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Essential structural features of (*2Z,4E*)-5-phenylpenta-2,4-dienoic acid for inhibition of root gravitropism

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Abstract

Previously, we found (*2Z,4E*)-5-phenylpenta-2,4-dienoic acid (**ku-76**) to be a selective inhibitor of root gravitropic bending of lettuce radicles at 5 μM, with no concomitant growth inhibition. Here, we describe a structure-activity relationship study of **ku-76** to determine the essential structural features for the inhibitory activity. A series of **ku-76** analogues was synthesized and the key features of **ku-76** that are necessary for inhibition of lettuce root gravitropic bending were determined. The (*2E,4E*)-, (*2Z,4Z*)- (*2E,4Z*)-analogues were inactive, and 4,5-saturated and 4,5-alkynyl analogues also did not show inhibitory activity, demonstrating the importance of the (*2Z,4E*) diene unit. The aromatic ring was also crucial and could not be replaced with an alkyl chain. Derivatives in which the carboxylic acid was replaced with amides, alcohols, or esters were much less potent. These results suggest that the (2*Z*,4*E*)-diene, the carboxylic acid moiety, and the aromatic ring are essential for potent inhibitory activity against gravitropic bending.

Key Words

Lactuca sativa, Compositae, gravitropism, inhibition, organic synthesis, structure-activity relationship study, *cis*-cinnamic acid

1. Introduction

Gravitropism is the developing curvature of a growing plant organ in response to gravity. Plant organs perceive gravity as a directional environmental cue to modulate their growth orientation. Generally, roots grow downward (positive gravitropism) and stems grow upward against gravity (negative gravitropism). Gravitropism comprises several sequential processes, which are gravity perception, signal transduction, intercellular signal transmission, and asymmetric organ growth (Morita and Tasaka, 2004; Morita, 2010). With regard to gravity perception, the starch-statolith hypothesis has been widely accepted based on abundant experimental evidence (Kiss et al., 1989)(Kiss et al., 1997)(Sack, 1997). Amyloplasts, which are plastids that accumulate high-density starch, sediment in the direction of gravity within specialized gravity sensing cell. In roots, columella cells in root caps at the root tip serve as gravity sensing cells (Blancaflor et al., 1998). Amyloplast sedimentation is thought to elicit signal transduction, leading to a change in the direction of auxin transport in columella cells (Friml et al., 2002)(Harrison and Masson, 2008). Auxins, a class of phytohormones exemplified by indole-3-acetic acid (IAA), play a number of key roles in the gravitropic responsive process from signal transmission to the asymmetric organ growth. Auxins are directionally transported from the columella cells to lower flank of the root through the lateral root cap and the epidermis, resulting in asymmetric growth between the upper and lower flanks. As such, higher concentrations of auxins exhibit inhibitory effects on cell elongation in root (Su et al., 2017)Sato et al., 2015; (Nakamura et al., 2019).

As gravitropism is a crucial and rapid plant growth response, it has been used for screening chemicals to identify plant growth modulators (Surpin et al., 2005)(Nishimura et al., 2012)(Nishimura et al., 2014). Several compounds that inhibit auxin transport or auxin synthesis have been identified using this methodology, since auxin plays an important role in gravitropism. Figure 1 shows examples of various gravitropism inhibitors that were also found to be auxin transport inhibitors, such as 1-*N*-naphthylphthalamic acid (Mentzer et al., 1950), pyrenoylbenzoic acid (Schwuchow et al., 2001), gravacin (Surpin et al., 2005) (Rojas-Pierce et al., 2007), alkoxy-auxins (Tsuda et al., 2011), and as auxin biosynthesis inhibitor, yucasin DF (Tsugafune et al., 2017). In conjunction with the observed auxin transport inhibition, these compounds were bioactive in other processes, including growth inhibition. Recently, natural products such as the antimalarial drug artemisinin (Yan et al., 2018), which can be regarded as an allelochemical, have also been reported to affect gravitropism as well as plant growth. These examples show the difficulty in developing selective gravitropism inhibitor, mainly due to insufficient clarification of relationship between chemical structure and mode of action. Selective inhibitors of gravitropism that do not affect other plant growth events, would be a very useful tool to research on the mechanism of this phenomenon. Furthermore, selective root gravitropism inhibitors would constitute a new class of agrochemicals, with potential uses as plant growth regulators or weed suppressors.

Insert Figure 1

1-*O*-*cis*-Cinnamoyl-β-D-glucopyranose (*cis*-CA glycoside) isolated from *Spiraea thunbergii* Sieb is a potent allelochemical (Hiradate et al., 2005) and shows growth-inhibitory activity on root elongation of germinated seedlings of lettuce (*Lactuca sativa* L.). The aglycone, *cis*-CA, is essential for phytotoxicity in lettuce root elongation test. *cis*-CA is believed to have growth-promoting activity in plants (Went, 1935; (Koepfli et al., 1938), and *cis*-CA was also reported to promote cell elongation, but was less active than IAA in the Avena straight growth test, in pea split-stem curvature test (Went, 1935)Koepfli et al., 1938; Åberg, 1961) and in the Avena curvature test (Went, 1935). It was also shown to inhibit root growth in wheat, flax (Åberg, 1961), and *Avena* (Ugochukwu and Wain, 1968). Recently, Boerjan and Vanholme reported that *cis*-CA is neither an auxin agonist nor antagonist, and that it is not an inhibitor of auxin biosynthesis, but instead it is an inhibitor of auxin efflux (Steenackers et al., 2017). However, a systematic approach to studying SAR (structure-activity relationship) of *cis*-CA, including its chemical modification, has not been reported prior to our initial investigations. In 2012, we carried out the SAR study of *cis*-CA with hundreds of synthetic analogues (Nishikawa et al., 2013a; Nishikawa et al., 2013b) to determine the essential structural features of *cis*-CA for growth inhibitory activity. To obtain insight into the mechanism of action, we designed and synthesized fluorescent probes of *cis*-CA. The fluorescent imaging experiments suggested that the probes were localized at root cap of lettuce radicles (Fukuda et al., 2016). Since the root cap includes columella cells, which play a key role in gravity sensing as described above, we hypothesized that *cis*-CA would affect the machinery for the gravity sensing. Of interest to this, Yang et al demonstrated that gaseous *cis*-CA, contaminated by *trans*-CA, inhibited the negative gravitropic response of stems of etiolated tomato seedlings and young plants (Yang et al., 1999). However, as *cis*-CA is also a strong inhibitor of root growth, this disturbed the observation of gravitropism effects because the primary root did not elongate enough. Therefore, we endeavored to discover inhibitors of gravitropic bending that did not suppress primary root elongation among our synthetic analogues of *cis*-CA. After screening the synthetic *cis*-CA analogues, especially the less potent elongation inhibitory compounds (Okuda et al., 2014), we found that (*2Z,4E*)-5-phenylpenta-2,4-dienoic acid (**ku-76**, Figure 2) inhibited root gravitropism at 5 μM, but did not repress primary root elongation at the 5-50 μM (Fujii et al., 2016). As such, **ku-76** was considered to specifically target regulatory component of root gravitropic bending. Herein, we describe a SAR study of **ku-76** to determine the essential structural features necessary for inhibitory activity of gravitropic bending.

