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Characterization, Synthesis and Self-Aggregation of (−)-Alternarlactam: A New Fungal Cytotoxin with Cyclopentenone and Isoquinolinone Scaffolds

Ai Hua Zhang,[a] Nan Jiang,[b] Wen Gu,[a] Jing Ma,[b] Yu Rong Wang,[a] Yong Chun Song,[a] and Ren Xiang Tan*[a]

Abstract: (−)-Alternarlactam [(−)-**1**], a new promising cytotoxin against two human cancer cell lines, was isolated from an endophyte culture and synthesized (along with (+)-**1**) from readily available starting materials. The absolute configuration, chirality–activity relevance and self-aggregation of (−)-**1** were assigned by a combination of synthetic, spectroscopic and computational approaches. The full characterization of the new fungal cytotoxin may provide valuable information in the discovery of new antitumor agents.

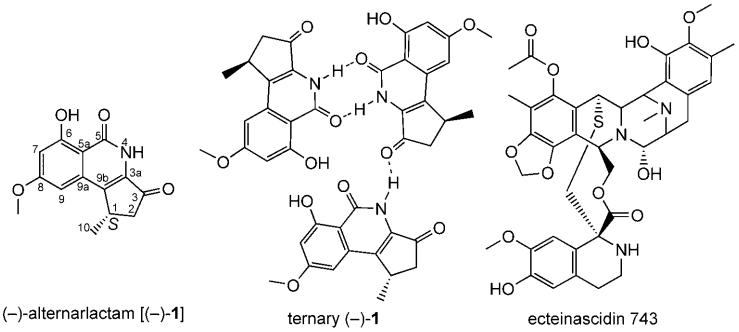
Keywords: alternarlactam · antifungal agents · antitumor agents · total synthesis

Introduction

Cancer remains one of the most severe threats to the public health worldwide, and unfortunately oncology retains one of the most pressing records in terms of investigational drug in clinical development with the success rate even below one-third of that for cardiovascular diseases.[1] This is worsened by the readily acquired resistance of carcinomas to the majority of the existing tumor-suppressing agents.[2] The identification of chemically unique and biologically promising molecules valuable for the antitumor drug discovery is therefore of increasing necessity.

Plants usually harbor in some organs or tissues a specific community of microorganisms called technically "endophyte", some of which have been ascertained to have gene flows with the host[3] and/or among different microbial species residing in the same niche.[4–5] This observation fundamentalizes that endophytes, as a big special group of microorganisms, are a rich source of functional biomolecules as substantiated by our characterization of new symbiont metabolites with antitumor,[6–8] antimicrobial,[9] and immunosuppressive activities.[10] In continuation of our bioassay for the antitumor secondary metabolites from symbiont cultures, the pronounced cytotoxicity against the test cancer cell line was discerned with the extract derived from the culture of *Alternaria* sp. HG1, a fungus isolated from inside the healthy leave of *Carex aridula* belonging to the Cyperaceae family.

The subsequent bioassay-directed fractionation afforded a new cytotoxic metabolite named trivially (−)-alternarlactam [(−)-**1**] (see below). After purification, the cytotoxicity of



(−)-**1** against two human cancer cell lines (cervix HeLa adenocarcinoma and hepatocellular carcinoma 7701) was confirmed to be comparable to those of doxorubicin (Table S1 in the Supporting Information), a marketed anticancer drug co-assessed as a positive control in the study.

[a] A. H. Zhang, Dr. W. Gu, Y. R. Wang, Dr. Y. C. Song, Prof. Dr. R. X. Tan
Institute of Functional Biomolecules
State Key Laboratory of Pharmaceutical Biotechnology
Nanjing University, Nanjing 210093 (P.R. China)
Fax: (+86) 25-8330-2728
E-mail: rxtan@nju.edu.cn

[b] Dr. N. Jiang, Prof. Dr. J. Ma
School of Chemistry and Chemical Engineering, Institute of Theoretical and Computational Chemistry, Lab of Mesoscopic Chemistry, Nanjing University, Nanjing 210093 (P. R. China)

