

Design and chemical synthesis of root gravitropism inhibitors: Bridged analogues of ku-76 have more potent activity

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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 μ M, with no concomitant growth inhibition, and revealed the structure–activity relationship in this inhibitory activity. The conformation of **ku-76** is flexible owing to the open-chain structure of penta-2,4-dienoic acid with freely rotating single bonds, and the (2Z)-alkene moiety may be isomerized by external factors. To develop more potent inhibitors and obtain insight into the target biomolecules, various analogues of **ku-76**, fixed through conformation and/or configuration, were synthesized and evaluated. Stereochemical fixation was effective in improving the potency of gravitropic bending inhibition. Finally, we found highly potent conformational and/or configurational analogues (**ku-257**, **ku-294** and **ku-308**), that did not inhibit root growth. The inhibition of root curvature by these analogues was comparable to that of naptalam.

1. Introduction

Gravitropism is a fundamental property of the plants, consisting of the curvature that develops in the growing organs of plants in response to gravity. Generally, roots grow downward (positive gravitropism) via several sequential processes (Morita and Tasaka, 2004; Morita, 2010). In roots, gravity-sensing cells are columella cells in root caps at the root tip (Blancaflor et al., 1998), where amyloplast sedimentation is thought to elicit signal transduction (the starch-statolith hypothesis) (Kiss et al., 1989, 1997; Sack, 1997), leading to a change in the direction of auxin transport (Friml et al., 2002) (Harrison and Masson, 2008). Auxins play a crucial role in the process from signal transmission to asymmetric organ growth (Su et al., 2017; Sato et al., 2015; Nakamura et al., 2019).

In order to identify plant growth regulators, gravitropism has been used to screen chemicals (Surpin et al., 2005; Nishimura et al., 2012, 2014). Using this methodology, several organic compounds have been found to be auxin transport inhibitors, such as 1-N-naphthylphthalamic acid (naptalam, NPA) (Mentzer et al., 1950), gravacin (Surpin et al., 2005; Rojas-Pierce et al., 2007), alkoxy-auxins (Tsuda et al., 2011), and auxin biosynthesis inhibitors, such as yucasin DF (Tsugafune et al.,

2017) (Fig. 1). While these chemicals can also be regarded as gravitropism inhibitors, they tend to inhibit growth. Selective gravitropism inhibitors, which suppress only gravitropic bending while not affecting other plant growth events, are valuable tools for research on the mechanism of gravitropism. Furthermore, inhibitors would be helpful for developing a new class of agrochemicals, with potential uses as plant growth regulators and weed suppressors.

During the study of allelochemicals (Nishikawa et al., 2013a, 2013b; Okuda et al., 2014; Fukuda et al., 2016), we found that *cis*-cinnamic acid (*cis*-CA) shows a weak gravitropic bending inhibition of lettuce roots as well as growth inhibition. Furthermore, we developed **ku-76**, a synthetic analogue of *cis*-CA, which inhibits gravitropic bending of lettuce roots at a concentration of 5 μ M (Fujii et al., 2016). Since **ku-76** did not show a significant suppression of growth (elongation) at this concentration, we recognized it as a new lead compound for a potent selective gravitropic bending inhibitor. In a previous paper, we revealed the structure–activity relationship, which designated the essential structural features to be (2Z,4E)-configuration, an aromatic ring, and a carboxylic acid for inhibitory activity (Shindo et al., 2020) (Fig. 2).

The conformation of **ku-76** would not be fixed, owing to the open-

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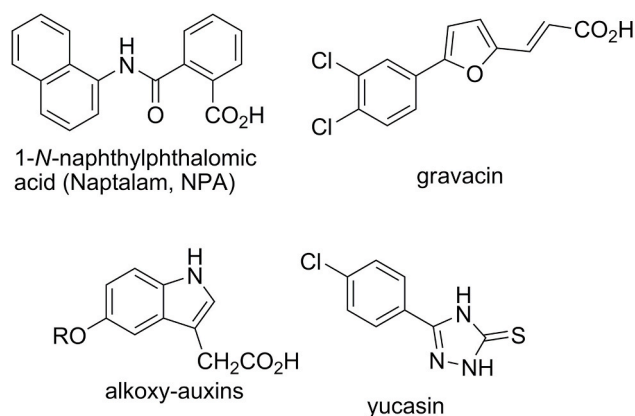


Fig. 1. Examples of non-selective gravitropism inhibitors.

chain structure of penta-2,4-dienoic acid with its freely rotating single bonds (C3–C4, C5–benzene), in spite of the conjugation between the aromatic ring and double bonds. Moreover, the (2*Z*)-alkene moiety may be isomerized into a more stable (*E*)-form by external factors such as UV light. To develop more potent inhibitors and to obtain insight into their pharmacophores, analogues of **ku-76**, fixed through conformation and/or configuration, will be useful. Herein, we describe the synthesis and evaluation of various stereochemically fixed synthetic analogues of **ku-76**.

2. Results and discussion

2.1. Design and synthesis of various bridged analogues of **ku-76**

Ring-closure by bridging between C4 and the aromatic *ortho*-position, designated in Fig. 3 by a black dashed curve line, constrains the C5–benzene single bond rotation.

The conformationally constrained analogues were designed as shown in Fig. 4. Naphthalene analogues **1a** and **1b**, five-, six- and seven-membered carbocyclic analogues **2a–c**, heterocyclic five membered-analogues **3a–d**, quinoline, and quinoxaline analogues (nitrogen-containing six membered-heterocycles: **4a–d**) were synthesized.

Furthermore, we planned to synthesize *ortho*-benzoic acid analogues **5a–d** (Fig. 5) with a benzene ring in place of (2*Z*)-alkene, indicated in Fig. 3 by a gray dashed curve line. While (*Z*)-alkenes are easily isomerized by UV or radical sources, the (2*Z*)-alkene in benzene is configurationally fixed to the external factors.

The naphthalene analogues **1** were synthesized according to our previous report (Nishikawa et al., 2013a). Other analogues having (*Z*)-alkene **2–4** were prepared by *cis*-selective olefination of the corresponding aldehydes, followed by hydrolysis (Fig. 6) (Fig. S1; Table S1). Some of the aldehydes were prepared according to the literature.

The styryl analogues (**5a** and **5b**) were prepared by Heck reaction of vinyl arenes with ethyl *o*-bromobenzoate, followed by hydrolysis (Fig. 7 (a)) (Shahzad et al., 2010). *o*-Arylbenzoates (**5c** and **5d**) were synthesized by the Suzuki coupling of arylboronic acids and *o*-bromobenzoate,

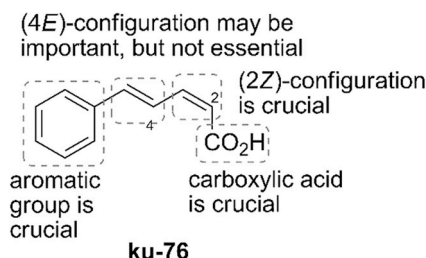


Fig. 2. Lead compound, **ku-76** and its SAR.

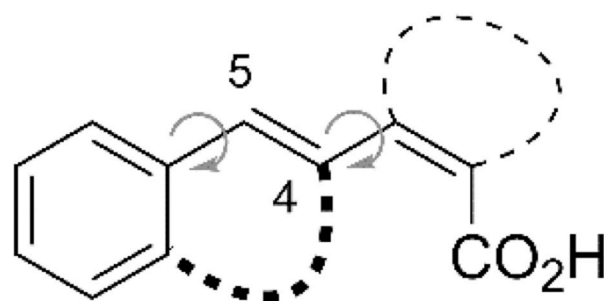


Fig. 3. Design of stereochemically fixed analogues of **ku-76**.

followed by hydrolysis (Fig. 7(b)).

2.2. Inhibitory activity test for root gravitropic bending

The inhibitory activity test for root gravitropic bending was conducted according to the method reported in our previous paper (Shindo et al., 2020): 2-day lettuce seedlings (*Lactuca sativa* L. cv. Great Lakes, Compositae) were transferred to agar plates containing test compounds (0.05 μ M–50 μ M) and arranged so that the roots were parallel to the gravity vector. The seedlings were preincubated vertically for 1 h and were gravistimulated by turning at 90°. After 24 h, root images were captured by camera, and the angles (θ , degree) of gravitropic curvature of the root as well as the extent of root growth during the 24 h period were measured (Fig. 8).

2.3. Evaluation and discussion

2.3.1. Naphthyl analogues (1)

The α - and β -naphthyl analogue (**1a** and **1b**) showed inhibitory activity at 0.05 μ M–50 μ M as well as **ku-76** for comparison, although significant elongation inhibition was also observed (Fig. 9). Therefore, naphthyl-type analogues were judged to be much less potent for *selective* root-bending inhibition. Thus, we did not perform further research on **1**.

2.3.2. Benzocarbocyclic analogues (2)

Benzocarbocyclic analogues with a styryl skeleton **2** inhibited gravitropic bending at 50 μ M and tended to inhibit elongation, except for **2b**, which was also potent for gravitropic bending inhibition at 5 μ M (Fig. 10). Consequently, the five-membered ring was thought to be a better bridging unit than the six- and seven-membered ones.

2.3.3. Benzoheterocyclic analogues (3)

As a bridging unit, five-membered heteroaromatics containing sulfur, oxygen, and nitrogen atoms were examined in a dose-response relationship study at 0.2–10 μ M. Benzothiophene, benzofuran, indole, and benzoxazoline analogues (**3a**, **3b**, **3c**, and **3d**) were found to be potent at a concentration of at least 5 μ M (Fig. 11). Benzoxazoline analogue (**3d**) did not exhibit elongation inhibitory activity, but rather elongated the roots, while the others somewhat inhibited elongation at 5 μ M. Among these analogues, **3a** (**ku-257**) exhibited the most potent inhibitory activity, which was highly effective even at 0.2 μ M for gravitropic bending inhibition. In total, the introduction of a heteroatom in the five-membered ring was linked with more potent inhibitory activity along with much less inhibition of elongation.

