Published in final edited form as:

Tetrahedron. 2012 April 15; 68(15): 3205–3209. doi:10.1016/j.tet.2012.02.048.

Nitrile-Containing Fischerindoles from the Cultured Cyanobacterium *Fischerella* sp

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Abstract

Chemical investigation of the cultured cyanobacterium *Fischerella* sp. (SAG strain number 46.79) led to the isolation of four nitrile-containing indole alkaloids, namely 12-*epi*-fischerindole I nitrile (**1**), deschloro 12-*epi*-fischerindole I nitrile (**2**), 12-*epi*-fischerindole W nitrile (**3**), and deschloro 12-*epi*-fischerindole W nitrile (**4**) along with a known metabolite hapalosin. The structures were determined by detailed spectroscopic analyses on the basis of 1D and 2D NMR and HRESIMS data. All isolates were evaluated for cytotoxicity against human cancer cells and for 20S proteasome inhibition. Deschloro 12-*epi*-fischerindole I nitrile (**2**) was found to be weakly cytotoxic against HT-29 cells with an ED₅₀ value of 23 μM. Hapalosin showed weak cytotoxicity against HT-29 and MCF-7 cells with ED₅₀ values of 22 and 27 μM, respectively, as well as moderate 20S proteasome inhibition with an IC₅₀ value of 12 μM. Compounds **1–4** all contain a nitrile moiety instead of the isonitrile found in all fischerindoles reported to date. Compounds **3** and **4** also display a new carbon skeleton, in which a six-membered ring replaces the five-membered ring normally found in fischerindole-type alkaloids.

1. Introduction

Over 70 indole alkaloids have been isolated from cyanobacteria (blue-green algae) of the order Stigonematales.¹ Representative classes of the indole alkaloids include hapalindoles,^{2–7} fischerindoles,^{8–9} welwitindolinones,⁹ ambiguines,^{10–13} hapalindolinones,¹⁴ hapaloxindoles,¹⁵ and fontonamides.¹⁵ These alkaloids possess diverse biological activities such as antifungal,⁴ antibacterial,⁵ and antialgal² activities. All of these alkaloids have a carbon skeleton derived from L-tryptophan and geraniol pyrophosphate.⁸ Of the more than 70 indole alkaloids, only five have been classified as fischerindoles (fischerindole L, 12-*epi*-fischerindole G and I, fischerindole U isonitrile, and fischerindole U isothiocyanate).^{1, 8–9}

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Supplementary data

¹H, gCOSY, gHSQC, and gHMBC spectra of **1–4**; DEPTQ spectra of **2** and **3**; 2D NOESY spectra of **1** and **3**; 1D selective NOESY spectra of **2**; ¹H spectrum of **5**. Supplementary data associated with this article can be found in the online version.

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The majority of these indole alkaloids contain an isonitrile or isothiocyanate moiety^{1, 16} while only two have been reported with a nitrile substituent (ambiguines G and Q).^{7, 13}

As part of our ongoing collaborative natural product drug discovery project,¹⁷ we evaluated organic extracts of cultured cyanobacteria for inhibition of the 20S proteasome, an established target for cancer treatment, as well as for cytotoxicity against a set of human cancer cell lines designated HT-29 colon, MCF-7 breast, NCI-H460 large lung, and SF268 glioblastoma. The organic extract of *Fischerella* sp. (SAG 46.79) displayed significant inhibition of the 20S proteasome, and bioassay-guided fractionation led to isolation of a previously known compound hapalosin as the active principle. Second culture of the same strain was prepared in an attempt to isolate hapalosin analogues. An extract from the second culture did not inhibit 20S proteasom activity. However, HPLC-ESIMS analysis of the second extract indicated a different chemical profile, and four nitrile-substituted fischerindole-type alkaloids were obtained from this extract. These alkaloids were named 12-*epi*-fischerindole I nitrile (**1**), deschloro 12-*epi*-fischerindole I nitrile (**2**), 12-*epi*-fischerindole W nitrile (**3**), and deschloro 12-*epi*-fischerindole W nitrile (**4**) in line with the tradition used for previously reported hapalindole-type alkaloids from cyanobacteria (Figure 1). We herein present the isolation, structure elucidation, and bioactivity evaluation of these alkaloids.