Insert Figure 2

2. Results and Discussion

2.1. Inhibitory activity test for root gravitropic bending

The inhibitory test for root gravitropic bending was conducted according to the following method: 3-day lettuce seedlings (*Lactuca sativa* L. cv. Great Lakes, Compositae) were transferred to agar plates containing test compounds (50 μM) and arranged so that the roots were parallel to the gravity vector. The seedlings were preincubated vertically for 1 h, and then were gravistimulated by turning by 90°. After 18 h, root images were captured by the camera and the angles (θ°) of gravitropic curvature as well as the length (cm) of the roots were measured (Figure 3).

Insert Figure 3

Figure 4 shows the effects of **ku-76** on lettuce gravitropism and Figure 5 presents an evaluation of the impact of **ku-76** on gravitropic bending inhibition. The curvature angles (θ) of the roots treated by **ku-76** (Fig. 4A(b) **A** and **B**) at 10 and 5 μM, respectively, were under 30° and between 30° and 60° at 1 μM (Fig. 4A(b) **C**), while those of the non-treated roots were over 60° (Fig. 4A(a)). The curvature angles of the roots were dependent on the concentration of the compound, while the root length seemed to be significantly elongated (near to 150-180% against mock), as shown on the horizontal axes of **A**, **B**, and **C** of Figure 5.

Insert Figure 4-1 and 4-2

2.2. Synthesis of ***ku-76*** *analogues*

To elucidate the essential structural features of **ku-76** that are important for inhibitory activity of gravitropic bending, we conducted a structure–activity relationship study using **ku-76** as a base structure for the synthesis of various analogues. To design the synthetic analogues, this compound was divided into three units (Figure 5): (a) conjugated diene (b) aromatic, and (c) carboxylic acid groups, which were converted into other moieties, including stereoisomers.

Insert Figure 5

The characteristic structural features of **ku-76** are the (*2Z,4E*)-diene, carboxylate and aromatic moieties. As such, preparation of analogues of **ku-76** including skeletal analogues comprising stereoisomers (**1a-c**), C4-alkyl and alkynyl analogues (**1d** and **1e**), an acyclic, non-aromatic analogue (**1f**), and analogues with different functional groups from carboxylic acid such as carboxamides (**2a** and **2b**), alcohol (**2c**) and ester (**2d**) was performed (Figure 6).

Insert Figure 6

Synthesis of the analogues is shown in Figure S1 (see supplemental information). **Ku-76** and **2d** were synthesized according to our previous report (Abe et al., 2012), and **1a** is also a known compound (Aung et al., 2011). The (2*E)*- and (2*Z)*-alkenes were constructed using the classical Horner-Emmons reaction and the Ando-modified methodology (Ando, 1997), respectively, using the corresponding aldehydes. **1b** and **1c** were synthesized starting from *cis*-cinnamaldehyde, prepared from ethyl *cis*-cinnamate. The 4,5-dihydro analogue **1d** and 4-alkynyl compound **1e** were synthesized from the corresponding commercially available aldehydes by the same protocol. The aliphatic analogues **1f** was also prepared via the same protocol.

The amides **2a** and **2b** were prepared by condensation of the corresponding acid chloride with amines. The alcohol, **2c**, was furnished by reduction of the ester (Figure S2, see supplemental information).

2.3. Evaluation and discussion

These analogues were evaluated for the gravitropic bending and elongation inhibitory activities of lettuce roots. As shown in Figure 7, the (*2E,4E*)-, (*2Z,4Z*)-, and (*2E,4Z*)-isomers (**1a**, **1b**, and **1c**, respectively) were not potent inhibitors of either gravitropic bending or elongation at 50 μM. The 4,5-dihydro analogue, **1d** did not show statistically significant activity, and the experimental results for the alkynyl **1e** showed a large degree of variation. From these experiments, it was concluded that (*2Z,4E*)-dienyl skeleton and stereochemistry are crucial for this inhibitory activity, but the (*4E*)-alkene may not be essential. The aliphatic analogue **1f** did not exhibit any gravitropic bending inhibition. This result suggested that the presence of the aromatic group is also critical for inhibitory activity.

The analogues with amides (**2a**, **2b**), alcohol (**2c**), and ester (**2d**) moieties in place of carboxylic acid in **ku-76** were inactive (Figure 8). While the tertiary amide (**2a**) was found to be an elongation inhibitor without gravitropic bending inhibition, the primary amide (**2b**), which inhibited neither gravitropic bending nor elongation, seemed to make the roots more sensitive to gravity stimulation as the root curvature was observed to be at almost a right angle.

From these experimental results, the essential structural features of **ku-76** that are necessary for its action as an inhibitor of gravitropic bending were found to be the benzene ring, a (*2Z*)-configuration of the diene unit, and the carboxylic acid group (Figure 9). The (*4E*)-olefin of the diene is important, but can be altered into alkynyl moiety.

3. Conclusion

Amongst a number of *cis*-CA synthetic analogues, **ku-76** was identified as a new lead selective inhibitor of gravitropic bending and does not exhibit concomitant growth inhibition. Based on the chemical structure of **ku-76**, three units, the benzene ring, the dienyl carbon chain and the carboxylic acid were targeted as points of interest to generate stereoisomeric or functional group derivatives to elucidate essential structural features. A set of new compounds exhibiting other functionalities or stereoisomers was generated via organic synthesis. Using these analogues, it was determined that the presence of the benzene ring, a (*2Z,4E*)-configuration of the alkene and the carboxylic acid are crucial for the inhibitory activity of gravitropic bending.

