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Original Article

Synthesis and structure-activity relationships of new pyrazole derivatives that induce triple response in *Arabidopsis* seedlings

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Twenty-seven analogues of pyrazole derivatives were synthesized and subjected to structure–activity relationship studies on inducing the triple response in *Arabidopsis* seedlings. We found that 3,4-Dichloro-*N*-methyl-*N*-[(1-allyl-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl]benzenesulfonamide (**C26**) exhibits potent activity on inducing the triple response in *Arabidopsis* seedlings. **C26** (10 μM) induced an exaggerated apical hook in *Arabidopsis* seedlings. The curvature of the hook of the *Arabidopsis* seedlings was found to be 300±23 degrees, while ethephon (10 μM), a prodrug of ethylene, and a non-chemically treated control were found to be 128±19 and 58±16 degrees, respectively. **C26** also exhibited potent activity on reducing stem elongation. The hypocotyl length of *Arabidopsis* seedlings treated with **C26** (10 μM) was found to be 0.25±0.02 cm, while those of ethephon-treated (10 μM) and treated controls were found to be 0.69±0.06 and 1.15±0.01 cm, respectively. **C26** displayed potency inhibiting the root growth of *Arabidopsis* seedlings similar to that of ethephon.

Keywords: plant hormone, plant growth regulators, triple response, pyrazole derivatives, structure–activity relationships.

Introduction

Plant responses to internal and external stimuli through activating the expression of genes are regulated by a complex mechanism of signal transduction networks.[1] Plant hormones are important signal mediators involved in signaling. Ethylene is a gaseous plant hormone that plays key roles in regulating broad aspects of physiological processes, including growth and development, as well as in defense responses to environmental cues.[2–4] Ethylene has been well characterized as a key hormone involved in the induction of release from seed dormancy,[5] formation of the apical hook in dark-grown seedlings,[6] flower opening,[7] control fruit ripening,[8] and senescence.[9] Ethylene has also been implicated in defense responses to flooding[10] and pathogen inflection.[11]

Because ethylene affects several important agronomy trials, such as the induction of release from seed dormancy, senescence, and plant defense, efforts have been made to use ethylene as a plant growth regulators.[12,13] Since ethylene is a flammable gas at normal atmosphere, this property greatly prevents the use of ethylene in the agricultural industry. Currently, the prodrug of ethylene, ethephon, which degrades and subsequently releases ethylene in plant tissues, is used in a major way to manipulate the ethylene levels in plant tissues.[14] Ethephon has been used for promoting fruit ripening[15] and abscission[16,17] and for weed control.[18] Ethephon has also been registered as a pesticide for a number of food, feed, and nonfood crops such as cotton.

To meet the demands for new chemicals that are non-gaseous at normal atmosphere but that have ethylene-like activity, we conducted a systemic search for chemicals that would be useful alternatives for ethylene and/or ethephon. In the previous work, we reported discovering through a chemical library screening a new synthetic pyrazole derivative (named **EH-1**, IUPAC name: *N*-[(1,3,5-trimethyl-1*H*-pyrazol-4-yl)methyl]-*N*-methyl-2-naphthalenesulfonamide) that displays ethylene-like activity.[19]

In the course of our work, we have used the triple response assay, which has been demonstrated to be quite useful in determining the effect of ethylene in plants.[20] Thus, compounds in a chemical library that caused *Arabidopsis* seedlings to exhibit short hypocotyls and with an exaggerated apical hook were marked as hits. We found that **EH-1** (the structure is shown in Fig. 1) displayed promising activity in inducing the triple response in *Arabidopsis* seedlings. Initial structure–activity relationship studies of the analogues with a phenyl moiety instead of a naphthalene moiety of **EH-1** indicated that introducing chlorine atom(s) on the phenyl ring (the benzenesulfonamide moiety) dramatically affects the biological activity of this synthetic

