JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Potential Allelopathic Indole Diketopiperazines Produced by the Plant Endophytic *Aspergillus fumigatus* Using the One Strain−Many Compounds Method

Qiang Zhang, Shi-Qiong Wang, Hao-Yu Tang, Xiao-Jun Li, Lu Zhang, Jian Xiao, Yu-Qi Gao, An-Ling Zhang, and Jin-Ming Gao*

Shaanxi Engineering Center of Bioresource Chemistry & Sustainable Utilization, College of Science, Northwest A&F University, Yangling 712100, Shaanxi, China

Supporting Information

ABSTRACT: On the basis of the OSMAC (one strain−many compounds) strategy, 14 indole diketopiperazine (DKP) alkaloids, including spirotryprostatins (**1**−**3**), tryprostatins (**4**−**6**), and cyclotryprostatins (**7**−**14**), were isolated from the endophyte *Aspergillus fumigatus* associated with *Melia azedarach* L. Their structures were identified by nuclear magnetic resonance and electrospray ionization mass spectrometry data. All the indole DKPs were evaluated for plant growth regulation using the lettuce (*Lactuca sativa*) seedling growth bioassay, which showed the plant growth influence of the seedling. Among these compounds tested, a tryprostatin-type compound, brevianamide F (**6**), was identified as a new type of natural potential plant growth inhibitor with a response index (RI) higher than that of the positive control glyphosate, a broad-spectrum systemic herbicide. **6** can also inhibit turnip (*Raphanus sativus*) shoot and root elongation with RIs of −0.76 and −0.70, respectively, at 120 ppm, and it strongly inhibits amaranth (*Amaranthus mangostanus*) seedling growth with a high RI of −0.9 at 40 ppm. The structure−allelopathic activity relationship analysis of these isolated alkaloids indicates that tryprostatin-type alkaloids without the C_5 prenyl and methoxy group give the most potent inhibition of seedling growth. Brevianamide F (**6**) could be used to develop a natural eco-friendly herbicide.

KEYWORDS: *Melia azedarach, Aspergillus fumigatus, herbicides, allelochemicals, indole diketopiperazines, phytotoxicity*

INTRODUCTION

In natural ecological systems, endophytic microorganisms play an important role by shaping plant communities and mediating ecological interactions.[1] Their influences on community biodiversity and microbial interactions have been shown to be important determinants of plant biodiversity.[2] Because of the ecological roles of endophytes, the chemical components in the endophytic microorganism become attractive and sustainable for agricultural pharmacology. In recent years, many allelopathic compounds were recognized from plant endophytic fungi.[3−5] The term allelopathy is generally considered as a form of negative chemical communication between organisms, whereby one participant releases metabolites into the environment to negatively impact other participants.[6] Those negative effects also play a major role in agricultural management such as weed control, crop protection, and crop reestablishment.[7] The incorporation of natural or natural-like allelopathic substances into agricultural management, which is a well-known practice, may reduce the level of use of toxic agrochemicals and ease environmental deterioration.[8−11]

As a common type of metabolite in those plant endophytes, especially *Aspergillus*, diketopiperazines (DKPs) were reported to have a broad bioactivity spectrum,[12−16] including cytotoxicity,[17] antibiotic activity,[18] and cancer invasion inhibitory activity.[19] Some DKPs, such as cyclo(L-Pro-L-Val), cyclo(L-Pro-L-Phe), and cyclo(L-Pro-L-Tyr), play an important role in plant−bacterium interactions by mediating prokaryote−eukaryote transkingdom signaling.[20] In recent years, DKPs were considered as a potential type of plant growth regulator,[21,22] such as brevicompanine C that accelerated the root growth of lettuce seedlings.[23] In our search for ecological metabolites from endophytes,[24−28] cyclo(L-Pro-L-Tyr) was found to potentially inhibit lettuce germination.[29] However, as the derivatives of indole acetic acid, indole DKPs were still not known for their allelopathic functions. Previously, we found that the endophytic fungi *Aspergillus fumigatus* associated with *Melia azedarach* can produce structurally diverse indole DKPs.[27] In fact, many microorganisms harbor significant numbers of secondary metabolite-encoding biosynthetic pathways, yet only a few of their metabolites are detected in the laboratory. Natural chemical diversity can be changed systematically by altering cultivation parameters to limit strains. This practical and costless approach allows the elucidation of a hidden reservoir of complex chemical diversity in the specific strain that is a so-called OSMAC (one strain−many compounds) strategy.[30] Thus, we subjected strain *A. fumigatus* LN-4 to the costless and pollution-free OSMAC approach to produce a series of indole DKPs, to explore their structures and allelopathic activity relationships (SAR). In this paper, we report the isolation of indole DKPs produced by *A. fumigatus* utilizing the OSMAC approach and a detailed investigation of

Received: July 25, 2013
Revised: October 31, 2013
Accepted: November 4, 2013
Published: November 4, 2013