2.3.4. Quinoline and quinoxaline analogues (4)

Quinoline and quinoxaline analogues **4** with six-membered heterocycles tended to be less potent than any of the five-membered heteroaromatics (**3**) tested at 50 μ M (Fig. 12). Quinoline analogue **4b** was stronger than the other analogues **4a**, **4c** and **4d**, indicating that the penta-2,4-dienoic carbon skeleton, not involving heteroatoms, is highly important for potency. Compared with the naphthalene analogue **1a**, **4b**

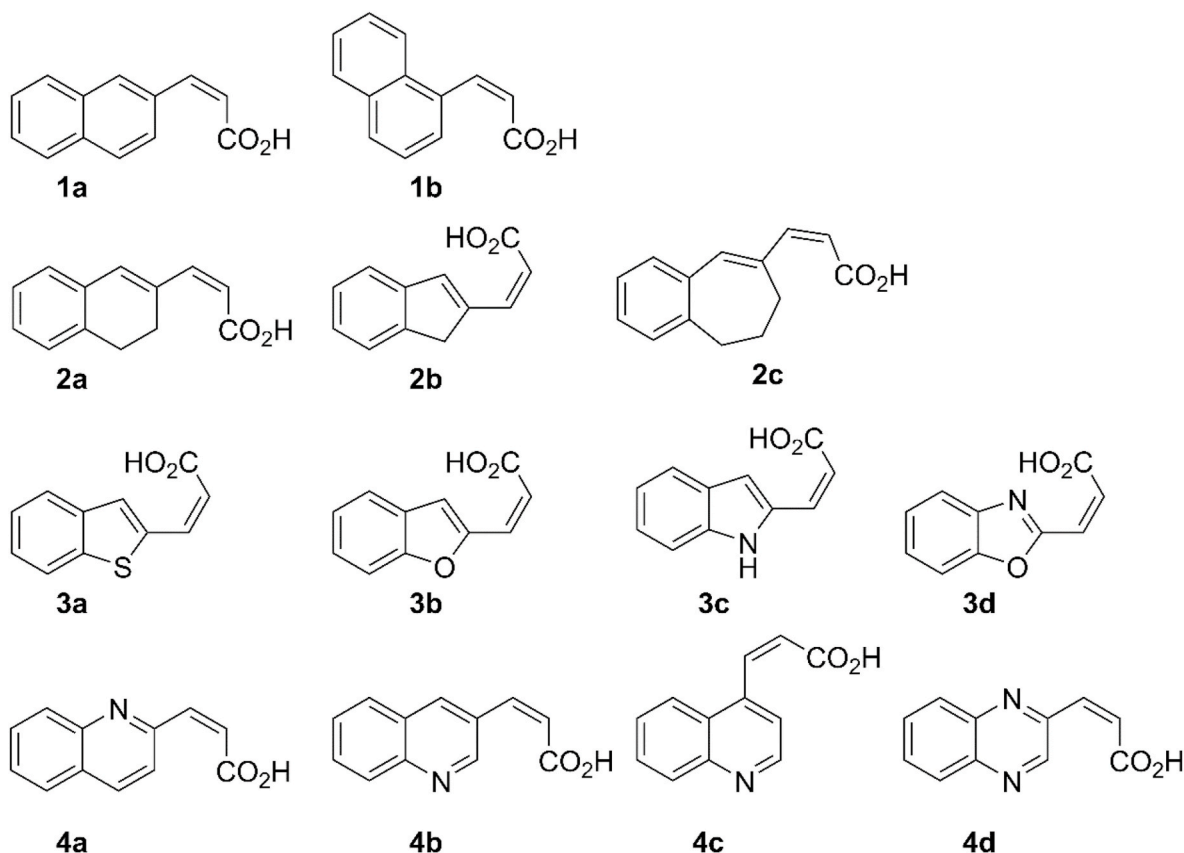


Fig. 4. Conformationally fixed analogues.

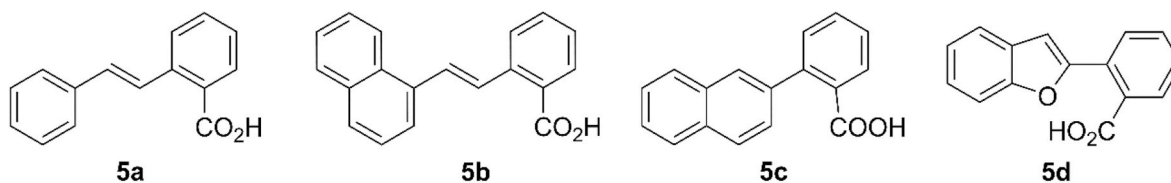
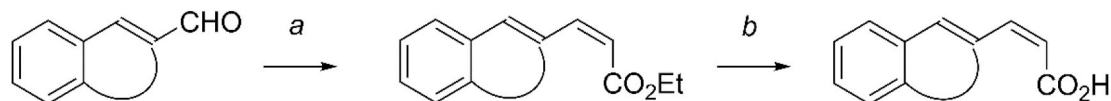


Fig. 5. Configurationally fixed (stilbene type) analogues.

Fig. 6. Synthesis of bridged carboxylate analogues from aldehydes. (a) ethyl 2-[bis(2-isopropylphenoxy)phosphoryl]acetate, Triton B, THF, -78°C , (b) 10% NaOH aq., EtOH, rt.

showed less elongation inhibitory activity.

2.3.5. *o*-Styrylbenzoic acid analogues (5)

o-Styrylbenzoic acid **5a** (**ku-294**) inhibited gravitropic bending even at $0.05\ \mu\text{M}$, while elongation was not inhibited. The bending inhibitory activity of (*E*)-2-(2-(naphthalen-1-yl)vinyl)benzoic acid **5b** was strong as well; however, inhibition of elongation was also detected. Benzoic acid analogues having a formal *cis*-alkene moiety seem to be more potent than **ku-76**. The double-bridged analogue **5c** (**ku-308**), having the fused structure of **1a** and **5a**, was found to be somewhat more potent with no concomitant elongation inhibition at $0.05\ \mu\text{M}$ although the results were variable. Similar analogue **5d**, which can be regarded as a hybrid compound of **3b** and **5a**, also exhibited strong inhibitory activity against bending, although elongation was also slightly inhibited at lower

concentrations. Consequently, these series of analogues were stronger inhibitors than **ku-76**, suggesting that their conformational and/or configurational fixation is effective for gravitropic bending inhibition. These structures, especially **5b**, reminded us of the structure of NPA with an amide bond instead of *E*-alkene in **5b** (Fig. 1). From the standpoint of structural similarity, NPA was employed in the inhibitory activity test for comparison (Fig. 13(e)). As a result, the bending inhibitory activity of NPA was stronger than that of **5b**, although it was virtually comparable to that of **5a** and **5c**; however, NPA also suppressed elongation. In terms of *selective* inhibitory activity without suppression of elongation, **5a** and **5c** seem to be equal or slightly more potent inhibitors than NPA.

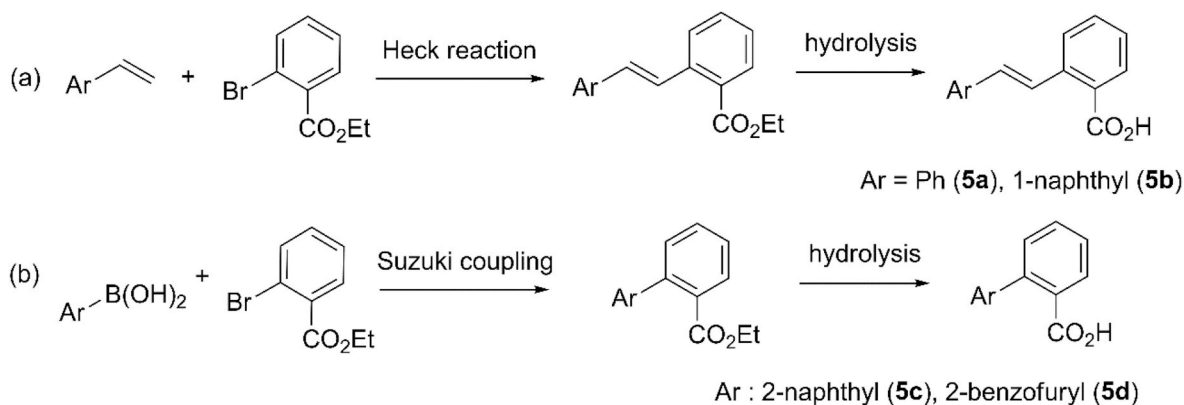
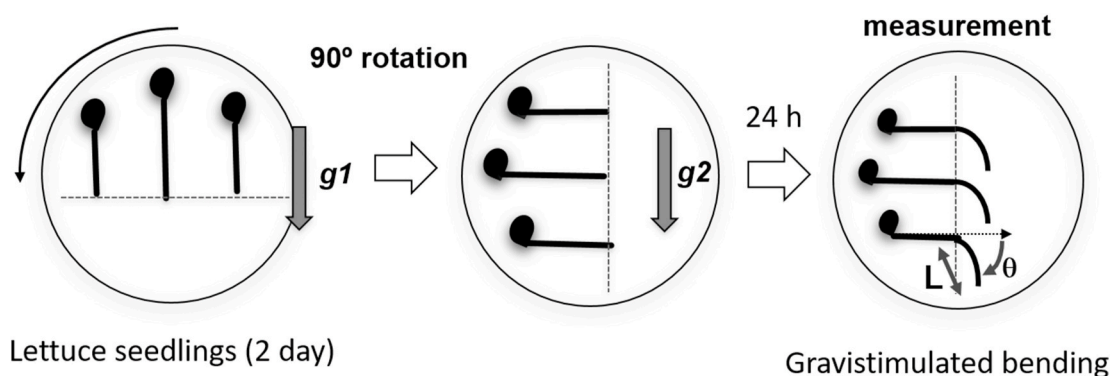


Fig. 7. Synthesis of configurationally fixed analogues 5.