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Results and Discussion

The structure determination of (−)-alternarlactam [(−)-**1**] was accommodated by a combination of spectral methods including IR (Figure S1), HR-ESI-MS (Figure S2), ^{1}H and ^{13}C NMR (Table 1; Figures S3 and S4), a set of 2D NMR experiments (^{1}H–^{1}H COSY, HMBC and HMQC; Figures S5–S7) and its NOE difference spectroscopy, coupled with experimental and computational electronic/vibrational circular dichroism (ECD/VCD) approaches. Briefly, the molecular formula ($C_{14}H_{13}NO_4$) of (−)-**1** was evidenced from the quasimolecular ion peak at *m/z* 282.0844 ($[M+Na]^+$) in its ESI mass spectrum, which was reinforced by a total of 13-proton integration and 14 resonance lines in its ^{1}H and ^{13}C NMR spectra, respectively. An *ortho*-substituted 2-hydroxy-4-methoxybenzoyl group was indicated by a hydroxyl singlet at δ 13.18 shifted downfield presumably via hydrogen bonding with carbonyl group, and from a pair of mutually *meta*-coupling doublets ($J=2.1$ Hz) at δ_H 6.67 and 6.71 ppm, which both showed NOE enhancements upon pre-saturation of the three-proton singlet at $\delta = 3.92$ PPM. Furthermore, the presence of an α,β-disubstituted 4-methylcyclopent-2-enone moiety was demonstrated by the three quaternary carbon resonance lines at $\delta_C = 197.8$, 165.6 and 134.2 ppm, as well as by a coupling sequence arising from a 2-substituted propyl group signifying at $\delta_H = 1.46$ (d, 3H, $J=7.2$ Hz), 3.53 (m, 1H), 3.01 (dd, 1H, $J=19.2$, 6.6 Hz) and 2.36 ppm (dd, 1H, $J=19.2$, 1.2 Hz). These two observations, compounding a nitrogen atom and nine degrees of unsaturation implied by the molecular formula, demonstrated that 2-hydroxy-4-methoxybenzoyl and 4-methylcyclopent-2-enone moieties had to combine through the δ-lactam ring to form the entire molecule. This elucidation was unambiguously confirmed collectively by ^{1}H–^{1}H COSY, HMBC and HMQC experiments (Figures S5–S7), which allowed the exact assignment of all ^{1}H and ^{13}C NMR signals of (−)-**1** (Table 1). Particularly, the elucidated framework was in agreement with the discerned HMBC correlation of H-1 with C-3, of H-2 with C-3a, C-9b and C-10, and of the NH singlet with C-5a and C-9b. This observation, along with the NOE enhancement of H-9 discerned upon irradiating H-10 signal, required that (−)-**1** was 6-hydroxy-8-methoxy-1-methyl-1*H*-cyclopenta[*c*]isoquinoline-3,5(2*H*,4*H*)-dione, which possessed a hitherto unreported framework.

(−)-Alternarlactam [(−)-**1**] was unique in its possession of an isoquinolinone moiety in conjunction with a cyclopentenone residue, which might be an ideal combination of cytotoxic pharmacophores as highlighted below. The isoquinolinone/isoquinoline moiety was recognized as a scaffold important for some antitumor and/or antiinflammatory drug candidates such as ecteinascidin 743 (see above).[11–13] On the other hand, the α,β-unsaturated cyclopentone, a soft electrophile, was recognized as an antitumor pharmacophore,[14] which was included as well in other bioactive molecules such as parthenin[15] and 1-deoxyrubralactone, a recently characterized eukaryotic DNA polymerase inhibitor.[16] And the biological functions of cyclopentenone-bearing compounds were shown to be stereochemically sensitive as summarized elsewhere.[14] It was therefore essential to understand the chirality of the cytotoxin. We thus acquired its CD spectrum that displayed clear Cotton effects at 218 (positive), 251 (negative), and 365 nm (negative) (Figure 1a), which depended solely on the absolute configuration of C-1, the only chiral carbon in the molecule. However, it could not be determined readily by the discerned CD bands since no model compound could be compared or consulted. This frustration was overcome by the quantum chemical approach. As illustrated in Figure 1, the experimentally recorded CD spectrum was well comparable with the theoretically predicted ECD curve for (−)-**1**, which was totally opposite to that calculated for its synthetic enantiomer (+)-**1** (see below). In particular, the two positive Cotton effects of (−)-**1** were calculated to center at 220 and 336 nm, which matched well with the observed bands at 218 and 333 nm (Figure 1a). Concerning the assignment of these distinctive ECD bands, the excitations from *n*-type (lone pair orbital on oxygen and nitrogen) and π-type (filled C=C orbital) molecular orbitals (designated as MO) to π*-type (antibonding C=C and C=O orbitals) MO, MO 77→80 and MO 76→78 (Table S3 and Figure S19), contributed dominantly to these absorbed bands. The first negative Cotton effect in the calculated ECD curve was located at 252 nm, corresponding to the broad band at 251 nm in the experimental spectrum. The $\pi_{C=C} \rightarrow \pi^*_{C=O}$ (MO 76→79) played an important role. The next calculated negative Cotton effect at 364 nm could be assigned to the experimental Cotton effect at 365 nm. Contributed to this band was the excitation of π→π* (MO 77→78), where π and π* were the filled C=C and antibonding C=C and C=O orbitals, respectively.