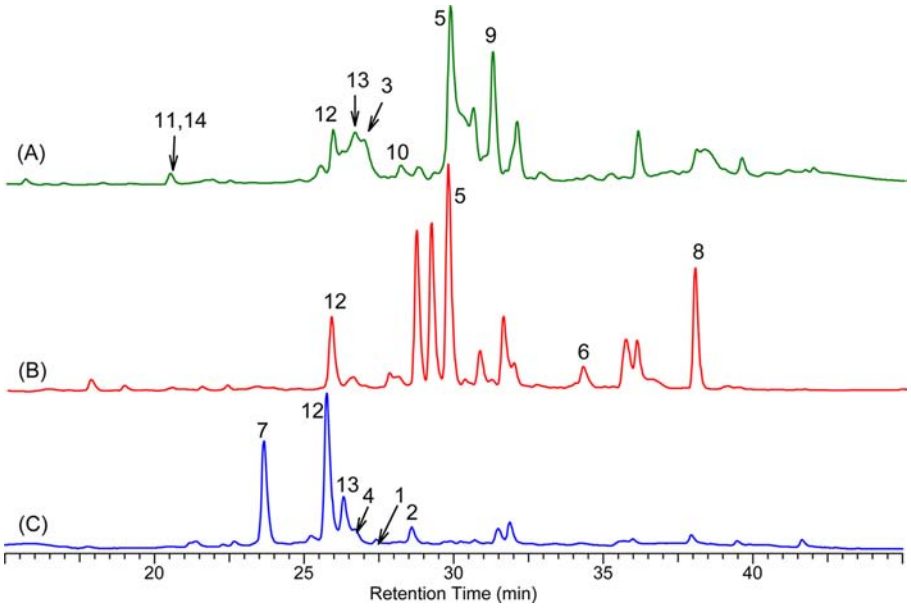

Figure 1. HPLC profiles of EtOAc extraction of *A. fumigatus* cultivated with (A) SP, (B) rice, and (C) PDB detected by UV absorption at 295 nm.

their allelopathy on three seedlings, lettuce, turnip, and amaranth.

MATERIALS AND METHODS

General Procedure. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE III (500 MHz) instrument. Chemical shifts were calculated using solvent residual as the internal standard. Electrospray ionization mass spectrometry (ESI-MS) was conducted with a Thermo LCQ Fleet instrument. Column chromatography (CC) was performed on silica gel (90−150 μm; Qingdao Marine Chemical Inc., Qingdao, China), MCI gel (75−150 μm; Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40−63 μm; Merck, Darmstadt, Germany). GF_{254} plates (Qingdao Marine Chemical Inc.) were used for thin-layer chromatography (TLC). High-performance liquid chromatography (HPLC) analysis was performed on an Angilent TC-C_{18} column (250 mm × 4.6 mm) on a Waters 1525 instrument (Waters Corp.). In the separation procedure, all the indole DKPs were inspected by TLC (*p*-dimethylaminobenzaldehyde as a color developing agent) and HPLC (UV at 258 and 295 nm) analysis.

Fungal Material. Fungal strain *A. fumigatus* LN-4 was isolated from a healthy stem bark of *M. azedarach* that was growing in Northwest A&F University and was deposited at the College of Science, Northwest A&F University.

Fermentation and HPLC Analysis. The seed strain was prepared in PDB medium (200 g of potato and 20 g of agar in 1000 mL of water) at 28 °C for 3 days on a rotary shaker at 140 rpm. The seed liquids were inoculated separately in three different media, PDB, sterilized rice, and SP solid medium. Fermentation was conducted in 500 mL Erlenmeyer flasks each containing 200 mL of medium at 28 °C. SP solid fermentation and production separation were reported previously.[27] The PDB fermentation (75 L) was kept on a rotary shaker at 140 rpm for 7 days, while the sterilized rice fermentation (12 L) was kept for 25 days.

Ethyl acetate extracts of the three fermentations were analyzed by HPLC eluted with a methanol gradient (from 10 to 100% in 40 min) and a further 100% methanol for 10 min. The chromatograms were detected by a UV detector, and compounds **1**−**14** are denoted in the respective spectrum (Figure 1).

Extraction and Isolation of Metabolites. The PDB culture broth was extracted with EtOAc three times to yield 48 g of extract. The crude extract was then subjected to a silica gel column and eluted with a chloroform/MeOH mixture (100:1, 50:1, and 20:1) to give three fractions (Fr.1−Fr.3). Fr.1 and Fr.2 containing indole DKP alkaloids were detected and chromatographed on an MCI gel eluted with a MeOH/H_2O mixture (1:9 to 9:1) separately. Fr.1 was further purified on Sephadex LH-20, silica gel, and preparative TLC to afford **7** (12 mg). Fr.2 was further chromatographed by CC on RP-18, Sephadex LH-20, silica gel, and PTCL to yield compounds **1** (5.8 mg), **2** (16 mg), **4** (2.3 mg), and **9** (12 mg).

The rice medium product was extracted with acetone four times. The extract was then scattered in 50% MeOH and further extracted with chloroform five times. The chloroform portion (57 g) was subjected to a silica gel and eluted with a chloroform/MeOH mixture (100:1 and 50:1) to afford two fractions, Fr.1 and Fr.2. Both fractions were further chromatographed sequentially on an MCI gel (MeOH/H_2O mixture from 10% to 90%), Sephadex LH-20 (MeOH), and silica gel to afford compounds **8** (100 mg) and **6** (15 mg).

