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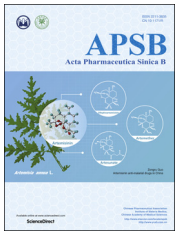

ORIGINAL ARTICLE

Three pairs of alkaloid enantiomers from the root of *Isatis indigotica*

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KEY WORDS

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2-Oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid;
Indolo[2,1-*b*]quinazolinone;
3-Thioxohexahydro-1-*H*-pyrrolo[1,2-c]imidazol-1-one

Abstract Three pairs of enantiomerically pure alkaloids with diverse structure features, named isatindigoticoic acid A and epiisatindigoticoic acid A [(−)-**1** and (+)-**1**], phaitanthrin A and epiphaitanthrin A [(−)-**2** and (+)-**2**], and isatindopyrromizol A and epiisatindopyrromizol A [(−)-**3** and (+)-**3**], respectively, were isolated from an aqueous extract of the roots of *Isatis indigotica*. Racemic and scalemic mixtures of these enantiomers were separated by HPLC on a chiral semi-preparative column. Their structures including absolute configurations were determined by extensive spectroscopic analysis in conjunction with the calculation of electronic circular dichroism (ECD) spectra. The enantiomer pairs possess parent structures of 2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid, indolo[2,1-*b*]quinazolinone, and 3-thioxohexahydro-1*H*-pyrrolo[1,2-*c*]imidazol-1-one, respectively. Except for phaitanthrin A [(−)-**2**] which the configuration was previously undetermined, these compounds are new enantiomeric natural products.

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1. Introduction

"Ban lan gen" (Radix Isatidis) is one of the most important traditional Chinese medicines used for the treatment of influenza and infection diseases. This medicine is derived from the dry roots of cultivated plant *Isatis indigotica* Fort. (Cruciferae)[1]. A literature survey shows that previous pharmacological and chemical investigations associated with this herbal medicine were mainly focused on methanol or ethanol extracts[2–9]. This differs from practical application of water decoctions of "ban lan gen", as well as formulations containing "ban lan gen". Therefore, as part of a program to assess the chemical and biological diversity of traditional Chinese medicines[10–22], we investigated a water decoction of "ban lan gen" and have reported characterization of 28 new alkaloids, including a pair of indole alkaloid enantiomers containing dihydrothiopyran and 1,2,4-thiadiazole rings, a pair of bisindole alkaloid enantiomers, seven glycosidic bisindole alkaloids, and 54 known compounds, as well as their antiviral and hepatocyte-protective activities[23–27]. A further in-depth investigation on the same extract led to the chiral separation of three pairs of alkaloid enantiomers (−)/(+)-**1**−(−)/(+)-**3** having diverse structural features (Fig. 1). This paper describes the details of isolation and structural elucidation of these enantiomers.

