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Structurally Diverse Alkaloids from the Seeds of *Peganum harmala*

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Abstract

Investigation of the alkaloids from *Peganum harmala* seeds yielded two pairs of unique racemic pyrroloindole alkaloids, (±)-peganines A–B (**1–2**); two rare thiazole derivatives, peganumals A–B (**3–4**); six new β-carboline alkaloids, pegaharmines F–K (**5–10**); and 12 known analogues. Their structures, including stereochemistry, were elucidated through spectroscopic analyses, quantum chemistry calculations, and single-crystal X-ray diffraction. Notably, the incorporation of pyrrole and indole moieties in peganines A–B, thiazole fragments in peganumals A–B, and a C-1 α,β-unsaturated ester motif in pegaharmine F (**5**) are all rare, and their presence in the genus *Peganum* were demonstrated for the first time. All isolates were tested for antiproliferative activities against the HL-60, PC-3, and SGC-7901 cancer cell lines, and compounds **9**, **11**, **12**, and **13** exhibited moderate cytotoxicity against HL-60 cancer cell lines with IC_{50} values in the range of 4.36–9.25 μM.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

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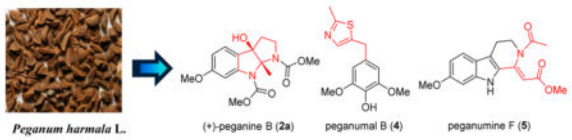
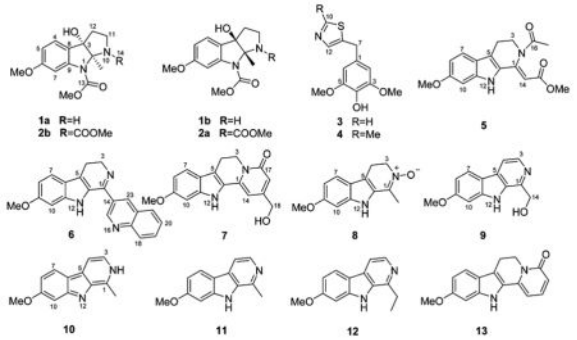
Experimental detail and characterization data (PDF)

X-ray data of compound **5** (CIF)



Cancer is a persistent global concern and leading cause of mortality. Therefore, the fight against cancer presents a significant challenge for modern science and technology.[1] With ongoing and valuable contributions, natural products are a consistent source of potential antitumor agents and lead compounds that have provided the inspiration for the semi or total synthesis of new effective drugs, such as vinca alkaloids, taxol, and etoposide.[2] Over the past few years, the β-carboline alkaloids have attracted the interest of both synthesis and natural product chemists because of their unique and fascinating structures, biosynthesis, and wide-ranging biological activities, most notably their antitumor activity.[3]

Peganum harmala L. (Zygophyllaceae) is an herb rich in β-carboline alkaloids that has been used traditionally for hundreds of years in northwestern China for the treatment of alimentary tract cancer and malaria.[4] We previously reported 12 new β-carboline alkaloids from *P. harmala* seeds with significant anticancer activities.[5] These exciting discoveries prompted us to pursue the minor anticancer alkaloids of this plant. The isolation of the crude alkaloids subsequently led to the identification of 12 known alkaloids and 12 structurally diverse new alkaloids: (±)-peganines A–B (**1–2**, in the optically pure form), peganumals A–B (**3–4**), and pegaharmines F–K (**5–10**). Their structures, including stereochemistry, were elucidated on the basis of extensive spectroscopic evidence, quantum chemistry calculations, and single-crystal X-ray diffraction. Herein, the isolation, structure identification, and biological evaluation of these alkaloids are discussed.



RESULTS AND DISCUSSION

Investigation of the alkaloids from *Peganum harmala* seeds afforded two pairs of unique racemic pyrroloindole alkaloids (**1–2**), two rare thiazole derivatives (**3–4**), six new β-carboline alkaloids (**5–10**), and the following known alkaloids: harmine (**11**),[6] 1-ethyl-7-methoxy-9*H*-pyrido[3,4-*b*]indole (**12**),[7] harmalanine (**13**),[8] harmaline (**14**),[9] arenarine C (**15**),[10] methyl harmate (**16**),[11] harmic amide (**17**),[12] harman (**18**),[13] 7-methoxy-1-oxo-1,2,3,4-tetrahydro-β-carboline (**19**),[14] harmine *N*-oxide (**20**),[11] 11-methoxyrutaecarpine (**21**),[15] and luotonin C (**22**).[16]

Peganine A (**1**) was isolated as a yellow, amorphous powder, and its molecular formula was determined to be $C_{14}H_{18}N_2O_4$ with seven indices of hydrogen deficiency via a positive HRESIMS ion at *m*/*z* 279.1325 $[M + H]^+$ (calcd for 279.1339). In the IR spectrum, bands from NH/OH (3429 cm^{-1}) and carbonyl (1715 cm^{-1}) functionalities were observed, and in the UV absorption spectrum, dihydroindole maxima at 216, 244, and 289 nm were detected.[5,17] The ^1H NMR data (Table 1) showed the presence of an aromatic ABX spin system (δ_H 7.22, 1H, d, J = 8.4 Hz; 6.61, 1H, dd, J = 8.4, 2.3 Hz; 7.34, 1H, broadening), two *O*-methyls (δ_H 3.77, 3H, s and 3.72, 3H, s), one methyl (δ_H 1.47, 3H, s), one broad OH singlet (δ_H 5.50, 1H, br.s), and two methylenes. The ^{13}C NMR data (Table 3) showed the presence of 14 carbon resonances that were classified by HSQC data as one carbamate carbonyl (δ_C 152.7), six aromatic carbons, two *O*-methyls, one methyl, two methylenes, one dinitrogenated secondary carbon, and one oxygenated tertiary carbon, which was consistent with the ^1H NMR data. Analysis of the 1D and 2D NMR data, especially the HMBC data (Figure 1), indicated the presence of a pyrroloindole motif. The key HMBC cross-peaks of H-4 (δ_H 7.22) with C-3 (δ_C 85.7), C-6 (δ_C 160.0), and C-9 (δ_C 142.3) and of 6-OCH_3 (δ_H 3.72) with C-6, revealed the presence of a 6-methoxydihydroindole substructure in **1**. Additionally, the deshielded carbon resonance at δ_C 85.7 indicated the oxygen substitution at this position, while the resonance at δ_C 91.2 was assigned to C-2, which was linked to N-1 and N-10.[17] The HSQC and ^1H NMR spectra defined the presence of an $–NCH_2CH_2–$ moiety that was connected to the dihydroindole at C-3 from the three-bond HMBC cross-peaks from H-12 to both C-2 and C-8 (δ_C 127.0). Therefore, the unique pyrroloindole skeleton was defined. The HMBC cross-peaks of 13-OCH_3 (δ_H 3.77) with C-13 (δ_C 152.7) and 2-CH_3 (δ_H 1.47) with C-2 and C-3 and the NOESY correlation of 13-OCH_3 with 2-CH_3 (δ_H 1.47) unambiguously assigned the methoxycarbonyl and the methyl groups at N-1 and C-2, respectively. The structure of **1** was thus determined (Figure 1). The broadening of the NMR signals of C(H)-7 and 9 presumably resulted from the “N-1 chirality inversion”. The NOESY (Figure 2) correlations of 2-CH_3 with 3-OH (δ_H 5.50), H-11β (δ_H 2.83), and H-12β (δ_H 2.04) and of 3-OH with 2-CH_3, H-11β, and H-12β indicated that 2-CH_3 and 3-OH were cofacial.