On the basis of the above information, the optimized 3D structure of (−)-**1** (Figure S20) was obtained with the Gaussian03 program[17] at B3LYP/6-31+G(d,p) level within the

Table 1. ^{1}H and ^{13}C NMR data of (−)-**1**.[a]

Position	δ_C	DEPT	δ_H (J in Hz)	Key HMBC (H→C)
1	30.2	CH	3.53 (m, 1H)	C-3
2α	44.1	CH_2	3.01 (dd, 1H, J = 19.2, 6.6)	C-9b, C-10
2β			2.36 (dd, 1H, J =19.2, 1.2)	C-3a, C-9b, C-10
3	197.8	C		
3a	134.2	C		
4			8.63 (brs, 1H)	C-5a, C-9b
5	165.6	C		
5a	107.4	C		
6	164.9	C		
7	102.6	CH	6.67 (d, J = 2.1, 1H)	C-9
8	165.1	C		
9	101.4	CH	6.71 (d, J =2.1, 1H)	C-5a, C-7, C-9b
9a	135.6	C		
9b	144.0	C		
10	21.2	CH_3	1.46 (d, J =7.2, 3H)	C-2, C-9b
6-OH			12.86 (brs, 1H)	C-5a, C-7
8-OCH_3	55.8	CH_3	3.92 (s, 3H)	C-8

[a] Acquired in $CDCl_3$ at 300 and 75 MHz, respectively.

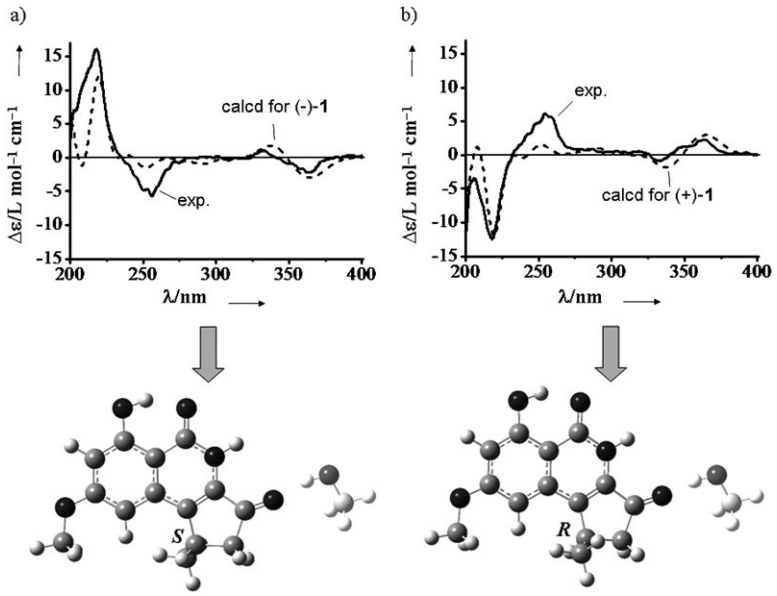


Figure 1. Attribution of the absolute configuration of (−)-**1** (natural) and (+)-**1** (synthetic) as 1*S* and 1*R* by comparing between the experimental (——) and calculated (•••••) ECD spectra. The computation was carried out at B3LYP/6-31+G(d,p) level within the combined explicit solvent/PCM model.

combined explicit solvent/polarized continuum model (PCM; CH_3OH solvent: dielectric constant $\varepsilon = 32.63$). The calculated distances between H-1/H-2α (2.348 Å), H-2β/H_α-10 (2.585 Å), H-1/H-9 (2.564 Å), H_β-10/H-9 (2.316 Å) and H-9/H_α-OCH_3 (2.329 Å) were all less than 4 Å, which was consistent with the NOEs observed for each of the proton pairs. Thus, the stereochemical structure of the fungal metabolite was established as (1*S*)-6-hydroxy-8-methoxy-1-methyl-1*H*-cyclopenta[*c*]isoquinoline-3,5(2*H*,4*H*)-dione [(−)-**1**].

In view of the dependence of the biological performance on its physical behavior of bioactive compounds,[18] we subsequently probed the aggregation of (−)-**1** in the solid state by utilizing the vibrational circular dichroism (VCD) technique that records the differential absorption between the left- and right-handed circularly polarized IR radiation via molecular vibration transition to provide the stereochemical and conformational information of the tested organic compounds.[19–25] As anticipated, the IR and VCD spectra acquired for (−)-**1** in a KBr disc was shown to be well comparable to that computerized for its ternary aggregate (Figure 2). This observation could be explained by assuming that (−)-**1** tends to pack through intermolecular hydrogen bonds. Subsequent scrutiny of the calculated IR and VCD spectra of the aggregates (Figure 2) showed that the ternary (−)-**1** involves various intermolecular non-covalent (C=O···H-N) linkages. The detailed geometries of the ternary (−)-**1** are given in Table S6. In addition, the VCD bands in the 1800–1200 cm^{-1} region could be resolved by Fourier self-deconvolution of the absorption spectrum. Aided by a comparison between the theoretically predicted and experimentally acquired VCD spectra of ternary (−)-**1**, the assignment was performed on the basis of the visual observation with Gaussview software (graphical interface for Gaussian 03, Gaussian Inc.). Harmonic vibrational frequency, dipole strength, and rotational strength as well as the assignment were given in Tables S7 and S8. As shown in Figure 2, the VCD signals at 1713(+)/1702(−) cm^{-1} was due to the C=O stretching vibrations in the cyclopentenone substructure. This band correlat-