Other compounds were isolated from SP solid medium. The detailed separation procedure was reported previously.[27]

Allelopathic Bioassay. The seeds of three herbaceous plants, lettuce (*Lactuca sativa*), turnip (*Raphanus sativus*), and amaranth (*Amaranthus mangostanus*), were used for the bioassay. The procedure was conducted according to the reported protocol.[31] The plant seeds was washed with running water for 2 h, soaked in 0.3% $KMnO_4$ for 15 min, and flushed until they were colorless. The compounds, positive control and blank solvent acetone, were added to 12-well plates with filter paper to final concentrations of 200 and 50 ppm. After the evaporation of acetone, the plant seeds were sown in the microdishes of 12-well plates and irrigated with deionized water. Triplicate experiments were conducted. The plates were then incubated at 24 °C for 90 h, and the germination rates were calculated according to eq 1. Allelopathic effects [response index (RI)] were calculated according to eq 2.[32]

$$\text{germination rate (\%)} = (\text{number of germinated seeds})/(\text{total number of seeds}) \quad (1)$$

$$\text{If } T > C, \text{ then RI} = 1 - C/T; \text{ if } T < C, \text{ then RI} = T/C - 1 \quad (2)$$

where T is the length of the treatment, C is the length of the blank control, and RI is the response index.

Germination rates and RIs were expressed as means ± the standard deviation (SD) for three replicates. All of the statistical differences

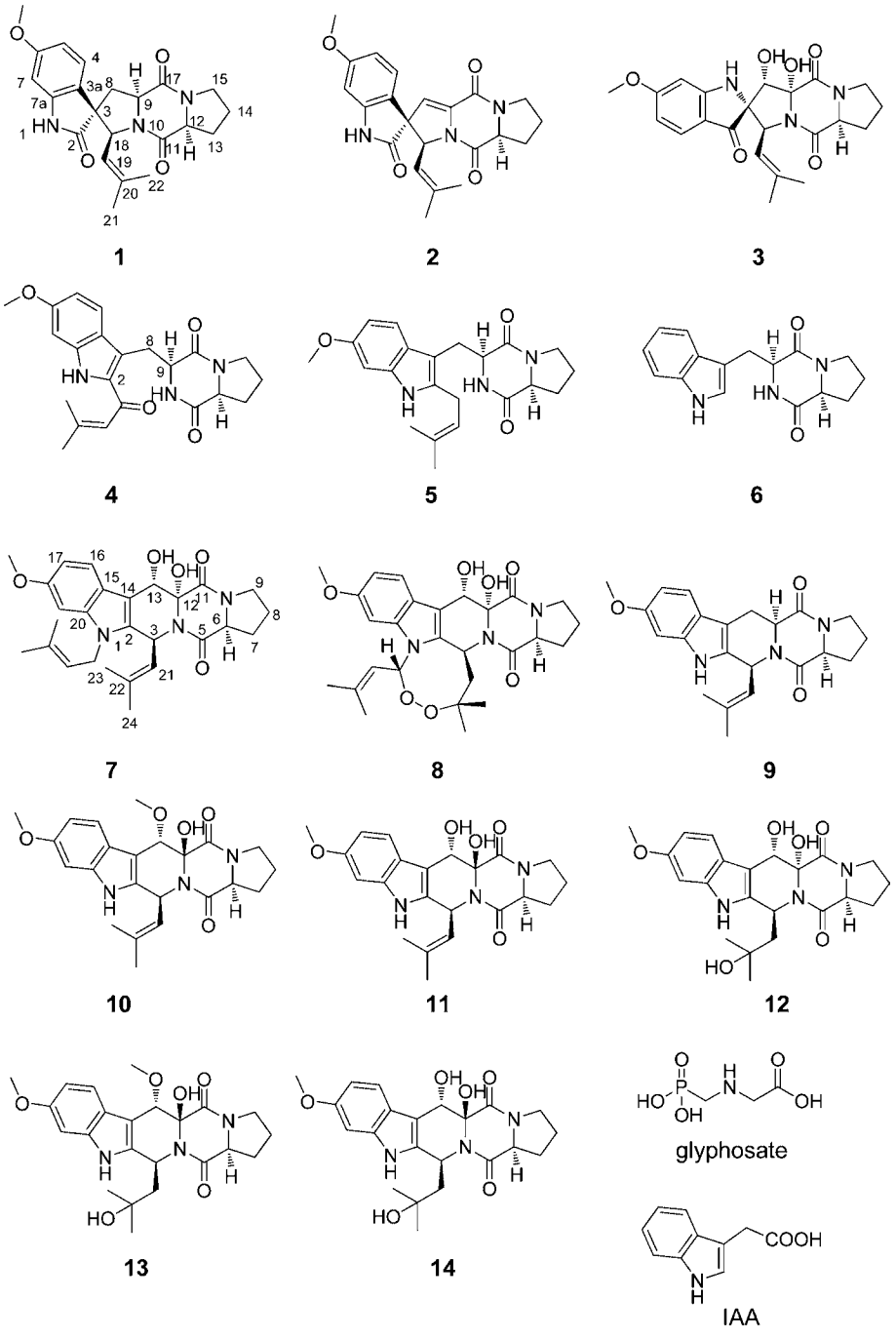

Figure 2. Structures of glyphosate, IAA, and metabolites **1**−**14** isolated from *A. fumigatus* LN-4.

were compared by SPSS software using one-way analysis of variance (ANOVA) at an α = 0.05 significance level.

RESULTS AND DISCUSSION

HPLC Analysis of Crude Extracts. Fungal strain *A. fumigatus* LN-4 was grown in three different media, SP, rice, and PDB, to provide three culture broths, and then the broths were extracted with EtOAc to offer three corresponding crude extracts (**A**, **B**, and **C**, respectively). All the crude extracts were analyzed by HPLC with indole characteristic UV absorption at 258 and 295 nm. The resulting chromatograms (Figure 1) showed significant differences in components among these crude extracts, such as components **3**, **10**, **11**, and **14** in **A**, **6** and **8** in **B**, and **1** and **7** in **C**. These results demonstrated that the OSMAC strategy could effectively produce the diversified natural compounds.