Peganine A (**1**) showed a specific rotation of $[\alpha]^{20}_D$ + 2 (*c* 0.1, MeOH), implying that it may be a racemic mixture. Subsequent chiral HPLC separation of **1** gave the corresponding enantiomers (−)-**1** (**1a**) and (+)-**1** (**1b**) in a 48:52 ratio (Figure S4, Supporting Information). The absolute configurations of (−)- and (+)-**1** were determined as (2*S*, 3*S*) (**1a**) and (2*R*, 3*R*) (**1b**), respectively, by the experimental and calculated ECD spectra using the Gaussian09 program (Figure 3).[18] The ECD spectra of the (2*R*, 3*S*) and (2*S*, 3*R*) stereoisomers were also calculated and did not correlate with the experimental data (Figure 3), thus further supporting the determined absolute configurations.

Peganine B (**2**) was obtained as a yellow, amorphous powder, and its molecular formula was determined as $C_{16}H_{20}N_2O_6$ with eight indices of hydrogen deficiency via a sodium adduct HRESIMS ion at *m*/*z* 359.1219 $[M + Na]^+$ (calcd for 359.1214). In the IR spectrum, bands due to hydroxyl (3429 cm^{-1}) and carbonyl (1710 cm^{-1}) groups were observed, and in the UV absorption spectrum, dihydroindole maxima at 216, 245, and 290 nm were detected.[5,17] The ^1H and ^{13}C NMR data (Tables 1 and 3) were similar to those of **1**, indicating that

compound **2** had the same scaffold as **1**, but that **2** had two methoxycarbonyl motifs (δ_C 154.0, 153.5, 52.2, and 51.9). The HMBC cross-peaks of 13-OCH_3 (δ_H 3.75) with C-13 (δ_C 154.0), 14-OCH_3 (δ_H 3.53) with C-14 (δ_C 153.5), 2-CH_3 (δ_H 1.75) with C-2 and C-3 (δ_C 84.5), and 3-OH (δ_H 5.81) with C-12 (δ_C 31.8), C-3, and C-8 (δ_C 124.9), and the NOESY correlations of 2-CH_3 with both 13-OCH_3 and 14-OCH_3 indicated that the methoxycarbonyl, methyl, and hydroxy groups were located at N-1, N-3, C-2, and C-6, respectively. Interpretation of the 2D NMR spectra confirmed the proposed structure of **2**. Moreover, ^{13}C NMR chemical shift calculation data further supported this structure, with all differences between experimental and calculated chemical shifts below 8.0 ppm (Table 4).[19] Similar to compound **1**, the broadening of the C-14 NMR signal is presumably attributable to the "N-10 chirality inversion". The NOESY (Figure 2) correlations of 2-CH_3 with 3-OH (δ_H 5.81), H-11β (δ_H 3.42), and H-12β (δ_H 2.13) and of 3-OH with 2-CH_3, H-11β, and H-12β implied that 2-CH_3 and 3-OH were cofacial.

Again, the small specific rotation of $[\alpha]^{20}_D$ − 3 (*c* 0.1, MeOH) indicated that compound **2** should also be a racemic mixture, which was subsequently separated by chiral HPLC into the enantiomers (+)-**2** (**2a**) and (−) -**2** (**2b**) in a 48:52 ratio (Figure S5), possessing the opposite ECD curves and specific rotations. The simulated ECD spectra of (2*R*, 3*R*)-**2** and (2S, 3S) -**2** matched those of the enantiomers (+)-**2** and (−) -**2**, respectively, thus, unambiguously assigning the absolute configurations of **2a** and **2b** (Figure 4). The calculated ECD spectra of enantiomers (2*R*, 3*S*)-**2** and (2*S*, 3*R*)-**2** did not correlate with the experimental data (Figure 4).

Peganumal A (**3**) was isolated as a white, amorphous powder. Its molecular formula was assigned as $C_{12}H_{13}NO_3S$ by a sodium adduct HRESIMS ion at *m*/*z* 274.0518 $[M + Na]^+$ (calcd for 274.0508). In the ^{13}C and ^1H NMR data (Table 3 and Experimental Section), a two-proton aromatic singlet at δ_H 6.52 (2H, s) and two magnetically equivalent *O*-methyls at δ_H 3.71 (6H, s), together with six aromatic carbon signals at δ_C 105.9 (× 2), 130.0, 134.2, and 148.0 (× 2) as well as two *O*-methyls at δ_C 56.0 (× 2) indicated that compound **3** has a 4-hydroxy-3,5-dimethoxyphenyl moiety. Considering the three remaining aromatic carbon signals at δ_C 139.4, 140.5, and 153.1 and a molecular formula of $C_{12}H_{13}NO_3S$, the presence of a thiazole fragment was readily deduced. The two moieties are connected by a methylene, which is supported by the HMBC cross-peaks of H_2-7 (2H, s) to C-1 (δ_C 130.0), C-2,6 (δ_C 105.9), and C-8 (δ_C 139.4). However, the substitution position of the methylene group on the thiazole fragment was not clear, and two possible structures **3a** and **3b** were proposed (Figure 5). Therefore, ^{13}C NMR chemical shift calculations were carried out by quantum chemical methods.[19] For structure **3a**, all differences between experimental and calculated chemical shifts were below 8.0 ppm (Table 4). However, structure **3b** shows chemical shift differences of 8.5, 20.4, and 23.3 ppm for C-7, C-8, and C-12, respectively (Table 4). Thus, the structure of compound **3** was defined as shown.

