the roots of *Isatis indigotica*

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ABSTRACT

Five new indole alkaloid glucosides named isatindigotindolosides A-E (**1–5**), along with three known analogs (**6–8**), were isolated from an aqueous extract of the *Isatis indigotica* roots. Their structures including the absolute configurations were determined based on comprehensive spectroscopic data analysis, combined with chemical methods and electronic circular dichroism spectra calculations. In the preliminary assays, compounds **1**, **6** and **7** showed antiviral activity against influenza virus A/Hanfang/359/95 (H3N2) with IC_{50} values of 14.6–33.3 μM. Compound **1** also exhibited inhibitory effect against nitric oxide (NO) production in microglial cell BV2 with an inhibition ratio of 93.0% at 10 μM.

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Cruciferae; *Isatis indigotica*; indole alkaloid glucoside; isatindigotindolosides A–E; antiviral activity; inhibition activity against nitric oxide production

1. Introduction

"Ban lan gen," the dried roots of *Isatis indigotica* Fort. (Cruciferae), is an important traditional Chinese medicine commonly used to treat influenza and infection diseases [1]. Decades of studies showed that extracts of this drug had extensive biological activities (antiviral, anti-endotoxic, antinociceptive, anti-inflammatory, antipyretic, and cytotoxic) and contained various active constituents [2–4], of which indole alkaloids are considered as the important ones [5]. However, previous chemical studies were mainly carried out on the ethanol and methanol extracts of the drug materials, which is not in accordance with the practical utilization of the herbal medicine by decocting with water. As part of a program to assess the chemical and biological diversity of traditional Chinese medicines, focusing on the minor components [6–18], we performed a detailed investigation of an aqueous extract of "ban lan gen." In our previous papers, the characterization of 28 new alkaloids and 33 constituents firstly isolated from *I. indigotica*, as well as their antiviral and hepatocyte-protective activities were described [19–23]. The continuous investigation on the same extract has afforded five new indole alkaloid glucosides named isatindigotindolosides A-E

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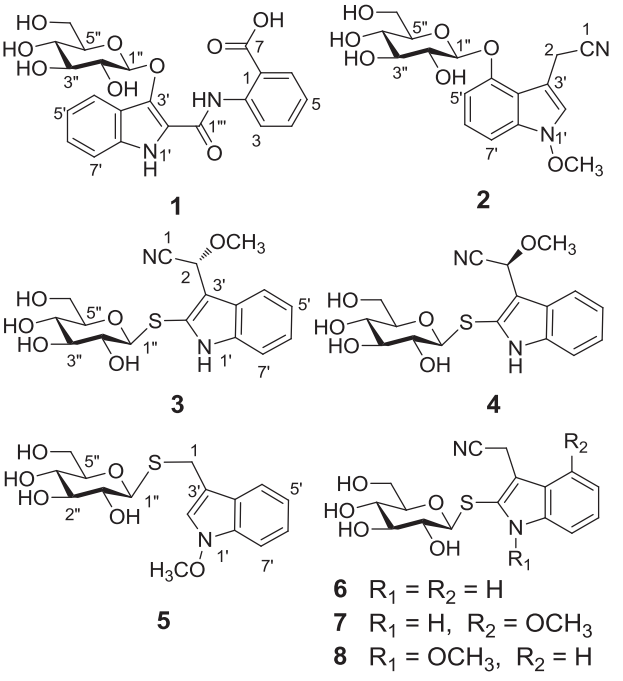

Figure 1. The structures of compounds **1–8**.

(**1–5**) and three known analogs (**6–8**) (Figure 1). Herein reported are details of the isolation, structure elucidation, and bioactivity assay of the new isolates.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder with $[\alpha]_D^{20}$ +17.9 (*c* 0.42, MeOH). Its IR spectrum indicated the presence of hydroxyl (3406 cm^{-1}), conjugated carbonyl (1653 cm^{-1}), and aromatic ring (1616 and 1508 cm^{-1}) functional groups. The molecular formula of **1** was elucidated as $C_{22}H_{22}N_2O_9$ by (+)-HR-ESI-MS at *m/z* 459.1407 $[M + H]^+$ combined with NMR spectral data (Tables 1 and 2). The ^{1}H NMR spectrum of **1** in DMSO-d_6 showed signals attributable to two *ortho*-disubstituted benzene rings [δ_H 8.52 (d, *J* = 8.5 Hz, H-3), 7.29 (dt, *J* = 1.5, 8.5 Hz, H-4), 6.97 (t, *J* = 8.5 Hz, H-5), and 7.91 (dd, *J* = 1.5, 8.5 Hz, H-6), and 7.70 (d, *J* = 8.0 Hz, H-4′), 6.94 (t, *J* = 8.0 Hz, H-5′), 7.14 (t, *J* = 8.0 Hz, H-6′), and 7.36 (d, *J* = 8.0 Hz, H-7′)] and three exchangeable nitrogen-bearing and carboxylic protons [δ_H 11.12 (s, N*H*-1′), 13.60 (s, N*H*-2), and 7.89 (COO*H*)], in addition to characteristic signals for a *β*-glucopyranosyl moiety (Table 1). The ^{13}CNMR and DEPT spectra of **1** exhibited carbon resonances corresponding to the above units (Table 2) and two additional carbonyl carbons resonated at δ_C 159.0 (C-1″) and 169.0 (C-7). These spectroscopic data indicate that **1** is an unusual aromatic alkaloid *β*-glucopyranoside, of which the structure was further elucidated by 2D NMR data analysis. In the ^{1}H-^{1}H COSY spectrum of **1,** the vicinal coupling correlations of H-3/H-4/H-5/H-6, H-4′/H-5′/H-6′/H-7′, and H-1″/H-2″/H-3″/H-4″/H-5″/H_2-6″, together with their chemical shifts and coupling constants, confirmed the presence of the *ortho*-disubstituted benzene and *β*-glucopyranosyl moieties.