2. Results and discussion

An enantiomeric mixture **1** was isolated as a white amorphous powder ($[\alpha]_D^{20}$ −35.3, *c* 0.25, MeOH), which is homogeneous as indicated by normal thin layer chromatography (TLC) and reversed phase high performance liquid chromatography (RP-HPLC) analyses. The IR spectrum of **1** showed the presence of hydroxy and amino (3233 cm^{-1}), conjugated carbonyl (1684 cm^{-1}), and aromatic ring (1586 and 1516 cm^{-1}) functional groups. The molecular formula of **1** was determined as $C_{17}H_{14}N_2O_5$ based on HR-ESI-MS (*m/z* 349.0803 $[M+Na]^+$) and NMR spectral data (Table 1). The ^1H NMR spectrum of **1** in DMSO-d_6 showed signals attributed to two *ortho*-disubstituted phenyl rings at δ_H 8.53 (brd, J=8.4 Hz, H-3), 7.47 (brdd, J=8.4 and 7.2 Hz, H-4), 7.10 (brt, J=7.2 Hz, H-5), and 8.01 (brd, J=7.2 Hz, H-6) and 7.42 (brd, J=7.2 Hz, H-5′), 6.96 (brt, J=7.2 Hz, H-6′), 7.21 (brt, J=7.6 Hz, H-7′), and 6.88 (brd, J=7.2 Hz, H-8′); an isolated methylene at δ_H 2.94 (1 H, d, J=16.2 Hz, H-3′a) and 2.72 (1 H, d, J=16.2 Hz, H-3′b); as well as signals due to a carboxylic proton at δ_H 13.63 (brs, COO*H*), two nitrogen-bearing protons at δ_H 12.32 (brs, N*H*-2), 10.12 (s, N*H*-1′), and a hydroxyl proton at δ_H 6.83 (brs, O*H*-4′). The ^{13}C NMR and DEPT spectra of **1** displayed 17 carbon resonances corresponding to the above structural units and two additional carbonyl carbons at δ_C 172.5 (C-1″) and 167.6 (C-2′). As compared with those of the previously reported compounds from *I. indigotica*[23–27], these spectroscopic data indicated that **1** is an uncommon alkaloid containing two aromatic rings, two amide carbonyls, one carboxylic group and an isolated methylene unit. To construct the final structure of **1**, connections among these units were further established by 2D NMR data analysis. Comprehensive analysis of the $^1H-^1H$ COSY and HSQC spectra of **1** confirmed the presence of the above structural units and unambiguously assigned the proton and proton-bearing carbon resonances in the NMR spectra. The HMBC spectrum of **1** showed two- and three-bond hetereonuclear correlations from H-3 to C-1 and C-5; from H-4 to C-2 and C-6; from H-5 to C-1 and C-3; and from H-6 to C-2, C-4 and C-7 (Fig. 2). These correlations, combined with their chemical shifts and the broadened and diminished resonance of C-7 (typical for carboxylic acid carbon with a dissociation property), indicated that there was an *N*-substituted 2′-aminobenzoic acid (anthranilic acid) moiety in **1**. Meanwhile, the HMBC spectrum exhibited the long-range correlations from N*H*-1′ to C-2′, C-3′, C-4′a, C-8′, and C-8′a; from H_2-3′ to C-2′, C-4′, and C-4′a; from H-5′ to C-4′, C-7′ and C-8′a; from

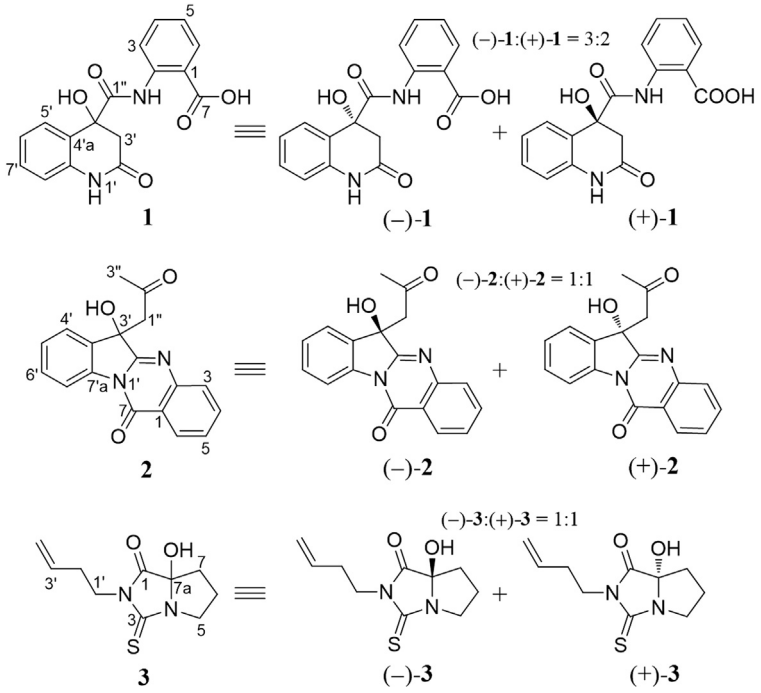


Figure 1 The structures of enantiomer mixtures **1**−**3** and compounds (−)-/(+)-**1**−(−)-/(+)-**3**.

Table 1 NMR spectral data (δ) for compounds (−)/(+)-**1**−(−)/(+)-**3**[a].

