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Alkaloids from the Root of *Isatis indigotica*

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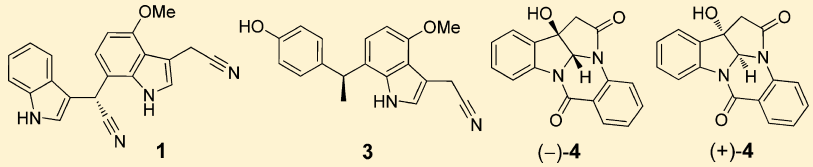
Supporting Information



ABSTRACT: Seventeen new alkaloids (**1**−**17**) and 14 known analogues have been isolated from an aqueous extract of the root of *Isatis indigotica*. The structures and absolute configurations of these compounds were determined by extensive spectroscopic data analysis, including 2D NMR, single-crystal X-ray crystallography using anomalous scattering of Cu Kα radiation, and electronic circular dichroism spectra calculations based on the quantum-mechanical time-dependent density functional theory. Compounds **1**, **2**, and **3** are the first examples of natural products with unique linkages between a molecule of 2-(4-methoxy-1*H*-indol-3-yl)acetonitrile and 2-(1*H*-indol-3-yl)acetonitrile, 2-(4-methoxy-1*H*-indol-3-yl)acetonitrile, and 4-hydroxyphenylethane, respectively. Compounds (−)-**4** and (+)-**4** represent the first natural products with the pyrrolo[2,3-*b*]indolo[5,5*a*,6-*b*,*a*]quinazoline skeleton. Some structural assignments for the new alkaloids suggest that the assignments made for certain previously reported alkaloids require revision. Compounds **1**−**3** and arvelexin (**18**) show antiviral activity against the influenza virus A/Hanfang/359/95 (H3N2), with IC_{50} values of 3.70−12.35 μM, and **17** inhibits Coxsackie virus B3 replication with an IC_{50} of 6.87 μM.

Isatis indigotica Fort. (Cruciferae) is a biennial herbaceous plant that is widely distributed and cultivated in China. Its dried roots and leaves, named "ban lan gen" and "da qing ye" in Chinese, respectively, are used in traditional Chinese medicine for the treatment of various diseases, especially for treating influenza, cold, fever, and infections.[1] Chemical and pharmacological studies of ethanol extracts of the roots and leaves of *I. indigotica* have aided the characterization of constituents with different structural features and biological activities, such as alkaloids,[2] lignans,[3] ceramides,[4] flavonoids,[5] epigoitrin, and 2-hydroxy-3-butenylthiocyanate.[6] Indole alkaloids are the main active constituents of these drugs, including more than 20 analogues. As part of a program to study the chemical diversity of traditional Chinese medicines and their biological effects,[7] we conducted detailed chemical analysis of the aqueous extract of the roots of *I. indigotica*, since the root decoction is practically used in the formulations. Chromatographic separation of the chemical components of the extract led to the isolation of 17 new alkaloids (**1**−**17**) and 14 known analogues. Compounds **1**, **2**, and **3** are the first examples of natural products with unique linkages between a 2-(4-methoxy-1*H*-indol-3-yl)acetonitrile molecule and 2-(1*H*-indol-3-yl)acetonitrile, 2-(4-methoxy-1*H*-indol-3-yl)acetonitrile, and 4-hydroxyphenylethane, respectively, though bisindole derivatives with various linkages, including indigotin, indirubin,[2a] (*E*)-2-[(1*H*-indol-3-yl)-cyanomethylene]-3-indolinone,[2e] and isatisine A from *I. indigotica*,[2g] cephalinone C from *Cephalanceropsis gracilis*,[8] and gelliusines D−F from deep-water marine sponge *Orina* sp.,[9] have been previously reported. Compounds (−)-**4** and (+)-**4** represent the first natural products with the pyrrolo[2,3-*b*]indolo[5,5*a*,6-*b*,*a*]quinazoline skeleton, which has been synthesized by the photoinduced cyclization of *N*-[2-(1-indolylmethyl)phenyl]chloroacetamide in a previous study.[10] Herein, we describe the detailed structure elucidation of the aqueous isolates of *I. indigotica* by extensive spectroscopic analysis, including 2D NMR, single-crystal X-ray crystallography, and electronic circular dichroism (ECD) spectral calculations based on the quantum-mechanical time-dependent density functional theory (TDDFT). The results obtained for some of the new alkaloids suggest that the structural and stereochemical assignments of previously reported compounds require revision. Biological assays of the *I. indigotica* isolates

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reveal interesting antiviral activity that may provide insights into the plant's use as a traditional remedy.

RESULTS AND DISCUSSION

Compound **1**, whose molecular formula was found to be $C_{21}H_{16}N_4O$ by high-resolution electrospray mass spectroscopy (HR-ESIMS) and NMR data, showed IR absorptions attributed to NH (3410 cm^{-1}), C≡N (2247 cm^{-1}), and aromatic ring (1615, 1601, and 1518 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** showed signals attributable to an ortho-disubstituted benzene ring at δ_H 7.49 (dd, J = 1 and 8 Hz, H-4′), 7.00 (dt, J = 1 and 8 Hz, H-5′), 7.13 (dt, J = 1 and 8 Hz, H-6′), and 7.43 (d, J = 8 Hz, H-7′); an ortho-tetrasubstituted benzene ring at δ_H 7.26 (d, J = 8 Hz, H-6‴) and 6.61 (d, J = 8 Hz, H-5‴); and two trisubstituted double bonds at δ_H 7.27 (brd, J = 1.5 Hz, H-2′) and 7.22 (brd, J = 2 Hz, H-2‴). In addition, resonances assignable to an uncoupled methine at δ_H 5.97 (s, H-2); an isolated methylene at δ_H 4.05 (s, H-2″); an aromatic methoxy group at δ_H 3.94 (3H, s, OCH_3-4‴); and two exchangeable protons at δ_H 10.35 (brs, NH-1′) and 10.26 (brs, NH-1‴) were observed. The ^{13}C NMR and DEPT spectra also indicated the presence of two quaternary cyano carbons at δ_C 120.3 and 119.7, which was supported by the IR data (2247 cm^{-1}). These spectroscopic data suggested that **1** was an unusual aromatic alkaloid with two cyano groups, which was confirmed by 2D NMR data analysis. The proton and protonated carbon resonances in the NMR spectra of **1** were unambiguously assigned by the HSQC experiment. $^1H-^1H$ gCOSY correlations of H-4′/H-5′/H-6′/H-7′ and NH-1′/H-2′ and HMBC correlations of H-2′/C-3′a, C-7′a, and C-2; H-4′/C-3′, C-3′a, C-6′, and C-7′a; H-5′/C-3′a and C-7′; H-6′/C-4′ and C-7′a; H-7′/C-3′a and C-5′; and H-2/C-1, C-3′, and C-3′a, in combination with the chemical shifts of these proton and carbon signals, revealed the presence of a 2-substituted 2-(1H-indol-3-yl)acetonitrile moiety[11] in **1**. Meanwhile, gCOSY correlations of H-5‴/H-6‴ and NH-1‴/H-2‴, together with HMBC correlations of H-2‴/C-3‴a, C-7‴a, and C-2″; H-5‴/C-3‴a, C-4‴, C-6‴, and C-7‴; H-6‴/C-4‴ and C-7‴a; H_2-2″/C-1″, C-3‴, and C-3‴a; and OCH_3/C-4‴, demonstrated the presence of a 3-cyanomethyl-4-methoxy-1H-indol-7-yl moiety.[12] In addition, HMBC correlations of H-2/C-6‴, C-7‴, and C-7‴a indicated that the above-mentioned two moieties were connected via a C-2−C-7‴ bond, thus giving **1** a planar structure, 2-(3-cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(1H-indol-3-yl)acetonitrile.

The absolute configuration at C-2 was determined by comparing the experimental circular dichroism (CD) spectrum with the ECD spectrum predicted from quantum-mechanical TDDFT calculations, a recent approach increasingly applied for the determination of absolute configurations of natural products.[13] In the 200−400 nm region, the calculated CD spectrum of (*R*)-**1** showed four peaks at 272 (−14), 254 (−7), 233 (+2), and 216 (+6) nm (Supporting Information, Figure S7), which corresponded to the experimental first negative (286 nm), second positive (261 nm), third negative (229 nm), and fourth positive (210 nm) Cotton effects, respectively. Therefore, compound **1** was determined to be (−)-(*R*)-2-(3-cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(1H-indol-3-yl)-acetonitrile.