Fig. 8. Inhibitory activity test for gravitropic bending. Gravitropic vectors before (g_1) and after (g_2) reorientation are indicated. Length (L , cm) of the root and the angle (θ , degree) of the curvature after reorientation were measured. This figure is representative of a control experiment where no treatment with chemicals was performed.

3. Experimental section

3.1. Materials

The ^1H 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 Me_4Si (TMS) used as the internal standard or relative to the residual protonated solvent peaks (acetone- d_6 : 2.05 and 29.8, TFA- d : 11.5 and 116.6). 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** (Abe et al., 2012), **1a** and **1b** (Nishikawa et al., 2013b) were reported previously. 3,4-Dihydronaphthalene-2-carbaldehyde, 1H-indene-2-carbaldehyde, 6,7-dihydro-5H-benzo[7]annulene-8-carbaldehyde and benzoxazole-2-carbaldehyde were synthesized according to the literature procedure (See supplemental information). 2-Benzofurancarboxaldehyde was purchased from Sigma-Aldrich Japan. Benzo[b]thiophene-2-carboxaldehyde, indole-2-carboxaldehyde, 2-quinolinecarboxaldehyde, 3-quinolinecarboxaldehyde and 4-quinolinecarboxaldehyde were purchased from Tokyo Chemical Industry. Quinoxaline-2-carbaldehyde was purchased from Apollo Scientific.

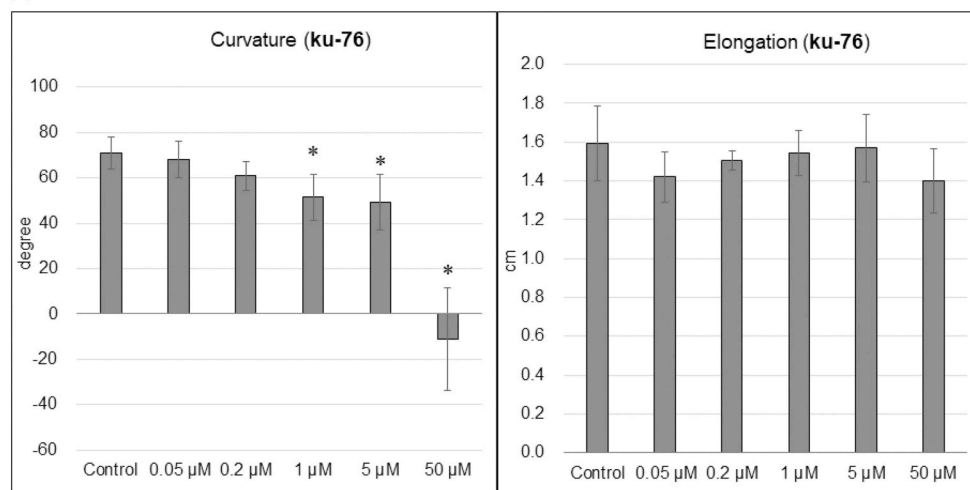
3.2. Synthesis

3.2.1. Synthesis of (Z)-3-(3,4-dihydronaphthalen-2-yl)acrylic acid (**2a**)

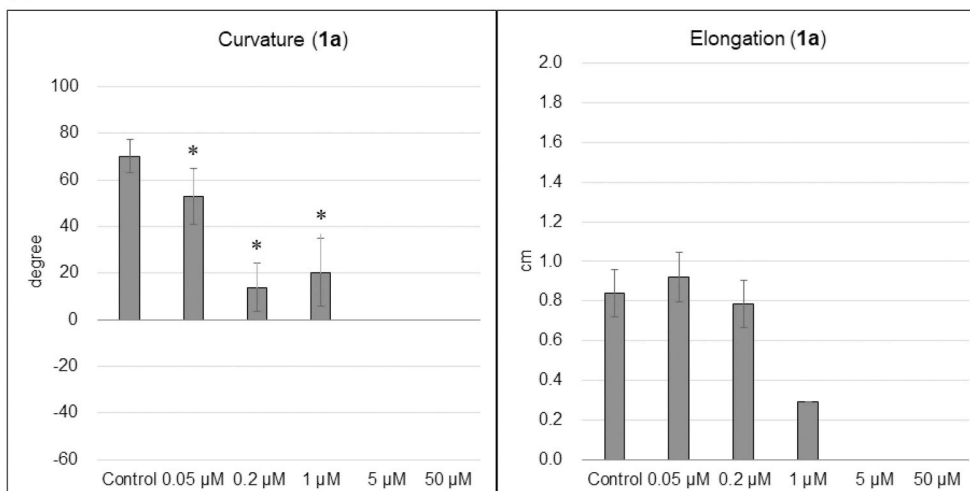
To a solution of 3,4-dihydronaphthalene-2-carbaldehyde (**7a**, 379 mg, 2.39 mmol) in THF (8.0 mL) was added Triton B (40% MeOH solution, 1.32 mL, 3.35 mmol) at -78°C under argon atmosphere. After stirring for 20 min, 2-[bis(2-isopropylphenoxy)phosphoryl] acetate (220 mg, 0.543 mmol) in THF (5.0 mL) was added to the mixture. The resulting mixture was stirred for 2.5 h at -78°C , and 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 purified by silica gel column chromatography (hexane/EtOAc = 97/3 to 95/5) to give ethyl ester of **2a** (466.9 mg, 86%, $Z:E = >99:1$) as a pale yellow oil; ^1H -NMR (270 MHz, CDCl_3) δ : 1.30 (t, $J = 7.2$ Hz, 3H), 2.60 (t, $J = 7.8$ Hz, 2H), 2.83 (t, $J = 7.8$ Hz, 2H), 4.20 (q, $J = 7.2$ Hz, 2H), 5.75 (d, $J = 12.7$ Hz, 1H), 6.56 (d, $J = 12.7$ Hz, 1H), 6.71 (s, 1H), 7.07–7.19 (m, 4H); ^{13}C -NMR (100 MHz, CDCl_3) δ : 14.2 (q), 25.8 (t), 28.1 (t), 60.3 (t), 117.9 (d), 126.5 (d), 127.1 (d), 127.2 (d), 128.1 (d), 133.8 (2C, apparent d), 136.1 (s), 136.3 (s), 142.9 (d), 166.7 (s); IR (NaCl) 1715 cm^{-1} ; EIMS m/z 228 (M^+), 153 (100%); Elem. Anal. calcd for $\text{C}_{15}\text{H}_{16}\text{O}_2$: C, 78.92; H, 7.06, found: C, 78.68; H, 7.07.

To a solution of the ester above (356 mg, 1.56 mmol) in EtOH (6.0 mL) was added 10% NaOH aq (8.0 mL) at room temperature. After being stirred for 1 h, the mixture was washed with hexane. After the water layer was acidified with 3M HCl, 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 (219.0 mg, 70%, $Z:E = >99:1$) was pure enough. The compound was recrystallized from hexane/EtOAc = 95/5 before used for the inhibitory test for gravitropic

(a)



(b)



(c)

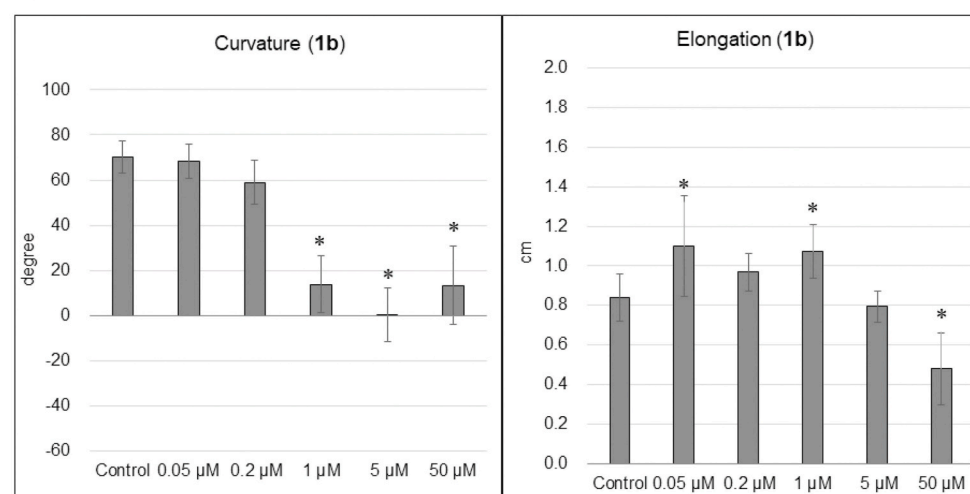


Fig. 9. Inhibitory activity tests of gravitropic bending (left) and elongation (right) for naphthyl analogues (a) **ku-76**, (b) **1a** and (c) **1b**. **1a** completely inhibited elongation at 5 and 50 μM . Data for gravitropic bending and elongation represent mean \pm SD. Asterisk indicates statistically significant differences between treatments and controls at $p < 0.05$ (Dunnnett's test, $n = 7$).