2. Results and discussion

2.1. Structure elucidation

12-*epi*-Fischerindole I nitrile (**1**) was obtained as a white amorphous powder. The HRESIMS pseudo-molecular $[M-H]^-$ ion at m/z 335.1331 indicated a molecular formula of $C_{21}H_{21}ClN_2$. The presence of a chlorine atom was confirmed by the 3:1 molecular ion cluster at m/z 335/337. Interpretation of the 1H NMR and COSY spectra of **1** (Table 1, Figure 2) revealed a 1,2-disubstituted indole moiety [δ_H 12.01 (H-1), 8.20 (H-4), 7.41 (H-7), 7.16 (H-6), and 7.10 (H-5)], a vinyl group [δ_H 5.94 (H-20), 5.29 (H-21*E*), and 5.05 (H-21*Z*)], and three methyl singlets [δ_H 1.49 (H₃-19), 1.42 (H₃-18), and 1.12 (H₃-17)]. Furthermore, an isolated spin system consisting of two methines [δ_H 4.46 (H-13) and 3.26 (H-15)] connected by a methylene [δ_H 2.13 (H-14_{eq}) and 2.00 (H-14_{ax})] was observed. Together, these findings indicated that **1** was a 2,3-disubstituted indole of the fischerindole class. The ^{13}C NMR spectrum of **1** displayed all 21 carbon resonances, required by the molecular formula. Thirteen resonances appeared in the range between 90 and 150 ppm, and eight of these (δ_C 163.2, 141.6, 122.7, 122.0, 121.7, 121.1, 113.1, and 112.8) were assigned to an indole moiety. The carbon resonances at δ_C 141.4 and 117.1 and at δ_C 156.0 and 96.6 were assigned to a vinyl group and a tetra substituted double bond, respectively. The final carbon resonance at δ_C 120.6 was attributed to a nitrile moiety. These fragments were connected based on correlations observed in the HMBC spectrum (Table 1 and Figure 2). The indole moiety was confirmed by correlations from H-4 to C-3, C-6, and C-8, and from H-5 to C-7 and C-9. This indole moiety was connected to the *gem*-dimethyl group at C-16 by correlations from both H₃-17 and H₃-18 to C-2. Correlations from both H₃-17 and H₃-18 to C-16 and C-15 further connected this group to the H₁₃-H₁₅ fragment. The vinyl and H₃-19 methyl groups were placed on C-12, and in turn connected to C-13 by correlations from H-20 to C-11, C-12, and C-19, and from H₃-19 to C-11, C-12, C-13, and C-20. Moreover, H-15 showed correlations to C-10, C-11, C-14, C-16, C-18, and C-22, establishing the fischerindole ring system, and placing the nitrile moiety at C-11. 1H and ^{13}C data of the structurally related 12-*epi*-fischerindole I isonitrile⁹ were available for comparison with those of **1**. However, only carbon chemical shifts were compared since spectra of the 12-*epi*-fischerindole I isonitrile were recorded in CD_2Cl_2 . A comparison of the ^{13}C NMR chemical shifts of **1** with those reported for the structurally related 12-*epi*-fischerindole I isonitrile indicated that the major differences were observed at C-22 (δ_C

164.8 vs 120.6 in **1**) and surrounding carbons [C-3 (δ_C 141.0 vs δ_C 112.8 in **1**), C-10 (δ_C 140.7 vs 156.0 in **1**), and C-11 (δ_C 113.0 vs 96.6 in **1**)]. This difference in carbon chemical shifts further indicated that a nitrile moiety had replaced the isonitrile moiety found in 12-*epi*-fischerindole I isonitrile. Based on these findings, the structure of **1** was established as 12-*epi*-fischerindole I nitrile.

The HRESIMS of deschloro 12-*epi*-fischerindole I nitrile (**2**) displayed a pseudo-molecular $[M-H]^-$ ion at m/z 301.1718, corresponding to the molecular formula $C_{21}H_{22}N_2$, indicating the absence of a chlorine atom. Examination of NMR spectra revealed **2** to be closely related to **1** (Table 1). The major difference being that the H-13 methine signal (δ_H 4.46) in the 1H NMR spectrum of **1** had been replaced by methylene signals [δ_H 1.84 (H-13_{eq}) and 1.61 (H-13_{ax})] in **2**. Similarly in the ^{13}C NMR spectrum, the C-13 methine (δ_C 66.4) in **1** had been replaced by a methylene carbon (δ_C 35.4) in **2**. Analysis of COSY spectrum of **2** further indicated the presence of a CH-CH₂-CH₂ spin system from C-15 to C-13, and the planar structure of **2** was determined to be the deschloro derivative of **1**, deschloro 12-*epi*-fischerindole I nitrile.