No.	(−)/(+)-**1**[b]		(−)/(+)-**2**[c]		(−)/(+)-**3**[d]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		122.0		123.0		172.6
2		140.2		148.6		
3	8.53 brd (8.4)	119.0	7.72 brd (1.0, 8.0)	128.5		186.8
4	7.47 brdd (7.2, 8.4)	132.5	7.84 dt (1.5, 8.0)	135.2		
5	7.10 brt (7.2)	122.6	7.59 dt (1.0, 8.0)	127.9	A: 3.89 dt (11.4, 8.4) B: 3.59 dt (3.0, 11.4)	48.6
6	8.01 brd (7.2)	131.3	8.35 dd (1.5, 8.0)	127.2	A: 2.40 m B: 2.21 m	25.5
7		169.2		160.0	A: 2.06 m B: 1.81 m	33.4
7a						93.3
1′	10.12				3.76 t (7.2)	41.1
2′		167.6		162.0	2.38 quin (7.2)	32.6
3′	A: 2.94 d (16.2) B: 2.72 d (16.2)	42.1		76.0	5.78 m	135.6
3′a				134.5		
4′		74.4	7.63 dd (1.0, 8.0)	124.4	A: 5.04 dd (16.8 1.8) B: 4.98 d (10.2)	117.3
4′a		125.5				
5′	7.42 brd (7.2)	125.8	7.34 dt (1.0, 8.0)	127.4		
6′	6.96 brt (7.2)	122.0	7.49 t (1.0, 8.0)	130.6		
7′	7.21 brt (7.2)	129.1	8.50 brd (8.0)	117.2		
7′a				140.9		
8′	6.88 brd (7.2)	115.4				
8′a		137.8				
1″		172.5	A: 3.84 d (18.0) B: 3.69 d (18.0)	52.5		
2″				205.5		
3″			2.08 s	30.3		

[a]Data were measured in DMSO-d_6 for (−)/(+)-**1** (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR) and in Me_2CO-d_6 for (−)/(+)-**2** (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) and (−)/(+)-**3** (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR), respectively. The assignments were based on DEPT, ^{1}H–^{1}H COSY, HSQC, HMQC, and HMBC experiments.

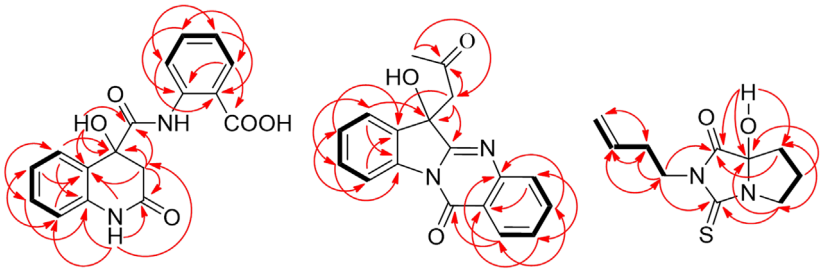
[b]Data for the amino and hydroxyl groups in (−)/(+)-**1**: δ_H 13.63 (1H, brs, COOH), 12.32 (1H, brs, NH-2), 6.83 (1H, brs, OH-4′).

[c]Data for the hydroxyl group in (−)/(+)-**2**: δ_H 5.40 (1H, brs, OH-3′).

[d]Data for the hydroxyl group in (−)/(+)-**3**: δ_H 6.10 (1H, brs, OH-7a).

H-6′ to C-4′a and C-8′; from H-7′ to C-5′ and C-8′a; and from H-8′ to C-6′ and C-4′a. These correlations, together with their chemical shifts, revealed the presence of a 4′,4′-disubstituted 2′-oxo-1′,2′,3′,4′-tetrahydroquinoline moiety in **1**. In addition, the HMBC correlations from H_2-3′ to C-1″ and from O*H*-4′ to C-4′, C-4′a, and C-1″, together with the chemical shifts and quaternary nature of C-4′ and C-1″, located both the hydroxy and carbonyl (C-1″) groups at the C-4′ of the tetrahydroquinoline moiety. To satisfy requirement of the molecular composition ($C_{17}H_{14}N_2O_5$), the two moieties must be connected *via* an amide bond between C-1″ and 2-amino to give a planar structure of 2-(4′-hydroxy-2′-oxo-1′,2′,3′,4′-tetrahydroquinoline-4′-carboxamido)benzoic acid for **1**. Although **1** had a relative large specific rotation value, to determine the absolute configuration, its optical purity was examined since several pairs of enantiomers had been found in the extracts of *I. indigotica*[23,24,27]. HPLC analysis of **1** on an analytical chiral column displayed two peaks with an about 3:2 integration ratio, indicating that **1** was indeed a mixture of enantiomers in inequivalent amounts. Subsequent HPLC separation of **1** using a semi-preparative chiral column afforded (−)-**1** {$[\alpha]_D^{20}$ −98.6 (*c* 0.09, MeOH)} and (+)-**1** {$[\alpha]_D^{20}$ +97.4 (*c* 0.05, MeOH)}, which had the identical ^{1}H NMR spectroscopic data with those of **1** prior to separation. The measured circular dichroism (CD) spectra of (−)-**1** and (+)-**1** displayed mirror curves, which were in good agreement with those of the calculated electronic circular dichroism (ECD) spectra of **1** with the preassigned *R* and *S* configurations (Fig. 3), respectively, using the time-dependent density functional theory (TDDFT) method[28]. Thus, compounds (−)-**1** and (+)-**1** were determined as (−)-(*R*)- and (+)-(*S*)-2-(4′-hydroxy-2′-oxo-1′,2′,3′,4′-tetrahydroquinoline-4′-carboxamido)benzoic acid and named isatindigoticoic acid A and epiisatindigoticoic acid A, respectively.