Ku-76 showed inhibitory activity of gravitropic bending in lettuce roots at a minimum concentration of 5 μM. In contrast to most known inhibitors that also tend to inhibit elongation of roots due to their auxin transport inhibitory activity, this side effect was not observed for **ku-76** up to 10 μM. Considering the phenotype of seedlings treated with **ku-76**, it is possible that **ku-76** inhibits gravity sensing, signaling, or auxin transport in root gravitropism. Although most inhibitors for auxin transport inhibit root gravitropism as well as root elongation, 1-NOA perturbed root gravitropism without inhibition of root elongation up to 30 μM in *Arabidopsis thaliana* (Parry et al., 2001), which is similar to the effect of **ku-76** on lettuce seedlings. Since 1-NOA is indicated to be an inhibitor of auxin influx mediated by *Arabidopsis* AUX1 (Marchant et al., 1999; Yang et al, 2006), **ku-76** might inhibit auxin influx in lettuce seedlings as well, although further study is needed to understand the mode-of-action of **ku-76**.

This study is a useful guide for future structural modifications to find more potent inhibitors, and also for preparation of molecular probes of **ku-76** for elucidation of the target biomolecules.

4. Experimental section

4.1. Materials

The ^{1}H and ^{13}C NMR spectra were recorded on a JNM EX-270 (270 and 67.5 MHz), AL-400 (400 and 100 MHz), and a JNM ECA-600 spectrometer (600 and 150 MHz). Chemical shifts were reported in ppm downfield from the peak of Me_4Si (TMS) used as the internal standard. Splitting patterns are designed as “br, s, d, t, q, and m,” indicating “broad, singlet, doublet, triplet, quartet, and multiplet,” respectively. The IR spectra were recorded on a SHIMADZU IRPrestige-21 FT-IR spectrophotometer using a KBr disk or a NaCl cell. Mass spectra were obtained on a JEOL JMS-700 or a JEOL JMS-T100CS. High-resolution mass spectra were obtained on a JEOL JMS-700 or a JEOL JMS-T100CS. Column chromatography was performed on silica gel (Kanto Chemical Co.). Thin-layer chromatography was performed on pre-coated plates (0.25 mm, silica gel Merck 60 F254). Reaction mixtures were stirred magnetically. Preparation of **ku-76** and **2d** were reported previously (Abe et al., 2012).

4.2. Synthesis

4.2.1. Synthesis of (2Z,4Z)-5-pheny-penta-2,4-dienoic acid ***(1b)***

To a solution of ethyl (*Z*)-3-phenylacrylate (881 mg, 5.00 mmol) in THF (20 mL) was added DIBAL (1.0 M in hexane, 12.0 mL, 12.0 mmol) at -78 °C under argon atmosphere. After 2.5 h, the reaction was quenched with H_2O, and extracted with CH_2Cl_2. The organic layer was washed with 3M HCl and brine, dried over $MgSO_4$, evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (hexane/EtOAc = 4/1 to 1/1) to give 502 mg (75%) of (*Z*)-3-phenylprop-2-en-1-ol as colorless oil (*Z*:*E* = 20:1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.62 (s, 1H), 4.44 (dd, *J* = 6.4, 1.6 Hz, 2H), 5.87 (dt, *J* = 11.7, 5.9 Hz, 1H), 6.57 (d, *J* = 11.7 Hz, 1H), 7.16-7.38 (m, 5H).

To a solution of (*Z*)-3-phenylprop-2-en-1-ol (100 mg, 0.748 mmol) in Et_2O (5.0 mL) was added Na_2CO_3 (795 mg, 7.48 mmol) and MnO_2 (addition in two portions, 649 mg and 1.30 g, total 1.95 g, 22.5 mmol). After being stirred for 2 h at room temperature, the mixture was filtered using celite and the filtrate was evaporated to afford the crude product (44.7 mg) of (*Z*)-3-phenyl-2-propenal as colorless oil (*Z*:*E* = 20:1): ^{1}H-NMR (270 MHz,$CDCl_3$) δ: 6.20 (dd, *J* = 11.5, 8.1 Hz, 1H), 7.38-7.45 (m, 5H), 7.63 (d, *J* = 11.5 Hz, 1H), 9.97 (d, *J* = 8.1 Hz, 1H). The spectral data were consistent with those reported in the literature (Pachali et al., 2009). The aldehyde was used in the next reactions without further purification.

To a solution of the above crude product in THF (5.0 mL) was added Triton B (40% MeOH solution, 76 μL, 0.48 mmol) at -78 °C under argon atmosphere. After being stirred for 30 min, 2-[bis(2-isopropylphenoxy)phosphoryl]acetate (220 mg, 0.543 mmol) in THF (3.0 mL) at -78 °C under argon atmosphere. After being stirred at -78 °C for 2.5 h, the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with EtOAc and the combined organic layer was washed with brine, dried over $MgSO_4$ filtered and concentrated *in vacuo*. The crude product was roughly purified by silica gel column chromatography (hexane/EtOAc = 95/5) to give 88.2 mg of 2*Z*- and 2*E*-mixture of ethyl (4*Z*)-5-phenylpenta-2,4-dienoate as pale yellow oil; Major product (2*Z*-isomer): ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.32 (t, J = 7.2 Hz, 3H), 4.23 (q, J = 7.2 Hz, 2H), 5.77 (dt, J = 11.5, 1.4 Hz, 1H), 6.89 (d, J = 11.5 Hz, 1H), 7.05 (ddd, J = 11.5, 11.5 1.2 Hz, 1H), 7.29-7.40 (m, 6H), 7.52 (ddd, J = 11.5, 11.5, 1.2 Hz, 1H).