Table 1. ^{1}H NMR spectral data (δ) for compounds **1–5**[a].

no	**1**[b,c]	**2**[d]	**3**	**4**	**5**[e]
1a/2a		/4.20 d (18.5)	/5.80 s	/5.78 s	4.19 d (13.2)/
1b/2b		/4.13 d (18.5)			3.98 d (13.2)/
1′/2′	11.12 s/	/7.53 s			/7.52 s
4′	7.70 d (8.0)		7.72 brd (7.8)	7.72 brd (7.8)	7.70 brd (7.8)
5′	6.94 t (8.0)	7.07 d (8.0)	7.08 dt (1.2, 7.8)	7.07 dt (1.2, 7.8)	7.07 dt (1.2, 7.8)
6′	7.14 t (8.0)	7.13 t (8.0)	7.18 dt (1.2, 7.8)	7.18 dt (1.2, 7.8)	7.21 dt (1.2, 7.8)
7′	7.36 d (8.0)	6.76 d (8.0)	7.35 brd (7.8)	7.34 brd (7.8)	7.40 brd (7.8)
1″	5.27 d (7.5)	4.90 d (7.5)	4.35 d (9.6)	4.36 d (9.6)	4.28 d (9.6)
2″	3.41 dd (7.5, 9.5)	3.37 m	2.87 dd (8.0, 9.6)	2.98 dd (8.0, 9.6)	3.26–3.36 m
3″	3.35 t (9.5)	3.28 m	3.27 t (8.0)	3.28 t (8.0)	3.26–3.36 m
4″	3.28 dd (8.5, 9.5)	3.19 m	3.05 t (8.0)	3.21 m	3.26–3.36 m
5″	3.43 m	3.32 m	3.26 m	3.21 m	3.26–3.36 m
6″a	3.61 brd (11.0)	3.71 ddd (3.0, 6.6, 12.0)	3.83 dd (2.4, 12.0)	3.83 dd (1.8, 12.0)	3.91 m
6″b	3.49 dd (4.5, 11.0)	3.49 ddd (6.0, 6.6, 12.0)	3.66 dd (7.2, 12.0)	3.64 dd (4.2, 12.0)	3.68 m
OCH_3		4.02 s	3.43 s	3.43 s	4.08 s

[a]^{1}H NMR data (δ) were measured at 500 MHz in DMSO-d_6 for **1** and **2** and at 600 MHz in CD_3OD for **3–4** and in acetone-d_6 for **5**, respectively. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on ^{1}H-^{1}H COSY, HSQC, and HMBC experiments.

[b]Data for protons of the anthranilic acid moiety in **1**: δ_H 13.60 (1H, s, N*H*-2), 8.52 (1H, d, *J* = 8.5 Hz, H-3), 7.29 (1H, dt, *J* = 1.5, 8.5 Hz, H-4), 6.97 (1H, t, *J* = 8.5 Hz, H-5), 7.91 (1H, dd, *J* = 1.5, 8.5 Hz, H-6), 7.89 (1H, brs, COO*H*).

[c]Data for hydroxyl protons in **1**: δ_H 5.05 (2H, brs, O*H*-3″, O*H*-4″), 4.44 (1H, brs, O*H*-6″), 4.10 (1H, brs, O*H*-2″).

[d]Data for hydroxyl protons in **2**: δ_H 5.35 (1H, brs, O*H*-2″), 5.14 (1H, brs, O*H*-3″), 5.01 (1H, brs, O*H*-4″), 4.59 (1H, t, *J* = 6.6 Hz, O*H*-6″).

[e]Data for hydroxyl protons in **5**: δ_H 4.33 (1H, brs, O*H*-3″), 4.21 (1H, brs, O*H*-4″), 4.11 (1H, brs, O*H*-2″), 3.80 (1H, dd, *J* = 5.4, 6.0 Hz, O*H*-6″).