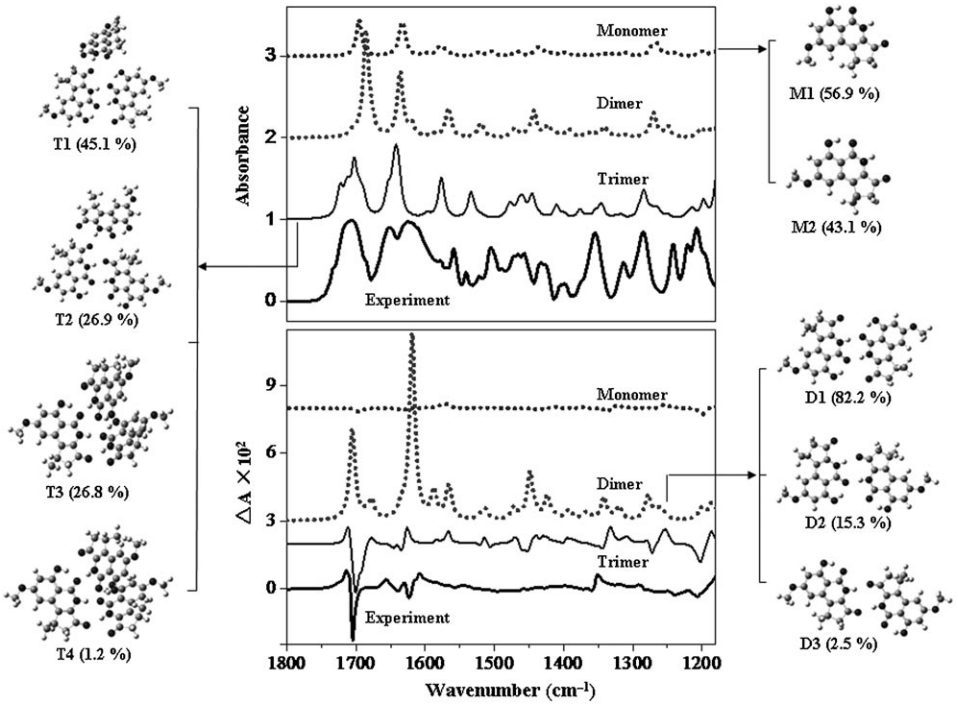
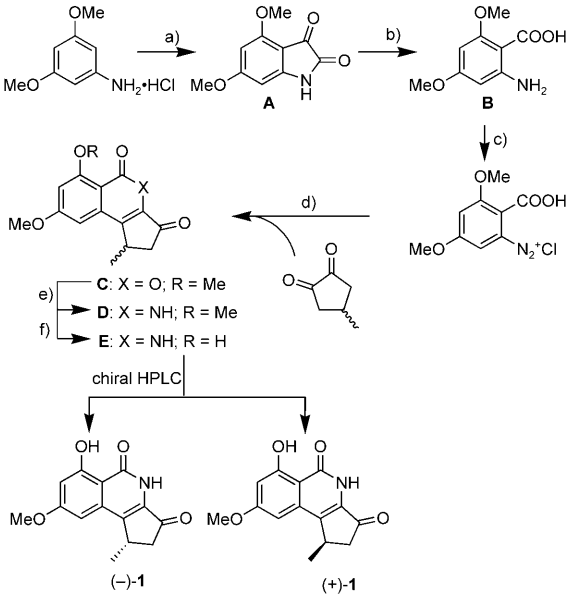


Figure 2. Comparisons of experimental IR (top) and VCD (bottom) spectra of (−)-**1** with those calculated for the aggregates of (−)-**1** at B3PW91/6-31+G(d,p) level in vacuum.

ed to those observed experimentally at 1712(+)/1704(−) cm^{-1}. The peaks at 1678(+) and 1645(−) cm^{-1} in the theoretical VCD curve corresponded to the experimentally intense band at 1657(+) and 1639(−) cm^{-1}, which were assigned to the C=O stretching vibrations in the δ-lactam. The signals at 1637(−)/1628(+) were ascribed to the C=C stretching vibrations. They could be correlated to the experimental bands at 1624(−) and 1608(+) cm^{-1}. On the other hand, the O–H, N–H, and C–H rocking vibrations gave rise to the bands at 1578 and 1569 cm^{-1}. The VCD signals ranging from 1517 to 1442 cm^{-1} in the theoretically predicted spectrum corresponded to the experimental bands around 1549–1449 cm^{-1}. They were attributed to the in-plane scissoring vibrations of CH_3 group. Finally, the bands in 1352–1200 cm^{-1} region in the experimental curve were reproduced by the theoretical calculations (in 1347–1200 cm^{-1}). In these bands, the coupled in-plane rocking vibrations of C–H, N–H, and O–H groups played a dominant role. It seems interesting that (−)-**1** present in its ternary form looks "similar" to ecteinascidin 743 (an anticancer drug at phase III clinical trial[12]) composed of three tetrahydroisoquinolinone units (see above).