To a solution of the above product (88.2 mg) in EtOH (2.0 mL) was added 3M NaOH aq (2.0 mL) at room temperature. After being stirred for 1 h, hexane was added to the mixture, and the mixture was extracted with water. The water layer was acidified with 3M HCl and the mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by recrystallization from hexane to give 34.6 mg (27% for 3 steps) of **1b** (*Z*:*E* = >99:1) as light purple needles: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.82 (dt, J = 11.5, 1.3 Hz, 1H), 6.96 (d, J = 11.5 Hz, 1H), 7.17 (td, J = 11.5, 1.3 Hz, 1H), 7.28-7.42 (m, 6H), 7.51 (td, J = 11.5, 1.3 Hz, 1H). The spectral data were consistent with those reported in the literature (Plevová et al., 2016)

*4.2.2 (2E,4Z)-5-phenylpenta-2,4-dienoic acid (**1c**)*

To a solution of (*Z*)-3-phenylprop-2-en-1-ol (100 mg, 0.748 mmol) in Et_2O (5.0 mL) was added MnO_2 in three portions (649 mg for each addition, total 1.95 g, 22.5 mmol). After being stirred for 2 h, the mixture was filtered using celite and the residue was washed with Et_2O. The filtrate was evaporated to afford the crude product containing (*Z*)-3-phenyl-2-propenal as colorless oil (*Z*:*E* = 20:1). The crude product was used in the next reactions without further purification.

To a solution of the above crude product in THF (3.0 mL) was added Triton B (188 mg, 1.13 mmol) at -78 °C under argon atmosphere. After being stirred for 15 min, the mixture was added triethyl phosphonoacetate (195 mg, 0.87 mmol) in THF (3.0 mL). After being stirred for 1.5 h, the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with EtOAc and the combined organic layer was washed with brine, dried over $MgSO_4$ filtered and concentrated *in vacuo*. The crude product was roughly purified by silica gel column chromatography (hexane/ether = 97/3) to give 92.3 mg of 2*E*- and 2*Z*-mixture of ethyl (4*Z*)-5-phenylpenta-2,4-dienoate as colorless oil; major product (2*E*-isomer): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.29 (t, *J* = 7.2 Hz, 3H), 4.21 (q, *J* = 7.2 Hz, 2H), 6.03 (d, *J* = 15.2 Hz, 1H), 6.36 (dd, *J* = 11.5 Hz, 1H), 6.81 (d, *J* = 11.5 Hz, 1H), 7.28-7.42 (m, 5H), 7.78 (ddd, *J* = 15.2, 11.5, 1.0 Hz, 1H).

To a solution of the above product (92.3 mg) in EtOH (2.0 mL) was added 3M NaOH aq (2.0 mL) at room temperature. After being stirred for 1.5 h, hexane was added to the mixture. The mixture was extracted with water and the water layer was acidified with 3M HCl. The mixture was extracted with EtOAc and the combined organic layer was washed with brine, dried over $MgSO_4$ filtered and concentrated *in vacuo*. The crude product was purified by recrystallization from hexane/EtOAc = 95:5 to give 57.0 mg (45% for 3 steps) of **1c** (*E*:*Z* = >99:1) as colorless needle; m.p. 105-107 °C (hexane/EtOAc = 95:5, colorless needle); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 6.04 (d, *J* = 15.2 Hz, 1H), 6.40 (dd, *J* = 11.5, 11.5 Hz, 1H), 6.88 (d, *J* = 11.5 Hz, 1H), 7.30-7.43 (m, 5H), 7.86 (ddd, *J* = 15.2, 11.5, 1.1 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 122.6 (d), 127.1 (d), 128.3 (d), 128.6 (d), 129.3 (d), 136.1 (s), 139.0 (d), 142.8 (d), 172.5 (s); IR (KBr) 1692 cm^{-1}; EIMS *m/z* 174 (M^+), 127 (100%); Elemental Analysis (%): calcd for $C_{11}H_{10}O_2$: C, 75.84; H, 5.79, found: C, 75.61; H, 5.79.

*4.2.3. (Z)-5-Phenylpent-2-enoic acid (**1d**)*

The procedure was the same as *4.2.1.* starting from the corresponding 3-phenylpropanal (402 mg, 4.00 mmol). Purification by silica gel column chromatography (hexane/AcOEt = 97/3) provided 295 mg (48%) of *(Z)*-ethyl 5-phenylpent-2-enoate ***(S1)*** (*Z*:*E* = >99:1) as colorless oil; ^{1}H-NMR

(270 MHz, $CDCl_3$) δ: 1.28 (t, *J* = 7.1 Hz, 3H), 2.76 (t, *J* = 7.7 Hz, 2H), 2.95-3.03 (m, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 5.77 (dt, *J* = 11.5, 1.6 Hz, 1H), 6.23 (dt, *J* = 11.5, 7.2 Hz, 1H), 7.16-7.35 (m, 5H). The spectral data were consistent with those reported in the literature (Yoshida et al., 2014). The procedure was the same as *4.2.1.* starting from **S1** (196 mg, 0.960 mmol). Purification by silica gel column chromatography and recrystallization from hexane provided 29.0 mg (17%) of **1d** (*Z*:*E* = >99:1) as colorless prism; m.p. 51.5-52.4 °C (hexane); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 2.78 (t, *J* = 7.6 Hz, 2H), 3.00 (m, 2H), 5.82 (dt, *J* = 11.5, 1.6 Hz, 1H), 6.38 (dt, *J* = 11.5, 7.4 Hz, 1H), 7.17-7.32 (m, 5H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 30.6 (t), 34.9 (t), 119.6 (d), 126.1 (d), 128.5 (d), 141.0 (s), 151.9 (d), 171.2 (d); IR (KBr) 1701 cm^{-1}. EIMS *m/z* 176 (M^+), 91 (100%); Elemental Analysis (%): calcd for $C_{13}H_{12}O_2$: C, 74.98; H, 6.86, found: C, 74.83; H, 6.86.

*4.2.4. (Z)-Ethyl 5-phenylpent-2-en-4-ynoate **(S2)***

The procedure was the same as *4.2.1.* starting from phenylpropargyl aldehyde (392 mg, 3.00 mmol). Purification by silica gel column chromatography (hexane/AcOEt = 97/3) provided 278 mg (46%) of **S2** (*Z*:*E* = >99:1) as colorless solid; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.34 (t, *J* = 7.1 Hz, 3H), 4.27 (t, *J* = 7.1 Hz, 2H), 6.13 (d, *J* = 11.4 Hz, 1H), 6.36 (d, *J* = 11.4 Hz, 1H), 7.33-7.37 (m, 3H), 7.51-7.55 (m, 2H); The spectral data were consistent with those reported in the literature (Takeuchi et al., 2000)