Identification of Fungal Metabolites. Chemical investigations of *A. fumigatus* LN-4 crude extracts obtained from three different media led to the isolation of a series of indole DKPs (Figure 2) by multiple chromatographic procedures: spirotryprostatin A (**1**),[33,34] 6-methoxyspirotryprostatin B (**2**),[35] compound **3**,[36] 18-oxotryprostatin A (**4**),[35] tryprostatin A (**5**),[37] brevianamide F (**6**),[38−40] fumitremorgin B (**7**),[41] verruculogen (**8**),[36,42] 12α-fumitremorgin C (**9**),[36,43]cyclotryprostatin B (**10**),[44] cyclotryprostatin A (**11**),[27] verruculogen TR-2 (**12**),[45]12β-hydroxy-13α-methoxyverruculogen TR-2 (**13**),[27] and 12β-hydroxyverruculogen TR-2 (**14**).[27] Their structures were identified on the basis of comparison of

Table 1. Allelopathic Effects on Lettuce (*L. sativa*) of Compounds 1−14[a]

	germination rate		shoot elongation (RI)		root elongation (RI)	
	200 ppm	50 ppm	200 ppm	50 ppm	200 ppm	50 ppm
1	0.85 ± 0.06 ab	0.70 ± 0.05 bcd	−0.24 ± 0.02 d	−0.25 ± 0.02 d	−0.28 ± 0.04 ef	0.30 ± 0.00 a
2	0.96 ± 0.06 a	0.76 ± 0.05 bc	−0.21 ± 0.04 d	−0.12 ± 0.01 c	0.13 ± 0.01 b	−0.35 ± 0.07 i
3	0.82 ± 0.06 b	0.93 ± 0.06 a	−0.13 ± 0.02 c	−0.14 ± 0.01 c	0.22 ± 0.04 b	0.28 ± 0.02 ab
4	0.82 ± 0.06 b	0.58 ± 0.05 e	−0.06 ± 0.02 b	−0.12 ± 0.02 c	−0.34 ± 0.09 f	−0.29 ± 0.02 h
5	0.82 ± 0.06 b	0.89 ± 0.00 a	−0.13 ± 0.00 c	0.05 ± 0.00 b	−0.17 ± 0.13 d	0.23 ± 0.04 bc
6	0.54 ± 0.08 e	0.92 ± 0.07 a	−0.91 ± 0.01 f	−0.65 ± 0.07 e	−0.88 ± 0.02 h	−0.57 ± 0.04 j
7	0.63 ± 0.06 de	0.70 ± 0.05 bcd	−0.32 ± 0.02 e	−0.08 ± 0.01 c	−0.36 ± 0.07 f	0.18 ± 0.00 cd
8	0.79 ± 0.08 b	0.63 ± 0.06 de	0.08 ± 0.03 a	−0.27 ± 0.09 d	0.41 ± 0.01 a	0.05 ± 0.01 e
9	0.63 ± 0.06 de	0.67 ± 0.05 cde	0.03 ± 0.01 a	−0.27 ± 0.04 d	0.20 ± 0.02 b	−0.08 ± 0.03 g
10	0.74 ± 0.06 bcd	0.61 ± 0.05 de	−0.33 ± 0.02 e	−0.17 ± 0.02 cd	0.00 ± 0.00 c	−0.02 ± 0.00 f
11	0.74 ± 0.06 bcd	0.76 ± 0.05 bc	0.03 ± 0.02 a	0.09 ± 0.07 b	−0.21 ± 0.07 de	0.05 ± 0.05 e
12	0.85 ± 0.06 ab	0.70 ± 0.05 bcd	−0.25 ± 0.01 d	−0.10 ± 0.03 c	0.21 ± 0.02 b	0.13 ± 0.03 d
13	0.85 ± 0.06 ab	0.64 ± 0.00 de	0.04 ± 0.01 a	−0.09 ± 0.03 c	0.19 ± 0.03 b	0.07 ± 0.02 e
14	0.78 ± 0.00 bc	0.67 ± 0.05 cde	−0.21 ± 0.01 d	0.47 ± 0.02 a	−0.05 ± 0.01 c	0.16 ± 0.03 d
gp	0.67 ± 0.08 cd	0.61 ± 0.05 de	−0.34 ± 0.10 e	−0.26 ± 0.16 d	−0.73 ± 0.08 g	−0.72 ± 0.06 k
ck	0.82 ± 0.06 b	0.78 ± 0.00 b				

[a]Means (±SD) within columns followed by the same letter are not significantly different at $p < 0.05$. gp stands for glyphosate and ck for the blank control.

their NMR and ESI-MS data with those reported. All the isolated structures can be divided into three types: spirotryprostatins (**1**−**3**) with the spiro atom at C-3, tryprostatins (**4**−**6**) with the *seco* B ring, and cyclotryprostatins (**7**−**14**).