The promising cytotoxicity of (−)-**1** was diminished by its low content in the fungal culture. It was apparently imperative to develop a reliable protocol that could supply it hopefully in an unlimited manner. We were therefore obligated to synthesize it from simple chemicals with an intention to afford meanwhile its enantiomer (+)-**1** that was highly desired for a detailed enantiomeric comparison between the functional and spectral features. For this reason, we pursued a synthetic approach toward (±)-alternarlactam which might give the corresponding single enantiomer after chiral HPLC separation. Accordingly, retrosynthetic analyses suggested that (±)-alternarlactam could be constructed by the C–C bondage between (±)-4-methyl-1,2-cyclopentanedione and a 2,4-dioxygenated benzoyl units. Thus, a five-step synthetic route was designed for its chemical generation from the two commercially available chemicals (Scheme 1). Starting from 3,5-dimethoxyaniline, the intermediate 2-amino-4,6-dimethoxybenzoic acid (**B**) was prepared in two steps.[28] After diazotizing **B**, the δ-lactone **C** formed upon addition of (±)-4-methyl-1,2-cyclopentanedione.[29] However, presumably owing to the stronger p–π conjugation of the ester oxygen with a 3-phenyl-cyclopentenone, the δ-lactone **C** could not be transformed into the expected δ-lactam **D** by the reported addition of formamide or gaseous ammonia.[11,26] This problem was solved eventually by a high-yield (91%) conversion of δ-lactone **C** to δ-lactam **D**, which we tried out by utilizing aqueous ammonia in the presence of formamide. The subsequent de-*O*-methylation using boron bromide in dichloromethane afforded (±)-alternarlactam (**E**), which was separated into (−)-**1** and (+)-**1** by the chiral HPLC procedure.

The IR, MS, ^1H and ^{13}C NMR data and specific rotation of the synthetic (−)-alternarlactam [(−)-**1**] were identical with those acquired for the natural product isolated from the fungal culture. Also as a confirmation for the aforementioned stereochemical assignment, the specific rotation and ECD/VCD bands were opposite (Figures 1, 3 and S22) between the two enantiomers [(−)-**1** and (+)-**1**] although both signified equally in their IR, MS, ^1H and ^{13}C NMR spectra.



Scheme 1. Total synthesis of (−)-**1** and (+)-**1**. a) Oxalyl chloride, 165 °C, 30 min (100%); b) 33% NaOH/30% H_2O_2 (59%); c) $NaNO_2$, HCl, 0 °C (100%); d) 50 °C, 12 h (48%); e) $NH_3{\cdot}H_2O$, formamide, 80 °C, 3 h (91%); f) BBr_3, CH_2Cl_2, 0 °C, 1 h (92%).

With the two single enantiomers of alternarlactam (**1**) in hand, we were able to compare the antitumor magnitude among the racemic and stereochemical isomers. The enantiomer (−)-**1** was shown to inhibit the growth of human cervix HeLa adenocarcinoma cell (IC_{50} = 1.10 μg mL^{-1}) and human hepatocellular carcinoma cell line QGY-7701 (IC_{50} = 1.52 μg mL^{-1}), with the magnitude close to those discerned with doxorubicin co-assayed as a positive control in the study (Figure 3). The enantiomer (+)-**1**, only moderately active to the QGY-7701 cell line (IC_{50} = 27.54 μg mL^{-1}), exhibited a weaker inhibition to the HeLa cell line with an

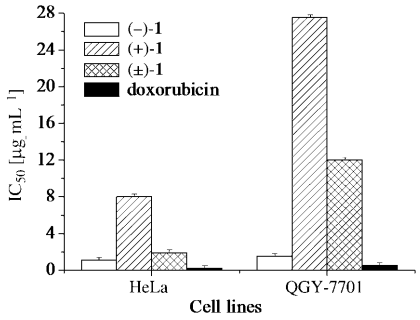


Figure 3. Comparisons of the cytotoxicity (in IC_{50} value) of (−)-**1** (blank), (+)-**1** (sparse), (±)-**1** (dense) and doxorubicin (solid, co-assayed as a positive control) against HeLa (human cervix HeLa adenocarcinoma) and QGY-7701 (human hepatocellular carcinoma QGY-7701) cell lines (see also Table S1).

IC_{50} value of 7.19 μg mL^{-1}, and those of the racemate [(±)-**1**] against the QGY-7701 and HeLa cell lines were 11.97 and 1.91 μg mL^{-1}, respectively. These data suggested that the cytotoxicity of alternarlactam is most likely chirality-sensitive (Figure 3).