*4.2.5. (Z)-5-phenylpent-2-en-4-ynoic acid **(1e)***

The procedure was the same as *4.2.1.* starting from **S2** (228 mg, 1.14 mmol). Purification by silica gel column chromatography (hexane/AcOEt = 7/3 to 1/1) and recrystallization from hexane/AcOEt = 95/5 provided 107 mg (54%) of **1e** (*Z*:*E* = >99:1) as yellow needle; m.p. 73-77 °C (hexane/AcOEt = 95/5, yellow needle); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 6.19 (d, *J* = 11.4

Hz, 1H), 6.49 (d, $J = 11.3$ Hz, 1H), 7.28-7.40 (m, 3H), 7.51-7.55 (m, 2H); EIMS *m/z* 172 (M^+, 100%). The spectral data were consistent with those reported in the literature. (Soltani et al., 2017)

*4.2.6. (2Z,4E)-Octa-2,4-dienoic acid (**1f**)*

The procedure for Emmons reaction was the same as *4.2.1.* starting from *trans*-2-hexenal (294 mg, 3.00 mmol). Purification by silica gel column chromatography (hexane/AcOEt = 98/2) provided 360 mg (71%) of ethyl (2*Z*,4*E*)-octa-2,4-dienoate (**S3**) as pale yellow oil; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.93 (t, $J = 7.3$ Hz, 3H), 1.30 (t, $J = 7.1$ Hz, 3H), 1.40-1.54 (m, 2H), 2.15-2.23 (m, 3H), 4.18 (q, $J = 7.1$ Hz, 2H), 5.56 (d, $J = 11.3$ Hz, 1H), 6.07 (dt, $J = 15.4, 6.8$ Hz, 1H), 6.55 (1H, t, $J = 11.3$ Hz), 7.32-7.42 (m, 1H); The spectral data were consistent with those reported in the literature (Ando, 1997).

The procedure for hydrolysis was the same as *4.2.1.* starting from **S3** (259 mg, 1.53 mmol). Purification by silica gel column chromatography (hexane/Et_2O = 9/1) provided 131 mg (61%) of **1f** (*Z*:*E* = >99:1) as pale yellow oil; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.94 (t, $J = 7.3$ Hz, 3H), 1.48 (m, 2H), 2.21 (tdd, $J = 7.2, 7.2, 1.4$ Hz), 5.59 (d, $J = 11.4$ Hz, 1H), 6.13 (dt, $J = 15.2, 7.2$ Hz, 1H,), 6.66 (t, $J = 11.4$ Hz, 1H), 7.29-7.40 (m, 1H); EIMS *m/z* 140 (M^+), 97 (100%); Elemental Analysis (%): calcd for $C_8H_{12}O_2$: C, 68.55; H, 8.63, found: C, 68.96; H, 8.92; The spectral data were consistent with those reported in the literature (Schmidt and Kunz, 2012)

*4.2.7. (2Z,4E)-5-Phenyl-1-(pyrrolidin-1-yl)penta-2,4-dien-1-one (**2a**)*

To a solution of **ku-76** (105 mg, 0.600 mmol) in CH_2Cl_2 (6.0 mL) was added DMF (1 drop) and oxalyl chloride (99.0 mg, 0.780 mmol) at room temperature. After being stirred for 1 h, a solution of pyrrolidine (55.5 mg, 0.781 mmol) and *i*-Pr_2NEt (101 mg, 0.780 mmol) was added. After being stirred for another 2 h, the reaction was quenched with H_2O (10 mL). The mixture was extracted with CH_2Cl_2 (20 mL ×3) and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexane/AcOEt = 4/1) and recrystallization from hexane afforded 131.0 mg (96%) of **2a** (*Z*:*E* = >99:1) as colorless needle; mp. 65-66 °C (hexane, colorless needle); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.85-2.02 (m, 4H), 3.54 (dt, *J* = 15.7, 6.6 Hz, 4H), 5.95 (d, *J* = 11.2 Hz, 1H), 6.60 (ddd, *J* = 11.2, 11.2, 0.6 Hz, 1H), 6.74 (d, *J* = 15.7 Hz, 1H), 7.22-7.35 (m, 3H), 7.49-7.53 (m, 2H), 8.17 (ddd, *J* = 15.7, 11.2, 1.0 Hz, 1H); ^{13}C-NMR (100 MHz, CD_3OD) δ: 25.3 (t), 27.0 (t), 46.9 (t), 48.3 (t), 120.4 (d), 126.4 (d), 128.2 (d), 129.7 (d), 129.8 (d), 138.1(s), 140.7 (d), 141.8 (d), 167.7 (s); IR (KBr) 1636 cm^{-1}. EIMS *m/z* 227 (M$^+$), 157 (100%); Elemental Analysis (%): calcd for $C_{15}H_{17}NO$: C, 79.26; H, 7.54; N, 6.16, found: C, 78.96; H, 7.57; N, 6.28.

*4.2.8. (2Z,4E)-5-Phenylpenta-2,4-dienamide (**2b**)*

The procedure was the same as *4.2.8.* starting from **ku-76** (105 mg, 0.600 mmol) using ammonium hydroxide solution (28% in water, 1.0 mL) instead of pyrrolidine and *i*Pr_2NEt. Purification by recrystallization from EtOAc provided 41.6 mg (40%) of **2b** (*Z*:*E* = >99:1) as colorless needles; m.p. 126-130 °C (AcOEt, colorless needle); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 5.41 (brs, 2H), 5.69 (d, *J* = 11.3 Hz, 1H), 6.65 (ddd, *J* = 11.3, 11.3, 2.5 Hz, 1H), 6.78 (dd, *J* = 15.7, 2.5 Hz), 7.29-7.37 (m, 3H), 7.50-7.55 (m, 2H), 8.16-8.26 (m, 1H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 118.91 (d), 118.94 (d), 124.8 (d), 127.3 (d), 127.3(d), 128.55 (d), 128.64 (d), 136.4 (s), 140.2 (d), 142.5 (d), 169.03 (s), 169.08 (s); IR (KBr) 1664, 3337, 3165 cm^{-1}. EIMS *m/z* 173 (M$^+$), 129 (100%); Elemental Analysis (%): calcd for $C_{11}H_{11}NO$: C, 76.28; H, 6.40; N, 8.09, found: C, 76.12; H, 6.41; N, 8.06.