Evaluation of Allelopathic Activity. All isolated compounds were evaluated for allelopathic activity against lettuce (*L. sativa*) seeds using our previously reported assay[31] by determining the germination rates and seedling growth (root and shoot elongation) with respect to the control, glyphosate, a broad-spectrum systemic herbicide.[25] The RI was selected as an evaluation indicator, which ranges from −1 to 1, with positive values indicating stimulation by the treatments and negative values indicating inhibition by them. The RI was considered to be superior to the *T*/*C* statistical method and easy to interpret because it is simply the proportional reduction of the treatment relative to the control.[32]

As shown in Table 1, spirotryprostatin-type DKPs **1**−**3** can inhibit the elongation of lettuce shoots (RI values ranging from −0.12 to −0.25 at 50 and 200 ppm), while being less active than the positive control glyphosate (RI values of −0.26 at 50 ppm and −0.34 at 200 ppm). Compared to that of the most active compound, **1**, the dehydrogenation or hydroxylation of C-8 and C-9 for **2** and **3** caused weak inhibition (RI values of −0.21 and −0.13 at 200 ppm). However, **1**−**3** had a weak to moderate influence (RI values of −0.28 to 0.22 at 200 ppm) on root elongation in comparison to glyphosate. Additionally, their stimulation or inhibition effects are dependent on their concentrations. For example, **1** showed inhibition (RI = −0.28) on root elongation at higher concentrations of 200 ppm and stimulation (RI = 0.30) at lower concentrations of 50 ppm.

Tryprostatin-type seco-DKPs (**4**−**6**) were found to exhibit inhibitory effects on the shoot and root elongation of lettuce. In particular, brevianamide F (**6**) showed the prominent inhibition of shoot and root elongation (−0.91 and −0.88, respectively) at 200 ppm compared to those of the control glyphosate (−0.34 and −0.73, respectively). **6** showed 3-fold stronger inhibition of shoot elongation (RI = −0.91 at 200 ppm) than the control (RI = −0.34). Even at a lower concentration of 50 ppm, **6** exhibits 2-fold inhibition (RI = −0.65) of shoot elongation compared to that of glyphosate. In contrast, **4** and **5** containing a prenyl side chain and a methoxy group in the indole ring showed weaker inhibition of shoot growth (RI values ranging from −0.06 to −0.13 at 200 ppm). These results indicate that both the prenyl side chain and the methoxy group in the indole ring are not favorable for inhibitory effects (**6** vs **4** and **5**).

Cyclotryprostatin-type DKPs **7**−**14** all have a pentacyclic ring skeleton. Cyclotryprostatins **7**−**14** (except **11** and **14**) displayed inhibition of shoot elongation. Compounds **7** and **10** showed high RI values of −0.32 and −0.33, respectively, similar to those of the positive control (RI = −0.34) at 200 ppm, while **8** and **9** also showed a high RI of −0.27 similar to that of the control (−0.26) at 50 ppm. On the other hand, **8**, **9**, **12**, and **13** stimulated root elongation with RI values ranging from 0.19 to 0.41. In particular, compound **8**, containing the peroxide bridge, showed strong stimulation (RI = 0.41) at 200 ppm. The stimulation of **7** and **11** on the root growth, containing the C5 unit at C-3 and hydroxyl groups at C-12 and C-13 in this type of scaffold, was reversed to moderate inhibition. In contrast to **9**, both **7** and **8** with the dihydroxylation at C-12 and C-13 decreased or reversed growth regulation (**7** and **8** vs **9**). Especially **8** reversed course to stimulate root and shoot elongation. Other cyclotryprostatin analogues influenced seedling growth to a certain extent, and their effects (including stimulation and inhibition) can be reversed upon concentration. Our observations suggest that tryprostatin-type DKPs could play an important role in plant growth regulation.

The difference among the three types of DKP structures is the binding site of the C_5 substructure (C-18), as shown in Figure 3. When the C_5 unit attached to C-3 and N-10 to form spirotryprostatins or C-2 and N-10 to form cyclotryprostatins, their inhibition of seedling growth could become weaker than that of **6**. In addition, the hydroxylations at C-8 and C-9 and the methoxy to the indole ring will also weaken inhibition. The inhibition SAR of the three types of DKPs is summarized as shown in Figure 3.

As stated above, brevianamide F (**6**) was shown to be the best growth inhibitor, so further investigation of its phytotoxic activity was conducted. Compound **6** was examined for

Figure 3. SAR of DKPs on plant growth inhibition. The peroxide bridge enhances the stimulation. The red substituents weaken seedling growth inhibition.

inhibition of shoot and root elongation of lettuce at concentrations ranging from 5 to 120 ppm, and the results are given in panels a and b of Figure 4. The compound