Table 2. ^{13}C NMR spectroscopic data (δ) for compounds **1–5**[a].

no	**1**[b]	**2**	**3**	**4**	**5**
1	127.1	119.8	119.1	119.0	24.2
2	139.8	14.9	66.5	66.3	
2′	116.8	121.8	125.9	126.9	123.6
3′	136.7	100.8	116.0	115.5	108.9
3′a	117.1	112.9	126.7	126.6	124.4
4′	121.5	152.2	120.4	120.2	120.4
5′	119.1	104.5	121.5	121.4	120.4
6′	123.9	124.1	124.7	124.6	123.2
7′	112.5	102.7	112.6	112.6	109.0
7′a	133.9	133.5	138.5	138.6	133.5
1″	102.3	101.5	88.7	89.2	84.8
2″	73.1	73.6	73.7	73.8	74.3
3″	75.6	76.7	79.4	79.5	79.7
4″	69.4	69.8	71.5	70.9	71.8
5″	77.0	77.2	82.7	82.1	81.7
6″	60.6	60.8	63.2	62.5	63.2
1‴/OCH_3	159.0/	/66.1	/57.2	/57.3	/66.1

[a]^{13}C NMR data (δ) were measured at 125 MHz in DMSO-d_6 for **1** and **2** and at 150 MHz in CD_3OD for **3–4** and in acetone-d_6 for **5**, respectively. The assignments were based on DEPT, ^{1}H-^{1}H COSY, HSQC, and HMBC experiments.

[b]Data for other carbon resonances of the anthranilic acid moiety in **1**: δ_C 120.2 (C-3), 129.4 (C-4), 121.6 (C-5), 130.9 (C-6), 169.0 (C-7).

The HMBC spectrum of **1** showed long-range correlations from H-3 to C-1, C-2, and C-5; from H-4 to C-2 and C-6; from H-5 to C-1, C-3, and C-6; from H-6 to C-2, C-4, and C-7; and from N*H*-2 to C-1 and C-3. These correlations, together with their chemical shifts, revealed the presence of an N-substituted anthranilic acid moiety in **1**. Meanwhile, the HMBC correlations from H-4′ to C-3′, C-6′, and C-7′a; from H-5′ to C-3′a and C-7′;

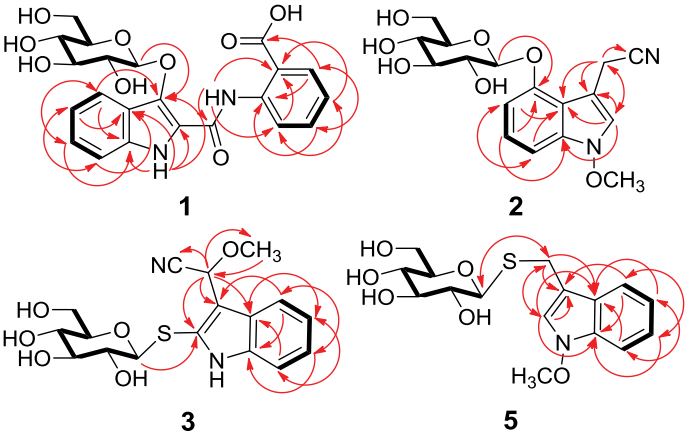

Figure 2. Main ^{1}H-^{1}H COSY (thick lines) and HMBC (arrows, from ^{1}H to ^{13}C) correlations of compounds **1–3** and **5**.

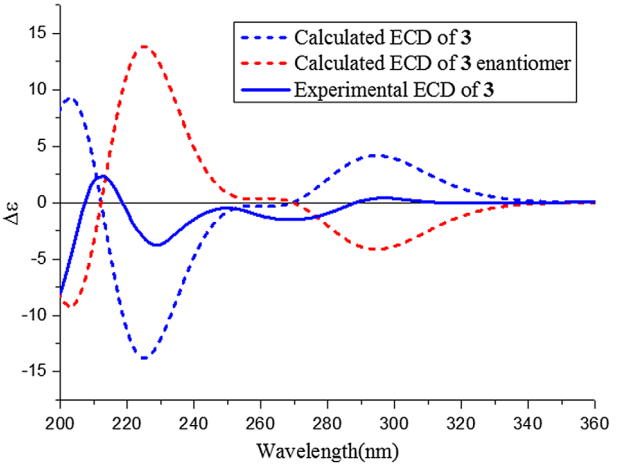

Figure 3. The experimental CD spectra (full line) of **3** (blue) and the calculated ECD spectra (dashed line) of **3** (blue) and its enantiomer (red).

from H-6′ to C-4′ and C-7′a; from H-7′ to C-3′a and C-5′; and from N*H*-1′ to C-2′, C-3′, C-3′a, and C-7′a; in combination with their chemical shifts, demonstrated that there was a 2′,3′-disubstituted indole moiety in **1**. The HMBC correlation from H-1″ to C-3′ indicated that the *β*-glucopyranosyloxy was located at C-3′ of the indole moiety. In addition, the HMBC correlation from N*H*-2 to the remaining carbonyl carbon (C-1‴) suggested that this carbonyl group was not only linked with N-atom of the anthranilic acid moiety to form an amide bond, but also connected to C-2′ of the 3′-O-*β*-glucopyranosyloxyindole moiety to match requirement of the molecular composition of **1**. Accordingly, the gross structure of **1** was established as shown. A literature survey indicates that **1** is the acid form of cephalandole C, which was isolated from a MeOH extract of *Cephalanceropsis gracilis* (Orchidaceae) [24]. The structure assignment of **1** was supported by comparison of its NMR spectroscopic data with the reported data of cephalandole C, though the configuration of