*4.2.9. (2Z,4E)-5-phenylpenta-2,4-dien-1-ol (**2c**)*

To a solution of **2d** (202 mg, 1.00 mmol) in THF (5.0 mL), cooled to -78 °C under argon atmosphere, was added DIBAL (1.0 M in hexane, 2.4 mL, 2.4 mmol). After being stirred for 2 h, the reaction was quenched with H_2O. The mixture was extracted with CH_2Cl_2 and the combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (hexane/AcOEt = 4/1) afforded 104.3 mg (65%) of **2c** (*Z*:*E* = >99:1) as colorless oil; ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.39 (brs, 1H), 4.43 (brd, *J* = 5.4 Hz, 2H), 5.70 (dtdd, *J* = 12.9, 5.4, 1.0, 1.0 Hz, 1H), 6.23-6.32 (m, 1H), 6.60 (d, *J* = 15.5 Hz, 1H), 7.06 (ddd, *J* = 15.5, 11.2, 1.0 Hz, 1H), 7.21-7.44 (m, 5H); EIMS *m/z* 160 (M^+), 104 (100%); The spectral data were consistent with those reported in the literature. (Dong et al., 2011)

4.2.10. Inhibitory test for gravitropic bending

The lettuce seeds (*Lactuca sativa* L. cv. Great Lakes, Compositae) were incubated on a solidified agar (2%w/v) in a Petri dish, where they were allowed to germinate and grow at 25 °C for 48 h in the dark. The well-grown seedlings (5-7 pieces) were transferred to solidified agar plate (1% (w/v)) containing test compounds (50 μM) in a single plate, and they were arranged parallel to gravity vector. The seedlings were preincubated vertically at the same conditions for 1 h, and then they were gravistimulated by 90°-reorientation of the plates and incubated for 18 h. Then, root images were captured by digital camera and the angles of gravitropic curvature as well as the length of the roots were analyzed by ImageJ (https://imagej.nih.gov/ij/download.html)(Figure 3).

4.3 Plant

Lettuce seeds (*Lactuca sativa* L. cv. Great Lakes, Compositae) were purchased from

Kuragi Co., Ltd.

Conflicts of interest

The authors have declared that there is no conflict of interest.

Acknowledgment

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Figure legends

Fig. 1. Examples of compounds having gravitropic inhibitory activity.

Fig. 2. Lead compound, **ku-76**

Fig. 3. Evaluation method for inhibitory activity against root gravitropic bending. Gravity vectors before (g1) and after (g2) reorientation are indicated. The length (l, cm) of the root length and the angle (θ, degree) of the curvature after reorientation were measured. The figure represents the lettuce seeding (mock treated).

Fig. 4 Inhibition tests of gravitropic bending at various concentrations of **ku-76** ((a) control, (b) [**ku-76]**; **A**: 10 μM, **B**: 5 μM, **C**: 1 μM), **(c)** Effect of **ku-76** on gravitropism, (d) elongation. Data represent mean ± SD. Asterisk indicates statistically significant differences between treatments and control at $p < 0.05$ (Welch t-test, $n = 7$).

Fig. 5. Three functional units for SAR study of **ku-76**.

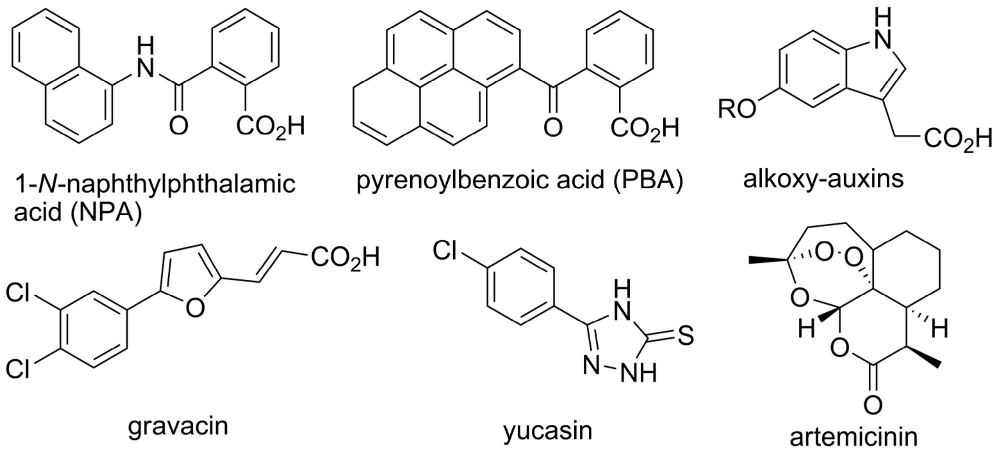
Fig. 6. Structures of the analogues of **ku-76**.

Fig. 7. Inhibitory activity tests of gravitropic bending and elongation for skeletal analogues (50 μM except for **ku-76** (10 μM). Data for (A) gravitropic bending and (B) elongation represent mean ± SD. Asterisk indicates statistically significant differences between treatments and control at $p < 0.05$ (Welch t-test, $n = 7$)

Fig. 8. Inhibitory activity tests for amide, alcohol, ester analogues (50 μM). Data of (A) gravitropic bending and (B) elongation represent mean ± SD. Asterisk indicates statistically significant differences between treatments and control at $p < 0.05$ (Welch t-test, $n = 7$)

Fig. 9. Essential structural features of **ku-76** in root gravitropic bending tests.

Fig. 1


1-*N*-naphthylphthalamic acid (NPA)

pyrenoylbenzoic acid (PBA)