Conclusion

In summary, *Alternaria* sp. HG1 from inside the normal *Carex aridula* leave was shown to produce in a trace amount the new antitumor polyketide (−)-alternarlactam [(−)-**1**] whose structure was determined rigorously by spectral and computational methods. (−)-Alternarlactam is unique in its co-possession of cyclopentenone and isoquinolinone scaffolds, both being important antitumor-related pharmacophores.[11–14] The usual structural character might be an implication for the new mode of action. The formation of (−)-**1** was rendered renewable through the synthesis from the commercially available chemicals. The lactamization procedure established in the work is useful for the construction of some lactam nuclei. The co-synthesis of both enantiomers [(−)-**1** and (+)-**1**] allowed both the disclosure of the chirality relevance to the antitumor action, and the robust validation of the computational ECD and VCD methods. Particularly, the correlative interpretation of the experimental and computational VCD spectra led to the recognition of the self-aggregation of (−)-**1** in KBr, which seems to be the first exemplification of the natural product-based supramolecule that may be of wider interest in chemistry. Finally, the new framework and biological profiles of both enantiomers of alternarlactam (**1**), along with the established synthetic protocol and its aggregative properties, provided collectively attractive and important information that may shed fresh light onto the elusive path towards the discovery of new antitumor agents, which has long been and continues to be one of the most urgent tasks in medicine.

Experimental Section

General reagents and instrumentation: As described earlier.[7,9,10]

Strain and cultivation: The alternarlactam-producing fungus, identified by Dr. Y. C. Song as *Alternaria* sp. HG1 according to its macro- and micro-graphic morphological characteristics,[27] was isolated from inside the fresh *Carex aridula* leaves collected in November 2001 from the suburb of Nanjing, China. The fresh mycelia grown on PDA plates were incubated into 1000 mL flasks preloaded with each 400 mL potato dextrose broth followed by shaking at 140 rpm at 25 °C for 5 d. After that, the liquid culture (10 mL) was transferred into each of 400 bottles containing millet medium,[28] before hatched quietly at 25 °C for 30 d. At the end of the solid-substrate fermentation, a total of 40 L fermented material was harvested, dehydrated, and then pulverized into dry powder (ca. 2 kg).

Fractionation of (−)-1: The powder derived from the solid substrate fermentation with *Alternaria* sp. HG1 was extracted exhaustively with $CHCl_3$/MeOH 1:1. In vacuo evaporation of the solvent yielded an oily crude extract (ca. 280 g), which was dissolved completely in the least amount of methanol prior to being kept at −20 °C overnight to get rid off the waxy material through precipitation. The filtrate was concentrated in vacuo to give an oily residue (ca. 108 g), which was then subjected to column chromatography (CC) on silica gel (1200 g, 200–300 mesh), eluting with $CHCl_3$/MeOH 1:0→0:1 to give nine fractions E1–E9. The bioactive fraction E2 was rechromatographed over silica gel. CC (170 g, 200–300 mesh) and then eluted again with $CHCl_3$/MeOH 1:0→0:1 to turn out five parts, one of which displayed bioactivity. The bioactive part was further separated through gel filtration over Sephadex LH-20 with $CHCl_3$/MeOH 1:1 to give a white solid of (−)-**1** (6.7 mg).

Spectral data of (−)-1: White solid; m.p. 234–236 °C; $[\alpha]_D^{25} = -20.6°$ (c = 0.04, MeOH); UV (MeOH): λ_{max} = 255, 358 nm; IR: $\tilde{\nu}$ = 3383, 3157, 3048, 1706, 1650 (s), 1625, 1559, 1503, 1466, 1284, 1207, 969, 841 cm^{-1}; CD (MeOH): λ ($\Delta\varepsilon$) = 355 (−0.84), 255 nm (−1.67); ESI-MS: m/z: 260.1021 $[M+H]^+$, 282.0844 $[M+Na]^+$, 298.0140 $[M+K]^+$, 541.2416 $[2M+Na]^+$, 800.3215 $[3M+Na]^+$; ^{1}H and ^{13}C NMR data: see Table 1.