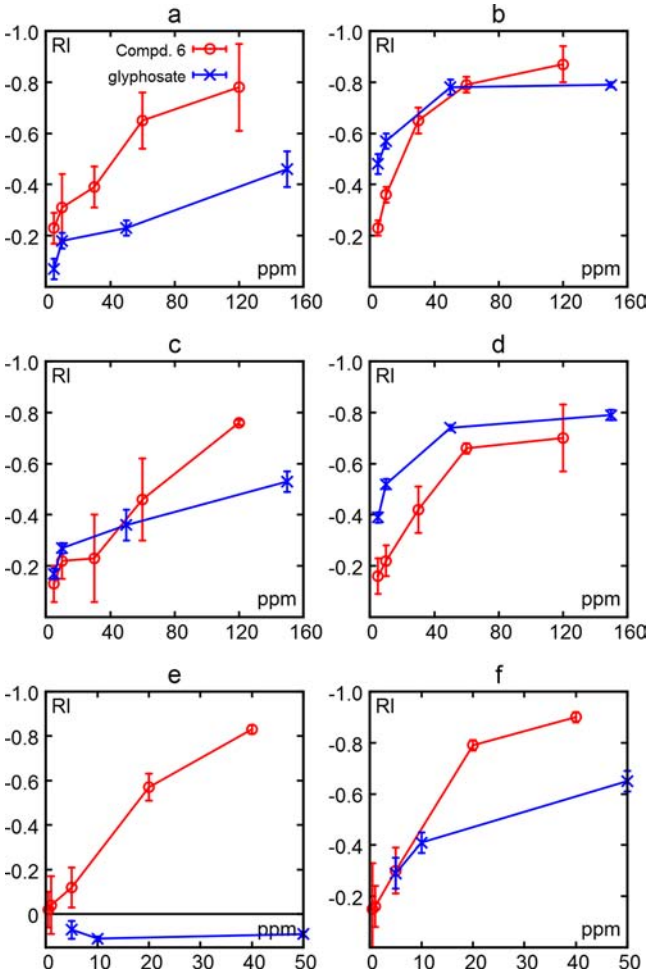

Figure 4. Inhibition by compound **6** and glyphosate of the elongation of *L. sativa* shoot (a) and root (b), *R. sativus* shoot (c) and root (d), and *A. mangostanus* shoot (e) and root (f) ($p < 0.05$).

inhibited lettuce seedling growth with RI values ranging from −0.23 to −0.87 in a dose-dependent manner. The observed inhibition by **6** of lettuce shoot growth was stronger than that of the control. At a lower concentration of 5 ppm, its RI was still up to −0.23. Furthermore, **6** exerted inhibition of root growth similar to that of glyphosate.

For a further allelopathic evaluation of **6**, its inhibition of the growth of other herbaceous seedlings was also assessed. Both turnip (*R. sativus*) and amaranth (*A. mangostanus*) were used in the bioassay because their seedlings were commonly used to evaluate allelopathic activities of organic molecules. Compound **6** showed potent turnip seedling inhibition (RI values ranging from −0.13 to −0.76) compared to that of glyphosate at concentrations ranging from 5 to 120 ppm (Figure 4c,d). In the case of amaranth seedlings, glyphosate showed no inhibition of shoot elongation, but **6** exhibited potentially potent inhibition of shoot and root elongation (−0.12 and −0.30, respectively), which is similar to that of the control (0.07 and −0.29, respectively) even at a quite lower concentration of 5 ppm. Its inhibition of shoot and root elongation reached dramatically up to an RI of −0.83 at a lower concentration of 40 ppm (Figure 4e,f). These findings revealed that **6** could be identified as a potential natural plant growth regulator.

Recently, three DKPs, cyclo(L-Pro-L-Val), cyclo(L-Pro-L-Phe), and cyclo(L-Pro-L-Tyr), were reported to have auxin-like activity in *Arabidopsis* because their heterocyclic system was similar to that of indole acetic acid [IAA (Figure 2)].[20] A reported molecular docking analysis indicated that DKPs might interact with the TIR1 auxin receptor.[20] However, because of the similar structure systems of IAA and compound **6**, the latter might block the interaction between IAA and the TIR1 auxin receptor to exhibit plant growth inhibition. Further studies in our lab aim to investigate the inhibition mechanism and ecological relevance of allelopathy with respect to the effective presence and persistence time.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Spectral characterizations (MS and NMR) of metabolites isolated and the data corresponding to Figure 4. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-29-87092515. E-mail: jinminggao@nwsuaf.edu.cn.

Author Contributions

Q.Z. and S.-Q.W. contributed equally to this work.

Funding

This work was financially supported by the National Natural Science Foundation of China (21102114 and 31371886) and the Chinese Universities Scientific Fund (QN2011118).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Zhang, H. W.; Song, Y. C.; Tan, R. X. Biology and chemistry of endophytes. *Nat. Prod. Rep.* **2006**, *23*, 753−771.

(2) Clay, K.; Holah, J. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* **1999**, *285*, 1742−1744.

(3) Gunatilaka, A. A. L. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *J. Nat. Prod.* **2006**, *69*, 509−526.

(4) Strobel, G.; Daisy, B.; Castillo, U.; Harper, J. Natural products from endophytic microorganisms. *J. Nat. Prod.* **2004**, *67*, 257−268.

(5) Gao, J. M.; Yang, S. X.; Qin, J. C. Azaphilones: Chemistry and biology. *Chem. Rev.* **2013**, *113*, 4755−4811.

(6) Cipollini, D.; Rigsby, C. M.; Barto, E. K. Microbes as targets and mediators of allelopathy in plants. *J. Chem. Ecol.* **2012**, *38*, 714−727.

(7) Gimenez, C.; Cabrera, R.; Reina, M.; Gonzalez-Coloma, A. Fungal endophytes and their role in plant protection. *Curr. Org. Chem.* **2007**, *11*, 707−720.