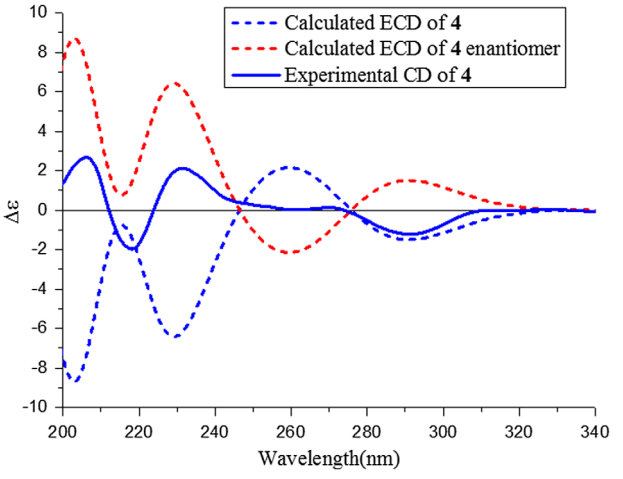

Figure 4. The experimental CD spectrum (full line) of **4** (blue) and the calculated ECD spectra (dashed line) of **4** (blue) and its enantiomer (red).

β-glucopyranosyl in the latter was undetermined. From the hydrolysate of **1** with snailase, D-glucose was isolated and identified by comparison of the retention factor (R_f) on TLC, specific rotation {$[\alpha]_D^{20}$}, and ^{1}H NMR spectroscopic data with those of an authentic sugar sample (Experimental Section and Supporting Information). Therefore, compound **1** was determined as (+)-2-[3′-*β*-D-glucopyranosyloxy-1′*H*-indol-2′-yl)carboxamido]benzoic acid and named isatindigotindoloside A.

Compound **2** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ −36.9 (*c* 0.17, MeOH). Its IR spectrum showed absorption bands for hydroxyl (3301 cm^{-1}), cyano (2243 cm^{-1}), and aromatic ring (1620 and 1503 cm^{-1}) functionalities. The (+)-HR-ESI-MS and the NMR spectral data of **2** indicated that it has the molecular formula $C_{17}H_{20}N_2O_7$. The NMR spectral data of **2** (Tables 1 and 2) showed signals assignable to an *ortho*-trisubstituted benzene ring, one trisubstituted double bond, a cyano group, and an isolated methylene, as well as a methoxy group and a *β*-glucopyranosyl moiety. The presence of these units was proved by cross peaks of H-1a/H-1b, H-5′/H-6′/H-7′, and H-1″/H-2″/H-3″/H-4″/H-5″/H_2-6″ in the ^{1}H-^{1}H COSY spectrum of **2** (Figure 2), as well as by their chemical shifts and coupling constants (Table 1). In the HMBC spectrum of **2**, correlations from H_2-2 to C-1, C-2′, C-3′, and C-3′a; from H-2′ to C-3′, C-3′a, and C-7′a; from H-5′ to C-3′a and C-7′; from H-6′ to C-4′ and C-7′a; and from H-7′ to C-3′a and C-5′ (Figure 2), together with the chemical shifts of these carbon resonances (Table 2), revealed a 4′-substituted 2-(1′*H*-indol-3′-yl) acetonitrile nucleus. Furthermore, the HMBC correlation from H-1″ to C-4′ indicated substitution of the *β*-glucopyranosyloxy group at C-4′. The remaining methoxy group was located at the nitrogen atom to satisfy the requirement of the molecular formula of **2**. This was supported by the chemical shift of the methoxy carbon resonance (δ_C 66.1) and the absence of a nitrogen-bearing proton resonance in the NMR spectrum of **2** in DMSO-d_6. Using the same protocols as described for **1**, D-glucose was isolated and identified from the enzymatic hydrolysate of **2**. Therefore, **2** was determined as (−)-2-[(1′-methoxy-4′-*β*-D-glucopyranosyloxy)-1′*H*-indol-3′-yl]acetonitrile and named isatindigotindoloside B.