Peganumal B (**4**) was isolated as a white, amorphous powder. Its molecular formula was determined to be $C_{13}H_{15}NO_3S$ by a positive HRESIMS ion at *m*/*z* 266.0855 $[M + H]^+$ (calcd for 266.0845). The ^{13}C and ^1H NMR data (Table 3 and Experimental Section) were similar to those of **3**, but the replacement of H-10 by a methyl group. Analyses of the NMR data defined the structure of compound **4** as shown (Figure 1). Compounds bearing thiazole

motifs, such as **3** and **4**, are rare in natural products and are now reported in the genus *Peganum* for the first time.

Pegaharmine F (**5**) was isolated as orange-red, block crystals. Its molecular formula was established as $C_{17}H_{18}N_2O_4$ based on a sodium adduct HRESIMS ion at *m*/*z* 337.1174 [M + Na]$^+$ (calcd for 337.1159). Its UV spectrum had absorption maxima at 209 and 374 nm, suggesting that an extended conjugation system was present. The IR spectrum showed absorption bands at 3357 and 1678 cm^{-1}, suggesting that NH and carbonyl functionalities were present. The ^{1}H, ^{13}C NMR (Tables 2 and 3), and HSQC data showed the presence of a 3,4-dihydro-*β*-carboline skeleton from the observation of an indole NH (δ_H 11.43, 1H, br.s), an *O*-methyl (δ_H 3.79, 3H, s), an aromatic ABX spin system (δ_H 7.40, 1H, d, *J* = 8.8 Hz; 6.69, 1H, dd, *J* = 8.8, 1.4 Hz; 6.81, 1H, d, *J* = 1.4 Hz), and a –CH_2CH_2N- moiety (the resonance of one methylene was not detected). The signals at δ_C 102.2, 165.2, and 51.0 were assigned to C-14, C-15, and 15-OCH_3, respectively, and corresponded to an acrylate moiety (though the resonance of C-1 was not detected), which is connected to C-13 based on the HMBC cross-peak of H-14 (δ_H 6.24) to C-13 (δ_C 127.9). Additionally, complete assembly required linking an acetyl group (δ_H 2.06, δ_C 21.9, 170.0) to N-2 to assign the full structure **5**. However, the undetected resonances of H_2-3, C-1, and C-3 in the ^{1}H and ^{13}C NMR spectra, which are necessary to reasonably elucidate the structure of compound **5**, prompted recourse to a crystallographic study. X-ray quality crystals were grown by the slow evaporation of a methanol solution and the X-ray crystallography analysis (Figure 6) unambiguously confirmed the proposed structure. The undetected NMR signals of C(H)-1, -3, -15, and -16 could be attributed to the “N-2 chirality inversion” (an unusual acetyl located at N-2), presumably initiated by the p-*π* conjugation between the N-2 electron pair and the 16-CO group (Scheme 1). A similar phenomenon was also observed in the case of chaetominine, a cytotoxic alkaloid possessing a quinazolinone moiety.[20]

Pegaharmine G (**6**) was isolated as a yellow, amorphous powder, and its molecular formula was determined as $C_{21}H_{17}N_3O$ based on a positive HRESIMS ion at *m*/*z* 328.1440 [M + H]$^+$ (calcd for 328.1444). The spectroscopic data of compound **6** were similar to trigonostemonine E.[21] Comparison of the ^{1}H and ^{13}C NMR data (Tables 2 and 3) with those of trigonostemonine E revealed that compound **6** had a similar 1-(quinolinyl)-3,4-dihydro-*β*-carboline skeleton. Unlike trigonostemonine E, the *O*-methyl group was located at C-9. Additionally, the key HMBC cross-peaks of H-15 (δ_H 9.25, 1H, d, *J* = 2.2 Hz) with C-1 (δ_C 156.4), C-14 (δ_C 130.0), C-23(δ_C 135.5), and C-17 (δ_C 147.8) and of H-23 (δ_H 8.67, 1H, d, *J* = 2.2 Hz) with C-1, C-15 (δ_C 149.7), C-21 (δ_C 129.2), and C-17 linked the 3,4-dihydro-*β*-carboline and quinoline moieties via C-1 and C-14. Thus, the structure of compound **6** was determined as shown.

Pegaharmine H (**7**) was isolated as a yellow, amorphous powder, and its molecular formula was determined as $C_{17}H_{16}N_2O_3$ based on a positive HRESIMS ion at *m*/*z* 297.1236 [M + H]$^+$ (calcd for 297.1234). Comparison of the ^{1}H and ^{13}C NMR data (Tables 2 and 3) of **7** with those of harmalanine[8] indicated that these two compounds had similar skeletons, but that compound **7** had an aromatic proton been replaced by a hydroxymethyl group. The HMBC cross-peaks of H-14 (δ_H 6.57) with both C-16 (δ_C 111.8) and C-18 (δ_C 61.5), of H-16 (δ_H 6.22) with both C-14 (δ_C 97.5) and C-18, and of H-18 (δ_H 4.38) with C-15 (δ_C

154.1), C-14, and C-16 revealed that the hydroxymethyl group was located at C-15. Therefore, the structure of compound **7** was elucidated as shown.

Pegaharmine I (**8**) was isolated as a yellow, amorphous powder, and its molecular formula was determined to be $C_{13}H_{14}N_2O_2$ via a positive HRESIMS ion at *m*/*z* 231.1133 $[M + H]^+$ (calcd for 231.1128). Comparison of ^1H and ^{13}C NMR data (Tables 2 and 3) of **8** with those of harmaline[9] indicated that they contained the identical 3,4-dihydro-β-carboline skeleton. However, the deshielded methylene carbon resonance at δ_C 59.4 (C-3) and the shielded imino carbon resonance at δ_C 134.5 (C-1) implied the presence of an *N*-oxide group in **8**, which was confirmed by the observation of an $[M-16]^+$ ion at *m*/*z* 214.1122. The structure of compound **8** was thus defined, and the key HMBC cross-peaks are displayed in Figure 1.