12-*epi*-Fischerindole W nitrile (**3**) was obtained as a white amorphous powder. The HRESIMS pseudo-molecular $[M-H]^-$ ion at m/z 333.1175 indicated a molecular formula of $C_{21}H_{19}ClN_2$. The presence of a chlorine atom was confirmed by the 3:1 molecular ion cluster at m/z 333/335. Unlike **1** and **2**, the NMR spectra of **3** were recorded in CDCl₃ since the structure was found to be unstable in DMSO-*d*₆. Despite the use of different solvents, some notable changes in chemical shifts between **3** and **1** were observed. A comparison of the 1H NMR spectra of **3** and **1** (Table 1 and 2) revealed that the methine (H-15) and one of the *gem*-dimethyl singlets (H₃-17) in **1** had disappeared, while two new methine singlets [δ_H 7.30 (H-16) and δ_H 4.79 (H-11)] had appeared. Furthermore, the chemical shift of H₃-18 (δ_H 1.42) had shifted downfield (δ_H 2.41). Similarly in the ^{13}C NMR spectra, two quaternary carbons C-11 (δ_C 96.6) and C-16 (δ_C 40.5) found in **1** had been replaced by two methines [δ_C 41.0 (C-11) and 112.4 (C-16)]. Moreover, the methine at C-15 (δ_C 58.1) found in **1** had been replaced by a new carbon at δ_C 125.0. In addition, a new carbon at δ_C 124.7 (C-17) in **3** had replaced one of the *gem*-dimethyls in **1**. Together, these findings indicated that the five-membered ring found in **1** had been rearranged in **3**. In the COSY spectrum of **3**, three spin systems assigned to H-4 to H-7, H-13 to H-14, and H-20 to H₂-21 were observed (Table 2 and Figure 3), indicating that the H₁₃-H₁₅ sequence observed in both **1** and **2** was shortened to a H₁₃-H₁₄ sequence in **3**. This finding along with chemical shift changes in the ^{13}C NMR spectra between **1** and **3** suggested that a new six-membered ring had been introduced in **3** by incorporation of one of the *gem*-dimethyls from **1** into the ring. The fragments from the COSY spectrum were connected by analysis of HMBC correlations to determine the planar structure of **3**. Similar as observed for both **1** and **2**, the connectivity of the indole moiety in **3** was established by correlations from H-4 to C-3, C-6, and C-8, from H-5 to C-7, and from H-7 to C-9. The vinyl and H₃-19 methyl groups were positioned on C-12 by correlations from H-20 to C-12 and C-19 as well as from H₃-19 to C-12 and C-20. Both of these groups were further connected to the H₁₃-H₁₄ fragment and to C-11 by correlations from both H-20 and H₃-19 to C-11 as well as from H₃-19 to C-13. The position of the nitrile moiety at C-11 and the connectivity from C-11 to C-10 and from C-10 to C-3 were determined by correlations from H-11 to C-3, C-12, C-13, C-19, C-20, and C-22. The planar structure of **3** was completed by the correlations observed from H₃-18 to C-2, C-17, C-16, from both H-14_{ax} and H-16 to C-10, and from H₂-14 to C-15. Together, these findings indicated that 12-*epi*-fischerindole W nitrile (**3**) possess a new carbon skeleton in which the five-membered ring, found in all previously known fischerindoles, has been replaced with a six-membered ring.

The HRESIMS of deschloro 12-*epi*-fischerindole W nitrile (**4**) displayed a pseudo-molecular $[M-H]^-$ ion at m/z 299.1556, corresponding to the molecular formula $C_{21}H_{20}N_2$, indicating the absence of a chlorine atom. Same as **3**, $CDCl_3$ was used to record NMR spectra for **4** since the structure was found to be unstable in $DMSO-d_6$. The NMR spectra of **4** were similar to that of **3**. A comparison of the 1H NMR spectra of **4** and **3** (Table 2) indicated that the H-13 methine signal (δ_H 4.72) found in **3** had been replaced by a new methylene [δ_H 2.23 (H-13_{ax}) and δ_H 1.98 (H-13_{eq})]. Analogously, this was also observed in the ^{13}C NMR spectra, where the C-13 methine signal (δ_C 62.7) found in **3** had been replaced by a new methylene carbon (δ_C 31.5). In the COSY spectrum of **4**, a CH_2-CH_2 spin system designated for C-13 and C-14 was also present. Together, these findings indicated **4** to be the deschloro derivative of **3**, deschloro 12-*epi*-fischerindole W nitrile.