Compound **3**, a white amorphous powder with $[\alpha]_D^{20}$ −34.3 (*c* 0.2, MeOH), has the molecular formula of $C_{17}H_{20}N_2O_6S$ as indicated by (+)-HR-ESI-MS at *m/z* 381.1113 $[M + H]^+$ and the NMR spectral data (Tables 1 and 2). The NMR spectra of **3** in CD_3OD exhibited typical resonances for a 2′,3′-disubstituted indole nucleus, a *β*-glucopyranosyl unit, a cyano, and a methoxy group, in addition to signals due to an unusual oxymethine (δ_H 5.80 and δ_C 66.5). The presence of the cyano group in **3** was confirmed by an absorption band at 2234 cm^{-1} in the IR spectrum. This suggests that **3** is an abnormal sulfur-containing indole *β*-glucopyranoside with cyano and methoxy substituents, of which the structure was further deduced by 2D NMR data analysis. Detailed analysis of ^1H-^1H COSY and HSQC spectra of **3** not only proved the occurrence of the above structural units, but also unambiguously assigned the resonances of protons and proton-bearing carbons in the NMR spectra. In the HMBC spectrum of **3**, besides the long-range correlations confirming the 2′,3′-disubstituted indole nucleus and *β*-glucopyranosyl units, the correlations from H-2 to C-1, C-2′, C-3′, C-3′a, and OCH_3 demonstrated the oxymethine carbon (C-2) was connected with both the methoxy and cyano groups, as well as with C-3′ of the indole nucleus. In addition, the HMBC correlations from H-1″ to C-2′, C-2″, C-3″, and C-5″, together with their chemical shifts and the molecular composition, indicated that the *β*-glucopyranosyl must be linked through the sulfur atom with C-2′ of the indole nucleus to give a gross structure of *β*-thioglucopyranoside for **3**. This was confirmed by comparison of the NMR spectral data of **3** with those of the related co-occurring indole *β*-thioglucopyranosides [5]. Compound **3** was hydrolyzed with 2 N HCl and the sugar isolated from the hydrolysate was identified as D-glucose using the same protocols as described for **1**. The circular dichroism (CD) spectrum of **3** displayed positive Cotton effects at 213 and 297 nm and negative ones at 229 and 270 nm. Using the time-dependent density functional theory (TDDFT) method [25], the calculated electronic circular dichroism (ECD) spectrum of **3** with the 2*R*-configuration gave the similar Cotton effects except for that at 270 nm, as compared with those in the experimental CD spectrum (Figure 3). This suggests the 2*R*-configuration for **3**. Therefore, compound **3** (isatindigotindoloside C) was determined as (−)-(2*R*)-2-methoxy-2-(2′-*β*-D-thioglucopyranosyl-1′*H*-indol-3′-yl)acetonitrile.

Compound **4** was obtained as a white amorphous powder with $[\alpha]_D^{20}$ +11.9 (*c* 0.2, MeOH). The spectroscopic data of **4** indicated that it was an isomer of **3**. 2D NMR spectroscopic data analysis revealed that **4** had the same planar structure as that of **3**. Because D-glucose was isolated and identified from the acid hydrolysate, **4** must be the C-2 epimer of **3**. This was supported by comparison of the CD spectra of **4** and **3**. The CD spectrum of **4** showed the positive Cotton effects at 232 and 270 nm and negative ones at 218 and 292 nm, of which the wavelengths are similar to those of **3**, but the corresponding signs are opposite. In addition, comparison of the experimental CD and calculated ECD spectra of **4** (Figure 4) also supported the 2*S*-configuration. Therefore, the structure of compound **4** was determined and named isatindigotindoloside D.

Compound **5**, a white amorphous powder with $[\alpha]_D^{20}$ −34.8 (*c* 0.38, MeOH), has the molecular formula of $C_{16}H_{21}NO_6S$ as determined based on (+)-HR-ESI-MS at *m/z* 378.0980 $[M + Na]^+$ and the NMR spectral data. The NMR spectral data of **5** in acetone-d_6 (Tables 1 and 2) indicated that this compound is another indole *β*-D-thioglucopyranoside with a methoxy group and an isolated methylene unit. The presence of a 1′*H*-indol-3′-yl nucleus was confirmed by the ^1H-^1H COSY cross peaks of H-5′/H-6′/H-7′ and the HMBC correlations from H-2′ to C-3′, C-3′a, and C-7′a; from H-4′ to C-3′, C-5′, and C-7′a; from

H-7′ to C-3′a and C-5′ (Figure 2). Additionally, the HMBC correlations from H_2-1 to C-1″, C-2′, C-3′, and C-3′a and from H-1″ to C-1, in combination with their chemical shifts, evidenced that the *β*-thioglucopyranosyl was connected via the isolated methylene to C-3′ of the indole nucleus in **5**. The chemical shift of the methoxy carbon resonance (δ_C 66.1) indicated that the methyl group was located at N-1′ of the nucleus to match the requirement of the molecular composition. This was supported by the absence of a nitrogen-bearing proton resonance in the ^{1}H NMR spectrum of **5** in acetone-d_6. The D-configuration of *β*-thioglucopyranosyl was verified by isolation and identification of D-glucose from the acid hydrolysate of **5**. Therefore, compound **5** was determined as (−)-(1′-methoxy-1′*H*-indole-3′-yl)methylthiol *S*-*β*-D-glucopyranoside and named isatindigotindoloside E.

By comparing the spectroscopic data with the reported data, the known compounds were identified as indole-3-acetonitrile-2-S-*β*-D-glucopyranoside (**6**), indole-3-acetonitrile-4-methoxy-2-S-*β*-D-gluco- pyranoside (**7**), and N-methoxy-indole-3-acetonitrile-2-S-*β*-D-glucopyranoside (**8**) [5], respectively.