Pegaharmine J (**9**) was isolated as a yellow, amorphous powder, and its molecular formula was assigned as $C_{13}H_{12}N_2O_2$ based on a positive HRESIMS ion at *m*/*z* 229.0975 $[M + H]^+$ (calcd for 229.0972). Its UV spectrum had absorption maxima at 242 and 301 nm, indicating that a β-carboline chromophore was present.[5,21] Analysis of the NMR data of **9** (Tables 2 and 3) revealed the presence of a β-carboline skeleton, which was in accord with an aromatic ABX spin system (δ_H 8.06, 1H, d, *J* = 8.6 Hz; 6.84, 1H, dd, *J* = 8.6, 2.1 Hz; 7.12, 1H, d, *J* = 2.1 Hz) and a pair of *ortho*-coupled protons (δ_H 8.19, 1H, d, *J* = 5.0 Hz; 7.89, 1H, d, *J* = 5.0 Hz). In addition, the key HMBC cross-peaks of H-14 (δ_H 4.92, 2H, d, *J* = 5.2 Hz) to C-13 (δ_C 133.6) and C-1 (δ_C 144.2) indicated that a hydroxymethyl group was located at C-1. Therefore, the structure of compound **9** was determined as shown.

Pegaharmine K (**10**) was isolated as a yellow, amorphous powder, and its molecular formula was assigned as $C_{13}H_{12}N_2O$ based on a positive HRESIMS ion at *m*/*z* 213.1018 $[M + H]^+$ (calcd for 213.1022). Analysis of NMR data of **10** (Tables 2 and 3), especially the HMBC cross-peaks (Figure 1), indicated that compound **10** was an isomer of harmine.[6] The most conspicuous characteristic of **10** was the deshielded chemical shift of the NH group at δ_H 11.93 (δ_H 11.42 in harmine). This deshielded chemical shift suggested that the NH group was located at N-2, which was confirmed by the COSY correlation of NH (δ_H 11.93) with H-3 (δ_H 7.56, 1H, *t*, *J* = 2.4 Hz) and the NOESY correlations of NH with both H-3 and 1-CH_3 (δ_H 2.77, 3H, s). Therefore, the structure of compound **10** was defined as shown.

The antiproliferative activities of the 12 new and 12 known alkaloids from *P. harmala* seeds were evaluated against HL-60, PC-3, and SGC-7901 cancer cell lines using the MTT and the trypan blue methods.[5,22] Compounds **9**, **11**, **12**, and **13** exhibited moderate cytotoxicity against HL-60 cancer cell lines with IC_{50} values of 4.36, 7.55, 9.05, and 9.25 μM, respectively. The other alkaloids showed weak or no activity (IC_{50} > 10 μM) against these three cell lines. These results indicated that the β-carboline skeleton is crucial for antitumor activity and a more in-depth biological evaluation of these alkaloids is ongoing.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were recorded on an Anton Paar MCP 200 polarimeter. UV spectra were measured on a Shimadzu UV-2201 spectrometer. ECD spectra were obtained on a Bio-Logic

MOS 450 spectrometer. IR spectra were measured on a Bruker IFS-55 spectrometer. 1D and 2D NMR spectra were acquired on Bruker AV-400 or AV-600 NMR spectrometers. Mass spectra were measured on Bruker micrOTOFQ-Q mass spectrometer. Single-crystal X-ray diffraction data was collected on a Rigaku Saturn 724 CCD diffractometer. Column chromatography (CC) were performed with D101 macroporous adsorption resin (D101 MAR), silica gel, ODS, and Sephadex LH-20. Semipreparative HPLC was performed with YMC ODS column, and the HPLC system was equipped with a Shimadzu SPD-20A UV–vis detector and LC-6AD pump.

Plant Material

The seeds were purchased from Anguo Medicine Co., Ltd. (Hebei, People's Republic of China) in 2012, and were identified as *P. harmala* L. seeds by Dr. Jincai Lu. The voucher sample (PH-20120705) has been deposited in Shenyang Pharmaceutical University.

Extraction and Isolation

P. harmala L. seeds (15.4 kg) were extracted under reflux with 95% ethanol (2 × 2 h × 100 L) and 75% ethanol (1 × 2 h × 100 L), respectively. The combined EtOH extract was concentrated to afford a residue (1.9 kg). The residue was suspended in acidic solution (13 L, pH 3, using 5% HCl). The acidic mixture was partitioned with CH_2Cl_2 (6 × 13 L) to give a CH_2Cl_2-soluble fraction, and the aqueous layer was alkalinized to pH 10 with 3 N NaOH, followed by exhaustive extraction with CH_2Cl_2 (6 × 13 L) to obtain crude alkaloids (420.2 g). The crude alkaloids were subjected to silica gel CC, eluting with CH_2Cl_2/MeOH (1:0 → 0:1) to yield nine fractions (Fr. A-Fr. I). Harmine (**11**, 600 mg) and harmaline (**14**, 400 mg) were obtained from fraction C (100:2) and F (100:7) via recrystallization in MeOH, respectively. Fraction B, eluted with CH_2Cl_2/MeOH (100:1), was subjected to silica gel CC (CH_2Cl_2/acetone 1:0 → 0:1) and afforded six subfractions (Fr. B1–Fr. B6). Fr. B1, B2, B3, and B4 were separated by ODS CC, semipreparative HPLC to yield **13** (54.6 mg), **18** (10.4 mg), **16** (3.2 mg), **6** (5.8 mg), **10** (2.2 mg), **12** (6.5 mg), **22** (29.6 mg), **17** (2.7 mg), **21** (6.5 mg),**15** (9.0 mg), **5** (5.0 mg), **3** (2.2 mg), and **4** (5.2 mg), respectively. Fraction C, eluted with CH_2Cl_2/MeOH (100:2), was chromatographed on silica gel CC (CH_2Cl_2/acetone 1:0 → 0:1) to afford six subfractions (Fr. C1–Fr. C7). Fr. C5 was separated by ODS CC, semipreparative HPLC to afford **19** (14.2 mg). Fraction D, eluted with CH_2Cl_2/MeOH (100:5–100:7), was chromatographed on D101 MAR CC (MeOH–H_2O 30, 60, 90%) to afford three subfractions (Fr. D1-Fr. D3). Fr. D1 was purified by ODS CC and semipreparative HPLC to obtain **8** (20.2 mg) and **20** (6.4 mg). Fr. D2 was subjected to ODS CC and semipreparative HPLC to afford **9** (6.0 mg) and **7** (2.0 mg). Fr. D3 was separated by silica gel CC (EtOAc/MeOH 1:0 → 0:1) to afford six subfractions (Fr. D3–1–Fr. D3–6). Fr. D3–1 was purified by ODS CC and semipreparative HPLC to yield **1** (4.1 mg) and **2** (8.2 mg). Racemic modifications of **1** and **2** were subjected to chiral HPLC (Lux Amylose-2, 250 × 4.6 mm, 5 μm, Phenomenex, U.S.A.) to yield the pure enantiomers, respectively. The mobile phase, *n*-hexane/2-propanol (90:10 and 40:60, respectively), was used.

