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(±)-Pyriindolin with a 2,2′-bipyridine-spiro[furan-3,3′-indoline] chimeric skeleton from the endophytic *Streptomyces albolongus* EA12432

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ABSTRACT

(±)-Pyriindolin (**1**) with a rare molecular backbone formed by fusing a 2,2′-bipyridine nucleus into a spiro[furan-3,3′-indoline] skeleton, was isolated from the *Streptomyces albolongus* EA12432. The constitution and the relative configuration of (±)-**1** were determined by extensive spectroscopic analyses, ^{13}C calculation and DP4+ probability analysis. The absolute configurations of optically pure (+)-**1** and (−)-**1** which were obtained after a chiral high performance liquid chromatography (HPLC) separation were further identified by electronic circular dichroism (ECD) calculations. (+)- and (−)-Pyriindolins displayed moderate cytotoxicity against HCT-116 cell line with the half-maximal inhibitory concentration (IC_{50}) values of 2.89 ± 0.17 μmol/L and 4.47 ± 0.26 μmol/L, respectively.

Natural 2,2′-bipyridine alkaloids, represented by caerulomycins, are a class of secondary metabolites with distinctive characteristics produced by actinomycetes [1]. Since the first example, caerulomycin A, was discovered from a *Streptomyces* strain in 1959 [2], a great number of analogs with different structural features have been isolated from actinomycetes, such as collismycins [3], pyrisulfoxins [4] and cyanogrisides [5]. These compounds generally possess favorable bioactivities, including cytotoxic, antimicrobial, immunosuppressive, neuroprotective and anti-inflammatory activities [1–5]. Our group has isolated dozens of 2,2′-bipyridine alkaloids from the actinomycete strain *Actinoalloteichus cyanogriseus* WH1–2216–6 and its mutants, some of which showed significant anticancer activity [4b,5a,5b,5c,6]. As part of our ongoing research on new bioactive 2,2′-bipyridine alkaloids from actinomycetes, *Streptomyces albolongus* EA12432 was obtained. This strain was isolated from the Chinese medicinal plant *Aconitum carmichaeli* (Ranunculaceae) [7]. From the culture of EA12432, we previously identified the new compounds pyrisulfoxin C, (±)-pyrisulfoxin D, pyrisulfoxin E and (±)-pyrisulfoxin F (Fig. S2 in Supporting information), among which (±)-pyrisulfoxin D exhibited significant cytotoxicity against a series of cancer cell lines [4b]. To obtain new pyrisulfoxin analogs with small amounts, we re-fermented the strain EA12432 in corn solid media in a large-scale and investigated the different secondary metabolites. As a result, we identified a pair of enantiomers, (±)-pyriindolin (**1**) (Fig. 1) possess a novel 2,2′-bipyridine-spiro[furan-3,3′-indoline] chimeric skeleton formed from 2,2′-bipyridine-6-carbaldehyde and 2-(7–hydroxy-2-oxoindolin-3-yl)acetic acid.

(±)-Pyriindolin (**1**) was obtained as a yellow amorphous powder. The molecular formula was established as $C_{23}H_{19}N_3O_5S$ according to its high resolution electrospray ionization mass spectroscopy (HRESIMS) peak (Fig. S3 in Supporting information) at *m/z* 450.1107 $[M + H]^+$ (calcd. for $C_{23}H_{20}N_3O_5S$, 450.1118). The ^{13}C nuclear magnetic resonance (NMR) spectra (Figs. S6 and S7 in Supporting information) showed 23 carbon signals which were classified by heteronuclear single quantum correlation (HSQC) (Fig. S8 in Supporting information) as two carbonyls, nine non-protonated carbons (one sp^3 and eight sp^2 ones), eight sp^2-methines, one sp^3-methine, one methylene and two methyls (one methoxyl). The ^1H NMR spectra (Figs. S4 and S5 in Supporting information) showed four coupled signals at δ_H 8.35 (H-3′, d, J = 8.0 Hz), 8.04 (H-4′, dd, J = 8.0, 7.7 Hz), 7.54 (H-5′, dd, J = 7.7, 4.7 Hz) and 8.76 (H-6′, d,

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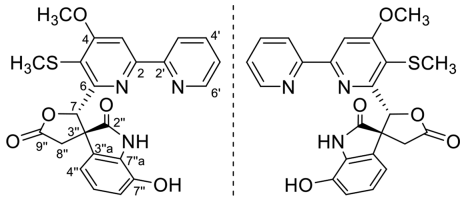


(+)-**1** 7*S*, 3"*R* (−)-**1** 7*R*, 3"*S*

Fig. 1. Structures of compounds (±)-**1**.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for (±)-pyriindolin (**1**) in DMSO-d_6.