The spectroscopic data of compound **2** (Table 1 and Experimental Section) indicated that it was an analogue of **1** with the molecular formula $C_{22}H_{18}N_4O_2$. Comparison of the NMR data of **2** and **1** demonstrated that the 2-(1H-indol-3-yl)acetonitrile moiety in **1** was replaced by a 2-(4-methoxy-1H-indol-3-yl)acetonitrile unit in **2**. This was confirmed by $^1H-^1H$ gCOSY correlations of H-5′/H-6′/H-7′ and NH-1′/H-2′ and HMBC correlations of H-2/C-1, C-2′, and C-3′a; H-2′/C-3′, C-3′a, and C-7′a; H-5′/C-3′a and C-7′; H-6′/C-4′ and C-7′a; H-7′/C-3′a and C-5′; and OCH_3/C-4′ in the 2D NMR spectra of **2**. In particular, HMBC correlations of H-2/C-6‴, C-7‴, and C-7‴a indicated the presence of the C-2−C-7‴ linkage in **2**. According to the same protocol as that described for **1**, in the 200−400 nm region, the calculated ECD spectrum of the *R*-enantiomer showed Cotton effects consistent with the experimental data. Therefore, compound **2** was determined to be (−)-(*R*)-2-(3-cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(4-methoxy-1H- indol-3-yl)acetonitrile.

Compound **3** had the molecular formula $C_{19}H_{19}N_2O_2$, as indicated by (+)-HR-ESIMS and NMR data. Comparison of the NMR data of **3** and **1** indicated substitution of the 2-(1H-indol-3-yl)acetonitrile moiety in **1** with a 1-(4-hydroxyphenyl)-ethyl moiety in **3**. This was further verified by 2D NMR data analysis of **3**. $^1H-^1H$ gCOSY correlations of H-2‴(H-6‴)/H-3‴(H-5‴) and H-1″/H_3-2″ and HMBC correlations of H-2‴(H-6‴)/C-1″ and C-4‴; H-3‴(H-5‴)/C-1‴; OH-4‴/C-3‴(C-5‴); H-1″/C-2″, C-1‴, and C-2‴(C-6‴); and H_3-2″/C-1″ and C-1‴ proved the existence of the 1-(4-hydroxyphenyl)ethyl moiety in **3**. Furthermore, HMBC correlations of H-6′/C-1″; H-1″/C-6′, C-7′, and C-7′a; and H_3-2″/C-7′ confirmed the C-1″−C-7′ linkage. Comparison of the experimental CD spectrum

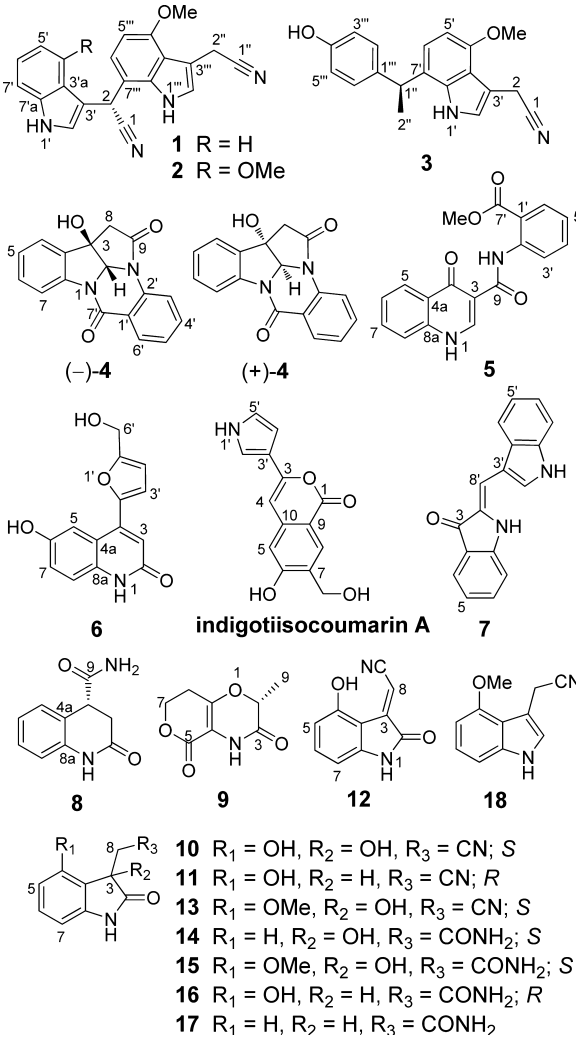

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Table 1. NMR Data for Compounds 1−3[a]

	1[b]		2[b]		3[b]	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		120.3		120.5		119.9
2	5.97 s	30.9	6.19 s	30.8	4.01 s	15.9
1′	10.35 brs		10.30 brs		9.84 brs	
2′	7.27 d (1.5)	124.8	6.99 brs	122.8	7.11 d (2.5)	122.9
3′		110.1		110.4		106.0
3′a		126.6		116.3		117.3
4′	7.49 dd (1, 8)	119.6		154.5		153.7
5′	7.00 dt (1, 8)	120.2	6.51 d (8)	100.1	6.52 d (8)	100.3
6′	7.13 dt (1, 8)	122.9	7.05 t (8)	123.4	6.97 d (8)	121.2
7′	7.43 d (8)	112.7	7.01 d (8)	105.3		124.0
7′a		138.1		138.8		137.3
1″		119.7		119.2	4.40 q (7.5)	39.4
2″	4.05 s	15.8	4.06 s	15.2	1.62 d (7.5)	22.1
1‴	10.26 brs		10.39 brs			137.6
2‴	7.22 d (2)	123.6	7.25 brs	122.9	7.08 d (8.5)	129.1
3‴		106.6		105.8	6.71 d (8.5)	115.8
3‴a		118.0		117.2		
4‴		155.4		154.5		156.5
5‴	6.61 d (8)	100.6	6.58 d (8)	99.9	6.71 d (8.5)	115.8
6‴	7.26 d (8)	123.7	7.16 d (8)	122.7	7.08 d (8.5)	129.1
7‴		113.1		113.9		
7‴a		136.4		135.8		

[a]Data (δ) were measured at 500 MHz for ^{1}H and at 125 MHz for ^{13}C in Me_2CO-d_6. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H gCOSY, gHSQC, and gHMBC experiments. [b]Data for O*Me*-4‴ in **1**: δ_H 3.94 (s) and δ_C 55.7; for O*Me*-4′ and O*Me*-4‴ in **2**: δ_H 3.80 (s) and 3.93 (s) and δ_C 54.9 and 55.1; for O*Me*-4′ and OH-4‴ in **3**: δ_H 3.90 (s) and 8.08 (brs) and δ_C 55.5.

of **3** with the calculated ECD spectra of the enantiomers suggested the *S*-configuration for **3**. Thus, compound **3** was assigned as (+)-(*S*)-2-{7-[1-(4-hydroxyphenyl)-ethyl]-4-methoxy-1*H*-indol-3-yl}acetonitrile.