Peganine A (1)—Yellow, amorphous powder; $[\alpha]_D^{20}$ + 2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.12), 244 (1.06), 289 (0.76) nm; IR (KBr) ν_{max} 3429, 2948, 2843, 1715,

1619, 1503, 1448, 1373, 1314, 1054, 1031, 1018, 764 $cm^{-1;}$ ^{1}H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 279.1325 $[M + H]^+$ (calcd for $C_{14}H_{19}N_2O_4$, 279.1339).

(−)-Peganine A (1a)—$[\alpha]_D^{20}$ − 65 (*c* 0.2, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 212 (+5.5), 217 (−8.5), 243 (+19.7), 287 (−9.6) nm.

(+)-Peganine A (1b)—$[\alpha]_D^{20}$ + 59 (*c* 0.2, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 212 (−7.3), 217 (+6.2), 243 (−18.4), 287 (+5.3) nm.

Peganine B (2)—Yellow, amorphous powder; $[\alpha]_D^{20}$ −3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.11), 245 (1.04), 290 (0.73) nm; IR (KBr) ν_{max} 3429, 3305, 2932, 2852, 1710, 1614, 1502, 1445, 1368, 1312, 1076, 1030, 763 $cm^{-1;}$ ^{1}H and ^{13}C NMR, see Tables 1 and 3; HRESIMS *m*/*z* 359.1219 $[M + Na]^+$ (calcd for $C_{16}H_{20}N_2O_6Na$, 359.1214).

(+)-Peganine B (2a)—$[\alpha]_D^{20}$ + 52 (*c* 0.2, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 210 (+11.1), 242 (−4.4) nm.

(−)-Peganine B (2b)—$[\alpha]_D^{20}$ − 60 (*c* 0.2, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 210 (−12.3), 242 (+4.8) nm.

Peganumal A (3)—White, amorphous powder; UV (MeOH) λ_{max} (log ε) 207 (3.84), 216 (4.14), 242 (2.01), 283 (0.82) nm; IR (KBr) ν_{max} 3386, 2937, 2839, 1612, 1516, 1460, 1428, 1335, 1244, 1216, 1115, 1026, 970, 828 $cm^{-1;}$ ^{1}H NMR (DMSO-d_6, 600 MHz) δ: 3.71 (6H, s, 3,5-OCH_3), 4.08 (2H, s, H_2-7), 6.52 (2H, s, H-2,6), 7.70 (1H, d, 0.5 Hz, H-12), 8.22 (1H, br.s, 4-OH), 8.91 (1H, d, 0.5 Hz H-10); ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 274.0518 $[M + Na]^+$ (calcd for $C_{12}H_{13}NNaO_3S$, 274.0508).

Peganumal B (4)—White, amorphous powder; UV (MeOH) λ_{max} (log ε) 207 (3.81), 216 (4.06), 241 (1.93), 282 (0.74) nm; IR (KBr) ν_{max} 3424, 2938, 2840, 1614, 1517, 1461, 1428, 1336, 1244, 1217, 1115, 1025, 971, 827 $cm^{-1;}$ ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.55 (3H, s, 10-CH_3), 3.71 (6H, s, 3,5-OCH_3), 3.98 (2H, s, H_2-7), 6.50 (2H, s, H-2,6), 7.38 (1H, s, H-12), 8.21 (1H, br.s, 4-OH); ^{13}C NMR data, see Table 3; HRESIMS *m*/*z* 266.0855 $[M + H]^+$ (calcd for $C_{13}H_{16}NO_3S$, 266.0845).

Pegaharmine F (5)—Orange-red, block crystals; UV (MeOH) λ_{max} (log ε) 209 (3.82), 374 (2.13) nm; IR (KBr) ν_{max} 3425, 2928, 2854, 1626, 1456, 1382, 1208, 1159, 1026, 756 $Cm^{-1;}$ ^{1}H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 337.1174 $[M + Na]^+$ (calcd for $C_{17}H_{18}N_2NaO_4$, 337.1159).

Pegaharmine G (6)—Yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 222 (4.02), 341 (1.73), 312 (0.92) nm; IR (KBr) ν_{max} 3427, 2930, 2853, 1650, 1569, 1456, 1383, 1270, 1202, 1143, 1029, 799 $cm^{-1;}$ ^{1}H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 328.1440 $[M + H]^+$ (calcd for $C_{21}H_{18}N_3O$, 328.1444).

Pegaharmine H (7)—Yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 205 (4.12), 220 (3.83), 271 (1.42), 376 (2.43), 394 (2.02) nm; IR (KBr) ν_{max} 3431, 2925, 2852, 1669,

1632, 1385, 1259, 1157, 1029, 818 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 297.1236 $[M + H]^+$ (calcd for $C_{17}H_{17}N_2O_3$, 297.1234).

Pegaharmine I (8)—Yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 207 (3.82), 217 (3.79), 264 (1.26), 388 (3.72) nm; IR (KBr) ν_{max} 3427, 2932, 2840,1630, 1588, 1508, 1445, 1384, 1262, 1203, 1150, 1076, 1026, 812, 511 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 231.1133 $[M + H]^+$ (calcd for $C_{13}H_{15}N_2O_2$, 231.1128).

Pegaharmine J (9)—Yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 242 (4.02), 301 (1.23) nm; IR (KBr) ν_{max} 3419, 2928, 1630, 1572, 1451, 1429, 1383, 1277, 1200, 1161, 1130, 1026, 802, 551 cm^{-1}; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 229.0975 $[M + H]^+$ (calcd for $C_{13}H_{13}N_2O_2$, 229.0972).