Position	δ_C	δ_H, mult. (*J* in Hz)
2	155.6, C	
3	103.0, CH	8.05, s
4	166.9, C	
4–OCH_3	56.5, CH_3	4.02, s
5	120.6, C	
5-SCH_3	15.8, CH_3	1.77, s
6	156.9, C	
7	79.7, CH	6.40, s
2′	154.1, C	
3′	120.6, CH	8.35, d (8.0)
4′	137.7, CH	8.04, dd (8.0, 7.7)
5′	124.9, CH	7.54, dd (7.7, 4.7)
6′	149.4, CH	8.76, d (4.7)
2″	175.7, C	
3″	54.1, C	
3″a	126.6, C	
4″	114.2, CH	5.19, d (7.6)
5″	122.0, CH	6.40, dd (7.6, 8.2)
6″	116.0, CH	6.61, d (8.2)
7″	141.5, C	
7″a	130.3, C	
8″	35.9, CH_2	3.20, d (17.7); 2.88, d (17.7)
9″	179.1, C	
1″-NH		10.64, s
7″-OH		9.74, brs

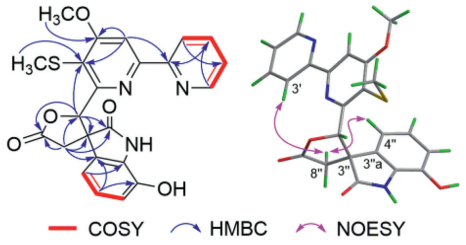


Fig. 2. Key 2D NMR correlations of **1**.

J = 4.7 Hz) (Table 1), suggesting the presence of a 2-substituted pyridine ring system. One methoxy signal (δ_H 4.02, s) and one methylthio signal (δ_H 1.77, s) were observed in the ^{1}H NMR spectra (Figs. S4 and S5). Careful comparison of the NMR data with those of pyrisulfoxins E and F [4b] indicated that pyriindolin (**1**) also contained a 4–methoxy-5-methylthio-2,2′-bipyridine structure, which could be confirmed by the key correlation spectroscopy (COSY) correlations of H-3′/H-4′/H-5′/H-6′ (Fig. 2 and Fig. S9 in Supporting information), and the key heteronuclear multiple bond correlation (HMBC) correlations of H-4′ to C-2′ (δ_C 154.1), H-3 to C-2′/C-5 (δ_C 120.6), 4–OCH_3 to C-4 (δ_C 166.9) and 5-SCH_3 to C-5 (Fig. 2 and Fig. S10 in Supporting information). In addition, the proton signals at δ_H 5.19 (H-4″, d, *J* = 7.6 Hz), 6.40 (H-5″, dd, *J* = 7.6, 8.2 Hz) and 6.61 (H-6″, d, *J* = 8.2 Hz) (Table 1) were assigned to a vicinal trisubstituted benzene ring, which was further supported by the COSY correlations of H-4″/H-5″/H-6″ (Fig. 2 and Fig. S9), and the key HMBC correlations of H-4″ to C-7″a (δ_C 130.3) and H-5″ to C-3″a (δ_C 126.6) and C-7″ (δ_C 141.5) (Fig. 2 and Fig. S10). The

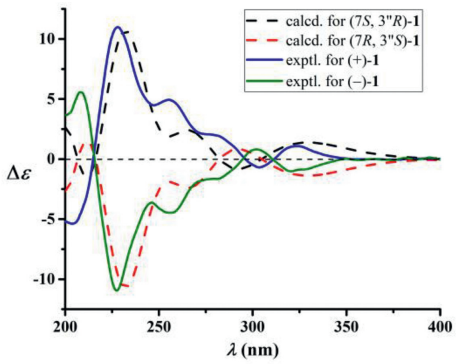
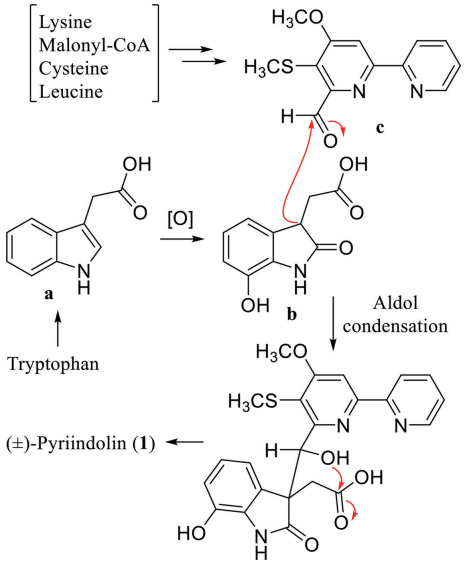


Fig. 3. Experimental and calculated ECD curves for (±)-**1**.