An enantiomeric mixture **2** was obtained as a white amorphous powder ($[\alpha]_D^{20} \approx 0.0$, *c* 0.1, DMSO). A comprehensive analysis of spectroscopic data including 2D NMR experiments revealed that **2** had the same planar structure as that of phaitanthrin A isolated from *Phaius mishmensis* (Orchidaceae)[29]. Because phaitanthrin A was reported as an optically active natural product with an $[\alpha]_D^{20}$ value of −25.1 (*c* 0.03, $CHCl_3$) and undetermined configuration, the optical inactivity of **2** indicated that it was obtained as a racemate. This was proved by the HPLC separation of **2** on a semi-preparative chiral column to yield (+)-**2** and (−)-**2** in a 1:1 ratio, which exhibited $[\alpha]_D^{20}$ +11.8 (*c* 0.12, MeOH) and −12.4 (*c* 0.1, MeOH), respectively, and their NMR spectra superimposed with



1 2 3

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

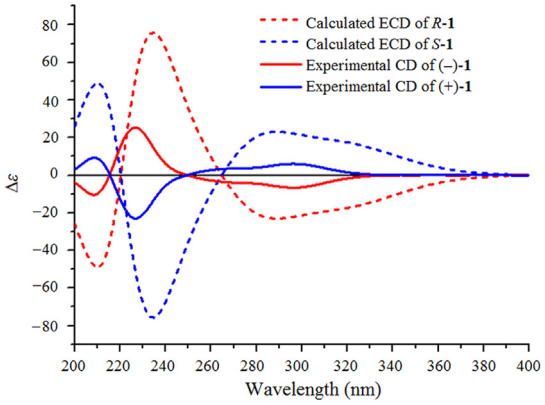


Figure 3 The measured CD spectra (full line) of (+)-**1** (blue) and (−)-**1** (red) in MeOH and the calculated ECD spectra (dashed line) of **1** with the preassigned configurations *S* (blue) and *R* (red).

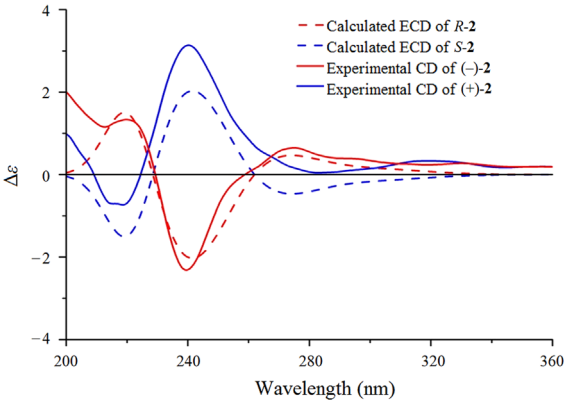


Figure 4 The measured CD spectra (full line) of (−)-**2** (red) and (+)-**2** (blue) in MeOH and the calculated ECD spectra (dashed line) of **2** with the preassigned configurations *S* (blue) and *R* (red).

that of **2** prior to separation. The calculated ECD with the preassigned *R*- and *S*-configurations of **2** were consistent with the experimental CD spectra of (−)-**2** and (+)-**2** (Fig. 4), respectively, indicating the *R*- and *S*-configurations for (−)-**2** and (+)-**2**. The negative specific rotations of (−)-**2** and phaitanthrin A suggested that these two compounds were identical. Therefore, phaitanthrin A was determined to have the *R*-configuration, and the structure of compound (+)-**2** was determined as the enantiomer of phaitanthrin A and named epiphaitanthrin A.