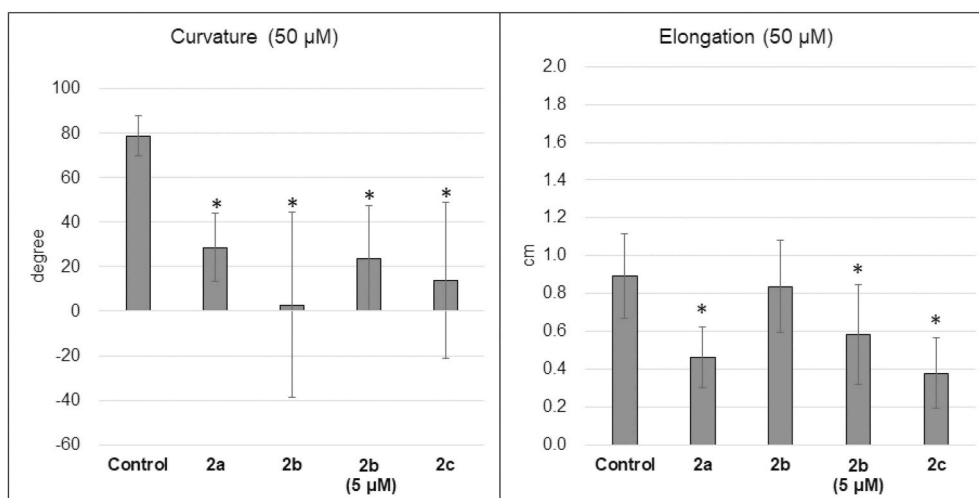


Fig. 10. Inhibitory activity tests of gravitropic bending and elongation for benzocarbocyclic analogues **2** (50 μ M, unless otherwise noted). Data for gravitropic bending (left) and elongation (right) represent mean \pm SD. Asterisk indicates statistically significant differences between treatments and controls at $p < 0.05$ (Dunnett's test, $n = 7$).

bending.

2a: colorless prism (219.0 mg, 70%, $Z:E = >99:1$), mp. 104–105 $^{\circ}$ C (hexane/EtOAc); $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ : 2.65 (t, $J = 7.8$ Hz, 2H), 2.84 (t, $J = 7.8$ Hz, 2H), 5.78 (d, $J = 12.5$ Hz, 1H), 6.69 (d, $J = 12.5$ Hz, 1H), 6.76 (s, 1H), 7.08–7.19 (m, 4H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 26.0 (t), 28.1 (t), 116.6 (d), 126.6 (d), 127.24 (d), 127.26 (d), 128.3 (d), 133.6 (s), 135.1 (d), 136.26 (s), 136.28 (s), 146.2 (d), 171.8 (s); IR (KBr) 1690 cm^{-1} ; EIMS m/z 200 (M^+ , 100%); Elem. Anal. calcd for $\text{C}_{13}\text{H}_{12}\text{O}_2$: C, 77.98; H, 6.04, found: C, 77.86; H, 6.00.

3.2.2. Synthesis of (Z)-3-(1H-inden-2-yl)acrylic acid (**2b**)

The title compound was synthesized according to the procedure of 4.2.1. from 1H-indene-2-carbaldehyde.

Ethyl ester of **2b**: starting from 22 mg of the aldehyde: yellow oil (22%, $Z:E = >99:1$); $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 1.34 (t, $J = 7.0$ Hz, 3H), 3.94 (s, 2H), 4.24 (q, $J = 7.0$ Hz, 2H), 5.75 (d, $J = 12.4$ Hz, 1H), 6.86 (d, $J = 12.4$ Hz, 1H), 7.22 (s, 1H), 7.42 (d, $J = 6.9$ Hz, 1H), 7.47 (d, $J = 6.9$ Hz, 1H); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ : 14.3 (q), 40.9 (t), 60.1 (t), 116.7 (d), 122.0 (d), 123.9 (d), 126.5 (2C, d), 138.0 (d), 140.7 (d), 143.2 (s), 143.5 (s), 145.4 (s), 166.3 (s); IR (NaCl) 1721 cm^{-1} ; EIMS m/z 214 (M^+), 168 (100%); HRMS (FAB) Calcd for $\text{C}_{14}\text{H}_{14}\text{O}_2$ (M^+): 214.0994, found: 214.0993.

2b: starting from 399 mg of the ester; pale yellow prism (50%, $Z:E = >99:1$); mp. 121–123 $^{\circ}$ C (hexane: AcOEt = 9:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 3.97 (s, 2H), 5.80 (d, $J = 12.6$ Hz, 1H), 6.99 (d, $J = 12.6$ Hz, 1H), 7.26–7.32 (m, 3H), 7.44–7.51 (m, 2H); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ : 40.9 (t), 115.2 (d), 122.2 (d), 124.0 (d), 126.6 (d), 126.9 (d), 140.5 (d), 142.3 (d), 143.0 (s), 143.3 (s), 145.7 (s), 171.0 (s); IR (KBr) 1686 cm^{-1} ; EIMS m/z 186 (M^+), 168 (100%); HRMS (FAB) Calcd for $\text{C}_{12}\text{H}_{10}\text{O}_2$ (M^+): 186.0681, found: 186.0685.

3.2.3. Synthesis of (Z)-3-(6,7-dihydro-5H-benzo[7]annulen-8-yl)acrylic acid (**2c**)

The title compound was synthesized according to the procedure of 4.2.1. from 6,7-dihydro-5H-benzo[7]annulene-8-carbaldehyde.

Ethyl ester of **2c**: starting from 399 mg of the aldehyde; pale yellow oil (29%, $Z:E = >99:1$); $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ : 1.27 (t, $J = 7.2$ Hz, 4H), 2.11–2.16 (m, 2H), 2.47 (t, $J = 6.6$ Hz, 2H), 2.79–2.83 (m, 2H), 4.18 (q, $J = 7.2$ Hz, 2H), 5.73 (d, $J = 12.6$ Hz, 1H), 6.56 (d, $J = 12.6$ Hz, 1H), 6.70 (s, 1H), 7.09–7.19 (m, 4H).

2c: starting from 159 mg of the ester; colorless needles (94%, $Z:E = >99:1$); mp. 58–59 $^{\circ}$ C (hexane); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 2.11–2.18 (m, 2H), 2.49 (t, $J = 6.5$ Hz, 2H), 2.80 (t, $J = 6.0$ Hz, 2H), 5.76 (d, $J =$

12.2 Hz, 1H), 6.71 (d, $J = 12.2$ Hz, 1H), 6.75 (s, 1H), 7.14–7.21 (m, 4H); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ : 29.9 (t), 31.6 (t), 34.7 (t), 116.8 (d), 125.9 (d), 127.6 (d), 129.2 (d), 131.1 (d), 136.2 (s), 137.1 (d), 139.1 (s), 142.1 (s), 148.9 (d), 171.6 (s); IR (KBr) 1686 cm^{-1} ; EIMS m/z 214 (M^+ , 100%); Elem. Anal. calcd for $\text{C}_{14}\text{H}_{14}\text{O}_2$: C, 78.48; H, 6.59, found: C, 78.28; H, 6.71.

3.2.4. Synthesis of (Z)-3-(benzo[b]thiophen-2-yl)acrylic acid (**3a**)

The title compound was synthesized according to the procedure of 4.2.1. from benzo[b]thiophene-2-carboxaldehyde.

3a: starting from 877 mg of the aldehyde; colorless needles (87% for 2 steps, $Z:E = >99:1$), mp. 154–156 $^{\circ}$ C (CH_3CN); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 5.92 (d, $J = 12.6$ Hz, 1H), 7.17 (d, $J = 12.6$ Hz, 1H), 7.33–7.40 (m, 2H), 7.67 (s, 1H), 7.78–7.83 (m, 2H); $^{13}\text{C-NMR}$ (150 MHz, Acetone- d_6) δ : 117.5 (d), 122.9 (d), 125.1 (d), 125.3 (d), 126.8 (d), 133.3 (d), 137.6 (d), 138.8 (s), 139.2 (s), 143.9 (s), 167.2 (s); IR (KBr) 1680 cm^{-1} ; EIMS m/z 204 (M^+ , 100%); Elem. Anal. calcd for $\text{C}_{11}\text{H}_8\text{O}_2\text{S}$: C, 64.69; H, 3.95, found: C, 64.58; H, 3.87.

3.2.5. Synthesis of (Z)-3-(benzofuran-2-yl)acrylic acid (**3b**)

The title compound was synthesized according to the procedure of 4.2.1. from 2-benzofurancarboxaldehyde.

Ethyl ester of **3b**: starting from 438 mg of the aldehyde; colorless oil (97%, $Z:E = >99:1$); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 1.35 (t, $J = 7.1$ Hz, 3H), 4.28 (q, $J = 7.1$ Hz, 2H), 5.96 (d, $J = 13.0$ Hz, 1H), 6.88 (d, $J = 13.0$ Hz, 1H), 7.21–7.25 (m, 1H), 7.31–7.35 (m, 1H), 7.44 (d, $J = 8.2$ Hz, 1H), 7.62 (d, $J = 7.7$ Hz, 1H), 7.95 (s, 1H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 14.3 (q), 60.5 (t), 111.2 (d), 112.5 (d), 118.6 (d), 122.2 (d), 123.1 (d), 126.1 (d), 128.8 (s), 130.2 (d), 151.6 (s), 154.8 (s), 165.8 (s); IR (NaCl) 1718 cm^{-1} ; EIMS m/z 216 (M^+ , 100%); Elem. Anal. calcd for $\text{C}_{13}\text{H}_{12}\text{O}_3$: C, 72.21; H, 5.59, found: C, 72.30; H, 5.51.