Pegaharmine K (10)—Yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 234 (4.02), 320 (1.12), 363 (1.08) nm; ^1H and ^{13}C NMR, see Tables 2 and 3; HRESIMS *m*/*z* 213.1018 $[M + H]^+$ (calcd for $C_{13}H_{13}N_2O$, 213.1022).

X-ray Crystallographic Analysis of Pegaharmine F (5)

Orange-red block crystals of **5** were obtained from a MeOH solution subjected to slow evaporation in a refrigerator for ca. 3 weeks. Single-crystal X-ray diffraction data were collected by a Rigaku Saturn 724 CCD diffractometer equipped with a multilayer-monochromator and a Mo Kα radiation (λ = 0.71073 Å) (Rigaku Co. Ltd., Japan). The structure was processed by direct methods, followed by Fourier techniques, and refined by SHELXL-97.[23] The block crystals of **5** are monoclinic, space group $p2(1)/c$ with cell dimensions a = 7.6992(15) Å, b = 7.9904(16) Å, c = 24.276(5) Å, V = 1489.3(5) $Å^3$, Z = 4, F (000) = 664, and goodness of fit on F^2 = 1.051. The R_1 and wR_2 values were 0.0644 [$I > 2\sigma(I)$] and 0.1361 (all data), respectively. Crystallographic data for compound **5** has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 1497011. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax, + 44 (0)-1223-336033; e-mail, deposit@ccdc.cam.ac.uk).

Computational Methods

Conformational searches using the MMFF94S force field were performed for **1** and **2** by CONFLEX software.[24] Subsequently, the selected stable conformers were subjected to geometry optimization by DFT theory at the B3LYP/6-31G* level. TDDFT ECD calculations for these optimized conformers were carried out at the B3LYP/6-31+G** level with a CPCM model in MeOH solvent. Finally, the overall ECD spectra were obtained by Boltzman weighting using SpecDis 1.5.[25] The optimized stable conformations of **2** were further used for ^{13}C NMR chemical shift calculations.[26] For the structures of **3a** and **3b**, four stable conformations (Figures S2 and S3, Supporting Information) were obtained after geometry optimizations and were used for ^{13}C NMR computations at the B3LYP/6-311+ +G** level, respectively. The chemical shifts were converted from magnetic shielding values with corrections.[27]

Cytotoxic Assays

Cytotoxicity of these isolates were evaluated by the MTT assay against the PC-3 (human prostate cancer) and the SGC-7901 (human gastric cancer) cell lines, and by the trypan blue method against the HL-60 (human leukemia) cell lines as described previously.[5,22]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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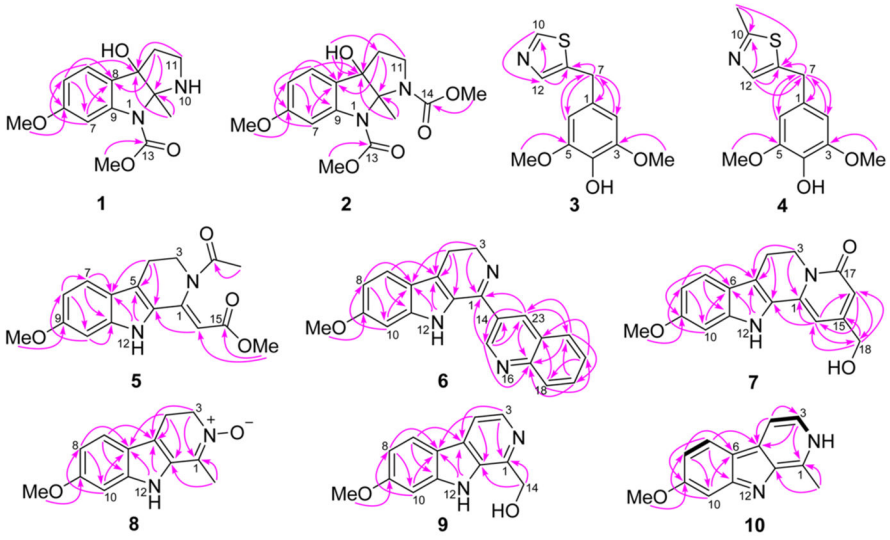


Figure 1.
Key HMBC and COSY correlations for **1–10**.

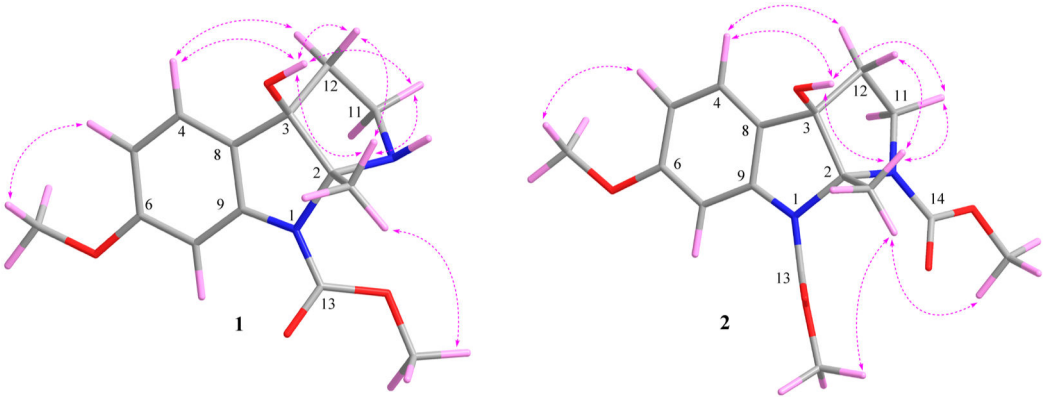


Figure 2.
Selected NOESY correlations for **1** and **2** (with hydrogens shown).

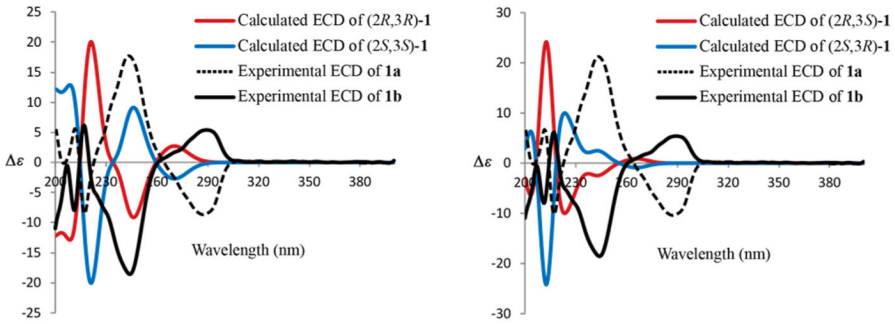


Figure 3.