An enantiomeric mixture **3** was obtained as a yellowish gum ($[\alpha]_D^{20} \approx 0.0$, *c* 0.1, MeOH), which is also homogeneous as shown by normal TLC and RP-HPLC analyses. Its IR spectrum showed absorption bands for hydroxy (3395 cm^{-1}), carbonyl (1755 and 1740 cm^{-1}), double bond (1645 cm^{-1}), and thiocarbonyl (1425 cm^{-1}) functionalities. The molecular formula of **3**, $C_{10}H_{14}N_2O_2S$ with 5 degrees of unsaturation, was determined by HR-ESI-MS (*m/z* 227.0848 $[M+H]^+$) in addition to the NMR spectroscopic data. The NMR spectral data of **3** in acetone-d_6 (Table 1) showed the presence of a terminal vinyl unit, five methylenes, one quaternary carbon bearing two heteroatoms (δ_C 93.3, C-7a), an amide carbonyl (δ_C 172.6, C-1), and a thiocarbonyl (δ_C 186.8, C-3), as well as a tertiary hydroxy group [δ_H 6.10 (brs, O*H*-7a)]. Together, information from the spectroscopic data revealed that **3** was a rear bicyclic alkaloid containing amide carbonyl and thiocarbonyl groups, which has never been reported for compounds isolated from *I. indigotica*. Accordingly, the structure was further deduced from 2D NMR data analysis. The ^{1}H–^{1}H COSY correlations of H_2-5/H_2-6/H_2-7 and the HMBC correlations from H_2-5 to C-7 and C-7a; from H_2-7 to C-5 and C-7a; from OH-7a to C-7a and C-7, together with their chemical shifts, demonstrated that C-7a and C-5 were connected *via* a nitrogen atom to form a *N*,7a-disubstituted 7a-hydroxypyrrolidine ring in **3**. Meanwhile, the ^{1}H–^{1}H COSY correlations of H_2-1′/H_2-2′ and H-3′/H_2-4′, combined with the HMBC correlations from H_2-1′ to C-2′ and C-3′ and from H_2-2′ to C-1′, C-3′ and C-4′, as well as the chemical shifts of these proton and carbon resonances, indicated the presence of a but-3′-en-1′-yl unit. In addition, the HMBC correlations from H_2-7 to C-1 and from OH-7a to C-1 located the carbonyl carbon (C-1) at C-7a. The HMBC correlations from H_2-1′ to C-1 and C-3 and from H_2-5 to C-3, along with the molecular formula and the degrees of unsaturation, suggested linkages of the remaining N-atom (N-1) with C-1, C-1′, and C-3 and of C-3 with the N atom of the pyrrolidine ring to afford a final planar structure of 2-(but-3′-en-1′-yl)-7a-hydroxy-3-thioxohexahydro-1*H*-pyrrolo[1,2-*c*]imidazol-1-one for **3**. Because of the presence of a chiral center in the structure, the optical inactivity of **3** suggested that it might be also a racemate. Using a semi-preparative chiral column, subsequent HPLC separation of **3** yielded (−)-**3** and (+)-**3** in a 1:1 ratio, of which the enantiomeric relationship was verified by measurement of their $[\alpha]_D^{20}$ values, as well as CD and ^{1}H NMR spectroscopic data (See in Experimental Section and Supplementary Information). Comparing the experimental CD and the calculated ECD spectra (Fig. 5), the absolute configurations of (−)-**3** and (+)-**3** were assigned as *R* and *S*, respectively. Therefore, the structure of compounds (−)-**3** and (+)-**3** were determined as (−)-(*R*)- and (+)-(*S*)-2-(but-3′-en-1′-yl)-7a-hydroxy-3-thioxohexahydro-1*H*-pyrrolo[1,2-c]imidazol-1-one and named isatindopyrromizol A and epiisatindopyrromizol A, respectively.