Compound **4** showed IR absorption bands attributed to OH (3331 cm^{-1}), conjugated carbonyl (1724 and 1644 cm^{-1}), and aromatic ring (1603 and 1489 cm^{-1}) functionalities. The molecular formula was indicated to be $C_{17}H_{12}N_2O_3$, with 13 degrees of unsaturation, by (+)-HR-ESIMS and NMR data. The NMR data of **4** (Table 2) demonstrated the presence of two ortho-disubstituted benzene rings, an uncoupled methine unit, an isolated methylene unit, an OH-substituted quaternary carbon (δ_C 77.4), and two carbonyl groups (δ_C 170.2 and 158.5). These spectroscopic data suggested that **4** had a highly fused pentacyclic skeleton containing the two ortho-disubstituted benzene rings, which was confirmed by 2D NMR data analysis. The proton and protonated carbon resonances in the NMR spectra of **4** were assigned by the HSQC experiment. The gCOSY cross-peaks of H-4/H-5/H-6/H-7, H-3′/H-4′/H-5′/H-6′, and H-8a/H-8b confirmed the presence of the ortho-disubstituted benzene rings and the isolated methylene unit in **4**. Two- and three-bond HMBC correlations of O*H*-3/C-2, C-3, and C-3a; H-2/C-3 and C-7a; H-4/C-3, C-6, and C-7a; and H-7/C-3a and C-5, in combination with shifts of these proton and carbon resonances, revealed the presence of a 1,2,3-trisubstituted indolin-3-ol moiety in **4**. HMBC correlations of O*H*-3/C-8; H-2/C-9; and H_2-8/C-2, C-3, C-3a, and C-9 demonstrated that the indolin-3-ol moiety was fused with a pyrrolidin-2-one ring via C-2 and C-3. In addition, HMBC correlations of H-6′/C-2′, C-4′, and C-7′ revealed that there was an ortho-substituted benzoyl moiety. Although N-bridged long-range correlations of H-2/C-2′ and C-7′ were not observed in the HMBC spectrum of **4**, the chemical shifts of C-2′ and C-7′ and the molecular composition suggested that the benzoyl moiety must be connected to the two N atoms of the fused pyrrolidin-2-one and indolin-3-ol moieties via C-2′ and C-7′, respectively. This supposition was supported by the absence of an amino proton resonance in the ^{1}H NMR spectrum. The shifts of H-7 and H-3′ indicated that the two protons were deshielded by the carbonyls, suggesting that the carbonyl group of the benzoyl moiety (C-7′) was connected to the N atom of the indolin-3-ol moiety and that C-2′ was connected to the N atom of the pyrrolidin-2-one moiety to give the planar structure of **4**. This was supported by the NOE difference spectrum, wherein the H-3′ resonance was not enhanced upon irradiation of H-7. Irradiation of the O*H*-3 resonance enhanced the H-2, H-4, and H-8b resonances, demonstrating that these protons were cofacial. Compound **4** was optically inactive, $[\alpha]^{20}_D \cong 0$ (*c* 0.1, DMSO), indicating that it was obtained as a racemate. This was proved by single-crystal X-ray crystallographic analysis using anomalous scattering of Cu Kα radiation. An ORTEP drawing with atom numbering is shown in Figure 1. In addition, the results of X-ray crystal structure analysis indicated that the crystals belonged to the *Pcab* space group and that two enantiomeric molecule pairs were arranged in the cell diagram (Supporting Information, Figure S1). Subsequent HPLC separation of **4** on a chiral column yielded two compounds whose NMR data were identical to those of **4** prior to HPLC separation. However, the isolated compounds showed opposite optical rotation, and their CD spectra displayed mirror curves. This confirmed the successful separation of enantiomers (−)-**4** and (+)-**4**; their ee values were determined to be >98.5% and >99.4%, respectively (Supporting Information, Figures S2−S4). As per the same ECD spectral calculation protocol described earlier, in the 200−400 nm region, the calculated ECD spectra of (2*R*,3*R*)-**4** and (2*S*,3*S*)-**4** were consistent with the experimental CD spectra of (−)-**4** and (+)-**4**, respectively (Supporting Information, Figure S13). The absolute configurations at C-3 in (−)-**4** and (+)-**4** were supported by using the bulkiness rule for the $Rh_2(OCOCF_3)_4$-induced circular dichroism data, wherein the E band (around 350 nm) was demonstrated to be useful for determining the absolute configuration of chiral secondary and tertiary alcohols.[14] The $Rh_2(OCOCF_3)_4$-induced CD spectra of (−)-**4** and (+)-**4** displayed negative and positive Cotton effects at 360 nm (the E band), respectively, which predicts the 3*R*- and 3*S*-configurations by applying the bulkiness rule (Supporting Information, Figure S54). Therefore, (−)-**4** and (+)-**4** were determined to be (−)-(2*R*,3*R*)- and (+)-(2*S*,3*S*)-3-hydroxy-2*H*-pyrrolo[2,3-*b*]indolo[5,5*a*,6-*b*,*a*]quinazoline-9(8*H*),7′-dione, respectively.

Compound **5** had the molecular formula $C_{18}H_{14}N_2O_4$, as indicated by HR-ESIMS. The NMR data (Table 2) indicated the presence of two ortho-disubstituted benzene rings, a trisubstituted double bond, three carbonyl groups, a methoxy group, and two exchangeable amino protons in the molecule.

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Table 2. NMR Data for Compounds 4–7[a]

	4		5		6		7	
no.	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.79 s	82.4	8.92 d (5.5)	145.5		169.3		131.6
3		77.4		112.8	7.22 s	119.1		184.6
3a		135.2						121.0
4	7.53 d (7.5)	124.5		177.2		122.3	7.56 d (7.5)	123.7
4a				127.8		122.0		119.1
5	7.22 dd (7.5, 8)	124.8	8.45 d (8)	126.9	7.79 brs	112.2	6.88 dd (7.5, 7)	135.2
6	7.40 dd (7.5, 8)	129.7	7.51 dd (7.5, 8)	125.8		152.1	7.48 dd (7, 8)	112.4
7	7.92 d (7.5)	114.7	7.78 dd (7.5, 8.5)	133.6	6.64 brd (8.5)	116.0	7.14 d (8)	152.6
7a		140.2						
8		45.7	7.76 d (8.5)	119.6	6.66 brd (8.5)	109.9		
8a	3.04 d (18.5)			140.3		134.9		
8b	3.14 d (18.5)							
9		170.2		164.4				
1′		123.3		120.9			11.97 brs	127.6
2′		136.3		140.6		149.8	8.19 s	110.0
3′	7.74 d (7.5)	121.9	8.72 d (8.5)	123.9	7.16 d (3.5)	121.5		127.2
3′a								118.3
4′	7.71 dd (7.5, 7.5)	133.4	7.55 dd (7.5, 8.5)	133.6	6.60 d (3.5)	110.4	7.84 d (7.5)	120.4
5′	7.45 dd (7.5, 8)	125.9	7.17 dd (7.5, 7.5)	123.6		160.3	7.16 dd (7.5, 7.5)	122.6
6′	8.02 d (8)	128.3	7.95 d (7.5)	131.7	4.64 d (6)	56.2	7.22 dd (7.5, 7.5)	112.0
7′		158.5		167.6			7.47 d (7.5)	136.1
7′a								104.1
8′							7.05 s	

[a]Data (δ) were measured at 500 MHz for ^{1}H and at 125 MHz for ^{13}C in DMSO-d_6 for **4**, **6**, and **7** and Me_2CO-d_6 for **5**. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H–^{1}H gCOSY, HSQC, and HMBC. Data for OH-3 in **4**: δ_H 6.69 (s); for O*Me*-7′ in **5**: δ_H 4.02 (s) and δ_C 52.6; for *N*H-1, OH-6, and OH-6′ in **6**: δ_H 10.21 (brs), 8.99 (s) and 5.47 (t, J = 6 Hz); for *N*H-1 in **7**: δ_H 9.35 (brs).

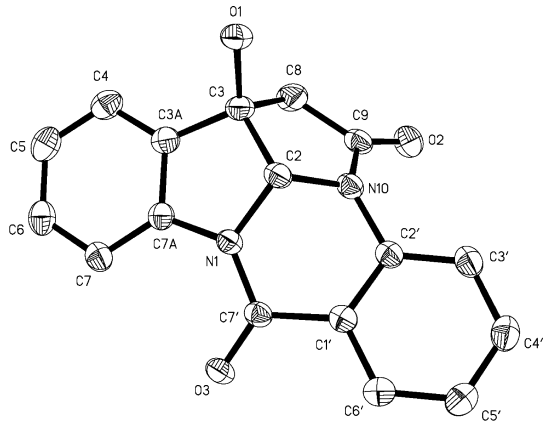

Figure 1. ORTEP diagram of compound **4**.

The ^{1}H–^{1}H gCOSY cross-peaks of H-5/H-6/H-7/H-8 and HMBC correlations of H-2/C-4, C-8a, and C-9; H-5/C-4, C-8a, and C-7; and H-8/C-4a and C-6 demonstrated the presence of a 4-oxo-1,4-dihydroquinoline-3-carbonyl moiety in **5**. This was supported by the quaternary nature of C-3 and the shifts of these proton and carbon resonances. In addition, ^{1}H–^{1}H gCOSY correlations of H-3′/H-4′/H-5′/H-6′ and HMBC correlations of H-3′/C-1′ and C-5′; H-6′/C-2′, C-4′, and C-7′; and OCH_3/C-7′ revealed the presence of a methyl-2-aminobenzoate moiety. Although no correlation of the N*H* resonance of the methyl-2-aminobenzoate moiety was observed in the HMBC spectrum of **5**, the shift of C-9 and the molecular composition suggested that the two moieties were connected via an amide bond. Thus, compound **5** was confirmed to be methyl 2-(4-oxo-1,4-dihydroquinoline-3-carboxamido)-benzoate. This compound (CAS No. 954769-04-1) can be found through a SciFinder search, but no references or chemical-physical data are indicated.