In the preliminary *in vitro* assays [22], compounds **1, 6**, and **7** showed antiviral activity against influenza virus A/Hanfang/359/95 (H3N2) with IC_{50} values of 14.6, 33.3, and 33.3 μM and SI values of 6.8, 3.0, and 3.0 (the positive control ribavirin gave an IC_{50} value of 1.4 μM and a SI value of 814.1), respectively. At 10 μM, compounds **1** and **2** reduced DL-galactosamine-induced hepatocyte (WB-F344 cell) damage with the same 39% inhibition as the positive control bicyclol, and compound **1** also exhibited inhibitory activity against the release of NO induced by LPS in microglial cell BV2 with an inhibition ratio of 93.0%, while the positive control curcumin showed 67.7% inhibition.

In summary, five new indole alkaloid glycosides, having diverse structure features, were isolated as the minor constituents from the aqueous extract of "ban lan gen." Among them, three showed antiviral activity against influenza virus A/Hanfang/359/95 (H3N2). This, together with our previous results [19–23], shows that diverse minor indole alkaloid components have contributions to pharmacological efficacy that supports the traditional applications of the herbal medicine. Especially, the bioactive new structures not only provide diverse scaffolds, entities, and hits for drug discovery, but also provide important clues for in-depth investigation of biomimetic and total syntheses, chemical transformation, structural modification, and structure–activity relationships, as well as for drug development based on the traditional herbal medicine.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a P-2000 polarimeter (JASCO, Tokyo, Japan). UV spectra were acquired on a V-650 spectrometer (JASCO, Tokyo, Japan). CD spectra were measured on a JASCO J-815 CD spectrometer (JASCO, Tokyo, Japan). IR spectra were obtained on a Nicolet 5700 FTIR microscope instrument (FTIR microscope transmission) (Thermo Electron Corporation, Madison, WI, USA). NMR spectra were recorded at 500 or 600 MHz for ^{1}H, and 125 or 150 MHz for ^{13}C, respectively, on Inova 500 or SYS 600 (Varian Associates Inc., Palo Alto, CA, USA) in DMSO-d_6, MeOH-d_4, or acetone-d_6, with solvent peaks used as references. ESIMS and HRESIMS data were taken on an AccuToFCS JMS-T100CS spectrometer (Agilent Technologies, Ltd., Santa Clara, CA, USA). Column chromatography

(CC) was carried out on macroporous adsorbent resin (HPD-110, Cangzhou Bon Absorber Technology Co. Ltd, Cangzhou, China), silica gel (200–300 mesh, Qingdao Marine Chemical Inc. Qingdao, China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), CHP 20P (Mitsubishi Chemical Inc., Tokyo, Japan), or reverse-phase C-18 silica gel (W. R. Grace & Co., Maryland, USA). HPLC separation was performed on an instrument equipped with an Agilent ChemStation for LC system, an Agilent 1200 pump, and an Agilent 1100 single-wavelength absorbance detector (Agilent Technologies, Ltd.) using a Grace semi-preparative column (250 × 10 mm i.d.) packed with C_{18} reverse-phase silica gel (5 μm) (W. R. Grace & Co., Maryland, USA) or a Chiralpak AD-H column (250 × 10 mm i.d.) packed with amylose tris(3,5-dimethylphenylcarbamate) coated on 5-μm silica gel (Daicel Chiral Technologies Co. Ltd. Shanghai, China). TLC was carried out on glass-precoated silica gel GF_{254} plates (Qingdao Marine Chemical Inc.). Spots were visualized under UV light or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating. Unless otherwise noted, all chemicals were purchased from commercially available sources and were used without further purification.

3.2. Plant material

The roots of Isatis indigotica were collected in December 2009 from Bozhou, Anhui Province, China. Plant identity was verified by Mr. Lin Ma (Institute of Materia Medica, Beijing 100050, China). A voucher specimen (No. ID-S-2385) was deposited at the herbarium of Natural Medicinal Chemistry, Institute of Materia Medica.

3.3. Extraction and isolation

The air-dried and pulvarized plant material (50 kg) was decocted with H_2O (150 L; 3 × 1 h). The aqueous extracts were combined and evaporated under reduced pressure to yield a dark-brown residue (32 kg). The residue was dissolved in H_2O (122 L), loaded on a macroporous adsorbent resin (HPD-110, 19 kg) column (20 × 200 cm), and eluted successively with H_2O (50 L), 50% EtOH (125 L), and 95% EtOH (100 L) to yield three corresponding fractions A, B, and C. After removing the solvent under reduced pressure, fraction B (0.9 kg) was separated by CC over MCI gel CHP 20P (5 L), with successive elution using H_2O (10 L), 30% EtOH (30 L), 50% EtOH (20 L), 95% EtOH (10 L), and Me_2CO (8 L) to give fractions B1-B5. Fraction B3 (165 g) was chromatographed over silica gel and eluted by a gradient of increasing MeOH (0–100%) in EtOAc to yield subfractions B3-1–B3-16. Subfraction B3-3 (7.5 g) was separated by CC over Sephadex LH-20, eluting with $CHCl_3$–MeOH (1:1) to yield subfractions B3-3-1–B3-3-4, of which subfraction B3-3-4 (3.0 g) was further chromatographed over silica gel, eluted by a gradient of increasing MeOH (0–100%) in $CHCl_3$ to yield subfractions B3-3-4-1–B3-3-4-14. Subfraction B3-3-4-10 (243.5 mg) was further fractionated by CC over Sephadex LH-20 (MeOH) to afford B3-3-4-10-1–B3-3-4-10-8, of which subfraction B3-3-4-10-1 (48.0 mg) was separated by RP-HPLC (39% MeOH in H_2O, 2.0 ml/min) to afford **8** (6.0 mg, t_R 90 min) and a mixture (7.0 mg, t_R 80 min). The mixture was further separated by HPLC on a semipreparative Chiralpak AD-H column using n-hexane-iPrOH (6:1) as the mobile phase (1.5 ml/min) to yield **3** (2.0 mg, t_R = 36.0 min) and **4** (2.5 mg, t_R = 40 min). Subfraction B3-3-4-11 (150.0 mg) was chromatographed over Sephadex LH-20 (MeOH) to yield B3-3-4-11-1–B3-3-4-11-7,