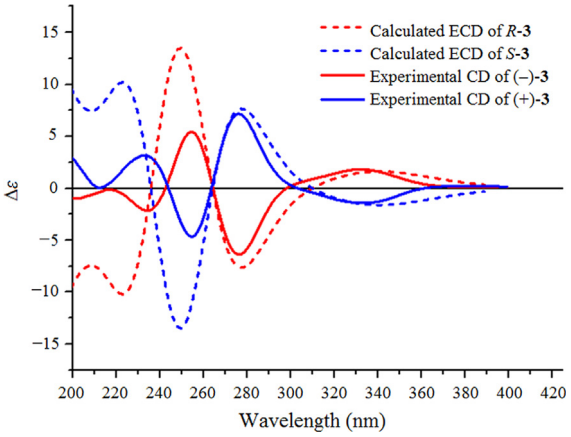


Figure 5 The measured CD spectra (full line) of (−)-**3** (red) and (+)-**3** (blue) in MeOH and the calculated ECD spectra of **3** (dashed line, blue shifted by 10 nm) with the preassigned configurations *R* (red) and *S* (blue).

3. Conclusions

In summary, three pairs of enantiomeric alkaloids (−)/(+)-**1**–(−)/(+)-**3** having diverse structure features were isolated from the water decoction of "ban lan gen". The enantiomers were separated by HPLC on a chiral semi-preparative column and their absolute configurations were determined by comparison of the experimental CD and the calculated ECD spectra. These compounds are new natural products except that (−)-**2** proved to be identical to the previously reported phaitanthrin A from the orchidaceous plant *P. mishmensis*[29]. The remaining problem with the previously undetermined absolute configuration of phaitanthrin A was solved in this study. Although these new isolates did not show activity in the *in vitro* preliminary assays carried out in our studies[23–27], their bioactivities, as well as possible contributions to the clinical effects of this traditional Chinese herbal medicine still deserve to be explored in-depth in other biological and pharmacological models including animal models, with the assistance of chemical synthesis to supply enough quantity of samples. Separation of these racemic and scalemic mixtures indicates that *I. indigotica* is an uncommon plant to produce diverse enantiomeric natural products[23–27], providing a fertile area for further inquiry for biogenetic mechanisms and pathways to create these distinctive enantiomers.

4. Experimental

4.1. 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). IR spectra were obtained on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission), (Thermo Electron Corporation, Madison, WI, USA). NMR spectra were recorded at 500 MHz or 600 MHz for ^{1}H NMR and 125 MHz or 150 MHz for ^{13}C NMR, respectively, on an Inova 500 or SYS 600 (Varian Associates Inc., Palo Alto, CA, USA) in DMSO-d_6 or acetone-d_6, using undeuterated solvent peaks as references. ESI-MS and HR-ESI-MS data were obtained 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 20 P (Mitsubishi Chemical Inc., Tokyo, Japan), or reversed 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 (250 mm × 10 mm) semi-preparative column packed with C18 reversed phase silica gel (5 μm) (W.R. Grace & Co., Maryland, USA) or a Chiralpak AD-H column (250 mm × 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 plates precoated with silica gel GF_{254} (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.

4.2. Plant material

The roots of *I. indigotica* were collected in December 2009 from 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.

4.3. Extraction and isolation

The air-dried and pulvarized plant material (50 kg) was decocted with H_2O (150 L; 1 h × 3). 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 (200 cm × 20 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 20 P (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 on silica gel, eluted by a gradient of increasing MeOH (0–100%) in EtOAc to yield subfractions B3-1–B3-16. Subfraction B3-1 (3.5 g) was subjected to CC over Sephadex LH-20 ($CHCl_3$–MeOH, 1:1, *v/v*) to yield B3-1-1–B3-1-5, of which B3-1-2 (1.79 g) was separated by reversed phase flash CC, eluted by a gradient of increasing MeOH (0–100%) in water to yield B3-1-2-1–B3-1-2-6. Further fractionation of B3-1-2-6 (282 mg) by CC over Sephadex LH-20, eluted with petroleum ether–$CHCl_3$–MeOH (5:5:1, *v/v/v*), to give B3-1-2-6-1–B3-1-2-6-6, of which B3-1-2-6-4 was separated by preparative TLC (petroleum ether–acetone, 3:1, *v/v*) then by RP-HPLC (C18 column, 55% MeOH in H_2O) to give **3** (4.3 mg). Subsequent separation of **3** by HPLC on a semi-preparative Chiralpak AD-H column (*n*-hexane-*i*PrOH, 1:1, 2.0 mL/min) afforded (+)-**3** (2.0 mg, t_R = 19.0 min) and (−)-**3** (2.1 mg, t_R = 25.3 min). Similarly, separation of B3-1-2-6-6 by preparative TLC (petroleum ether–acetone, 3:1, *v/v*) followed by RP-HPLC (C18 column, 54% MeOH in H_2O) yielded **2** (5.4 mg),