Synthesis of 4,6-dimethoxyanthranilic acid (B): 3,5-Dimethoxyaniline hydrochloride (12 g, 63 mmol) was dissolved in oxalyl chloride (20 mL, 230 mol) at room temperature, and the resultant solution was stirred at 165 °C for 30 min. In vacuo removal of the excess oxalyl chloride gave a green-yellow residue, which was refluxed for 10 min with methanol (50 mL). Subsequent filtration gave 4,6-dimethoxyindole-2,3-dione **A** (13 g, 63 mmol, 100 %), which was added into an oversized flask and dissolved with 33 % NaOH solution (60 mL), followed by dropwise addition of 30 % solution of H_2O_2 (30 mL) with the reaction mixture maintained at 100 °C for an additional 10 min. The solution was then adjusted to pH 3 with concentrated HCl. The solid that formed was filtered, washed with water and dried to yield **B** (7.3 g, 37 mmol, 59 %).[29] Compound **A**: m.p. 302–306 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.93 (br s, 1H), 6.18 (d, J = 1.8 Hz, 1H), 6.00 (d, J = 1.8 Hz, 1H), 3.89 (s, 3H), 3.87 nm (s, 3H); EI-MS: m/z: calcd for $C_{10}H_9NO_4$: 207.1; found: 208.1 $[M+H]^+$. Compound **B**: m.p. 123–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 11.09 (br s, 1H), 6.45 (br s, 2H), 5.83 (d, J = 2.1 Hz, 1H), 5.81 (d, J = 2.1 Hz, 1H), 3.97 (s, 3H), 3.79 ppm (s, 3H); EI-MS: m/z: calcd for $C_9H_{11}NO_4$: 197.1; found: 198.1 $[M+H]^+$.

Synthesis of 6,8-dimethoxy-1-methyl-1,2-dihydrocyclopenta[*c*]isochromene-3,5-dione (C): Similar to the procedure detailed elsewhere,[30] concentrated HCl (2 mL) was added to the mixture of **B** (394 mg, 2 mmol) and water (2 mL), to which, sodium nitrite (144 mg, 2.1 mmol) in water (1 mL) was added at 0 °C, followed by addition of 4-methyl-1,2-cyclopentanedione (280 mg, 2.5 mmol). After stirred at 50 °C for 12 h, the reaction mixture was diluted with water (5 mL), extracted with CH_2Cl_2 (30 mL) and dried over Na_2SO_4. The solid residue afforded after evaporation was purified over silica gel column chromatography eluted with $CHCl_3$/MeOH 100:1 to give **C** (263 mg, 0.96 mmol, 48 %) as a yellowish solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.67 (d, J = 2.1 Hz, 1H), 6.65 (d, J = 2.1 Hz, 1H), 4.00 (s, 3H), 3.99 (s, 3H,), 3.44 (m, 1H), 2.93 (dd, J = 19.2,6.3 Hz, 1H), 2.28 (dd, J = 18.9,0.9 Hz, 1H), 1.44 ppm (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 195.9, 165.6, 164.4, 157.0, 149.1, 142.9, 137.1, 104.3, 100.8, 100.2, 56.4, 55.9, 42.8, 28.1, 21.1; ESI-MS: m/z: calcd for $C_{15}H_{14}O_5$: 274.10: 297.0834 $[M+Na]^+$; found: 275.1001 $[M+H]^+$.

Synthesis of 6,8-dimethoxy-1-methyl-1*H*-cyclopenta[*c*]isoquinoline-3,5-(2*H*,4*H*)-dione (D): A solution of **C** (274 mg, 1 mmol) in formamide (5 mL) was refluxed for 3 h at 80 °C with concentrated ammonia (2 mL) added in every 30 min. The progress of the reaction was monitored by TLC developed with petrol ether/acetone 2:1. After evaporated in vacuo, the residue was subjected to gel filtration over Sephadex LH-20 with $CHCl_3$/MeOH 1:1 to give **D** (248 mg, 0.91 mmol, 91 %) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.74 (br s, 1H), 6.73 (d, J = 2.0 Hz, 1H), 6.68 (d, J = 2.0 Hz, 1H), 4.00 (s, 3H), 3.97 (s, 3H,), 3.52 (m, 1H), 3.00 (dd, J = 19.1,6.5 Hz, 1H), 2.34 (dd, J = 19.1,1.1 Hz, 1H), 1.45 ppm (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.7, 163.8, 163.7, 160.4, 141.3, 138.1, 135.4, 112.1, 100.7, 99.5, 56.5, 55.7, 44.3, 30.1, 21.5 ppm; ESI-MS: m/z: calcd for $C_{15}H_{15}NO_4$: 273.09; found: 296.0868 $[M+Na]^+$, 274.1051 $[M+H]^+$.

Synthesis of 6-hydroxy-8-methoxy-1-methyl-1*H*-cyclopenta[*c*]isoquinoline-3,5(2*H*,4*H*)-dione [(±)-1]: Boron(III) bromide (150 mg, 0.6 mmol) was added dropwise at 0 °C under a nitrogen atmosphere to a solution of 6,8-dimethoxy-1-methyl-1H-cyclopenta[*c*]isoquinoline-3,5(2H,4H)-dione **D** (28 mg, 0.1 mmol) in dry dichloromethane (2 mL). One hour later, the