The relative stereoconfigurations of **1-4** were deduced based on $^1H-^1H$ coupling constant analysis and/or correlations observed in 1D selective or 2D NOESY spectra. Large coupling constants between H-14_{ax} and H-13 ($J=12.0$ Hz) and H-14_{ax} and H-15 ($J=12.0$ Hz) were observed for 12-*epi*-fischerindole I nitrile (**1**), indicating both H-13 and H-15 to be in axial orientations. The NOE correlations between H-13 and H-14_{eq}, H-15, and H₃-19 in the 2D NOESY spectrum further supported all to be in the same plane. Based on these findings, the relative configuration of **1** was determined as 12*R**, 13*R**, and 15*R**. The NOE correlation between H-15 and H₃-18 was supported H₃-18 also to be in the same plane. Deschloro 12-*epi*-fischerindole I nitrile (**2**) also displayed a large coupling constant between H-14_{ax} and H-15 ($J=12.6$ Hz), indicating the H-15 to be in the axial orientation. 1D selective NOESY experiments were further performed to firmly determine the relative stereoconfiguration of **2**. Irradiation at the resonance frequency of H-15 (δ_H 2.92) produced NOE correlations with H-14_{eq} and H-13_{ax}. Irradiation of the H-13_{ax} (δ_H 1.61) produced NOE correlations to H-15 and H₃-19, indicating these to be in the same plane, and the relative stereoconfiguration of **2** was determined as 12*R** and 15*R**. 12-*epi*-Fischerindole W nitrile (**3**) displayed a large coupling constant between H-14_{ax} and H-13 ($J=11.5$ Hz), suggesting that H-13 was in the axial orientation. In addition, the 2D NOESY spectrum of **3** displayed NOE correlations between H-11 and H₃-19 and H-13_{ax}, indicating all to be in the same plane. Thus, the relative stereoconfiguration of **3** was established as 11*R**, 12*R**, and 13*R**. Due to decomposition of 12-*epi*-fischerindole W nitrile (**4**), no NOESY NMR experiments were obtained. However, we assume that the relative stereoconfiguration of **4** is 11*R** and 12*R** as depicted since **3** and **4** likely share a common biosynthesis.

2.2. Biological evaluation

Cytotoxicity of the isolates was evaluated using a set of human cancer cell lines designated HT-29 colon, NCI-H460 large lung, MCF-7 breast, and SF268 glioblastoma. Deschloro 12-*epi*-fischerindole I nitrile (**2**) was found to be weakly cytotoxic against HT-29 cells with an IC_{50} value of 23 μM , and hapalosin also showed similar IC_{50} values of 22 and 27 μM against HT-29 and MCF-7 cells, respectively. However, both **2** and **5** were found to be inactive against all other cancer cells displaying IC_{50} values of higher than 40 μM . All isolates were also evaluated for the inhibition of 20S proteasome activity, and only hapalosin showed mild activity with an IC_{50} value of 12 μM . It should be noted that 12-*epi*-fischerindole W nitrile (**3**) and deschloro 12-*epi*-fischerindole W nitrile (**4**) were found to be structurally unstable in $DMSO-d_6$. Thus, it is inevitable that degradation products of **3** and **4** were evaluated in all biological assays.

3. Conclusions

The bioassay-guided chemical investigation of the cyanobacterium *Fischerella* sp. led to the isolation of four new fischerindoles. Most hapalindole-type alkaloids reported to date have either isonitrile or isothiocyanate substituents. The only report of a nitrile moiety is in