The spectroscopic data of compound **6** were identical to those of indigotiisocoumarin A, which was previously reported from *I. indigotica*.[15] Detailed comparison of the ^{1}H NMR data of **6** with those of indigotiisocoumarin A in the same solvent (DMSO-d_6) indicated that the partially overlapped AB coupling of H-7 and H-8 (corresponding to H-5 and H-2′ assigned for indigotiisocoumarin A[15]) was ignored in the structure elucidation of the latter. In addition, the O*H* and N*H* protons were inversely assigned in indigotiisocoumarin A, which was proved by the enhancements of the H-5 and H-7 resonances upon irradiation of O*H*-6 and the enhancement of the H-8 resonance upon irradiation of N*H*-1 in the NOE difference spectrum of **6**. This finding prompted us to examine the detailed structure by using the 2D NMR data. The correlations of H-3/C-2, C-4, and C-4a; H-5/C-4, C-7, and C-8a; H-8/C-6 and C-4a; N*H*-1/C-2, C-4a, and C-8a; and O*H*-6/C-5, C-6, and C-7 in the HMBC spectrum of **6**, together with their shifts, revealed that there was a 4-substituted 6-hydroxyquinolin-2(1*H*)-one moiety. HMBC correlations of H-3′/C-2′, C-4′, and C-5′; H-4′/C-2′, C-3′, and C-5′; H_2-6′/C-4′ and C-5′; and O*H*-6′/C-5′ and C-6′, in combination with the coupling constant between H-3′ and H-4′ and the shifts of C-2′ and C-5′, confirmed the presence of a 5-(hydroxymethyl)furan-2-yl unit in **6**. In addition, HMBC correlations of H-3/C-2′ and H-3′/C-4 showed that the furan-2-yl unit was located at the C-4 position of the quinolin-2(1*H*)-one moiety. Therefore, compound **6** was determined to be 6-hydroxy-4-(5-hydroxymethylfuran-2-yl)quinolin-2(1*H*)-one. The structure and NMR data assignment of indigotiisocoumarin A[15] should be

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Table 3. ^{1}H NMR Data (δ) for Compounds 8−17[a]

no.	8[b]	9[c]	10[d]	11[d]	12[d]	13[e,f]	14[b,e]	15[b,e,f]	16[b,d]	17[b]
1	9.91 brs		10.38 brs	10.56 brs	9.98 brs	10.50 brs	10.12 brs	10.0 brs	10.27 brs	10.33 brs
2		4.76 q (7)								
3 a	2.50 dd (16.5, 5)			3.74 dd (5, 5)					3.51 dd (4, 7.5)	3.63 dd (4.5, 9)
3b	2.58 dd (16.5, 6.5)									
4	3.72 t (5.5)						7.24 d (7.5)			7.15 d (7.5)
5	7.29 d (7.5)		6.44 d (8)	6.52 d (8)	6.57 d (8)	6.67 d (10.5)	6.90 ddd (7.5, 8, 1)	6.54 d (8)	6.35 d (8)	6.89 dd (7.5, 8)
6	6.91 dd (7, 7.5)		7.05 dd (8, 8)	7.02 dd (7.5, 8)	7.21 dd (8, 8)	7.24 dd (8, 10.5)	7.15 ddd (7.5, 8, 1)	7.10 dd (8, 8)	6.97 dd (7.5, 8)	7.14 dd (7.5, 8)
7	7.14 dd (7.5, 7.5)	4.43 t (6.5)	6.29 d (8)	6.34 d (7.5)	6.45 d (8)	6.47 d (8)	6.74 d (7.5)	6.35 d (8)	6.28 d (7.5)	6.79 d (7.5)
7a										
8	6.81 d (7.5)	2.71 dd (6.5, 12.5)								
8a			3.02 d (16.5)	3.04 dd (4.5, 17)	6.54 s	3.02 d (16)	2.63 d (15)	2.74 d (15)	2.59 dd (7.5, 16.5)	2.37 dd (9, 15.5)
8b			3.19 d (16.5)	3.17 dd (5.5, 17)		3.14 d (16)	2.74 d (15)	3.02 d (15)	2.87 dd (4, 16.5)	2.71 dd (4.5, 15.5)

[a]Data (δ) were measured at 500 MHz in DMSO-d_6 for **8** and **10**−**17** and Me_2CO-d_6 for **9** and **12**. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on ^{1}H−^{1}H COSY, HSQC, and HMBC. [b]Data for NH_2-9: δ_H 6.99 and 7.50 (brs) in **8**; 6.72 and 7.30 (brs) in **14**; 6.58 and 7.20 (brs) in **15**; 7.22 and 7.75 (brs) in **16**; and 6.91 and 7.41 (brs) in **17**. [c]Data for N*H*-4 and CH_3-9 in **9**: δ_H 8.44 (brs) and 1.49 (d, J = 7 Hz). [d]Data for O*H*-4: δ_H 9.85 (brs) in **10**, 10.04 (brs) in **11**, 9.67 (brs) in **12**, and 10.36 (brs) in **16**. [e]Data for O*H*-3: δ_H 6.41 (brs) in **13**, 6.08 (brs) in **14**, and 5.80 (brs) in **15**. [f]Data for OCH_3-4: δ_H 3.80 (s) in **13** and 3.74 (s) in **15**.

Table 4. ^{13}C NMR Data (δ) for Compounds 8−17[a]

no.	8	9	10	11	12	13[b]	14	15[b]	16	17
2	168.9	75.5	176.6	176.6	165.5	176.4	178.3	178.5	178.2	178.9
3	33.0	164.3	72.6	40.4	143.7	72.6	73.3	73.4	40.4	42.2
3a			113.3	111.1	108.8	114.5	131.5	116.9	113.7	130.1
4	42.2		155.1	154.2	156.9	156.8	123.9	156.6	153.7	124.1
4a	122.2	106.9								
5	128.0	161.9	110.5	109.9	110.7	105.8	121.2	105.2	109.6	121.4
6	121.9		131.1	129.7	135.2	131.6	129.0	130.3	128.6	127.9
7	127.9	65.5	101.4	101.3	102.8	103.2	109.4	102.9	100.6	109.4
7a			143.4	144.2	144.9	143.1	142.6	144.2	144.0	142.9
8	115.3	26.2	23.4	16.5	97.7	23.6	42.4	40.7	34.8	35.7
8a	138.4	149.9								
9	173.6	17.2	116.8	118.0	116.3	116.6	170.6	170.9	174.1	171.9

[a]Data (δ) were measured at 125 MHz in DMSO-d_6 for **8** and **10**−**17** and Me_2CO-d_6 for **9** and **12**. The assignments were based on ^{1}H−^{1}H COSY, HSQC, and HMBC. [b]Data for OCH_3-4: δ_C 55.4 in **13** and 55.4 in **15**.

revised because it had the same HRMS and ^{1}H and ^{13}C NMR spectroscopic data as **6**.

Compound **7** showed spectroscopic data similar to those of (*E*)-2-(1*H*-indol-3-ylmethylidene)-1,2-dihydro-3*H*-indol-3-one (the *Z*-configuration was described, but the *E*-configuration was shown in the literature).[16] 2D NMR data analysis also proved that **7** has the same planar structure as the reported compound and that the reported NMR data assignment of C-2 and C-8 should be reversed in the literature.[16] However, comparison of the NMR data of **7** with those reported in the literature indicated that the C-2 resonance in **7** was shielded by $\Delta\delta_C$ −3.6 ppm, whereas the C-3′ resonance was deshielded by $\Delta\delta_C$ +4.8 ppm. This suggested that **7** had the *Z*-configuration, which was further supported by the enhancement of the N*H*-1 resonance upon irradiation of H-2′ in the NOE difference spectrum of **7**. Thus, compound **7** was confirmed to be (*Z*)-2-(1*H*-indol-3-ylmethyldene)-1,2-dihydro-3*H*-indol-3-one.

The NMR data of compound **8** (Tables 3 and 4) indicated the presence of an ortho-disubstituted benzene ring, a $CHCH_2$ unit, two carbonyls, and three exchangeable amino protons. This, combined with HR-ESIMS data, indicated that the molecular formula of **8** was $C_{10}H_{10}N_2O_2$. HMBC correlations of H_2-3/C-2, C-4, C-4a, and C-9; H-4/C-2, C-5, and C-8a; H-5/C-4, C-8a, and C-7; and N*H*-1/C-3, C-4a, and C-8, together with their shifts and the molecular formula, revealed that **8** was 2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxamide. The CD spectrum of **8** showed positive and negative Cotton effects at 257 nm ($\Delta\varepsilon$ +1.75) and 224 nm ($\Delta\varepsilon$ −5.41), respectively, which were consistent with those in the calculated ECD spectrum of the *R*-enantiomer but opposite of those in the case of the *S*-enantiomer, as determined using the quantum-mechanical TDDFT methodology. Therefore, compound **8** was confirmed to be (+)-(*R*)-2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxamide.

Compound **9** had the molecular formula $C_8H_9NO_4$, as indicated by HR-ESIMS and NMR data. The NMR data of **9** demonstrated the presence of a secondary methyl, two vicinal methylenes (one oxygen-bearing), an oxymethine, a tetrasub-

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stituted double bond, two carboxylic and/or amide carbonyl groups, and one amino or OH proton. $^1H-^1H$ gCOSY correlations of H-2/H_3-9 and H_2-7/H_2-8 and HMBC correlations of H-2/C-3, C-8a, and C-9; H_3-9/C-2 and C-3; H-7/C-5, C-8, and C-8a; and H-8/C-4a, C-7, and C-8a, in combination with their shifts, revealed that **9** was 2-methyl-7,8-dihydropyrano[4,3-*b*][1,4]oxazine-3,5(2*H*,4*H*)-dione. Comparison of the specific rotation of **9**, $[\alpha]^{20}_D$ +120.6 (*c* 0.2, $CHCl_3$), with that of (*R*)-2-methyl-3-oxo-perhydro-1,4-oxazin[17,18] suggested that **9** had the *R*-configuration. Therefore, **9** was proposed to be (+)-(*R*)-2-methyl-7,8-dihydropyrano[4,3-*b*]-[1,4]oxazine-3,5(2*H*,4*H*)-dione.