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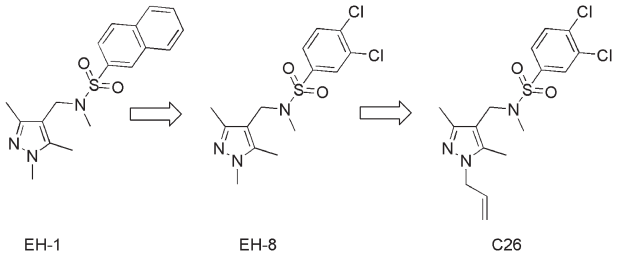


Fig. 1. Chemical structure of pyrazole derivatives described in the present work. Development of new compounds that induce the triple response in *Arabidopsis* seedlings: **EH-1** was discovered as a lead compound through a compound library, **EH-8** is a derivative of **EH-1** reported in our previous work,[19] and **C26** is the most potent compound reported in this work.

series. We also found that a 3,4-dichlorophenyl derivative (**EH-8**, the structure is shown in Fig. 1) is the most potent analogue among the synthesized compounds.[19]

In order to gain understanding on the structure–activity relationships of this synthetic series, we report herein the synthesis of 27 analogues with different substitutions on the phenyl ring of the benzenesulfonamide moiety as well as three analogues with different substitutions at position 1 of the 3,5-dimethylpyrazole moiety. Structure–activity relationships and future directions of the application use of this synthetic series were discussed.

Materials and Methods

1. General

^{1}H-NMR spectra were recorded with a JEOL ECP-400 spectrometer (Tokyo, Japan), with chemical shifts being expressed in ppm downfield from TMS as an internal standard. High-resolution electrospray ionization Fourier transform ion cyclotron resonance (ESI-FTICR) mass spectra were recorded on an Exactive MS system (Thermo Fisher Scientific, Waltham, MA, USA).

2. Reagents

Chemicals for synthesis were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Reagents were of the highest grade commercially available. *N*-methyl-1-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-methanamine was purchased from Sigma-Aldrich. *N*-methyl-1-(1-allyl-3,5-dimethyl-1*H*-pyrazol-4-yl)-methanamine and *N*-methyl-1-(1-*tert*-butyl-3,5-dimethyl-1*H*-pyrazol-4-yl)methanamine were purchased from Aldlab Chemicals (Woburn, MA, USA).

3. Chemical synthesis

3.1. 2-Fluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)-*methyl*]*benzenesulfonamide* (**C1**)

2-Fluorobenzenesulfonyl chloride (0.26 mmol) was added to a methylene chloride (0.2 M) solution of trimethylamine (1.0 mmol) and *N*-methyl-1-(1,3,5-trimethyl-1*H*-pyrazol-4-yl) methanamine (0.26 mmol), and stirred at room temperature for 12 hr. The reaction mixture was washed with 5% $NaHCO_3$, 1 M HCl, and saturated NaCl. The organic layer was dried, and the solvent was removed under reduced pressure. The residue was purified by chromatography using $CHCl_3$: MeOH = 9 : 1 (v : v) as an elution solution to obtain 30.7 mg of the target compound (yield: 38%), m.p. 72–74°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.20 (s, 3H), 2.25 (s, 3H), 2.61 (d, J = 1.4 Hz, 3H), 3.75 (s, 3H), 4.10 (s, 2H), 7.23–7.26 (m, 1H), 7.28–7.33 (m, 1H), 7.58–7.64 (m, 1H), 7.89–7.93 (m, 1H). The HRMS-ESI calculated for $C_{14}H_{19}FN_3O_2S$ $[M+H]^+$ was 312.1182, and we found 312.1179.

Other compounds, **C2–C24**, were prepared in a similar way, by the reaction of *N*-methyl-1-(1,3,5-trimethyl-1*H*-pyrazol-4-yl)-methanamine with the corresponding sulfonyl chloride.

Syntheses of compounds **C26** and **C27** were carried out using commercially available *N*-methyl-1-(1-allyl-3,5-dimethyl-1*H*-pyrazol-4-yl)-methanamine **(B2)** and *N*-methyl-1-(1-*tert*-butyl-3,5-dimethyl-1*H*-pyrazol-4-yl)-methanamine **(B3)** as starting materials. The method used was similar to that used in the preparation of compound **C1**.