ambiguine G and Q nitriles, and both are thought to be the products of rearrangements of ambiguine isonitrile precursors.^{7, 13} All four isolates reported here are unusual fischerindole-type indole alkaloids in that they all contain a nitrile moiety. Interestingly, the only difference between the previously reported 12-*epi*-fischerindole I isonitrile⁹ and compound **1** is that the isonitrile has been replaced by a nitrile. This placement indicates a different biosynthetic origin of the nitrile moiety in compound **1**, rather than it being the product from a rearrangement of an isonitrile precursor as suggested for ambiguine G and Q nitriles. 12-*epi*-Fischerindole W nitrile (**3**) and deschloro 12-*epi*-fischerindole W nitrile (**4**) both contain a new ring system for fischerindole-type alkaloids, where the five-membered ring of the fischerindole skeleton has been expanded to a six-membered ring. Thus, the finding of compounds **3** and **4** suggests that a new biosynthetic pathway may be present in this cyanobacterium.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Varian Cary 50 Bio spectrophotometer. 1D and 2D NMR spectra were obtained at room temperature on a Bruker Avance DRX 600 MHz spectrometer with a 5 mm CPTXI Z-gradient probe. ¹³C NMR spectra were obtained on a Bruker AV 900 MHz NMR spectrometer with a 5 mm ATM CPTCI Z-gradient probe. ¹H and ¹³C chemical shifts were referenced to a residual proton, 7.27 and 77.2 ppm in CDCl₃, 2.50 and 39.5 ppm in DMSO-*d*₆, respectively. HRESIMS were obtained using a Shimadzu IT-TOF spectrometer.

4.2. Biological material

Fischerella sp. was acquired from the Culture Collection of Algae at the University of Gottingen (SAG strain number 46.79). The cyanobacterium was grown in a 2.8 L Fernback flask containing 2 L of inorganic media (Z media) under aeration.¹⁸ Cultures were illuminated with fluorescent lamps at 1.93 klx with an 18/6 hours light/dark cycle. The temperature of the culture room was maintained at 22 °C. After eight weeks, the biomass of the cyanobacterium was harvested by centrifugation and then lyophilized. The same conditions were used for all three cultures.

4.3. Extraction and isolation

The lyophilized biomass (1.1 g) from 2 L of culture was extracted with CH₂Cl₂/MeOH (1:1 v/v) at room temperature and the solvent was evaporated *in vacuo*. The extract (147.8 mg) was redissolved in CH₂Cl₂/MeOH (1:1 v/v) and mixed with Diaion® HP20SS resin. The mixture was dried *in vacuo* and the dried mixture was fractionated on a Diaion® column using a step gradient with increasing amount of 2-propanol in water to afford eight fractions. Fraction 5, eluting with 70% 2-propanol, displayed 75% inhibition of 20S proteasome at 50 ppm. Fraction 5 was subjected to a reversed-phase HPLC column (Varian C₈ Dynamax, 10 × 250 mm, flow rate 3 mL/min) with 75-95% MeOH/H₂O gradient over 45 min to yield hapalosin (0.7 mg, *t*_R 36 min). To obtain more of hapalosin, an extract (176.9 mg) was prepared from a second culture (2.5 g from 2 × 2L of culture). By the procedure described above, eight subsequent fractions were obtained. Fractions 5 and 6, eluted with 70% and 80% 2-propanol, respectively, were subjected to HPLC-ESI-TOF-MS analysis (Varian Microsorb C₈, 2.0 × 250 mm, flow rate 0.2 mL/min) with 30-100% MeOH gradient over 35 min containing 0.1% acetic acid. The analysis of MS spectra indicated the presence of potentially new indole alkaloids. Fraction 5 was subjected to reversed-phase HPLC column (Varian C₈ Dynamax, 10 × 250 mm, flow rate 3 mL/min) with 70-85% MeOH/H₂O over 80 min to yield **1** (0.3 mg, *t*_R 25 min), **2** (0.6 mg, *t*_R 29 min), and **4** (0.4 mg, *t*_R 22 min). Fraction 6 was further subjected to reversed-phase HPLC with a 70-80% MeOH/H₂O

gradient over 80 min and yielded **1** (0.5 mg, t_R 29 min), **2** (0.2 mg, t_R 34 min), **3** (0.6 mg, t_R 34 min), and **4** (0.5 mg, t_R 24 min). Due to the degradations of **3** and **4** in DMSO- d_6 , a third extract (299.5 mg extract from 2×2 L culture) was purified by the same procedure to produce **1** (0.3 mg) and **2** (0.1 mg), **3** (0.5 mg) and **4** (0.7 mg). Material obtained for **3** and **4** was used to complete the structure elucidations in $CDCl_3$.

4.3.1. 12-epi-Fischerindole I nitrile (1)—Amorphous white powder; $[\alpha]_D$ -44.0° (c 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 223 (6.10) 277 (5.76) 323 (5.84); IR γ_{max} 3283, 2962, 2924, 2852, 2204, 1715, 1616, 1474, 1453 cm^{-1} ; 1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 335.1331 ($[M-H]^+$, calcd for $C_{21}H_{20}ClN_2$, 335.1393).