The spectroscopic data of compound **10** indicated that it is a 4- or 7-OH derivative of the co-occurring (−)-(*S*)-2-(3-hydroxy-2-oxoindolin-3-yl)acetonitrile.[19,20] The HMBC spectrum of **10** showed long-range correlations of H-5/C-3a and C-7; H-6/C-4 and C-7a; H-7/C-5 and C-3a; H_2-8/C-2, C-3, C-3a, and C-9; and O*H*/C-3a and C-4, confirming that the OH group was located at C-4. The CD spectrum of **10** showed positive and negative Cotton effects at 240 nm (Δε +2.33) and 266 nm (Δε −0.53), respectively, which were consistent with those of the co-occurring (−)-(*S*)-2-(3-hydroxy-2-oxoindolin-3-yl)-acetonitrile and its synthetic analogues, which also have the *S*-configuration.[20,21] This suggested the *S*-configuration for **10**, which was supported by the positive Cotton effect at 350 nm in the $Rh_2(OCOCF_3)_4$-induced CD spectrum based on the empirical bulkiness rule proposed by Snatzke.[14] The configuration of **10** was further confirmed by single-crystal X-ray crystallographic analysis using anomalous scattering of Cu Kα radiation. An ORTEP drawing with atom numbering is shown in Figure 2. On the basis of these results, **10** was determined to

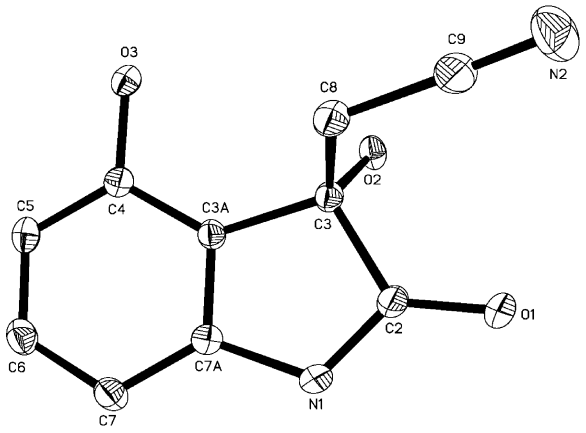

Figure 2. ORTEP diagram of compound **10**.

be (+)-(*S*)-2-(3,4-dihydroxy-2-oxoindolin-3-yl)acetonitrile. A compound with the same planar structure was recently reported from this plant.[2h] However, in the same solvent (DMSO-d_6), the reported NMR data differed from those of **10**, suggesting that the reported structure should be reassigned.

Compound **11** showed spectroscopic data identical to those of 2-(4-hydroxy-2-oxoindolin-3-yl)acetonitrile.[2d] However, the configuration of this compound was not reported in the literature. The CD spectrum of **11** showed positive and negative Cotton effects at 200 nm (Δε +18.51) and 246 nm (Δε −1.02), respectively, consistent with those in the calculated ECD spectrum of the *R*-enantiomer but opposite of those for the *S*-enantiomer. Thus, compound **11** was concluded to be (−)-(*R*)-2-(4-hydroxy-2-oxoindolin-3-yl)-acetonitrile.

Compound **12** showed spectroscopic features similar to those of **11**, but HR-ESIMS indicated that it had the molecular formula $C_{10}H_6N_2O_2$. Comparison of the NMR data of **12** and **11** demonstrated the replacement of the $CHCH_2$ unit in **11** by a trisubstituted double bond [δ_H 6.54 (s, H-8) and δ_C 97.7 (C-8) and 143.7 (C-3)] in **12**, indicating that the latter was 2-(4-hydroxy-2-oxoindolin-3-ylidene)acetonitrile. In the NOE difference spectrum of **12**, the O*H*-4 resonance was not enhanced upon irradiation of H-8, suggesting the *E*-configuration of the double bond. This was supported by the shift of H-8 in the synthetic *Z*-analogue: H-8 resonated at a lower field (about δ_H 6.94) than did the corresponding proton in the *E*-analogue (about δ_H 6.51) because of the deshielding effect of the carbonyl group.[22] Therefore, compound **12** was determined to be (*E*)-2-(4-hydroxy-2-oxoindolin-3-ylidene)acetonitrile, which was also proved by the 2D NMR data.

The spectroscopic data of compound **13** indicated that it is an analogue of **10** with the molecular formula $C_{11}H_{10}N_2O_3$. Comparison of the NMR data of **13** and **10** indicated that the 4-OH group in **10** was replaced by a 4-OCH_3 group (δ_H 3.80 and δ_C 55.4) in **13**. This was confirmed by the 2D NMR data of **13**, especially by the HMBC correlations of H-6 and OCH_3/C-4. The *S*-configuration was indicated by positive and negative Cotton effects at 241 nm (Δε +3.59) and 269 nm (Δε −0.86), respectively, in the CD spectrum of **13**, which was supported by the $Rh_2(OCOCF_3)_4$-induced CD data based on the empirical bulkiness rule.[14] Therefore, compound **13** was assigned as (+)-(*S*)-2-(3-hydroxy-4-methoxy-2-oxoindolin-3-yl)acetonitrile.

Compound **14** is another analogue of **10**, as indicated by spectroscopic data. Comparison of the NMR data of **14** and **10** demonstrated the absence of the 4-OH group in **14** and the replacement of the cyano group in **10** by an aminocarbonyl group in **14**. Therefore, compound **14** was assigned as (−)-(*S*)-2-(3-hydroxy-2-oxoindolin-3-yl)acetamide, which was confirmed by the 2D NMR and $Rh_2(OCOCF_3)_4$-induced CD data.

Comparison of the spectroscopic data of compounds **15** and **13** indicated that the only difference between these two compounds was the substitution of the cyano group in **13** by an aminocarbonyl group in **15**. The specific rotation and CD data of **15** indicated that it had the same *S*-configuration as **13**. Thus, compound **15** was determined to be (+)-(*S*)-2-(3-hydroxy-4-methoxy-2-oxoindolin-3-yl)acetamide, which was confirmed by 2D NMR data.

Compound **16** is an isomer of **14**, as indicated by spectroscopic data. The NMR data of **16** indicated the presence of an ortho-trisubstituted benzene ring, a $CHCH_2$ unit, and a phenolic OH group. This demonstrated that the 3-OH group in **14** was at C-4 or C-7 in **16**. HMBC correlations of H-3, H-5, H-7, and H_2-8/C-3a; H_2-8/C-2, C-3, and C-9; O*H*-4/C-3a, C-4, and C-5; and N*H*-1/C-2, C-3, C-3a, and C-7a in the spectrum of **14** confirmed that **16** was 2-(4-hydroxy-2-oxoindolin-3-yl)acetamide. The CD spectrum of **16** showed a negative Cotton effect at 235 nm (Δε −0.52) and a positive effect at 195 (Δε +6.18), which were consistent with those in the case of **11**. This suggested the *R*-configuration for **16**. Therefore, compound **16** was determined to be (−)-(*R*)-2-(4-hydroxy-2-oxoindolin-3-yl)acetamide.

The spectroscopic data of **17** indicated that it is the dehydroxy analogue of **14**, which was confirmed by 2D NMR data analysis. Compound **17** was optically inactive, $[\alpha]^{20}_D \cong 0$ (*c* 0.1, MeOH), indicating that it was obtained as a racemate.

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This was proved by HPLC separation of **17** on a chiral column that yielded two compounds, whose NMR data were identical to those of **17** prior to HPLC separation. However, the isolated compounds were also optically inactive due to easy racemization, as indicated by HPLC reanalysis of the isolates on the chiral column. Thus, compound **17** was determined to be (±)-2-(2-oxoindolin-3-yl)acetamide. The synthesis of the racemate has been reported, but no spectroscopic data are available in the literature.[23]

The known compounds were identified by comparing their spectroscopic data with the reported data for 3-formylindole, indole-3-acetonitrile-6-*O*-β-D-glucopyranoside, 2,5-dihydroxyindole,[2d] 1-methoxy-3-indoleacetonitrile,[3c] 3-indoleacetonitrile,[11] arvelexin (**18**),[12] (−)-(*S*)-cyanomethyl-3-hydroxyoxindole,[19,20] 2-aminobenzoic acid β-D-glucopyranosyl ester,[24] dihydroascorbigen,[25] indican,[26] 3-indoleacetamide,[27] tryptanthrin, indirubin,[28] and indigo.[29]