HMBC correlations of H-7 (δ_H 6.40, s) to C-2″ (δ_C 175.7)/C-3″a/C-9″ (δ_C 179.1) and H-8″ to C-7 (δ_C 79.7)/C-2″/C-3″ (δ_C 54.1)/C-3″a/C-9″ (Fig. 2 and Fig. S10) indicated that the vicinal trisubstituted benzene ring was further fused to form a 7″–hydroxy-7*H*-spiro[furan-3″,3″-indoline]−2″,9″ (4*H*)–dione skeleton moiety. This moiety and the 2,2′-bipyridine unit were connected to form compound **1** through the C–C sigma bond between C-6 and C-7, confirmed by the HMBC correlation of H-7 to C-5 (Fig. 2 and Fig. S10).

The relative configuration of **1** was assigned by the NOESY correlations of H-8″a (δ_H 3.20, d) to H-3′ and H-4″ (Fig. 2 and Fig. S11 in Supporting information). Furthermore, δ_C values of two plausible epimers, (7*S*,3″*R*)-**1** and (7*R*,3″*R*)-**1** (Fig. S12 in Supporting information), were calculated at the B3LYP/6–311++*G*(2d, p) level [8]. The DP4+ probability analysis (Fig. S12) [9] supported the relative configuration of **1** as (7*S**, 3″*R**). To determine its absolute configuration, we measured the electronic circular dichroism (ECD) spectrum. The result showed that compound **1** was not optically active, indicating pyriindolin (**1**) as a racemic mixture. This was supported by a chiral high performance liquid chromatography (HPLC) analysis (Fig. S1 in Supporting information). Then (±)-**1** was successfully resolved *via* a chiral HPLC separation into two optically pure enantiomers, (+)-**1** and (−)-**1**. To determine their absolute configurations, the predicted ECD spectrum was obtained by the TDDFT [B3LYP/6–31G(d)] method [10]. The measured ECD spectrum of (+)-**1** matched well with the calculated ECD curve of (7*S*,



Scheme 1. The postulated biosynthesis of (±)-**1**.

3′′*R*)-**1** (Fig. 3). Thus, the absolute configurations of (+)- and (−)-pyriindolins were unambiguously assigned as (7*S*, 3′′*R*) and (7*R*, 3′′*S*).

The plausible biosynthetic pathways for (±)-**1** were postulated (Scheme 1). Tryptophan was utilized to form the intermediate, indole-3-acetic acid (**a**), which further underwent oxidation to form intermediate, 2-(7–hydroxy-2-oxoindolin-3-yl)acetic acid (**b**). Meanwhile, another biosynthetic precursor, 4–methoxy-5-methylthio-2,2′-bipyridine-6-carbaldehyde (**c**) was formed from lysine, malonyl-CoA, cysteine and leucine [11]. The two precursors b and c underwent an intermolecular aldol condensation followed by an intramolecular esterification to generate the racemic products, (±)-pyriindolin (**1**).

Compounds (+)-**1** and (−)-**1** were evaluated for cytotoxicity against the cancer cell lines HCT-116, A549, Hela and K562. (+)-**1** and (−)-**1** exhibited inhibitory activity against HCT-116 cell line with the IC_{50} values of 2.89 ± 0.17 μmol/L and 4.47 ± 0.26 μmol/L, respectively, while no obvious cytotoxic activity was observed for other cell lines at the concentration of 10 μmol/L.

In summary, we identified a pair of enantiomers containing a novel 2,2′-bipyridine-spiro[furan-3,3′-indoline]chimeric skeleton, (+)- and (−)-pyriindolins. The formation mechanism of this spirocyclic scaffold could provide objects for the study of condensation reactions involving aldehyde in the biosynthesis of chimeric natural products.

Declaration of competing interest

The authors declare no competing financial interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2021.05.028.

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