of which B3-3-4-11-1(88.0 mg) was isolated by PTLC (ethyl acetate:MeOH:H_2O, 8:1:0.5, *v/v/v*) to yield B3-3-4-11-1-1–B3-3-4-11-1-4. Subfraction B3-3-4-11-1-4 (16.0 mg) was purified by RP-HPLC (45% MeOH in H_2O, 1.5 ml/min) to afford **2** (3.8 mg, t_R = 25 min), **5** (1.6 mg, t_R = 51 min), and **8** (3.1 mg, t_R = 58 min). Subfraction B3-3-4-12 (481.0 mg) was chromatographed over Sephadex LH-20 (MeOH) to yield B3-3-4-12-1–B3-3-4-12-10, of which B3-3-4-12-2 (380.0 mg) was further separated by PTLC (ethyl acetate:MeOH:H_2O, 8:1:0.5, *v/v/v*) to yield B3-3-4-12-2-1–B3-3-4-12-2-3. Subfraction B3-3-4-12-2-2 (300.0 mg) was further isolated by RP-HPLC (57% MeOH in H_2O, 1.5 ml/min) to afford **6** (64.0 mg, t_R = 44 min) and **7** (202.0 mg, t_R = 63 min). Subfraction B3-4 (11.0 g) was fractionated by CC over Sephadex LH-20 (MeOH) to yield B3-4-1–B3-4-6, of which B3-4-5 (2.5 g) was separated by RP-MPLC and eluted with a gradient of increasing MeOH (20–100%) in H_2O to afford subfractions B3-4-5-1–B3-4-5-30. Subfraction B3-4-5-17 (15.0 mg) was purified by RP-HPLC (25% MeOH, 1.5 ml/min) to yield **1** (7.0 mg, t_R = 28 min).

3.3.1. Isatindigotindoloside A (1)

White amorphous solid; $[\alpha]_D^{20}$ +17.9 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.21), 233 (4.00, sh), 263 (3.71), 315 (3.93) nm; IR ν_{max} 3406, 2931, 1653, 1616, 1585, 1508, 1453, 1431, 1379, 1346, 1291, 1246, 1158, 1066, 1045, 957, 891, 828, 764, 741, 706, 663, 624 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) spectral data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) spectral data, see Table 2; (−)-ESI-MS: *m/z* 457 $[M-H]^-$; (+)-HR-ESI-MS: *m/z* 459.1407 $[M+H]^+$ (calcd for $C_{22}H_{23}N_2O_9$, 459.1398), 481.1222 $[M+Na]^+$ (calcd for $C_{22}H_{22}N_2O_9Na$, 481.1218).

3.3.2. Isatindigotindoloside B (2)

White amorphous solid; $[\alpha]_D^{20}$ −36.9 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.10), 269 (3.43), 298 (3.41) nm; IR ν_{max} 3505, 3301, 2921, 2884, 2243, 1659, 1620, 1582, 1503, 1459, 1408, 1391, 1365, 1319, 1252, 1093, 1044, 999, 978, 887, 763, 729, 663, 640, 580 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) spectral data, see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) spectral data, see Table 2; (+)-ESI-MS: *m/z* 387 $[M+Na]^+$; (−)-ESI-MS: 399 $[M+Cl]^-$; (+)-HR-ESI-MS: *m/z* 387.1168 $[M+Na]^+$ (calcd for $C_{17}H_{20}N_2O_7$ Na, 387.1163).

3.3.3. Isatindigotindoloside C (3)

White amorphous solid; $[\alpha]_D^{20}$ −34.3 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.81), 281 (4.37), 290 (4.37) nm; CD (MeOH) $\Delta\varepsilon_{213\ nm}$ +2.29, $\Delta\varepsilon_{229\ nm}$ −3.78, $\Delta\varepsilon_{270\ nm}$ −1.53, $\Delta\varepsilon_{297\ nm}$ +0.42; IR ν_{max} 3417, 1990, 2927, 2825, 2575, 2519, 2454, 2234, 1632, 1589, 1538, 1447, 1417, 1357, 1335, 1291, 1245, 1189, 1133, 1103, 1077, 1031, 992, 960, 900, 871, 847, 800, 743, 678, 613, 578, 550 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) spectral data, see Table 1; ^{13}C NMR (CD_3OD, 150 MHz) spectral data, see Table 2; (+)-ESI-MS: *m/z* 403 $[M+Na]^+$; (−)-ESI-MS: 379 $[M-H]^-$, 415 $[M+Cl]^-$; (+)-HR-ESI-MS: *m/z* 381.1113 $[M+H]^+$ (calcd for $C_{17}H_{21}N_2O_6S$, 381.1115), 403.0939 $[M+Na]^+$ (calcd for $C_{17}H_{20}N_2O_6SNa$, 403.0934).