The indole ring is a privileged substructure, with representation in over 3000 natural products and 40 medicinal agents of diverse therapeutic action.[30] This, together with the traditional use of the plant, prompted us to test the bioactivities of all the isolates. In the preliminary in vitro assays, compounds **1**−**3** and **18** showed antiviral activity against influenza virus A/Hanfang/359/95 (H3N2), with IC_{50} values of 3.70−12.35 μM and SI values of 2.2−4.0, respectively (the positive control, oseltamivir, gave IC_{50} = 1.23 μM and SI = 1024.4). Compound **17** inhibited Coxsackie virus B3 replication, with an IC_{50} value of 6.87 μM (the positive control, pleconaril, gave an IC_{50} value of 0.41 μM). Compounds **4**, **7**, **8**, **11**, and **13** reduced DL-galactosamine-induced hepatocyte (WB-F344 cell) damage with 44−55% inhibition at 10 μM, while the positive control, bicyclol, gave 42% inhibition. The isolates were also assessed for activities against HIV-1, HSV-1, and several human cancer cell lines but were found to be inactive at 10 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Rudolph Research Autopol III automatic polarimeter. UV, CD, and IR spectra were recorded using a Cary 300 spectrometer, a JASCO J-815 CD spectrometer, and a Nicolet 5700 FT-IR spectrometer (FT-IR microscope transmission), respectively. ^1H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, using an Inova or Bruker 500 MHz spectrometer, with solvent peaks used as references. EIMS and HR-EIMS data were measured using a Micromass Autospec−Ultima ETOF spectrometer. ESIMS data were measured using a Q-Trap LC/MS/MS (turbo ionspray source) spectrometer. Column chromatography (CC) was performed using silica gel (200−300 mesh, Qingdao Marine Chemical Inc., China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and MCI gel (CHP20P). HPLC separation was performed using a system comprising a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual-wavelength absorbance detector with an Alltima (250 × 10 mm) preparative column packed with C_{18} (5 μm) and a Chiralpak AD-H column (250 × 10 mm) packed with amylose tris(3,5-dimethylphenylphenylcarbamate) coated on 5 μm silica gel. TLC was carried out on precoated silica gel GF_{254} plates. Spots were visualized under UV light (254 or 356 nm) or by spraying with 7% H_2SO_4 in 95% EtOH followed by heating.

Plant Material. The roots of *Isatis indigotica* were collected in December 2009 from Anhui Province, People's Republic of 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 the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China.

Extraction and Isolation. The air-dried and pulvarized plant material (50 kg) was extracted 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 B2 (547 g) was subjected to CC over silica gel, with elution by a gradient of increasing MeOH concentration (0−100%) in EtOAc and then with 30% EtOH, to yield fractions B2-1−B2-5 based on TLC analysis. Fraction B2-1 (16.3 g) was chromatographed over Sephadex LH-20 with elution by a petroleum ether/chloroform/methanol (5:5:1) mixture to yield B2-1-1−B2-1-10. Subsequent separation of B2-1-3 (200 mg) by reversed-phase (RP) HPLC (45% CH_3OH in H_2O) gave **9** (2.7 mg). Fraction B2-1-6 (500 mg) was chromatographed over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to give fractions B2-1-6-1 and B2-1-6-2, of which B2-1-6-2 (20.3 mg) was purified by RP HPLC (28% MeOH in H_2O) to afford **13** (6.1 mg). Fraction B2-1-9 (190.5 mg) was separated by silica gel CC ($CHCl_3$/MeOH, 50:1) to give B2-1-9-1−B2-1-9-4. Purification of B2-1-9-2 (20.4 mg) by RP HPLC (40% CH_3CN in H_2O) yielded **12** (12.3 mg), separation of B2-1-9-4 (50.6 mg) by RP flash CC (20−80% MeOH in H_2O) afforded **6** (3.8 mg) and **7** (2.4 mg), and purification of B2-1-10 (210 mg) by RP HPLC (20% MeOH in H_2O) gave **10** (30.2 mg). Fraction B2-2 (11.4 g) was chromatographed over Sephadex LH-20 ($CHCl_3$/MeOH, 5:1) to give B2-2-1−B2-2-4, of which B2-2-1 (7.3 g) was further fractioned by CC over Sephadex LH-20 (petroleum ether/chloroform/MeOH, 5:5:1) to yield B2-2-1-1−B2-2-1-6. Separation of B2-2-1-5 (700 mg) by silica gel CC ($CHCl_3$/MeOH, 20:1 to 10:1) gave subfractions B2-2-1-5-1−B2-2-1-5-5, of which B2-2-1-5-3 (234 mg) was purified by RP HPLC (27% MeOH in H_2O) to give **11** (15.4 mg). B2-2-1-5-4 (179 mg) was separated by CC over Sephadex LH-20 (MeOH/H_2O, 1:1) to obtain B2-2-1-5-4-1−B2-2-1-5-4-3, and subsequent purification of B2-2-1-5-4-1 (50 mg) and B2-2-1-5-4-2 (35 mg) by RP HPLC (30% MeOH in H_2O) yielded **17** (21.0 mg) and **8** (10.5 mg), respectively. B2-2-1-5-5 (600 mg) was fractionated by RP flash CC with a gradient of increasing MeOH concentration (5−60%) in H_2O to yield B2-2-1-5-5-1−B2-2-1-5-5-6, and purification of B2-2-1-5-5-1 (80 mg) by RP HPLC (10% MeCN in H_2O) afforded **14** (13.1 mg) and **15** (6.5 mg). Fraction B2-2-1-6 (100.5 mg) was chromatographed over silica gel ($CHCl_3$/MeOH, 10:1) to give **16** (15.6 mg). Fraction C (88.0 g) was subjected to CC over silica gel, with elution using a gradient of increasing acetone (0−100%) in petroleum ether, to afford fractions C1−C11. Separation of C7 by CC over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) gave fractions C7-1−C7-5, of which C7-5 (2.7 g) was fractionated by silica gel CC (petroleum ether/EtOAc, 200:1) to yield C7-5-1−C7-5-3. Separation of C7-5-2 (1.9 g) by RP flash CC (55% MeOH in H_2O), followed by RP HPLC (59% MeOH in H_2O), afforded **3** (0.7 mg). Fraction C7-5-3 (204 mg) was separated by RP flash CC (55% MeOH in H_2O) to give fractions C7-5-3-1−C7-5-3-3, of which C7-5-3-2 (23 mg) was successively isolated by preparative TLC (mobile phase: $CHCl_3$/Me_2CO, 30:1), followed by RP HPLC (59% MeOH in H_2O), to yield **1** (5.7 mg) and **2** (2.2 mg). Fraction C8 (2.5 g) was chromatographed over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to yield C8-1−C8-4, of which C8-3 (30 mg) was separated by preparative TLC (mobile phase: $CHCl_3$/Me_2CO, 30:1) to yield **4** (10.2 mg). Fraction C10 (3.5 g) was subjected to CC over Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) to give C10-1−C10-4, of which C10-3 (1.5 g) was fractionated by RP flash CC (30−70% MeOH in H_2O) to give fractions C10-3-1−C10-3-5. Purification of C10-3-1 (500 mg) by RP flash CC (10−50% MeOH in H_2O), followed by RP HPLC (67% MeOH in H_2O), afforded **5** (0.8 mg).

(−)-(R)-2-(3-Cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(1H-indol-3-yl)acetonitrile (1): colorless gum; $[\alpha]^{20}_D$ −26.5 (*c* 0.07, CH_3CN); UV (MeOH) λ_{max} (log ε) 203 (5.27), 221 (5.29), 269 (4.59), 285

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(4.51) nm; CD (MeOH) 210 ($\Delta\varepsilon$ +1.74), 229 ($\Delta\varepsilon$ −3.75), 261 ($\Delta\varepsilon$ +1.59), 286 ($\Delta\varepsilon$ −0.88) nm; IR (KBr) ν_{max} 3410, 2930, 2838, 2247, 1615, 1601, 1518, 1458, 1359, 1268, 1079, 746 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; (−)-ESIMS m/z 339 $[M - H]^-$; (+)-HR-ESIMS m/z 341.1399 $[M + H]^+$ (calcd for $C_{21}H_{17}N_4O$, 341.1397).

*(−)-(R)-2-(3-Cyanomethyl-4-methoxy-1H-indol-7-yl)-2-(4-methoxy-1H-indol-3-yl)acetonitrile (**2**):* colorless gum; $[\alpha]^{20}_D$ −30.2 (c 0.08, CH_3CN); UV (MeOH) λ_{max} (log ε) 202 (5.39), 221 (5.22), 268 (4.50), 291 (4.41) nm; CD (MeOH) 203.5 ($\Delta\varepsilon$ +3.88), 222 ($\Delta\varepsilon$ −3.30), 243.5 ($\Delta\varepsilon$ +1.11), 266.5 ($\Delta\varepsilon$ −0.82) nm; IR (KBr) ν_{max} 3392, 2958, 2920, 2847, 2249, 1619, 1515, 1461, 1361, 1265, 1086, 736 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; (−)-ESIMS m/z 369 $[M - H]^-$; (+)-HR-ESIMS m/z 393.1317 $[M + Na]^+$ (calcd for $C_{22}H_{18}N_4O_2Na$, 393.1322).