3b: starting from 500 mg of the ester; colorless prism (93%, $Z:E = >99:1$), mp. 147–149 $^{\circ}$ C (CH_3CN); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 5.99 (d, $J = 13.0$ Hz, 1H), 6.91 (d, $J = 13.2$ Hz, 1H), 7.24 (t, $J = 7.6$, 7.6 Hz, 1H), 7.34 (dd, $J = 7.6$, 7.6 Hz, 1H), 7.46 (d, $J = 7.6$ Hz, 1H), 7.62 (d, $J = 7.6$ Hz, 1H), 7.89 (s, 1H); $^{13}\text{C-NMR}$ (100 MHz, acetone- d_6) δ : 111.9 (d), 112.9 (d), 120.2 (d), 123.1 (d), 124.1 (d), 127.1 (d), 129.7 (s), 130.7 (d), 152.8 (s), 155.5 (s), 167.0 (s); IR (KBr) 1701 cm^{-1} ; EIMS m/z 188 (M^+ , 100%); Elem. Anal. calcd for $\text{C}_{11}\text{H}_8\text{O}_3$: C, 70.21; H, 4.29, found: C, 69.91; H, 4.24.

3.2.6. Synthesis of (Z)-3-(1H-indol-2-yl) acrylic acid (**3c**)

The title compound was synthesized according to the procedure of

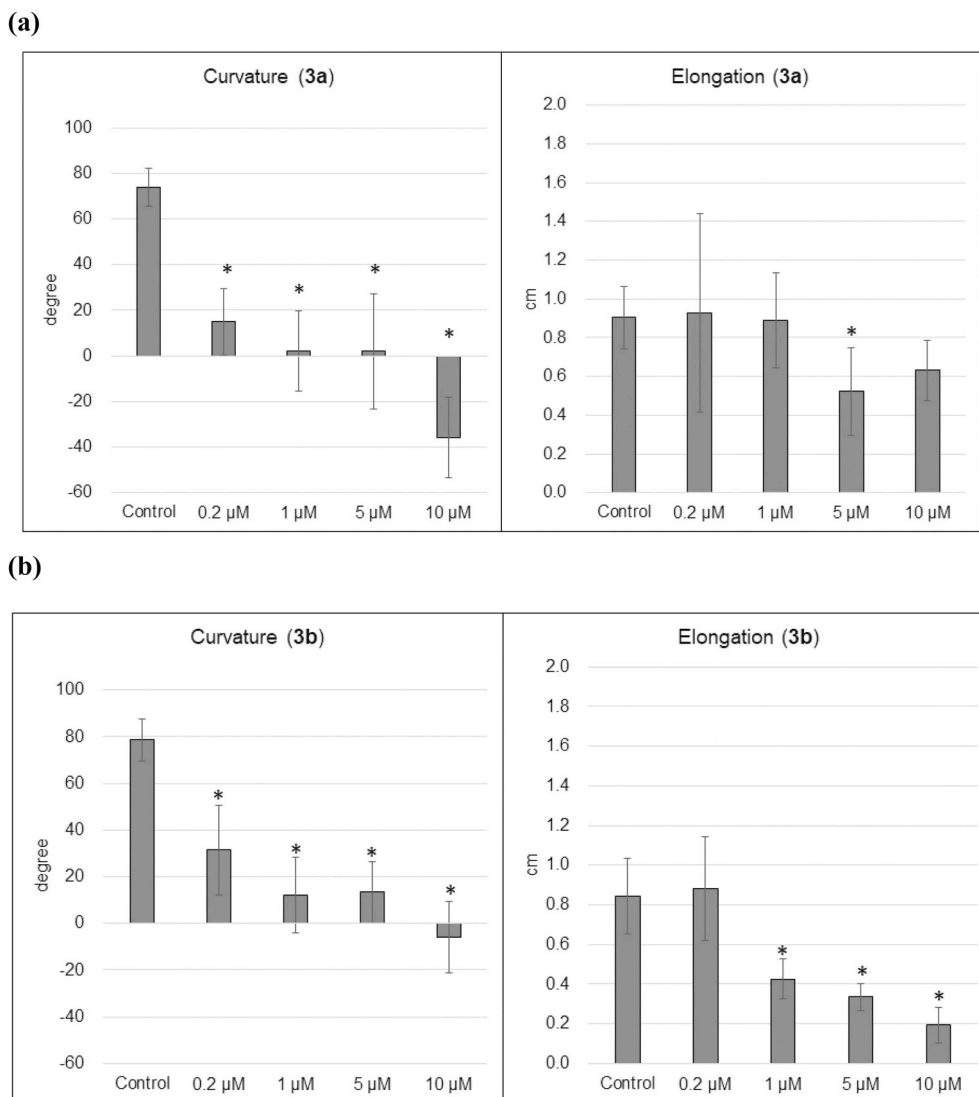


Fig. 11. Inhibitory activity tests of gravitropic bending and elongation for benzoheterocyclic analogues. Dose-response relationship study for (a) **3a**, (b) **3b**, (c) **3c**, and (d) **3d** are shown. Data for gravitropic bending and elongation represent the mean \pm SD. Asterisk indicates statistically significant differences between treatments and controls at $p < 0.05$ (Dunnett's test, $n = 7$).

4.2.1. from indole-2-carboxaldehyde.

Ethyl ester of **3c**: starting from 145 mg of the aldehyde; pale yellow solid (77%, $Z:E = >99:1$); $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ : 1.36 (t, $J = 7.0$ Hz, 3H), 4.29 (q, $J = 7.0$ Hz, 2H), 5.79 (d, $J = 12.7$ Hz, 1H), 6.77 (s, 1H), 6.94 (d, $J = 12.7$ Hz, 1H), 7.06–7.12 (m, 1H), 7.22–7.29 (m, 1H), 7.44 (d, $J = 8.2$ Hz, 1H), 7.62 (d, $J = 8.2$ Hz, 1H), 11.87 (s, 1H).

3c: starting from 166 mg of the ester; yellow prisms (98%, $Z:E = >99:1$); mp. 133–135 °C (benzene); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 5.85 (d, $J = 12.6$ Hz, 1H), 6.84 (s, 1H), 7.07 (d, $J = 12.6$ Hz, 1H), 7.11 (apparent t, $J = 7.7$ Hz, 1H), 7.27–7.31 (m, 1H), 7.45 (d, $J = 8.2$ Hz, 1H), 7.64 (d, 8.2 Hz, 1H), 11.6 (s, 1H); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD) δ : 112.2 (d), 112.7 (d), 114.4 (d), 121.0 (d), 122.3 (d), 125.5 (d), 129.0 (s), 135.1 (s), 136.1 (d), 138.9 (s), 171.2 (s); IR (KBr) 1676, 3329 cm^{-1} . EIMS m/z 187 (M^+), 169 (100%); Anal. calcd for $\text{C}_{11}\text{H}_9\text{NO}_2$: C, 70.58; H, 4.85; N, 7.48, found: C, 70.38; H, 4.79; N, 7.39.

3.2.7. Synthesis of (Z)-3-(benzo[d]oxazol-2-yl)acrylic acid (**3d**)

The title compound was synthesized according to the procedure of 4.2.1. from benzoxazole-2-carbaldehyde.

Ethyl ester of **3d**: starting from 292 mg of the aldehyde; pale yellow

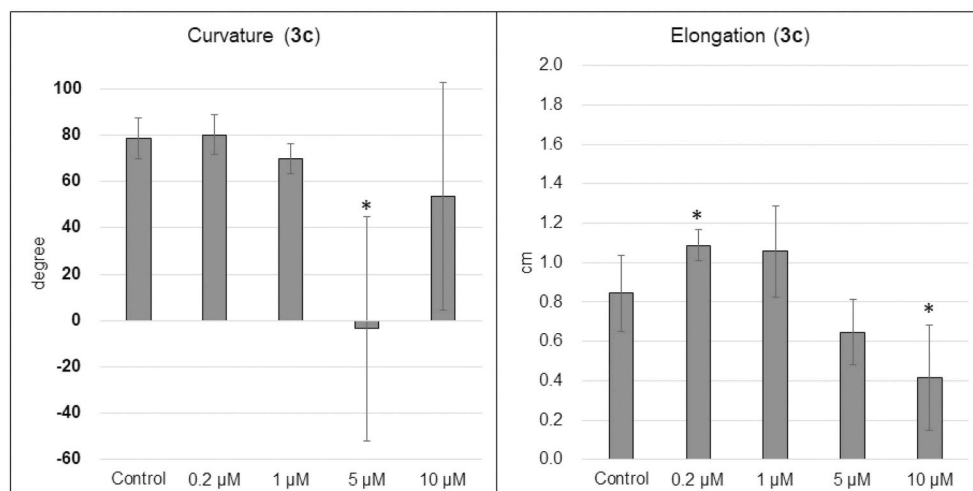
oil (79%, $Z:E = >99:1$); $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 1.35 (t, $J = 7.2$ Hz, 3H), 4.37 (q, $J = 7.2$ Hz, 2H), 6.44 (d, $J = 12.7$ Hz, 1H), 6.78 (d, $J = 12.7$ Hz, 1H), 7.34–7.39 (m, 2H), 7.51 (d, $J = 7.7$ Hz, 1H), 7.75 (d, $J = 7.7$ Hz, 1H); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ : 14.0 (q), 61.3 (t), 110.6 (d), 120.6 (d), 122.4 (d), 124.7 (d), 126.1 (d), 129.0 (d), 141.4 (s), 150.4 (s), 159.3 (s), 165.6 (s); IR (KBr) 1719 cm^{-1} ; EIMS m/z 217 (M^+ , 100%); Elem. Anal. calcd for $\text{C}_{12}\text{H}_{11}\text{NO}_3$: C, 66.35; H, 5.10; N, 6.45, found: C, 66.20; H, 5.08; N, 6.24.