3.3.4. Isatindigotindoloside D (4)

White amorphous solid; $[\alpha]_D^{20}$ +11.9 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.81), 281 (4.37), 290 (4.37) nm; CD (MeOH) $\Delta\varepsilon_{218\,nm}$ −1.98, $\Delta\varepsilon_{232\,nm}$ +2.12, $\Delta\varepsilon_{270\,nm}$ +0.13, $\Delta\varepsilon_{292\,nm}$ −1.23; IR ν_{max} 3367, 2925, 2853, 2245, 1653, 1617, 1528, 1448, 1421, 1347, 1275, 1247, 1189, 1099, 1071, 957, 920, 890, 846, 799, 748, 687, 590 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) spectral

data, see Table 1; ^{13}C NMR (CD_3OD, 150 MHz) spectral data, see Table 2; (+)-ESI-MS: *m/z* 403 $[M + Na]^+$, 419 $[M + K]^+$; (−)-ESI-MS: 379 $[M - H]^-$, 415 $[M + Cl]^-$; (+)-HR-ESI-MS: *m/z* 381.1122 $[M + H]^+$ (calcd for $C_{17}H_{21}N_2O_6S$, 381.1115), 403.0943 $[M + Na]^+$(calcd for $C_{17}H_{20}N_2O_6S$ Na, 403.0934).

3.3.5. Isatindigotindoloside E (5)

White amorphous solid; $[\alpha]_D^{20}$ −34.8 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.27, sh), 223 (4.44), 275 (3.63), 291 (3.67) nm; CD (MeOH) $\Delta\varepsilon_{222\ nm}$ −4.96, $\Delta\varepsilon_{268\ nm}$ +0.23; IR ν_{max} 3357, 2933, 2863, 1612, 1600, 1546, 1454, 1418, 1355, 1322, 1278, 1231, 1116, 1096, 1058, 1031, 984, 961, 925, 889, 832, 741, 652, 569 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 600 MHz) spectral data, see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data, see Table 2; (+)-ESI-MS: *m/z* 378 $[M + Na]^+$, 394 $[M + K]^+$; (+)-HR-ESI-MS: *m/z* 378.0980 $[M + Na]^+$ (calcd for $C_{16}H_{21}NO_6SNa$, 378.0982).

3.3.6. Enzymatic hydrolysis of 1–2 and acidic hydrolysis of 3–5

Compounds **1** and **2** (~1.0 mg) were separately hydrolyzed in H_2O (3 ml) with snailase (3.0 mg, CODE S0100, Beijing Biodee Biotech Co., Ltd., Beijing, China) at 37 °C for 24 h. Compounds **3–5** (~1.2 mg) were separately hydrolyzed with 2 N HCl (3 ml) at 80 °C for 6 h. The hydrolysate was concentrated under reduced pressure and the residue was isolated by CC over silica gel eluting with CH_3CN–H_2O (8:1) to afford sugar. The sugar (0.3–0.6 mg) showed a retention factor ($R_f \approx 0.38$) on TLC (EtOAc-MeOH-AcOH-H_2O, 12:3:3:2), with $[\alpha]_D^{20}$ values of +44.2–+47.5 (*c* 0.03–0.06, H_2O), and ^{1}H NMR (D_2O) data in agreement with those of an authentic D-glucose.

3.3.7. ECD calculation of 3–4

For details, see Supporting Information. Briefly, conformational analysis was performed by Monte Carlo searching method with the MMFF94 molecular mechanics force field using the Spartan 10 software for both **3** and **4**. The lowest energy conformers with relative energies lower than 2 kcal/mol were reoptimized using the density functional theory (DFT) at the B3LYP/6-31 + G (d, p) level via the Gaussian 09 program. The conductor-like polarizable continuum model (CPCM) was employed to consider the solvent effects using the dielectric constant of MeOH (ε = 32.6). The energies, oscillator strengths, and rotational strengths of excitations were calculated using the TDDFT methodology at the B3LYP/6-311++G (2d, 2p) level in vacuum. The reoptimized conformers having relative Gibbs free energies (ΔG) within 2 kcal/mol were used to simulate the ECD spectra with the Gaussian function (σ = 0.28 eV). To obtain the final spectrum of each compound, the simulated spectra of its lowest energy conformers were averaged based on the Boltzmann distribution theory and their relative Gibbs free energy (ΔG). All quantum computations were conducted using Gaussian 09 program package on an IBM cluster machine located at the High Performance Computing Center of Peking Union Medical College.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Note

We dedicate this paper to Prof. Zhong-Jian Jia on the occasion of her 85th birthday.

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