3.2. 3-Fluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)-*methyl*]*benzenesulfonamide* (**C2)**

Yield: 29%, m.p. 86–87°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.16 (s, 3H), 2.23 (s, 3H), 2.53 (s, 3H), 3.74 (s, 3H), 3.92 (s, 2H), 7.32–7.37 (m, 1H), 7.53–7.64 (m, 3H). The HRMS-ESI calculated for $C_{14}H_{19}FN_3O_2S$ $[M+H]^+$ was 312.1182, and we found 312.1179.

3.3. 4-Fluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)-*methyl*]*benzenesulfonamide* (**C3**)

Yield: 30%, m.p. 127–128°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.16 (s, 3H), 2.23 (s, 3H), 2.50 (s, 3H), 3.74 (s, 3H), 3.90 (s, 2H), 7.24–7.29 (m, 2H), 7.83–7.87 (m, 2H). The HRMS-ESI calculated for $C_{14}H_{19}FN_3O_2S$ $[M+H]^+$ was 312.1182, and we found 312.1179.

3.4. 2,4-Difluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C4**)

Yield: 40%, m.p. 86–87°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.19 (s, 3H), 2.25 (s, 3H), 2.61 (s, 3H), 3.74 (s, 3H), 4.08 (s, 2H), 6.98–7.06 (m, 2H), 7.90–7.96 (m, 1H). The HRMS-ESI calculated for $C_{14}H_{18}F_2N_3O_2S$ $[M+H]^+$ was 330.1088, and we found 330.1085.

3.5. 3,4-Difluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C5)**

Yield: 26%, m.p. 111–112°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.20 (s, 3H), 2.26 (s, 3H), 2.53 (s, 3H), 3.79 (s, 3H), 3.93 (s, 2H), 7.33–7.42 (m, 1H), 7.60–7.64 (m, 1H), 7.65–7.70 (m, 1H). The HRMS-ESI calculated for $C_{14}H_{18}F_2N_3O_2S$ $[M+H]^+$ was 330.1088, and we found 330.1086.

3.6. 2,4,5-Trifluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C6**)

Yield: 45%, m.p. 80–81°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.18 (s, 3H), 2.24 (s, 3H), 2.63 (s, 3H), 3.73 (s, 3H), 4.09 (s, 2H), 7.10–7.17 (m, 1H), 7.73–7.79 (m, 1H). The HRMS-ESI calculated for $C_{14}H_{16}F_3N_3O_2SNa$ $[M+Na]^+$ was 370.0813, and we found 370.0812.

3.7. 3-Chloro-2-fluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C7**)

Yield: 58%, m.p. 123–124°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.20 (s, 3H), 2.25 (s, 3H), 2.64 (s, 3H), 3.74 (s, 3H), 4.13 (s, 2H), 7.24–7.28 (m, 2H), 7.64–7.67 (m, 1H), 7.79–7.83 (m, 1H). The HRMS-ESI calculated for $C_{14}H_{18}FClN_3O_2S$ $[M+H]^+$ was 346.0792, and we found 346.0791.

3.8. 3-Chloro-4-fluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C8**)

Yield: 20%, m.p. 101–102°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.19 (s, 3H), 2.25 (s, 3H), 2.56 (s, 3H), 3.77 (s, 3H), 3.96 (s, 2H), 7.73 (d, *J*=8.2 Hz, 1H), 7.93 (dd, *J*=2.1, 6.4 Hz, 1H), 8.12 (d, *J*=2.1 Hz, 1H). The HRMS-ESI calculated for $C_{15}H_{17}F_3ClN_3O_2SNa$ $[M+Na]^+$ was 418.0580, and we found 418.0578.

3.9. 4-Chloro-N-*methyl-3-trifluoromethyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C9**)

Yield: 20%, m.p. 101–102°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.19 (s, 3H), 2.25 (s, 3H), 2.56 (s, 3H), 3.77 (s, 3H), 3.96 (s, 2H), 7.73 (d, *J*=8.2 Hz, 1H), 7.93 (dd, *J*=2.1, 6.4 Hz, 1H), 8.12 (d, *J*=2.1 Hz, 1H). The HRMS-ESI calculated for $C_{15}H_{17}F_3ClN_3O_2SNa$ $[M+Na]^+$ was 418.0580, and we found 418.0578.