alkoxy-auxins

gravacin

yucasin

artemicinin

Fig. 2

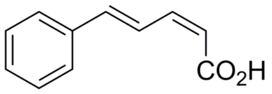


Fig. 3

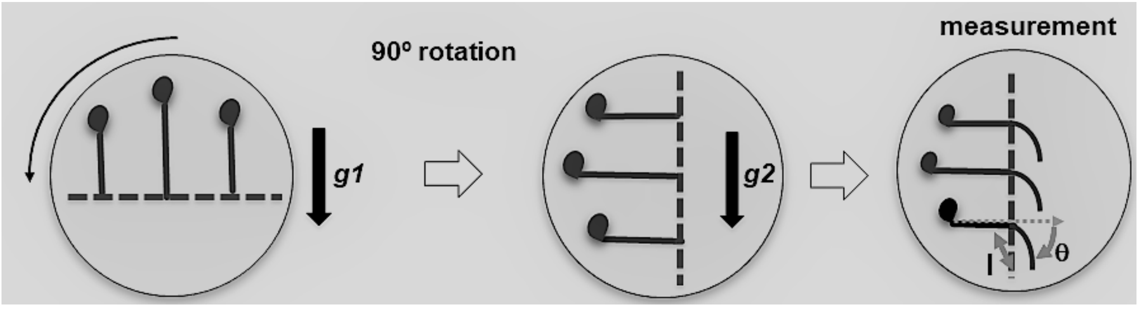
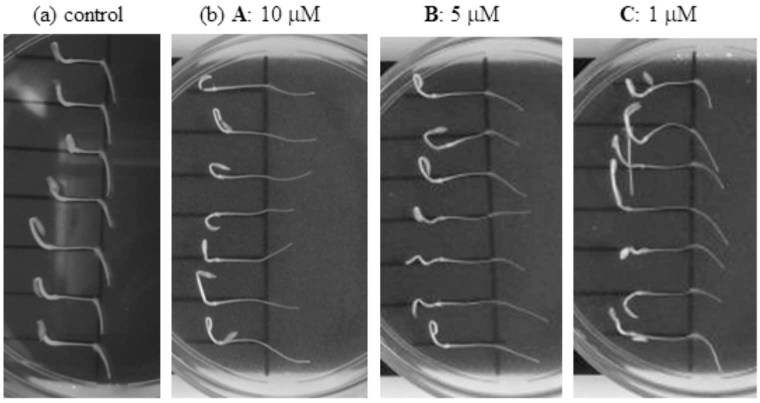
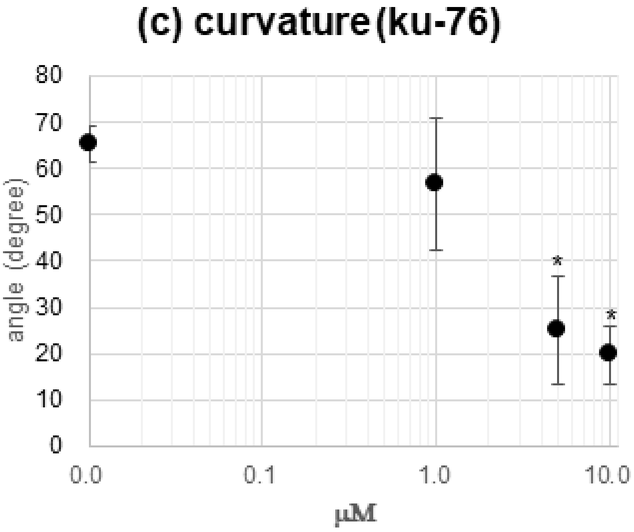


Fig. 4


(a) control
(b) **A**: 10 μM
B: 5 μM
C: 1 μM


(c) curvature (ku-76)
angle (degree)
80
70
60
50
40
30
20
10
0
0.0
0.1
1.0
10.0
μM
*
*

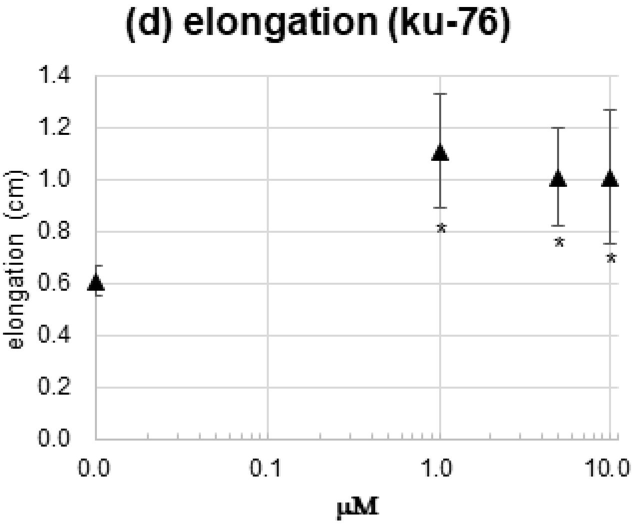

(d) elongation (ku-76)
elongation (cm)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.1
1.0
10.0
μM
*
*
*

Fig. 5

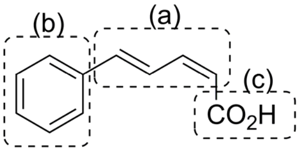
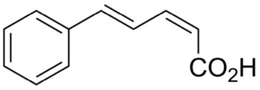
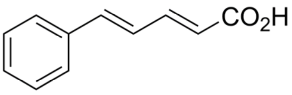