*(+)-(S)-2-{7-[1-(4-Hydroxyphenyl)ethyl]-4-methoxy-1H-indol-3-yl}acetonitrile (**3**):* colorless gum; $[\alpha]^{20}_D$ +15.6 (c 0.09, CH_3CN); UV (MeOH) λ_{max} (log ε) 204 (5.29), 219 (5.31), 268 (4.65), 284 (4.56) nm; CD (MeOH) 201 ($\Delta\varepsilon$ +4.46), 222 ($\Delta\varepsilon$ −1.68), 248.5 ($\Delta\varepsilon$ +0.40), 267.5 ($\Delta\varepsilon$ −0.46) nm; IR (KBr) ν_{max} 3368, 2964, 2931, 2256, 1611, 1514, 1444, 1372, 1266, 1082, 836, 802 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; (−)-ESIMS m/z 305 $[M - H]^-$, 611 $[2M - H]^-$; (+)-HR-ESIMS m/z 307.1441 $[M + H]^+$ (calcd for $C_{19}H_{19}N_2O_2$, 307.1441).

*3-Hydroxy-2H-pyrrolo[2,3-b]indolo[5,5a,6-b,a]quinazoline-9-(8H),7′-dione (**4**):* colorless needles (acetone); mp 209−210 °C; UV (MeOH) λ_{max} (log ε) 203 (4.06), 223 (4.09), 244 (3.72), 297 (3.61) nm; IR (KBr) ν_{max} 3331, 2941, 1724, 1644, 1602, 1489, 1469, 1429, 1365, 1329, 1283, 825, 789, 762 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 2; (−)-ESIMS m/z 291 $[M - H]^-$, 327 $[M + Cl]^-$, 583 $[2M - H]^-$; EIMS m/z 292 $[M]^{+\bullet}$ (60), 274 (13), 246 (100), 222 (28); (+)-HR-ESIMS m/z 293.0930 $[M + H]^+$ (calcd for $C_{17}H_{13}N_2O_3$, 293.0921); (−)-(2*R*,3*R*)-**4**: $[\alpha]^{20}_D$ −194.5 (c 0.2, MeOH); CD (MeOH) 217 ($\Delta\varepsilon$ +14.98), 240 ($\Delta\varepsilon$ −16.04), 260 ($\Delta\varepsilon$ −13.67), 326 ($\Delta\varepsilon$ +1.54) nm; $Rh_2(OCOCF_3)_4$-induced CD (CH_2Cl_2) 360 ($\Delta\varepsilon$ −0.47) nm; (+)-(2*S*,3*S*)-**4**: $[\alpha]^{20}_D$ +194.2 (c 0.2, MeOH); CD (MeOH) 216 ($\Delta\varepsilon$ −12.13), 242 ($\Delta\varepsilon$ +15.90), 262 ($\Delta\varepsilon$ +13.36), 324 ($\Delta\varepsilon$ −1.37) nm; $Rh_2(OCOCF_3)_4$-induced CD (CH_2Cl_2) 360 ($\Delta\varepsilon$ +0.50) nm.

*X-ray Crystallography of Compound **4**.* $C_{17}H_{12}N_2O_3$, M = 292.29, orthorhombic, *Pcab*, a = 11.145(3) Å, b = 14.533(5) Å, c = 16.506(4) Å, $\alpha = \beta = \gamma$ = 90°, V = 2673.5(2) Å^3, Z = 8, D_{calcd} = 1.452 g/cm^{-3}, 2565 reflections independent, 2259 reflections observed ($|F|^2 \geq 2\sigma|F|^2$), R_1 = 0.0457, wR_2 = 0.1110, S = 1.107.

The data were collected on a Rigaku MicroMax 002+ diffractometer with Cu Kα radition by using the ω and κ scan technique to a maximum 2θ value of 143.98°. The crystal structure was solved by direct methods by using SHELXS-97, and all non-hydrogen atoms were refined anisotropically using the least-squares method. All hydrogen atoms were positioned by geometric calculations. Crystallographic data for the structure of **4** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CCDC 866442. Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

*Methyl 2-(4-oxo-1,4-dihydroquinoline-3-carboxamido)benzoate (**5**):* colorless gum; UV (MeOH) λ_{max} (log ε) 202 (4.58), 215 (4.50), 312 (4.24), 322 (4.22) nm; IR (KBr) ν_{max} 3405, 3218, 1697, 1669, 1628, 1573, 1516, 1446, 1255, 1206, 1136, 756 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 2; (+)-ESIMS m/z 345 $[M + Na]^+$, 667 $[2M + Na]^+$; (+)-HR-ESIMS m/z 323.1027 $[M + H]^+$ (calcd for $C_{18}H_{15}N_2O_4$, 323.1026).

*6-Hydroxy-4-(5-hydroxymethylfuran-2-yl)-quinolin-2(1H)-one (**6**):* purple, amorphous powder; UV (MeOH) λ_{max} (log ε) 204 (5.17), 235 (4.77), 265 (4.65), 359 (5.01), 438 (4.09) nm; IR (KBr) ν_{max} 3183, 2873, 2805, 1691, 1612, 1467, 1397, 1207, 1021, 807 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 2; EIMS m/z 257 $[M]^{+\bullet}$ (10), 71 (62), 57 (100); (+)-HR-ESIMS m/z 258.0761 $[M + H]^+$ (calcd for $C_{14}H_{12}NO_4$, 258.0761).

*(Z)-2-(1H-Indol-3-ylmethyldene)-1,2-dihydro-3H-indol-3-one (**7**):* red, amorphous powder; UV (MeOH) λ_{max} (log ε) 205 (5.98), 271 (5.72), 389 (5.56), 493 (5.78) nm; IR (KBr) ν_{max} 3314, 1668, 1621, 1563, 1525, 1487, 1462, 1429, 1327, 1229, 1134, 1109, 745 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 2; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 2; EIMS m/z 260 $[M]^{+\bullet}$ (100); (+)-HR-ESIMS m/z 261.1016 $[M + H]^+$ (calcd for $C_{17}H_{13}N_2O$, 261.1022).

*(+)-(R)-2-Oxo-1,2,3,4-tetrahydroquinoline-4-carboxamide (**8**):* white, amorphous powder; $[\alpha]^{20}_D$ +52.9 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.26), 253 (3.72), 283 (3.11) nm; CD (MeOH) 224 ($\Delta\varepsilon$ −5.41), 257 ($\Delta\varepsilon$ +1.75) nm; IR (KBr) ν_{max} 3389, 3220, 2920, 1652, 1597, 1493, 1401, 1283, 1241, 1211, 751, 653 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 4; (+)-ESIMS m/z 403 $[2M + Na]^+$, 213 $[M + Na]^+$; (+)-HR-ESIMS m/z 213.0635 $[M + Na]^+$ (calcd for $C_{10}H_{10}N_2O_2Na$, 213.0634), 191.0815 $[M + H]^+$ (calcd for $C_{10}H_{11}N_2O_2$, 191.0815).

*(+)-(R)-2-Methyl-7,8-dihydropyrano[4,3-b][1,4]oxazine-3,5-(2H,4H)-dione (**9**):* colorless crystals; mp 135−136 °C; $[\alpha]^{20}_D$ +120.6 (c 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 204 (4.35), 223 (4.27), 284 (4.10) nm; CD (MeOH) 229 ($\Delta\varepsilon$ −1.27), 277 ($\Delta\varepsilon$ +0.27) nm; IR (KBr) ν_{max} 3207, 3123, 2942, 1716, 1678, 1464, 1403, 1297, 1184, 1093, 1053, 1035, 921 cm^{-1}; ^1H NMR (Me_2CO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 4; EIMS m/z 183 $[M]^{+\bullet}$ (86), 155 (12), 139 (17), 56 (100); (+)-HR-ESIMS m/z 206.0415 $[M + Na]^+$ (calcd for $C_8H_9NO_4Na$, 206.0424).

*(+)-(S)-2-(3,4-Dihydroxy-2-oxoindolin-3-yl)acetonitrile (**10**):* colorless prisms (MeOH); mp 135−137 °C; $[\alpha]^{20}_D$ +58.5 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.64), 251 (3.65), 298 (3.87) nm; CD (MeOH) 240 ($\Delta\varepsilon$ +2.34), 266 ($\Delta\varepsilon$ −0.53), 284 ($\Delta\varepsilon$ +0.54) nm; $Rh_2(OCOCF_3)_4$-induced CD (CH_2Cl_2) 350 ($\Delta\varepsilon$ +0.69) nm; IR (KBr) ν_{max} 3270, 2972, 2937, 2251, 1701, 1622, 1502, 1468, 1408, 1329, 1280, 1185, 1100, 1039, 972, 815, 791, 747 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 4; (+)-ESIMS m/z 227 $[M + Na]^+$, 431 $[2M + Na]^+$; (−)-ESIMS m/z 203 $[M - H]^-$, 407 $[2M - H]^-$; (+)-HR-ESIMS m/z 205.0613 $[M + H]^+$ (calcd for $C_{10}H_9N_2O_3$, 205.0608).