3.10. 5-Chloro-2-methoxy-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C10**)

Yield: 36%, m.p. 101–102°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.19 (s, 3H), 2.26 (s, 3H), 2.64 (s, 3H), 3.75 (s, 3H), 3.95 (s, 3H), 4.13 (s, 2H), 6.97–6.99 (m, 1H), 7.47–7.50 (m, 1H), 7.91 (t, *J*=2.5 Hz, 1H). The HRMS-ESI calculated for $C_{15}H_{21}ClN_3O_3S$ $[M+H]^+$ was 358.0992, and we found 358.0991.

3.11. 4-Methyl-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C11**)

Yield: 34%, m.p. 90–91°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.17 (s, 3H), 2.25 (s, 3H), 2.47 (s, 3H), 2.48 (s, 3H), 3.78 (s, 3H), 3.88 (s, 2H), 7.37 (d, *J*=8.2 Hz, 2H), 7.72 (d, *J*=8.2 Hz, 2H). The HRMS-ESI calculated for $C_{15}H_{21}N_3O_2SNa$ $[M+Na]^+$ was 330.1252, and we found 330.1250.

3.12. 4-Ethyl-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)-*methyl*]*benzenesulfonamide* (**C12**)

Yield: 52%, m.p. 84–85°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 1.27–1.32 (m, 3H), 2.17 (s, 3H), 2.25 (s, 3H), 2.49 (s, 3H), 2.73–2.79 (m, 2H), 3.75 (s, 3H), 3.89 (s, 2H), 7.39 (d, *J*=7.3 Hz, 2H), 7.74 (d, *J*=6.6 Hz, 2H). The HRMS-ESI calculated for $C_{16}H_{23}N_3O_2SNa$ $[M+Na]^+$ was 344.1409, and we found 344.1406.

3.13. N-*Methyl-4-propyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C13**)

Yield: 50%, oil, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 0.97 (t, *J*=7.6 Hz, 3H), 1.65–1.74 (m, 2H), 2.15 (s, 3H), 2.23 (s, 3H), 2.49 (s, 3H), 2.69 (t, *J*=7.8 Hz, 2H), 3.73 (s, 3H), 3.89 (s, 2H), 7.37 (d, *J*=7.7 Hz, 2H), 7.73 (d, *J*=8.0 Hz, 2H). The HRMS-ESI calculated for $C_{17}H_{25}N_3O_2SNa$ $[M+Na]^+$ was 358.1565, and we found 358.1563.

3.14. 4-tert-*Butyl*-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C14**)

Yield: 45%, m.p. 117–118°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 1.37 (d, *J*=1.4 Hz, 9H), 2.20 (s, 3H), 2.27 (s, 3H), 2.51 (s, 3H), 3.79 (s, 3H), 3.92 (s, 2H), 7.57 (dd, *J*=1.4, 7.1 Hz, 2H), 7.75 (dd, *J*=1.4, 7.3 Hz, 2H). The HRMS-ESI calculated for $C_{18}H_{28}N_3O_2S$ $[M+H]^+$ was 350.1902, and we found 350.1900.

3.15. Biphenyl-4-sulfonic acid methyl-(*1,3,5-trimethyl-1*H-*pyrazol-4-ylmethyl*)*amide* (**C15**)

Yield: 40%, m.p. 126–127°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.19 (s, 3H), 2.26 (s, 3H), 2.55 (s, 3H), 3.76 (s, 3H), 3.95 (s, 2H), 7.42–7.46 (m, 1H), 7.50(t, *J*=7.1 Hz, 2H), 7.64 (d, *J*=8.2 Hz, 2H), 7.78 (d, *J*=7.6 Hz, 2H), 7.90 (d, *J*=8.2 Hz, 2H). The HRMS-ESI calculated for $C_{20}H_{24}N_3O_2S$ $[M+H]^+$ was 370.1589, and we found 370.1588.