reaction mixture was quenched by the addition of 1 M HCl (3 mL). The resulting solution was extracted with CH_2Cl_2 (20 mL), washed sequentially with H_2O (40 mL), dried over Na_2SO_4, and evaporated to give a residue that was chromatographed over Sephadex LH20 with MeOH/H_2O 1:1 to furnish (±)-**1** (24 mg, 0.092 mmol, 92%) as a white solid. $[\alpha]_D^{25} = 0°$; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 12.85 (br s, 1H), 8.70 (br s, 1H), 6.71 (d, J = 2.1 Hz, 1H), 6.67 (d, J = 2.1 Hz, 1H), 3.92 (s, 3H), 3.52 (m, 1H), 3.00 (dd, J = 19.2,6.6 Hz, 1H), 2.36 (dd, J = 19.2,1.2 Hz, 1H), 1.45 ppm (d, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 197.8, 165.6, 165.1, 164.9, 144.0, 135.6, 134.3, 107.5, 102.6, 101.4, 55.8, 44.2, 30.2, 21.2 ppm; HR-ESI-MS: m/z: calcd for $C_{14}H_{13}NO_4$: 259.08: found: 282.0751 $[M+Na]^+$.

Chiral HPLC separation of (±)-1: The racemic alternarlactam [(±)-**1**] was separated over a Chiralpak AS-H column (4.6×250 mm, Daicel Chemical Ltd) at 40 °C using methanol/DEA (100:0.1, v/v) as mobile phase at a flow rate of 1.0 mL min^{-1}. The retention time of the two single enantiomers was 8.67 [(+)-**1**] and 11.81 min [(−)-**1**], respectively, with the optical purity (>99% *ee*) checked also by the chiral HPLC. The ESI-MS, ^{1}H and ^{13}C NMR spectra of (+)-**1** ($[\alpha]_D^{25} = +20.3°$) was identical with those of (−)-**1**.

Cytotoxicity evaluation: The cytotoxicity was tested on the HeLa (human cervix HeLa adenocarcinoma) and QSG-7701 (human hepatocellular carcinoma 7701) cell lines by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[31] Briefly, the test cells at the exponential growth phase were collected and transferred into 96-well plates. After incubation for 24 h, compound dilutions were dispensed to the established culture plates. Two days (48 h) later, the MTT solution (0.1 mg per well) was then added to each well. After further incubation for 4 h, the supernatant was removed, the crystals were fully dissolved in DMSO (150 μL), and the absorbance of each well was read at 570 nm. The IC_{50} value was determined as the concentration, at which a half of the test cell growth was inhibited. The experiment was performed in triplicate, and the data expressed as means ± SD.[32]

ECD calculations: Density functional theory (DFT) at B3LYP/6-31+G(d,p) level was employed to optimize the geometry of (−)-**1** and (+)-**1**. Subsequently, the corresponding TDDFT calculations with the same basis set were performed on the basis of the ground-state optimized structures. The ECD calculation was accomplished by utilizing the combined explicit solvent/PCM (polarized continuum model; CH_3OH solvent: dielectric constant ε = 32.63), which was demonstrated to be effective in describing solvent effects.[33,34] The final ECD spectra were obtained according to the following equations:[35]

$$\Delta\varepsilon(\lambda) = \sum_n \Delta\varepsilon_n \exp\left[-\left(\frac{\lambda-\lambda_n}{\Delta\lambda_n}\right)^2\right] \quad (1)$$

$$\Delta\varepsilon_n = \frac{\lambda_n R_n}{22.94\sqrt{\pi}\Delta\lambda_n} \times 10^{40} \quad (2)$$

where $\Delta\varepsilon_n$ was the peak intensity given in L mol^{-1} cm^{-1}, R_n was the rotatory strength in unit of 10^{-40} cgs, λ_n was the wavelength of the nth transition, and $\Delta\lambda_n$ was the half-width at 1/*e* of peak maximum. Here we used a half-width $\Delta\lambda_n = \lambda_n^2\Delta\tilde{\nu}$ with $\Delta\tilde{\nu}$ = 1200 cm^{-1} for the studied systems. All calculations were performed with the Gaussian03 program.[17]

VCD calculations: Since VCD spectrum is conformationally sensitive, systematic QM conformation search for the ternary aggregates of (−)-**1** are firstly carried out. Then the VCD calculations are performed to get the frequencies and rotational strengths. For the vibrational frequencies, an empirical scaling factor of 0.945 was used to achieve the better agreement with the experimental results. Such a scaling factor is comparable to the reported values.[36] The IR absorption and VCD intensity were converted to the corresponding spectra using a Lorentzian function (with the full line width at half maximum of 8 cm^{-1}). The calculated dipole strengths and rotational strength are scaled 7.1×10^{-4} and 4.3×10^{-4} to get better agreement with the experiments. Since the three-parameter hybrid approach using the Perdew and Wang correction functional (B3PW91) works well in predicting the IR and VCD spectra for many chiral molecules with chainlike, cylic, and even spherical cage structures,[37,38] the B3PW91/6-31+G(d,p) is employed here. The IR and VCD calculations are also performed with the Gaussian03 program.[17]

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