4.3.2. Deschloro 12-epi-fischerindole I nitrile (2)—Amorphous white powder; $[\alpha]_D$ -115.6° (c 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 221 (6.75) 277 (5.30) 321 (5.46); IR γ_{max} 3743, 3290, 2953, 2926, 2856, 2200, 1686, 1617, 1455 cm^{-1} ; 1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 301.1718 ($[M-H]^+$, calcd for $C_{21}H_{21}N_2$, 301.1783).

4.3.3. 12-epi-Fischerindole W nitrile (3)—Amorphous white powder; $[\alpha]_D$ -22.0° (c 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 237 (5.49) 262 (5.15) 298 (5.03) 328 (4.73); 1H and ^{13}C NMR data, see Table 2; HRESIMS m/z 333.1175 ($[M-H]^+$, calcd for $C_{21}H_{18}ClN_2$, 333.1236).

4.3.4. Deschloro 12-epi-fischerindole W nitrile (4)—Amorphous white powder; $[\alpha]_D$ -18.3° (c 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 237 (5.67) 262 (5.32) 298 (5.22) 328 (4.91); 1H and ^{13}C NMR data, see Table 2; HRESIMS m/z 299.1556 ($[M-H]^+$, calcd for $C_{21}H_{19}N_2$, 299.1626).

4.3.5. Hapalosin—Amorphous white powder; $[\alpha]_D$ -37.4° (c 0.09, CH_2Cl_2); Other spectroscopic data as previously reported.¹⁹

4.4. 20S proteasome assay

Evaluation of inhibition of the 20S proteasome was performed as previously described.²⁰

4.5. Cytotoxicity assay

Cytotoxicity against HT-29 cells was performed using a commercially available kit according to the manufacturer's instructions (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega Corp, Madison, WI, USA). Cytotoxicity against NCI-H460, MCF-7, and SF-268 cells was performed as previously described.²¹

Supplementary Material

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Acknowledgments

This research was supported by the National Cancer Institute (PO1CA125066). We thank the Research Resources Center (RRC) at UIC for high-resolution mass spectrometry.

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1 R = Cl
2 R = H

3 R = Cl
4 R = H

Figure 1.
Structures of the compounds **1-4**

Figure 2.
Key COSY and HMBC correlations of 12-*epi*-fischerindole I nitrile (1)

Figure 3.
Key COSY and HMBC correlations of 12-*epi*-fischerindole W nitrile (3)

Figure 4.
Key NOESY correlations of 12-*epi*-fischerindole I nitrile (**1**, left) and 12-*epi*-fischerindole W nitrile (**3**, right)

Table 1

NMR data of 12-*epi*-fischerindole I nitrile (**1**) and deschloro 12-*epi*-fischerindole I nitrile (**2**) in DMSO-*d*₆.

Position		2				
1	δ_C , mult. ^a	δ_H , mult. (<i>J</i> in Hz) ^b	HMBC ^b	δ_C , mult. ^c	δ_H , mult. (<i>J</i> in Hz) ^b	HMBC ^b
1		12.01, br s	2, 7, 8, 9		12.01, br s	
2	163.2, C			163.0, C		
3	112.8, C			113.3, C		
4	121.7, CH	8.20, d (7.9)	3, 6, 8	121.3, CH	8.21, d (8.1)	3, 6, 8
5	121.1, CH	7.10, dd (7.9, 7.9)	7, 9	120.4, CH	7.07, dd (8.1, 8.1)	7, 9
6	122.7, CH	7.16, dd (7.9, 7.9)	4, 8	122.0, CH	7.13, dd (8.1, 8.1)	4, 8
7	113.1, CH	7.41, d (7.9)	5	112.5, CH	7.38, d (8.1)	5
8	141.6, C			141.1, C		
9	122.0, C			121.49, C		
10	156.0, C			156.3, C		
11	96.6, C			96.8, C		
12	44.9, C			39.8, C		
13 _{ax}	66.4, CH	4.46, dd (12.0, 3.0)	20		1.61, ddd (12.6, 2.4)	15, 16, 19, 20
13 _{eq}				35.4, CH ₂	1.84, ddd (12.6, 5.4, 2.4)	11, 14, 15, 16
14 _{ax}	29.7, CH ₂	2.00, ddd (12.0)			1.48, dddd (12.6, 2.4)	15, 16
14 _{eq}		2.13, ddd (12.0, 5.4, 3.0)		18.1, CH ₂	1.67, m	13, 16
15	58.1, CH	3.26, dd (12.0, 5.4)	10, 11, 14, 16, 18, 22	58.5, CH	2.92, dd (12.6, 5.4)	10, 11, 14, 16, 18, 22
16	40.5, C			40.1, C		
17	25.3, CH ₃	1.12, s	2, 15, 16, 18	25.0, CH ₃	1.07, s	2, 15, 16, 18
18	24.9, CH ₃	1.42, s	2, 15, 16, 17	24.7, CH ₃	1.40, s	2, 15, 16, 17
19	24.0, CH ₃	1.49, s	11, 12, 13, 20	27.2, CH ₃	1.32, s	11, 12, 13, 20
20	141.4, CH	5.94, dd (17.2, 10.5)	11, 12, 19	146.1, CH	5.80, ddd (17.2, 10.4)	11, 12, 13, 19
21E		5.29, d (10.5)	12		5.45, d (10.4)	12
21Z	117.1, CH ₂	5.05, d (17.2)	12, 20	113.6, CH ₂	4.91, d (17.2)	12, 20
22	120.6, C			121.40, C		