Experimental ECD spectra of **1a** and **1b**, and the calculated ECD spectra of four stereoisomers of **1**.

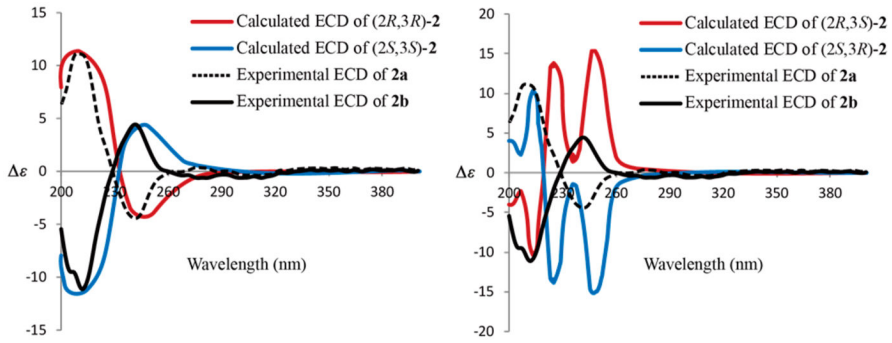


Figure 4.

Experimental ECD spectra of **2a** and **2b**, and the calculated ECD spectra of four stereoisomers of **2**.

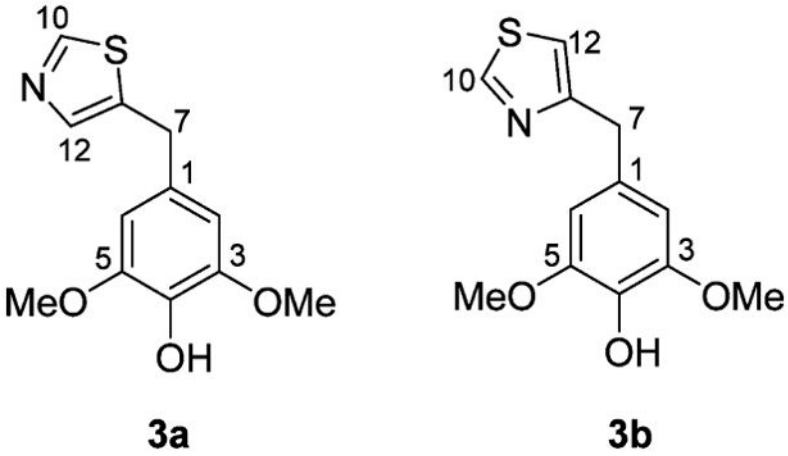


Figure 5.
Two possible structures of **3** (**3a** and **3b**).

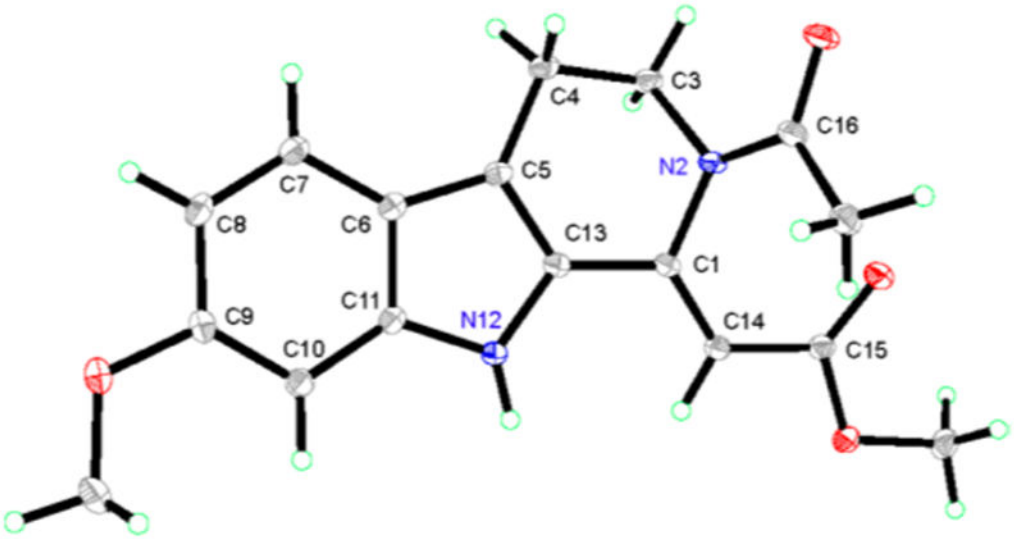
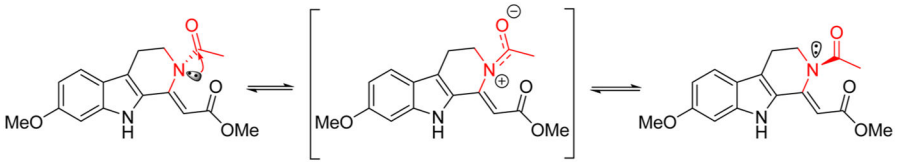


Figure 6.

Oak Ridge thermal ellipsoid plot (ORTEP) drawings of pegaharmine F (**5**) based on single X-ray crystallographic analysis.



Scheme 1.

Proposed “N-2 Chirality Inversion” of 5 due to p–π Conjugation

Table 1

^{1}H NMR Data of Compounds 1 and 2

Position	1[a] δ_H (*J* in Hz)	2[a] δ_H (*J* in Hz)
4	7.22 d (8.4)	7.33 d (8.4)
5	6.61 dd (8.4, 2.3)	6.65 dd (8.4, 2.4)
7	7.34 (broadening)	7.35 d (2.4)
11α	2.45 ddd (12.0, 9.2, 5.2)	2.69 dt (6.5, 12.0)
11β	2.83 dd (9.2, 7.2)	3.42 dd (12.0, 8.9)
12α	1.92 dd (12.0, 5.2)	2.31 dd (12.0, 6.5)
12β	2.04 dt (7.2, 12.0)	2.13 dt (8.9, 12.0)
2-CH_3	1.47 s	1.75 s
3-OH	5.50, br.s	5.81, br.s
6-OCH_3	3.72 s	3.74 s
13-OCH_3	3.77 s	3.75 s
14-OCH_3		3.53 s

[a]Run at 400 MHz, DMSO-d_6.