which was separated into (+)-**2** (2.5 mg, t_R = 31.6 min) and (−)-**2** (2.4 mg, t_R = 33.2 min) by semi-preparative HPLC (Chiralpak AD-H column, *n*-hexane-*i*PrOH, 3:1, 2.0 mL/min). Fraction of B3-3 (7.5 g) by CC over Sephadex LH-20, eluting with $CHCl_3$–MeOH (1:1), yielded B3-3-1–B3-3-4, of which B3-3-4 (3.0 g) was further separated by CC over silica gel, eluted by a gradient of increasing MeOH (0–100%) in $CHCl_3$, to afford B3-3-4-1–B3-3-4-14. Subfraction B3-3-4-14 (413.0 mg) was chromatographed on Sephadex LH-20 (MeOH) to yield B3-3-4-14-1–B3-3-4-14-10, of which B3-3-4-14-5 (30.0 mg) was purified by RP-HPLC (45% MeOH in H_2O containing 0.3% AcOH, *v/v/v*, 2.0 mL/min) to afford **1** (2.8 mg). Separation of **1** by HPLC on the semi-preparative Chiralpak AD-H column eluting with *n*-hexane-*i*PrOH (6:1, containing 0.1% TFA, *v/v/v*, 1.3 mL/min) yielded (−)-**1** (1.2 mg, t_R = 18.2 min) and (+)-**1** (0.8 mg, t_R = 48.1 min).

4.3.1. *Isatindigoticoic acid A and epiisatindigoticoic acid A [(−)-**1** and (+)-**1**]*

Mixture of (−)-**1** and (+)-**1** in a 3:2 ratio (**1**), a white amorphous solid; $[\alpha]_D^{20}$ −35.3 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (logε) 211 (4.28), 250 (3.95), 292 (3.43) nm; CD (MeOH) 226 ($\Delta\varepsilon$ +10.32), 295 ($\Delta\varepsilon$ −2.98); IR ν_{max} 3233, 2975, 2912, 2258, 1782, 1684, 1586, 1516, 1450, 1379, 1296, 1251, 1165, 1142, 1088, 1047, 1025, 1003,915, 880, 825, 760, 701, 656 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (DMSO-d_6, 150 MHz) data, see Table 1; ESI-MS *m/z* 325 $[M-H]^-$, 651 $[2M-H]^-$; HR-ESI-MS *m/z* 349.0803 $[M+Na]^+$ (Calcd. for $C_{17}H_{14}N_2O_5Na$, 349.0795). (−)-**1**: $[\alpha]_D^{20}$ −98.6 (*c* 0.09, MeOH); CD (MeOH) 209 ($\Delta\varepsilon$ −10.49), 227 ($\Delta\varepsilon$ +25.32), 297 ($\Delta\varepsilon$ −6.65); (+)-**1**: $[\alpha]_D^{20}$ +97.4 (*c* 0.05, MeOH); CD (MeOH) 209 ($\Delta\varepsilon$ +9.30), 227 ($\Delta\varepsilon$ −23.03), 296 ($\Delta\varepsilon$ +6.02). ^1H NMR (DMSO-d_6, 600 MHz) data of (−)-**1** and (+)-**1** were identical with those of **1**.