^aChemical shift determined from gHSQC and gHMBC experiments recorded at 600 MHz.

1 H Recorded at 600 MHz. 13 C DEPTQ experiment recorded at 226 MHz.

Table 2

NMR data of 12-*epi*-fischerindole W nitrile (3) and deschloro 12-*epi*-fischerindole W nitrile (4) in CDCl₃.

Position		3	4			
	δ_{C} , mult. ^a	δ_{H} , mult. (<i>J</i> in Hz) ^b	HMBC ^b	δ_{C} , mult. ^c	δ_{H} ^b , mult. (<i>J</i> in Hz) ^b	HMBC ^b
1		8.15, br s			8.08, br s	
2	134.8, C			135.6, C		
3	124.8, C			124.4, C		
4	122.3, CH	8.15, d (7.8)	3, 6, 8	122.4, CH	8.21, d (7.8)	3, 8
5	120.2, CH	7.34, dd (7.8, 7.8)	7	119.8, CH	7.32, dd (7.8, 7.8)	7, 9
6	125.8, CH	7.48, dd (7.8, 7.8)	4, 8	125.3, CH	7.45, dd (7.8, 7.8)	8
7	111.0, CH	7.50, d (7.8)	9	110.7, CH	7.49, d (7.8)	9
8	139.7, C			139.7, C		
9	122.0, C			121.4, C		
10	118.0, C			118.8, C		
11	41.0, CH	4.79, s	3, 12, 13, 19, 20, 22	39.4, CH	4.57, s	12, 13, 15, 22
12	42.6, C			37.8, C		
13 _{ax}	62.7, CH	4.72, dd (11.5, 5.8)	20	31.5, CH ₂	2.23, m	
13 _{eq}					1.98, dd (13.2, 6.4)	
14 _{ax}	36.0, CH ₂	2.97, dd (16.6, 11.5)	10, 13, 15	24.3, CH ₂	2.90, dd (17.2, 6.4)	15
14 _{eq}		3.46, dd (16.6, 5.8)	12, 13, 15, 20		2.71, m	
15	125.0, C ^d			126.0, C		
16	112.4, CH	7.30, s	10, 17, 18	111.7, CH	7.27, s	10, 15, 18
17	124.7, C			125.7, C		
18	20.6, CH ₃	2.41, s	2, 16, 17	20.6, CH ₃	2.39, s	2, 16, 17
19	24.8, CH ₃	1.76, s	11, 12, 13, 20	27.4, CH ₃	1.59, s	11, 12, 13, 20
20	135.6, CH	6.00, dd (17.5, 11.0)	11, 12, 19	141.3, CH	5.80, dd (17.5, 11.0)	
21 ^E	117.3, CH ₂	5.12, d (11.0)	12	114.0, CH ₂	4.94, d (11.0)	12
21 ^Z		5.02, d (17.5)	12, 20		4.09, d (17.5)	12
22	118.6, C			119.1, C		

^a DEPTQ experiment recorded at 226 MHz.

^b Recorded at 600 MHz.

^c Chemical shift determined from gHSQC and gHMBC experiments recorded at 600 MHz.

^d Chemical shift determined from gHMBC experiment recorded at 600 MHz.