3d: starting from 240 mg of the ester; pale brown needles (77%, $Z:E = >99:1$); mp. 92–94 °C (hexane, EtOAc); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 6.59 (d, $J = 13.5$ Hz, 1H), 6.97 (d, $J = 13.5$ Hz, 1H), 7.48–7.56 (m, 2H), 7.65 (d, $J = 7.6$ Hz, 1H), 7.81 (dd, $J = 7.6$ Hz, 1H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 111.5 (d), 120.0 (d), 121.7 (d), 126.4 (d), 127.8 (d), 133.3 (d), 138.3 (s), 149.6 (s), 159.8 (s), 164.3 (s); IR (KBr) 1719 cm^{-1} ; EIMS m/z 189 (M^+), 145 (100%); Elem. Anal. calcd for $\text{C}_{10}\text{H}_7\text{NO}_3$: C, 63.49; H, 3.73; N, 7.40, found: C, 63.56; H, 3.74; N, 7.45.

3.2.8. Synthesis of (Z)-3-(quinolin-2-yl)acrylic acid (**4a**)

The title compound was synthesized according to the procedure of 4.2.1. from 2-quinolinecarboxaldehyde.

(c)



(d)

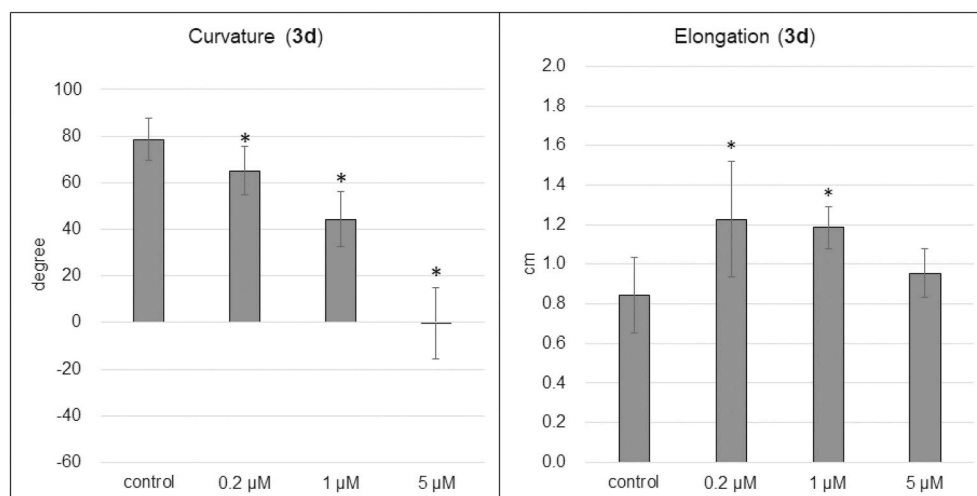


Fig. 11. (continued).

Ethyl ester of **4a**: starting from 451 mg of the aldehyde; pale orange oil (quant, *Z:E* = >99:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 1.23 (t, J = 7.6 Hz, 3H), 4.23 (q, J = 7.6 Hz, 2H), 6.26 (d, J = 12.4 Hz, 1H), 7.16 (d, J = 12.4 Hz, 1H), 7.54 (dd, J = 7.6, 7.6 Hz, 1H), 7.68–7.72 (m, 2H), 7.81 (d, J = 7.6 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 8.13 (d, J = 8.7 Hz, 1H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 14.0 (q), 60.5 (t), 121.9 (d), 124.0 (d), 126.8 (d), 127.3 (s), 127.4 (d), 129.4 (d), 129.5 (d), 135.6 (d), 140.3 (d), 147.6 (s), 154.1 (s), 166.4 (s); IR (NaCl) 1721 cm^{-1} . EIMS m/z 227 (M^+), 182 (100%); Elem. Anal. calcd for $\text{C}_{14}\text{H}_{13}\text{NO}_2$: C, 73.99; H, 5.77; N, 6.16, found: C, 73.74; H, 5.83; N, 6.03.

4a: starting from 500 mg of the ester; colorless needles (23%, *Z:E* = >99:1); mp. 72–74 °C (toluene); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 6.48 (d, J = 13.0 Hz, 1H), 7.01 (d, J = 13.0 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.70 (ddd, J = 7.6, 7.6, 1.1 Hz, 1H), 7.89 (m, 1H), 8.11 (d, J = 7.6 Hz, 1H), 8.42 (d, J = 8.2 Hz, 1H); $^{13}\text{C-NMR}$ (150 MHz, CD_3OD) δ : 125.4 (d), 126.4 (d), 129.4 (s), 129.5 (d), 130.1 (d), 132.0 (d), 133.6 (d), 137.6 (d), 142.1 (d), 144.3 (s), 152.7 (s), 170.0 (s); IR (KBr) 1707 cm^{-1} . EIMS m/z 199 (M^+), 155 (100%); Elem. Anal. calcd for $\text{C}_{12}\text{H}_9\text{NO}_2$: C, 72.35; H, 4.55; N, 7.03, found: C, 72.46; H, 4.49; N, 6.92.

3.2.9. Synthesis of (Z)-3-(quinolin-3-yl)acrylic acid (**4b**)

The title compound was synthesized according to the procedure of

4.2.1.1. from 3-quinolinecarboxaldehyde.

Ethyl ester of **4b**: starting from 471 mg of the aldehyde; pale yellow oil (96%, *Z:E* = 17:1); $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ : 1.25 (t, J = 7.1 Hz, 4H), 4.21 (q, J = 7.1 Hz, 2H), 6.15 (d, J = 12.6 Hz, 1H), 7.10 (d, J = 12.6 Hz, 1H), 7.53–7.59 (m, 1H), 7.70–7.76 (m, 1H), 7.85 (d, J = 8.1 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 8.58 (d, J = 2.1 Hz, 1H), 8.96 (d, J = 2.1 Hz, 1H).

4b: starting from 654 mg of the ester; colorless prisms (88%, *Z:E* = >99:1); mp. 175–177 °C (EtOH); $^1\text{H-NMR}$ (270 MHz, CD_3OD) δ : 6.21 (d, J = 12.5 Hz, 1H), 7.19 (d, J = 12.5 Hz, 1H), 7.63 (ddd, J = 8.2, 6.9, 1.2 Hz, 1H), 7.79 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 8.4 Hz, 1H), 8.58 (s, 1H), 9.01 (d, J = 2.3 Hz, 1H); $^{13}\text{C-NMR}$ (150 MHz, acetone- d_6) δ : 122.8 (d), 127.7 (d), 128.2 (s), 129.3 (d), 129.9 (d), 130.8 (d), 137.2 (d), 140.7 (d), 145.5 (s), 148.6 (s), 152.3 (d), 167.2 (s); IR (KBr) $1686, 1637\text{ cm}^{-1}$; FAB-MS m/z 200 ($\text{M} + \text{H}^+$); Elem. Anal. calcd for $\text{C}_{12}\text{H}_9\text{NO}_2$: C, 72.35; H, 4.55; N, 7.03, found: C, 71.93; H, 4.51; N, 7.01.

3.2.10. Synthesis of (Z)-3-(quinolin-4-yl)acrylic acid (**4c**)

The title compound was synthesized according to the procedure of 4.2.1. from 4-quinolinecarboxaldehyde.