Table 2

^{1}H NMR Data of Compounds 5–10

Position	5[b] δ_H (*J* in Hz)	6[b] δ_H (*J* in Hz)	7[b] δ_H (*J* in Hz)	8[a] δ_H (*J* in Hz)	9[a] δ_H (*J* in Hz)	10[b] δ_H (*J* in Hz)
3	_[c]	3.98 t (8.6)	4.22 t (6.9)	4.07 t (8.4)	8.19 d (5.0)	7.56 t (2.4)
4	2.80 m	2.89 t (8.6)	2.99 t (6.9)	3.05 t (8.4)	7.89 d (5.0)	7.00 dd (2.4, 1.0)
7	7.40 d (8.8)	7.54 d (8.8)	7.46 d (8.7)	7.37 d (8.8)	8.06 d (8.6)	8.11 d (8.8)
8	6.69 dd (8.8, 1.4)	6.78 dd (8.8, 2.3)	6.72 dd (8.7, 2.2)	6.70 dd (8.8, 2.2)	6.84 dd (8.6, 2.1)	7.14 dd (8.8, 2.6)
10	6.81 d (1.4)	6.92 d (2.3)	6.85 d (2.2)	6.83 d (2.2)	7.12 d (2.1)	7.38 d (2.6)
14	6.24 s		6.57 d (1.5)		4.92 d (5.2)	
15		9.25 d (2.2)				
16			6.22 d (1.5)			
18		8.11 br.d (8.3)	4.38 s			
19		7.85 ddd (8.3, 8.3, 1.4)				
20		7.69 ddd (8.3, 8.3, 1.4)				
21		8.12 dd (8.3, 1.4)				
23		8.67 d (2.2)				
–CH_3	2.06 s			2.25 s		2.77
–OCH_3	3.79, 3.63 s	3.80 s	3.80 s	3.77 s	3.86 s	3.87 s
–OH			5.42 br.s		5.51 t (5.2)	
–NH	11.43 br.s	11.09 br.s	11.56 br.s	11.17 br.s	11.22 br.s	11.93 br.s

[a]Run at 400 MHz, DMSO-d_6.

[b]Run at 600 MHz, DMSO-d_6.

[c]Not detected.

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Table 3

^{13}C NMR Data of Compounds 1–10

position	1[a]	2[b]	3[b]	4[a]	5[a]	6[b]	7[b]	8[a]	9[a]	10[b]
1			130.0	130.1	_[c]	156.4	137.4	134.5	144.2	127.8
2	91.2	87.6	105.9	105.8						
3	85.7	84.5	148.0	148.0	_[c]	48.5	40.0	59.4	137.1	127.3
4	124.8	124.5	134.2	134.1	21.4	19.1	19.2	19.5	113.0	100.4
5	108.4	108.8	148.0	148.0	120.4	117.3	113.5	106.5	128.3	127.0
6	160.0	160.5	105.9	105.8	117.9	119.3	119.7	120.0	114.3	117.0
7	100.2	102.6	31.9	32.3	120.4	120.6	120.1	119.5	122.4	124.0
8	127.0	124.9	139.4	138.8	110.4	110.9	110.2	110.2	109.0	115.7
9	142.3	143.7			158.0	157.5	157.4	156.3	160.1	157.2
10			153.1	164.1	94.1	94.7	94.4	94.6	94.9	108.5
11	42.1	44.3			139.0	138.1	139.3	138.7	142.2	143.4
12	42.8	31.8	140.5	139.1						
13	152.7	154.0			127.9	126.6	126.6	129.0	133.6	146.2
14		153.5			102.2	130.0	97.5		63.6	
15					165.2	149.7	154.1			
16					170.0		111.8			
17					21.9	147.8	161.6			
18						128.7	61.5			
19						130.4				
20						127.1				
21						129.2				
22						127.0				
23						135.5				
–CH_3	21.1	20.0		18.8				12.3		20.9
3,5-OCH_3			56.0	56.0						
9-OCH_3					55.2	55.1	55.2	55.2	55.3	55.1
6/13-OCH_3	55.2/52.2	55.2/52.2								

position	1[a]	2[b]	3[b]	4[a]	5[a]	6[b]	7[b]	8[a]	9[a]	10[b]
14/15-OCH_3		51.8			51.0					

[a]Run at 100 MHz, DMSO-*d*6.

[b]Run at 150 MHz, DMSO-*d*6.

[c]Not detected.

Table 4

Experimental and Calculated ^{13}C NMR Data for Compounds 2, 3a, and 3b

	2			3	3a		3b	
no.	**exptl**	**calcd**	**error**	**exptl**	**calcd**	**error**	**calcd**	**error**
1				130.0	131.0	1.0	131.7	1.7
2	87.6	90.2	2.6	105.9	112.4	6.5	106.6	0.7
3				148.0	148.6	0.6	148.5	0.5
4	44.3	45.9	1.6	134.2	139.3	5.1	137.3	3.1
5	31.8	32.4	0.6	148.0	148.5	0.5	147.8	−0.2
6	84.5	88.6	4.1	105.9	102.9	−3.0	101.8	−4.1
7	124.9	121.9	−3.0	31.9	36.4	4.5	40.4	8.5
8	124.5	122.2	−2.3	139.4	145.2	5.8	159.8	20.4
9	108.8	109.7	0.9					
10	160.5	161.8	1.3	153.1	155.3	2.2	155.2	2.1
11	102.6	100.5	−2.1					
12	143.7	146.0	2.3	140.5	140.8	0.3	117.2	−23.3
13	154.0	154.0	0					
14	153.5	152.5	−1.0					
2-CH_3	20.0	21.9	1.9					
3-OCH_3/10-OCH_3	55.2	53.0	−2.2	56.0	53.7	−2.3	53.8	−2.2
5-OCH_3/13-OCH_3	52.2	51.0	−1.2	56.0	56.2	0.2	54.3	−1.7
15-OCH_3	51.9	51.3	−0.6					