(8) Fischer, T. C.; Gosch, C.; Mirbeth, B.; Gselmann, M.; Thallmair, V.; Stich, K. Potent and specific bactericidal effect of juglone (5-

hydroxy-1,4-naphthoquinone) on the fire blight pathogen *Erwinia amylovora*. *J. Agric. Food Chem.* **2012**, *60*, 12074−12081.
(9) Queiroz, S. C. N.; Cantrell, C. L.; Duke, S. O.; Wedge, D. E.; Nandula, V. K.; Moraes, R. M.; Cerdeira, A. L. Bioassay-directed isolation and identification of phytotoxic and fungitoxic acetylenes from *Conyza canadensis*. *J. Agric. Food Chem.* **2012**, *60*, 5893−5898.
(10) Zhao, X.; Zheng, G.-W.; Niu, X.-M.; Li, W.-Q.; Wang, F.-S.; Li, S.-H. Terpenes from *Eupatorium adenophorum* and their allelopathic effects on *Arabidopsis* seed germination. *J. Agric. Food Chem.* **2009**, *57*, 478−482.
(11) Mai, F.; Glomb, M. A. Isolation of phenolic compounds from *Iceberg Lettuce* and impact on enzymatic browning. *J. Agric. Food Chem.* **2013**, *61*, 2868−2874.
(12) Borthwick, A. D. 2,5-Diketopiperazines: synthesis, reactions, medicinal chemistry, and bioactive natural products. *Chem. Rev.* **2012**, *112*, 3641−3716.
(13) Borthwick, A. D.; Liddle, J. The design of orally bioavailable 2, 5-diketopiperazine oxytocin antagonists: From concept to clinical candidate for premature labor. *Med. Res. Rev.* **2011**, *31*, 576−604.
(14) Cornacchia, C.; Cacciatore, I.; Baldassarre, L.; Mollica, A.; Felician, F.; Pinnen, F. 2,5-Diketopiperazines as neuroprotective agents. *Mini-Rev. Med. Chem.* **2012**, *12*, 2−12.
(15) de Carvalho, M. P.; Abraham, W. R. Antimicrobial and biofilm inhibiting diketopiperazines. *Curr. Med. Chem.* **2012**, *19*, 3564−3577.
(16) Li, X. J.; Tang, H. Y.; Duan, J. L.; Gao, J. M.; Xue, Q. H. Bioactive alkaloids produced by *Pseudomonas brassicacearum* subsp. Neoaurantiaca, an endophytic bacterium from *Salvia miltiorrhiza*. *Nat. Prod. Res.* **2013**, *27*, 496−499.
(17) Wang, Y.; Li, Z.-L.; Bai, J.; Zhang, L.-M.; Wu, X.; Zhang, L.; Pei, Y.-H.; Jing, Y.-K.; Hua, H.-M. 2,5-Diketopiperazines from the marine-derived fungus *Aspergillus fumigatus* YK-7. *Chem. Biodiversity* **2012**, *9*, 385−393.
(18) Park, H. B.; Kwon, H. C.; Lee, C.-H.; Yang, H. O. Glionitrin A, an antibiotic-antitumor metabolite derived from competitive interaction between abandoned mine microbes. *J. Nat. Prod.* **2009**, *72*, 248−252.
(19) Park, H. B.; Kim, Y.-J.; Park, J.-S.; Yang, H. O.; Lee, K. R.; Kwon, H. C. Glionitrin B, a cancer invasion inhibitory diketopiperazine produced by microbial coculture. *J. Nat. Prod.* **2011**, *74*, 2309−2312.
(20) Ortiz-Castro, R.; Diaz-Perez, C.; Martinez-Trujillo, M.; del Rio, R. E.; Campos-Garcia, J.; Lopez-Bucio, J. Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 7253−7258.
(21) Degrassi, G.; Aguilar, C.; Bosco, M.; Zahariev, S.; Pongor, S.; Venturi, V. Plant growth-promoting *Pseudomonas putida* WCS358 produces and secretes four cyclic dipeptides: Cross-talk with quorum sensing bacterial sensors. *Curr. Microbiol.* **2002**, *45*, 250−254.
(22) Cronan, J. M., Jr.; Davidson, T. R.; Singleton, F. L.; Colwell, R. R.; Cardellina, J. H., II Plant growth promoters isolated from a marine bacterium associated with *Palythoa* sp. *Nat. Prod. Lett.* **1998**, *11*, 271−278.
(23) Kimura, Y.; Sawada, A.; Kuramata, M.; Kusano, M.; Fujioka, S.; Kawano, T.; Shimada, A. Brevicompanine C, cyclo-(D-Ile-L-Trp), and cyclo-(D-Leu-L-Trp), plant growth regulators from *Penicillium brevicompactum*. *J. Nat. Prod.* **2005**, *68*, 237−239.
(24) Xue, M.; Zhang, Q.; Gao, J.-M.; Li, H.; Tian, J.-M.; Pescitelli, G. Chaetoglobosin V_b from endophytic *Chaetomium globosum*: absolute configuration of chaetoglobosins. *Chirality* **2012**, *24*, 668−674.
(25) Yang, S.-X.; Gao, J.-M.; Laatsch, H.; Tian, J.-M.; Pescitelli, G. Absolute configuration of fusarone, a new azaphilone from the endophytic fungus *Fusarium* sp. isolated from *Melia azedarach*, and of related azaphilones. *Chirality* **2012**, *24*, 621−627.