Ethyl ester of **4c**: starting from 471 mg of the aldehyde; pale pink oil

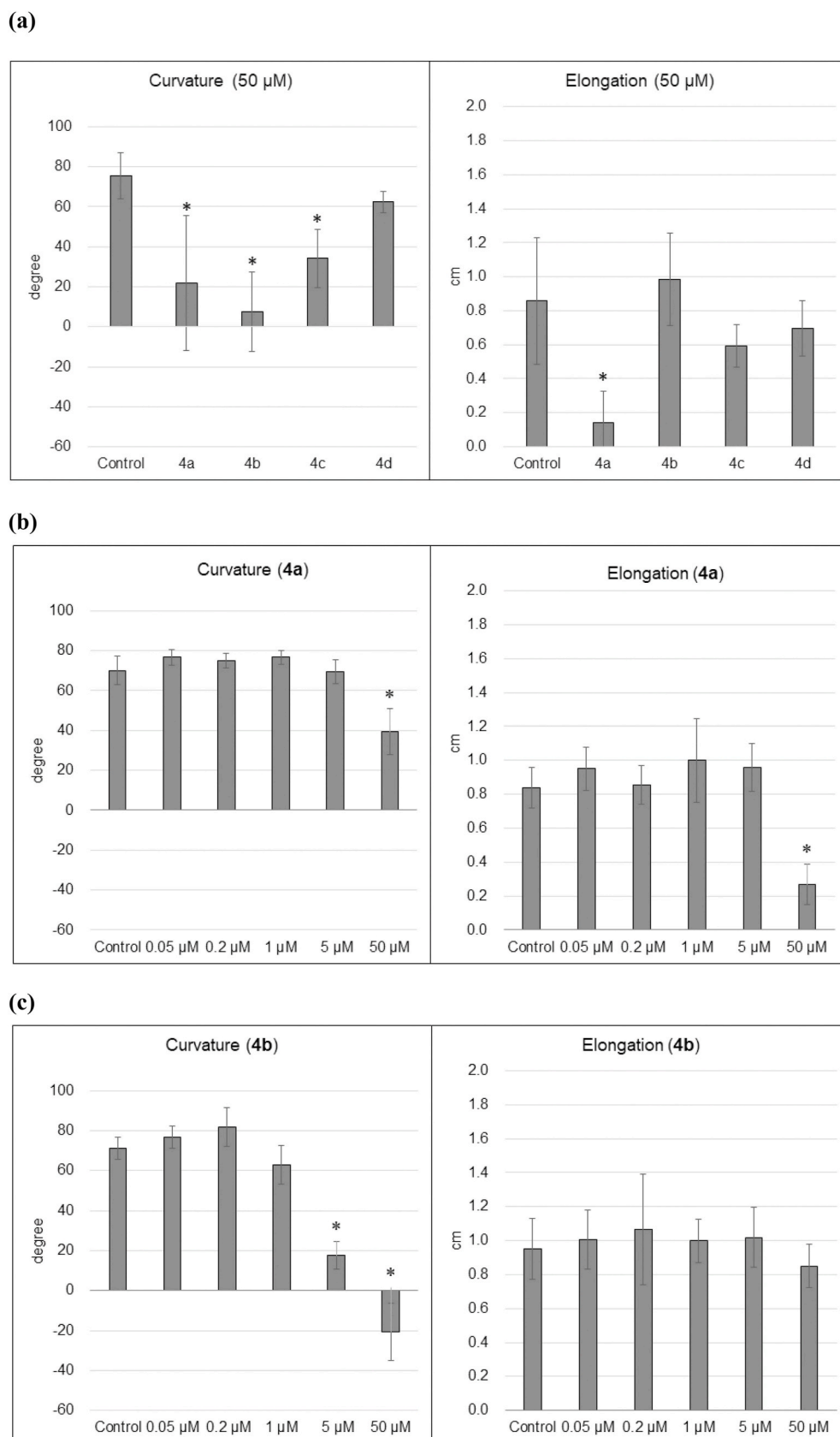


Fig. 12. Inhibitory activity tests of gravitropic bending and elongation for quinoline and quinoxaline analogues **4**: (a) 50 μM for **4a-4d**, (b) 0.05–50 μM for **4a**, (c) 0.05–50 μM for **4b**. Data for gravitropic bending (left) and elongation (right) represent the mean \pm SD. Asterisk indicates statistically significant differences between treatments and controls at $p < 0.05$ (Dunnett's test, $n = 7$).

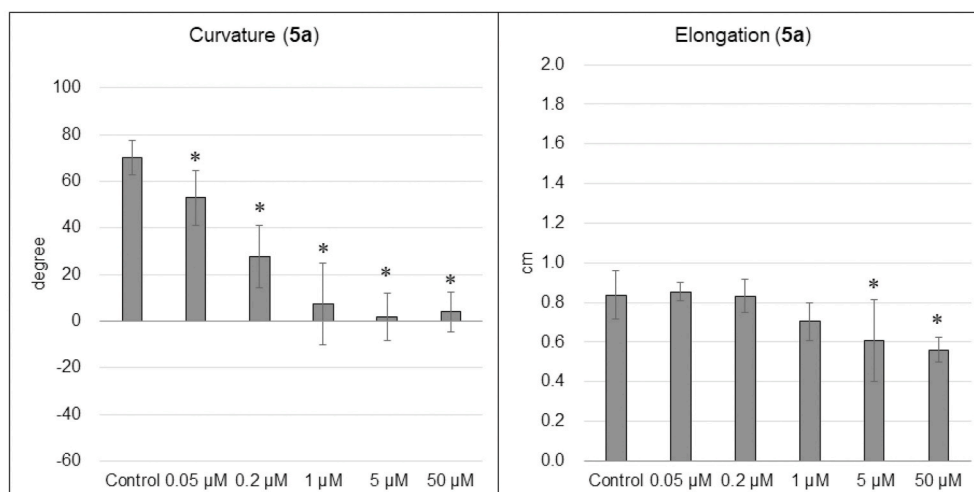
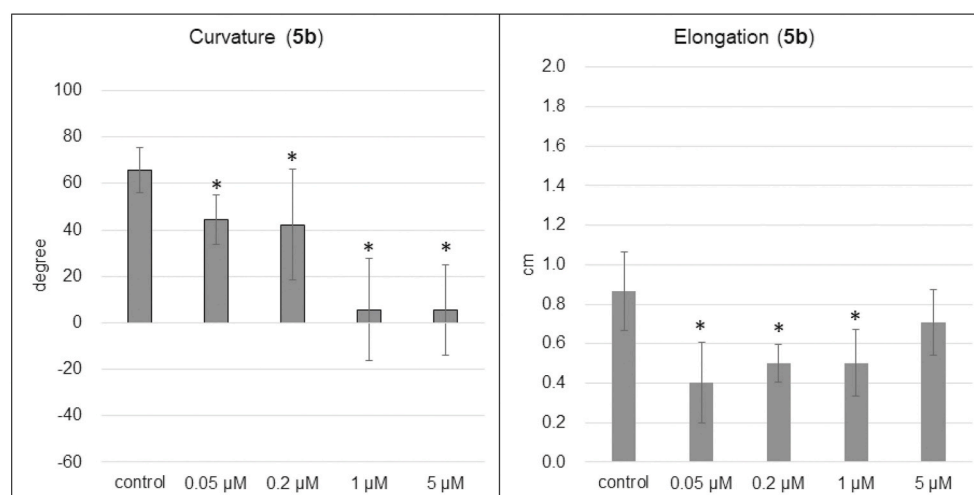
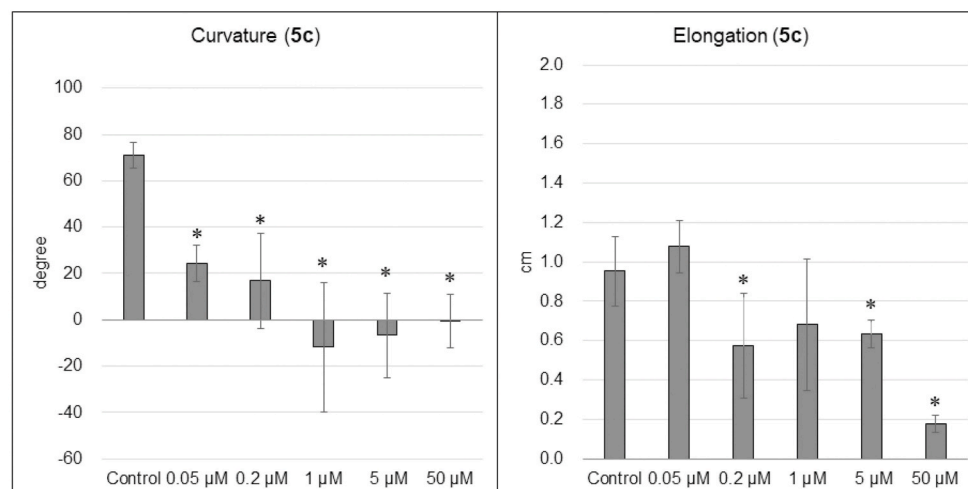
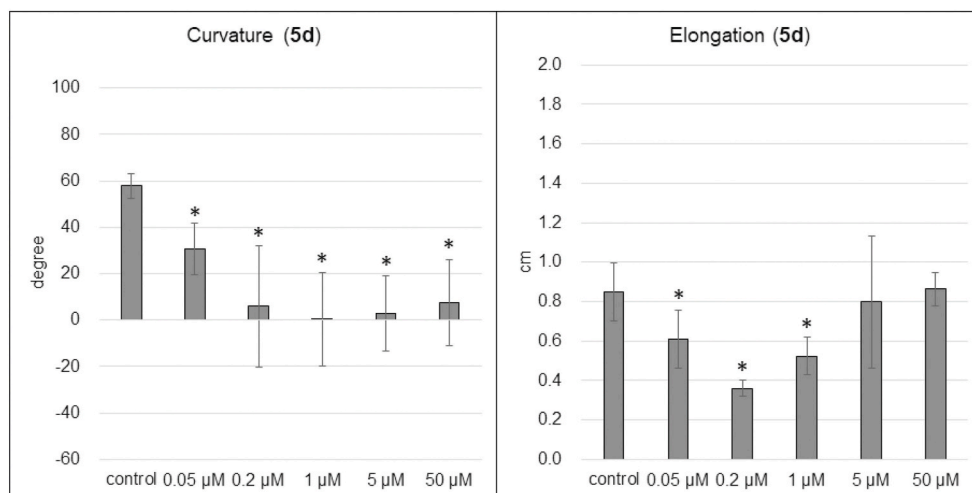
(a)**(b)****(c)**

Fig. 13. Inhibitory activity tests of gravitropic bending and elongation for *o*-styrylbenzoic acid analogues **5** and NPA (0.05–5 μ M). Data for gravitropic bending and elongation represent the mean \pm SD. Asterisk indicates statistically significant differences between treatments and controls at $p < 0.05$ (Dunnett's test, $n = 7$).

(d)



(e)

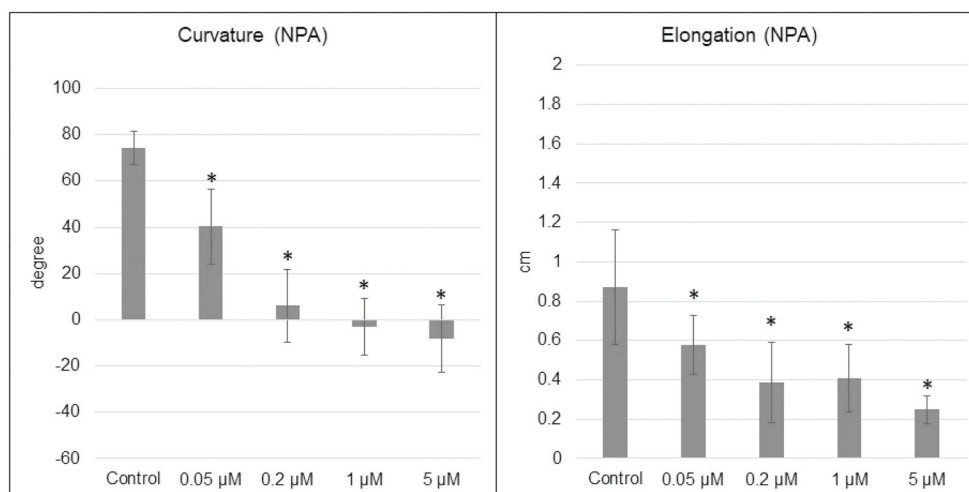


Fig. 13. (continued).