*X-ray Crystallography of Compound **10**.* $C_{10}H_8N_2O_3$, M = 204.19, orthorhombic, $P2_12_12_1$, a = 7.031(5) Å, b = 7.138(8) Å, c = 17.970(12) Å, $\alpha = \beta = \gamma$ = 90°, V = 901.0(11) Å^3, Z = 4, D_{calcd} = 1.504 g/cm^{-3}, 1532 reflections independent, 1467 reflections observed ($|F|^2 \geq 2\sigma|F|^2$), R_1 = 0.0346, wR_2 = 0.0935, S = 1.058. The data were collected on a Rigaku MicroMax 002+ diffractometer with Cu Kα radition by using the ω and κ scan technique to a maximum 2θ value of 136.18°. The crystal structure was solved by direct methods by using SHELXS-97, and all non-hydrogen atoms were refined anisotropically using the least-squares method. All hydrogen atoms were positioned by geometric calculations. Crystallographic data for the structure of **10** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CCDC 866443. Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

*(−)-(R)-2-(4-Hydroxy-2-oxoindolin-3-yl)acetonitrile (**11**):* yellowish gum; $[\alpha]^{20}_D$ −20.3 (c 1.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.17), 215 (4.10), 250 (3.60), 291 (3.24), 353 (3.00) nm; CD (MeOH) 209 ($\Delta\varepsilon$ +1.91), 261.5 ($\Delta\varepsilon$ −0.12), 284 ($\Delta\varepsilon$ +0.15), 310.5 ($\Delta\varepsilon$ −0.15) nm; ^1H NMR (DMSO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 4; (+)-ESIMS m/z 211 $[M + Na]^+$; (−)-ESIMS m/z 187 $[M - H]^-$.

*(E)-2-(4-Hydroxy-2-oxoindolin-3-ylidene)acetonitrile (**12**):* yellowish needles; mp 201−203 °C; UV (MeOH) λ_{max} (log ε) 205 (4.26), 253 (3.72), 283 (3.11) nm; IR (KBr) ν_{max} 3211, 2229, 1717, 1615, 1462, 1416, 1301, 1212, 1056, 837, 774 cm^{-1}; ^1H NMR (Me_2CO-d_6,

500 MHz) data, see Table 3; ^{13}C NMR (Me_2CO-d_6, 125 MHz) data, see Table 4; (−)-ESIMS m/z 185 $[M - H]^-$, 371 $[2\ M - H]^-$; (+)-HR-ESIMS m/z 187.0501 $[M + H]^+$ (calcd for $C_{10}H_7N_2O_2$, 187.0502).

*(+)-(S)-2-(3-Hydroxy-4-methoxy-2-oxoindolin-3-yl)acetonitrile (**13**):* colorless needles (MeOH); mp 140−142 °C; $[\alpha]^{20}_D$ +42.8 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.19), 250 (3.21), 296 (3.30) nm; CD (MeOH) 241 ($\Delta\varepsilon$ +3.59), 264 ($\Delta\varepsilon$ −0.86), 292 ($\Delta\varepsilon$ +0.40) nm; Rh_2 $(OCOCF_3)_4$-induced CD (CH_2Cl_2) 350 ($\Delta\varepsilon$ +1.83) nm; IR (KBr) ν_{max} 3444, 3207, 3034, 2987, 2948, 2254, 1728, 1692, 1618, 1502, 1467, 1414, 1368, 1286, 1106, 998, 769, 727, 684 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 4; (+)-ESIMS m/z 241 $[M + Na]^+$; (+)-HR-ESIMS m/z 219.0767 $[M + H]^+$ (calcd for $C_{11}H_{11}N_2O_3$, 219.0764).

*(−)-(S)-2-(3-Hydroxy-2-oxoindolin-3-yl)acetamide (**14**):* colorless needles (MeOH); mp 144−146 °C; $[\alpha]^{20}_D$ −54.0 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 208 (4.97), 253 (4.29), 290 (3.67) nm; CD (MeOH) 209 ($\Delta\varepsilon$ −8.99), 239 ($\Delta\varepsilon$ +6.12), 264 ($\Delta\varepsilon$ −1.79) nm; Rh_2 $(OCOCF_3)_4$-induced CD (CH_2Cl_2) 350 ($\Delta\varepsilon$ +1.11) nm; IR (KBr) ν_{max} 3449, 3260, 3178, 1696, 1655, 1623, 1470, 1410, 1223, 1117, 799, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 4; (+)-ESIMS m/z 229 $[M + Na]^+$, 435 $[2\ M + Na]^+$; (+)-HR-ESIMS m/z 207.0761 $[M + H]^+$ (calcd for $C_{10}H_{11}N_2O_3$, 207.0764).

*(+)-(S)-2-(3-Hydroxy-4-methoxy-2-oxoindolin-3-yl)acetamide (**15**):* colorless prisms; mp 140−142 °C; $[\alpha]^{20}_D$ +39.8 (c 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.23), 253 (3.11), 296 (3.32) nm; CD (MeOH) 241 ($\Delta\varepsilon$ +4.61), 265 ($\Delta\varepsilon$ −0.49), 291 ($\Delta\varepsilon$ +1.17) nm; IR (KBr) ν_{max} 3592, 3408, 3316, 3205, 1707, 1667, 1622, 1501, 1468, 1424, 1400, 1280, 1204, 1098, 1046, 1006, 778 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 4; (+)-ESIMS m/z 259 $[M + Na]^+$; (+)-HR-ESIMS m/z 237.0870 $[M + H]^+$ (calcd for $C_{11}H_{13}N_2O_4$, 237.0870).

*(−)-(R)-2-(4-Hydroxy-2-oxoindolin-3-yl)acetamide (**16**):* white, amorphous powder; $[\alpha]^{20}_D$ −18.1 (c 0.54, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.29), 216 (4.27), 250 (3.68), 292 (3.40) nm; CD (MeOH) 195 ($\Delta\varepsilon$ +6.18), 235 ($\Delta\varepsilon$ −0.52), 259.5 ($\Delta\varepsilon$ −0.38) nm; IR (KBr) ν_{max} 3206, 2850, 1715, 1668, 1632, 1470, 1409, 1287, 782 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 4; (+)-ESIMS m/z 229 $[M + Na]^+$; (−)-ESIMS m/z 205 $[M - H]^-$; (+)-HR-ESIMS m/z 229.0580 $[M + Na]^+$ (calcd for $C_{10}H_{10}N_2O_3Na$, 229.0584).

*(±)-2-(2-Oxoindolin-3-yl)acetamide (**17**):* colorless needles; mp 171−172 °C; $[\alpha]^{20}_D \cong 0$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.32), 248 (3.81), 290 (3.20) nm; IR (KBr) ν_{max} 3392, 3350, 3225, 2881, 2816, 1700, 1642, 1624, 1473, 1415, 1343, 1233, 1183, 1103, 751 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) data, see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) data, see Table 4; (+)-ESIMS m/z 213 $[M + Na]^+$, 403 $[2\ M + Na]^+$; (+)-HR-ESIMS m/z 191.0814 $[M + H]^+$ (calcd for $C_{10}H_{11}N_2O_2$, 191.0815).

ECD Calculation. Conformational analysis of the *S*-enantiomer was performed with the MMFF94 molecular mechanics force field using Spartan 08 software.[31] The lowest-energy conformers having relative energies within 2 kcal/mol were optimized with the Gaussian 09 program[32] at the B3LYP/6-31+G(d) level in the gas phase. The stabilities of these conformers were confirmed by harmonic vibrational frequency calculations at the B3LYP/6-31+G(d) level. The energies, oscillator strengths, and rotational strengths of the electronic excitations of the lowest-energy conformers were calculated using the TDDFT method at the B3LYP/6-311++G(2d,2p) level in the gas phase, and ECD spectra were then simulated by the GaussSum 2.25 program.[33] The final ECD spectrum of the *S*-enantiomer was obtained according to Boltzmann weighting of each conformer.

Anti-influenza Virus, Coxsackie Virus, and Herpes Simplex Virus (HSV) Assay. See ref 34.

Protective Effect on Cytotoxicity Induced by DL-Galactosamine in WB-F344 Cells. See ref 35.

Anti-HIV Activity Assay. See ref 36.

Cytotoxicity Assay. See ref 37.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Crystal cell diagram of **4** and **10**; CIF files, tables of atomic coordinates and equivalent isotropic displacement parameters for the oxygen and carbon atoms, bond lengths, and bond angles for **4** and **10**. ECD spectra calculation details of **1**−**4**, **8**, **11**, and **17**. Copies of IR, MS, 1D and/or 2D NMR, and CD spectra for compounds **1**−**17**. This can be accessed free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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