(26) Li, X.-J.; Gao, J.-M.; Chen, H.; Zhang, A.-L.; Tang, M. Toxins from a symbiotic fungus, *Leptographium qinlingensis* associated with *Dendroctonus armandi* and their in vitro toxicities to *Pinus armandi* seedlings. *Eur. J. Plant Pathol.* **2012**, *134*, 239−247.
(27) Li, X.-J.; Zhang, Q.; Zhang, A.-L.; Gao, J.-M. Metabolites from *Aspergillus fumigatus*, an endophytic fungus associated with *Melia azedarach*, and their antifungal, antifeedant, and toxic activities. *J. Agric. Food Chem.* **2012**, *60*, 3424−3431.
(28) Zhang, L.; Wang, S.-Q.; Li, X.-J.; Zhang, A.-L.; Zhang, Q.; Gao, J.-M. New insight into the stereochemistry of botryosphaeridione from a *Phoma* endophyte. *J. Mol. Struct.* **2012**, *1016*, 72−75.
(29) Wang, L.; Zheng, C.-D.; Li, X.-J.; Gao, J.-M.; Zhang, X.-C.; Wei, G.-H. Cyclo(PRO-TYR) from an endophytic rhizobium isolated from *Glycyrrhiza uralensis*. *Chem. Nat. Compd.* **2012**, *47*, 1040−1042.
(30) Bode, H. B.; Bethe, B.; Höfs, R.; Zeeck, A. Big effects from small changes: Possible ways to explore nature's chemical diversity. *ChemBioChem* **2002**, *3*, 619−627.
(31) Zhang, H.; Gao, J.-M.; Liu, W.-T.; Tang, J.-C.; Zhang, X.-C.; Jin, Z.-G.; Xu, Y.-P.; Shao, M.-A. Allelopathic substances from walnut (*Juglans regia* L.) leaves. *Allelopathy Journal* **2008**, *21*, 426−432.
(32) Bruce Williamson, G.; Richardson, D. Bioassays for allelopathy: Measuring treatment responses with independent controls. *J. Chem. Ecol.* **1988**, *14*, 181−187.
(33) Onishi, T.; Sebahar, P. R.; Williams, R. M. Concise, asymmetric total synthesis of spirotryprostatin A. *Org. Lett.* **2003**, *5*, 3135−3137.
(34) Cui, C.-B.; Kakeya, H.; Osada, H. Novel mammalian cell cycle inhibitors, spirotryprostatins A and B, produced by *Aspergillus fumigatus*, which inhibit mammalian cell cycle at G2/M phase. *Tetrahedron* **1996**, *52*, 12651−12666.
(35) Zhang, M.; Wang, W.-L.; Fang, Y.-C.; Zhu, T.-J.; Gu, Q.-Q.; Zhu, W.-M. Cytotoxic alkaloids and antibiotic nordammarane triterpenoids from the marine-derived fungus *Aspergillus sydowi*. *J. Nat. Prod.* **2008**, *71*, 985−989.
(36) Wang, F.; Fang, Y.; Zhu, T.; Zhang, M.; Lin, A.; Gu, Q.; Zhu, W. Seven new prenylated indole diketopiperazine alkaloids from holothurian-derived fungus *Aspergillus fumigatus*. *Tetrahedron* **2008**, *64*, 7986−7991.
(37) Cui, C.-B.; Kakeya, H.; Osada, H. Novel mammalian cell cycle inhibitors, tryprostatins A, B and other diketopiperazines produced by *Aspergillus fumigatus*. II. Physico-chemical properties and structures. *J. Antibiot.* **1996**, *49*, 534−540.
(38) Liu, Y.-X.; Ma, S.-G.; Wang, X.-J.; Zhao, N.; Qu, J.; Yu, S.-S.; Dai, J.-G.; Wang, Y.-H.; Si, Y.-K. Diketopiperazine alkaloids produced by the endophytic fungus *Aspergillus fumigatus* from the Stem of *Erythrophloeum fordii* Oliv. *Helv. Chim. Acta* **2012**, *95*, 1401−1408.
(39) Birch, A. J.; Russell, R. A. Studies in relation to biosynthesis. XLIV: Structural elucidations of brevianamides-B, -C, -D and -F. *Tetrahedron* **1972**, *28*, 2999−3008.
(40) Han, W. J.; Lu, X. L.; Xu, Q. Z.; Liu, X. Y.; Jiao, B. H. Isolation, identification and biological characterization of secondary metabolites produced by a marine *Bacillus subtilis*. *Dier Junyi Daxue Xuebao* **2008**, *29*, 1234−1238.
(41) Yamazaki, M.; Fujimoto, H.; Akiyama, T.; Sankawa, U.; Iitaka, Y. Crystal structure and absolute configuration of fumitremorgin B, a tremorgenic toxin from *Aspergillus fumigatus* fres. *Tetrahedron Lett.* **1975**, 27−28.
(42) Fayos, J.; Lokensgard, D.; Clardy, J.; Cole, R. J.; Kirksey, J. W. Structure of verruculogen, a tremor producing peroxide from *Penicillium verruculosum*. *J. Am. Chem. Soc.* **1974**, *96*, 6785−6787.
(43) Hino, T.; Kawate, T.; Nakagawa, M. A synthesis of so-called fumitremorgin C. *Tetrahedron* **1989**, *45*, 1941−1944.
(44) Cui, C.-B.; Kakeya, H.; Osada, H. Novel mammalian cell cycle inhibitors, cyclotroprostatins A−D, produced by *Aspergillus fumigatus*, which inhibit mammalian cell cycle at G2/M phase. *Tetrahedron* **1997**, *53*, 59−72.
(45) Cole, R. J.; Kirksey, J. W.; Cox, R. H.; Clardy, J. Structure of the tremor-producing indole, TR-2. *J. Agric. Food Chem.* **1975**, *23*, 1015−1018.