(quant, *Z:E* = 20:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 0.98 (t, J = 7.0 Hz, 3H), 3.99 (q, J = 7.0 Hz, 2H), 6.36 (d, J = 12.1 Hz, 1H), 7.35 (d, J = 4.3 Hz, 1H), 7.45 (d, J = 12.1 Hz, 1H), 7.56 (apparent t, J = 7.6 Hz, 1H), 7.73 (apparent t, J = 7.6 Hz, 1H), 7.88 (d, J = 8.2 Hz, 1H), 8.14 (d, J = 8.7 Hz, 1H), 8.90 (d, J = 4.3 Hz, 1H); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ : 13.6 (q), 60.5 (t), 120.1 (d), 124.4 (d), 125.1 (d), 125.9 (s), 126.7 (d), 129.4 (d), 130.0 (d), 139.0 (d), 142.2 (s), 148.0 (s), 149.6 (d), 165.0 (s); IR (KBr) 1722 cm^{-1} ; EIMS m/z 227 (M^+), 154 (100%); HRMS (FAB) Calcd for $\text{C}_{14}\text{H}_{14}\text{O}_2\text{N}$ ($\text{M} + \text{H}^+$): 228.1025, found: 228.1027.

4c: starting from 500 mg of the ester; colorless prism (45%, *Z:E* = 20:1); mp. 210–212 °C (EtOH); $^1\text{H-NMR}$ (600 MHz, TFA- d) δ : 6.65 (d, J = 12.4 Hz, 1H), 7.69 (d, J = 12.4 Hz, 1H), 7.86 (d, J = 5.8 Hz, 1H), 7.93 (apparent t, J = 7.6 Hz, 1H), 8.13 (apparent t, J = 7.6 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.20 (d, J = 8.2 Hz, 1H), 8.91 (d, J = 5.8 Hz, 1H); $^{13}\text{C-NMR}$ (150 MHz, TFA- d) δ : 122.6 (d), 122.7 (d), 128.2 (d), 128.4 (d), 129.4 (s), 133.4 (d), 138.6 (d), 139.6 (s), 142.1 (d), 144.6 (d), 159.3 (s), 171.7 (s); IR (KBr) 1697 cm^{-1} ; FAB-MS m/z 200 ($\text{M} + \text{H}^+$); Elem. Anal. calcd for $\text{C}_{12}\text{H}_9\text{NO}_2$: C, 72.35; H, 4.55; N, 7.03, found: C, 72.14; H, 4.33; N, 6.98.

3.2.11. Synthesis of (*Z*)-3-(quinoxalin-2-yl)acrylic acid (**4d**)

The title compound was synthesized according to the procedure of

4.2.1. from quinoxaline-2-carbaldehyde.

Ethyl ester of **4d**: starting from 474 mg of the aldehyde; pale purple oil (92%, *Z:E* = >99:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 1.23 (t, J = 7.0 Hz, 3H), 4.23 (q, J = 7.0 Hz, 2H), 6.39 (d, J = 12.1 Hz, 1H), 7.16 (d, J = 12.1 Hz, 1H), 7.75–7.79 (m, 2H), 8.04 (dd, J = 6.3, 3.4 Hz, 1H), 8.10 (dd, J = 6.3, 3.4 Hz, 1H), 9.03 (s, 1H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 14.0 (q), 60.9 (t), 125.9 (d), 129.2 (d), 129.3 (d), 130.1 (d), 130.2 (d), 137.5 (d), 141.4 (s), 141.7 (s), 145.6 (d), 149.5 (s), 165.9 (s); IR (KBr) 1721 cm^{-1} ; EIMS m/z 228 (M^+), 199 (100%); HRMS (FAB) Calcd for $\text{C}_{13}\text{H}_{13}\text{O}_2\text{N}_2$ ($\text{M} + \text{H}^+$): 229.0977, found: 229.0973.

4d: starting from 400 mg of the ester; pale pink needles (85%, *Z:E* = >99:1); mp. 105 °C (decomposed, EtOH); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 6.59 (d, J = 13.3 Hz, 1H), 7.15 (d, J = 13.3 Hz, 1H), 7.93–7.97 (m, 2H), 8.10 (dd, J = 6.2, 3.5 Hz, 1H), 8.21 (dd, J = 6.2, 3.5 Hz, 1H), 9.04 (s, 1H); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 127.1 (d), 129.6 (d), 132.2 (d), 132.3 (d), 132.5 (d), 132.9 (d), 137.6 (s), 142.7 (s), 145.7 (s), 147.4 (d), 165.6 (s); IR (KBr) 1678 cm^{-1} ; FAB-MS m/z 201 ($\text{M} + \text{H}^+$); Anal. calcd for $\text{C}_{11}\text{H}_8\text{N}_2\text{O}_2$: C, 66.00; H, 4.03; N, 13.99, found: C, 65.87; H, 3.97; N, 13.98.

3.2.12. Synthesis of (*E*)-2-styrylbenzoic acid (**5a**)

The title compound was synthesized according to the literature

procedure (Shahzad et al., 2010): $^1\text{H-NMR}$ (270 MHz, CDCl_3) δ : 7.03 (d, $J = 16.3$ Hz, 1H), 7.24–7.31 (m, 2H), 7.34–7.40 (m, 3H), 7.55–7.61 (m, 3H), 7.74–7.76 (m, 1H), 8.05–8.13 (m, 2H); The spectral data were consistent with those reported in the literature.

3.2.13. Synthesis of (E)-2-(2-(naphthalen-1-yl)vinyl)benzoic acid (5b)

The title compound was synthesized according to the literature procedure (Shahzad et al., 2010): $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 7.41 (t, $J = 7.7$ Hz, 1H), 7.52 (m, 3H), 7.64 (dd, $J = 7.7$, 7.7 Hz, 1H), 7.78 (d, $J = 16.4$ Hz, 1H), 7.82 (d, $J = 7.7$ Hz, 2H), 7.87 (d, $J = 8.7$ Hz, 2H), 8.08 (d, $J = 16.4$ Hz, 1H), 8.12 (dd, $J = 7.7$, 1.9 Hz, 1H), 8.26 (d, $J = 7.7$ Hz, 1H); The spectral data were consistent with those reported in the literature.

3.2.14. Synthesis of 2-(naphthalen-2-yl)benzoic acid (5c)

The title compound was synthesized according to the literature procedure (Wang et al., 2013): $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 7.45–7.51 (m, 5H), 7.61 (td, $J = 7.6$, 1.4 Hz, 1H), 7.81–7.87 (m, 4H), 7.99 (dd, $J = 8.2$, 1.4 Hz, 1H); The spectral data were consistent with those reported in the literature.

3.2.15. Synthesis of 2-(benzofuran-2-yl)benzoic acid (5d)

The title compound was synthesized according to the literature procedure (Yamaguchi et al., 1995): $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 6.99 (s, 1H), 7.23–7.30 (m, 2H), 7.48–7.51 (m, 2H), 7.62 (apparent t, $J = 8.6$ Hz, 2H), 7.76 (d, $J = 7.6$ Hz, 1H), 7.91 (d, $J = 7.6$ Hz, 1H); The spectral data were consistent with those reported in the literature.

3.3. Inhibitory test for gravitropic bending

The lettuce seeds (*Lactuca sativa* cv. Great Lakes 366) 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 at the concentrations specified 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 24 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>) (Fig. 3).

4. Conclusion

Considering **ku-76**, developed by the authors as a lead compound in selective gravitropism inhibition, analogues fixed through conformation and/or configuration were designed, synthesized, and evaluated for gravitropic bending inhibitory activity along with elongation inhibitory activity using lettuce roots. The rotatable C5-aromatic single bond was fixed by bridging the C4-ortho-position of the aromatic ring. Although the naphthyl-type analogues (**1a** and **1b**) were highly potent for bending, significant growth inhibition was also observed. The benzo-carbocycle analogues (**2a-c**) showed less elongation inhibition than naphthyl analogues, but were less potent. Among the carbocycles, the five-membered one (**2b**) was the most potent, and replacing the bridging unit of five-membered carbocycles with five-membered heterocycles was much more effective in selective gravitropic bending. In particular, thio-analogue **3a** (**ku-257**) inhibited bending at 0.2 μM without growth inhibition. Quinoline analogues **4** were less potent than five-membered ones (**3**). These results suggest that the conformational fixation of the C5-aromatic ring of **ku-76** would be effective for selective gravitropic bending.

As the *cis*-alkene of **ku-76** is labile to external factors, the styryl analogues of the *cis*-alkene were replaced with a benzene ring. This was also effective in the potency of gravitropic bending inhibition. Among the analogues described here, the conformational and/or

configurational fixed analogues **5a** (**ku-294**) and **5c** (**ku-308**) as well as **3a** (**ku-257**) were the most potent inhibitors without inhibition of growth. The inhibitory activity of the potent analogues was comparable to that of NPA.

These highly potent analogues would be very useful tools in the elucidation of the mechanism of gravitropic inhibitory activity and yield important clues for the design of new types of agrochemicals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.phytochem.2020.112508>.

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