Fig 6

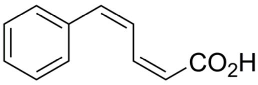
skeletal analogues



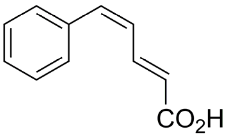
ku-76



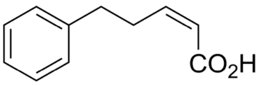
1a



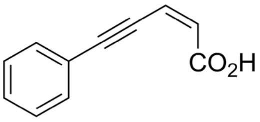
1b



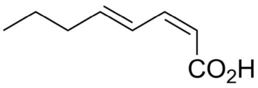
1c



1d

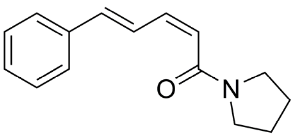


1e

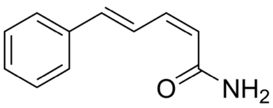


1f

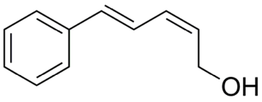
functional group



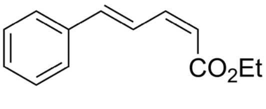
2a



2b



2c



2d

Fig 7

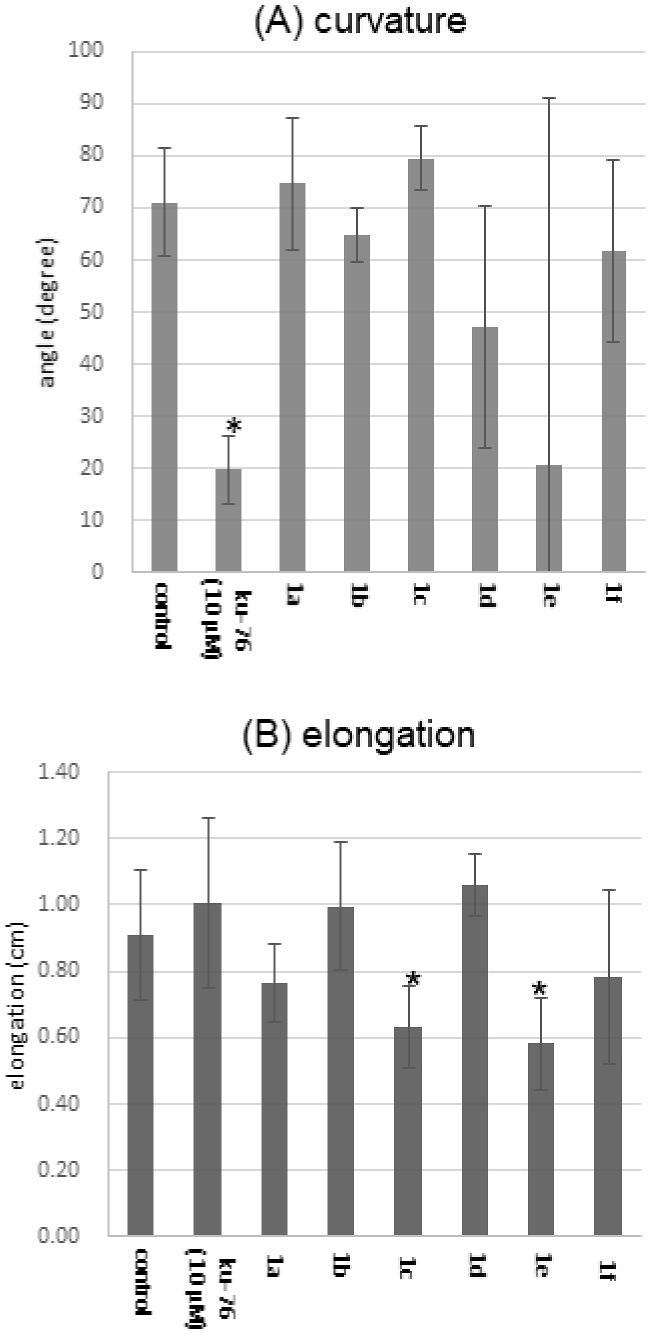

(A) curvature
angle (degree)
0
10
20
30
40
50
60
70
80
90
100
*
control
ku-76 (10 μM)
1a
1b
1c
1d
1e
1f
(B) elongation
elongation (cm)
0.00
0.20
0.40
0.60
0.80
1.00
1.20
1.40
*
*
control
ku-76 (10 μM)
1a
1b
1c
1d
1e
1f

Fig 8

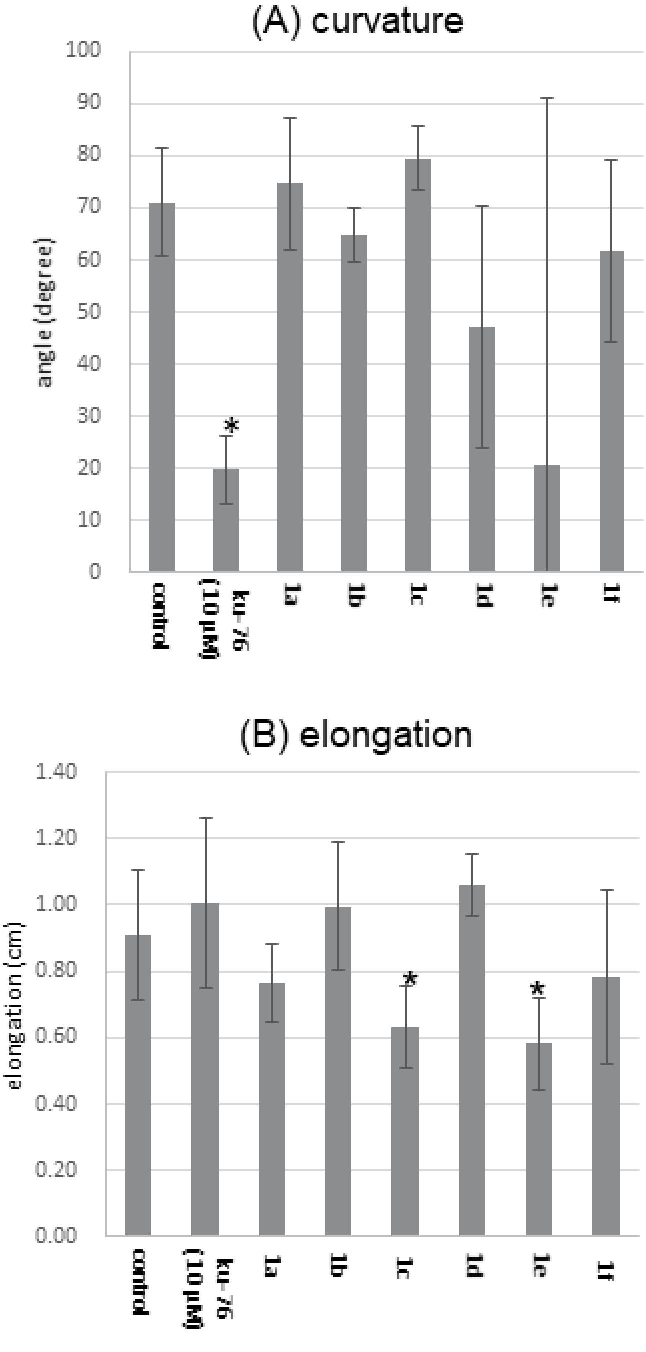
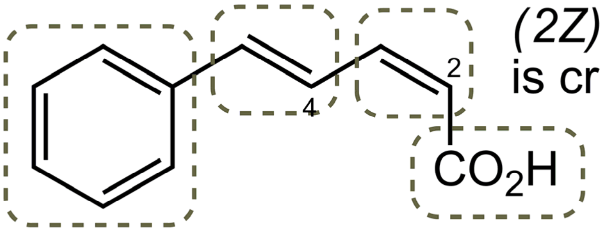

(A) curvature
angle (degree)
100
90
80
70
60
50
40
30
20
10
0
*
control
ku-76 (10 μM)
1a
1b
1c
1d
1e
1f
(B) elongation
elongation (cm)
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
*
*
control
ku-76 (10 μM)
1a
1b
1c
1d
1e
1f

Fig 9



(4*E*)-configuration may be important, but not essential

(2Z)-configuration is crucial

aromatic group is crucial

carboxylic acid is crucial