4.3.2. *Phaitanthrin A and epiphaitanthrin A [(−)-**2** and (+)-**2**]*

Mixture of (−)-**2** and (+)-**2** in a 1:1 ratio (**2**), a white amorphous solid, $[\alpha]_D^{20} \approx 0.0$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 226 (5.47), 258 (4.88), 314 (4.25), 327 (4.06) nm; IR ν_{max} 3395, 3194, 3074, 2922, 2850, 1713, 1647, 1604, 1565, 1466, 1419, 1360, 1324, 1241, 1181, 1119, 1065, 1024, 987, 961, 877, 776, 756, 713, 693, 661, 557, 479 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 1; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 1; ESI-MS *m/z* 329 $[M+Na]^+$; HR-ESI-MS *m/z* 307.1075 $[M+H]^+$ (Calcd. for $C_{18}H_{15}N_2O_3$, 307.1077), 329.0894 $[M+Na]^+$ (Calcd. for $C_{18}H_{14}N_2O_3Na$, 329.0897). (−)-**2**: $[\alpha]_D^{20}$ −12.4 (*c* 0.1, MeOH); CD (MeOH) 221 ($\Delta\varepsilon$ +1.33), 240 ($\Delta\varepsilon$ −2.32); (+)-**2**: $[\alpha]_D^{20}$ +11.8 (*c* 0.12, MeOH); CD (MeOH) 217 ($\Delta\varepsilon$ −0.61), 241 ($\Delta\varepsilon$ +2.91). ^1H NMR (Me_2CO-d_6, 600 MHz) data of (−)-**2** and (+)-**2** were identical with those of **2**.

4.3.3. *Isatindopyrromizol A and epiisatindopyrromizol A [(−)-**3** and (+)-**3**]*

Mixture of (−)-**3** and (+)-**3** in a 1:1 ratio (**3**), a yellowish gum, $[\alpha]_D^{20} \approx 0.0$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (logε) 211 (3.64), 269 (5.00) nm; IR ν_{max} 3395, 3188, 3078, 2922, 2850, 1755, 1740, 1645, 1425, 1376, 1348, 1325, 1279, 1227, 1193, 1123, 1063, 1026, 996, 918, 872, 814, 752, 723, 660, 647, 583 cm^{-1}; ^1H NMR (Me_2CO-d_6, 600 MHz) data, see Table 1; ^{13}C NMR (Me_2CO-d_6, 150 MHz) data, see Table 1; HR-ESI-MS *m/z* 227.0848 $[M+H]^+$ (Calcd. for $C_{10}H_{15}N_2O_2S$, 227.0849). (−)-**3**: $[\alpha]_D^{20}$ −13.1 (*c* 0.15, MeOH); CD (MeOH) 235 ($\Delta\varepsilon$ −2.15), 254 ($\Delta\varepsilon$ +5.40), 277 ($\Delta\varepsilon$ −6.36), 332 ($\Delta\varepsilon$ +1.82); (+)-**3**: $[\alpha]_D^{20}$ +13.6 (*c* 0.12, MeOH); CD (MeOH) 233 ($\Delta\varepsilon$ +3.16), 255 ($\Delta\varepsilon$ −4.65), 276 ($\Delta\varepsilon$ +7.15), 335 ($\Delta\varepsilon$ −1.43). ^1H NMR (Me_2CO-d_6, 600 MHz) data of (−)-**3** and (+)-**3** were identical with those of **3**.

4.4. *ECD calculation of (−)-/(+)-**1**−(−)-/(+)-**3***

For details, see Supplementary Information. Briefly, conformational analysis was performed in the MMFF94 molecular mechanics force field using the Spartan 10 software. The lowest-energy conformers with relative energy under 2 kcal/mol were re-optimized using the density functional theory (DFT) at the B3LYP/6-31+G (d, p) level for **1** and **3** and at B3LYP/6-31 G (d) level for **2** using the Gaussian 09 program. The solvent effects were evaluated using the conductor-like polarizable continuum model (CPCM) with the dielectric constant of MeOH (ε = 32.6). The energy, oscillator strengths, and rotational strengths of the excitations were calculated using the TDDFT methodology at the B3LYP/6-311++G (2d, 2p) level for **1** and **3** and at B3LYP/6-31 G (d) level for **2** in vacuum. The re-optimized conformers having relative Gibbs free energy (ΔG) within 2 kcal/mol were used to simulate the ECD spectra with the Gaussian function (σ = 0.28 eV). To obtain the final spectra, the simulated spectra of the lowest energy conformers were averaged on the basis of the Boltzmann distribution theory and their relative Gibbs free energy (ΔG). All quantum computations were conducted using the 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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Appendix A. Supplementary Information

Supplementary data associated with this article can be found in the online version at: http://dx.doi.org/10.1016/j.apsb.2016.01.003.

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