3.16. 2,5-N-*Dimethyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)-*methyl*]*benzenesulfonamide* (**C16**)

Yield: 43%, m.p. 78–80°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.13 (s, 3H), 2.18 (s, 3H), 2.39 (s, 3H), 2.60 (d, *J*=3.2 Hz, 6H), 3.75 (s, 3H), 4.04 (s, 2H), 7.22 (d, *J*=7.8 Hz, 1H), 7.27–7.29 (m, 1H), 7.70 (s, 1H). The HRMS-ESI calculated for $C_{16}H_{24}N_3O_2S$ $[M+H]^+$ was 322.1589, and we found 322.1587.

3.17. N-*Methyl-2,4,6-trimethyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C17**)

Yield: 53%, m.p. 88–89°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.12 (s, 3H), 2.14 (s, 3H), 2.32 (s, 3H), 2.53 (s, 3H), 2.64 (s, 6H), 3.80 (s, 3H), 4.02 (s, 2H), 6.97 (d, *J*=7.3 Hz, 2H). The HRMS-ESI calculated for $C_{17}H_{26}N_3O_2S$ $[M+H]^+$ was 336.1746, and we found 366.1743.

3.18. 4-Methoxy-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C18**)

Yield: 40%, m.p. 94–95°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.17 (s, 3H), 2.24 (s, 3H), 2.48 (s, 3H), 3.75 (s, 3H), 3.88 (s, 2H), 3.91 (t, *J*=1.4 Hz, 3H), 7.03–7.06 (m, 2H), 7.76–7.79 (m, 2H). The HRMS-ESI calculated for $C_{15}H_{21}N_3O_3SNa$ $[M+Na]^+$ was 346.1201, and we found 346.1199.

3.19. 4-Isopropoxy-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C19**)

Yield: 22%, oil, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 1.39 (s, 3H), 1.40 (s, 3H), 2.21 (s, 3H), 2.28 (s, 3H), 2.48 (s, 3H), 3.81 (s, 3H), 3.88 (s, 2H), 4.62–4.70(m, 1H), 6.98–7.02 (m, 2H), 7.72–7.77 (m, 2H). The HRMS-ESI calculated for $C_{17}H_{26}N_3O_3S$ $[M+H]^+$ was 352.1695, and we found 352.1693.

3.20. N-*Methyl-4-trifluoromethyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C20**)

Yield: 57%, m.p. 116–117°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.18 (s, 3H), 2.25 (s, 3H), 2.54 (s, 3H), 3.76 (s, 3H), 3.94 (s, 2H), 7.85 (d, *J*=8.5 Hz, 2H), 7.96 (d, *J*=8.5 Hz, 2H). The HRMS-ESI calculated for $C_{15}H_{19}F_3N_3O_2S$ $[M+H]^+$ was 362.1150, and we found 362.1149.

3.21. N-*Methyl-2-trifluoromethyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C21**)

Yield: 20%, m.p. 80–81°C, ^{1}H NMR (400 MHz, $CDCl_3$); *δ*: 2.15 (d, *J*=2.1 Hz, 3H), 2.21 (d, *J*=2.1 Hz, 3H), 2.64 (d, *J*=1.6 Hz,

3H), 3.72 (d, J=1.8 Hz, 3H), 4.19 (s, 2H), 6.97–6.99 (m, 1H), 7.71–7.75 (m, 2H), 7.92–7.93 (m, 1H), 8.03–8.05 (m, 1H). The HRMS-ESI calculated for $C_{15}H_{18}F_3N_3O_2SNa$ $[M+Na]^+$ was 384.0970, and we found 384.0969.

3.22. 2,4,5-Trichloro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C22**)

Yield: 32%, m.p. 85–86°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.19 (s, 3H), 2.24 (s, 3H), 2.70 (s, 3H), 3.74 (s, 3H), 4.21 (s, 2H), 7.64 (s, 1H), 8.10 (s, 1H). The HRMS-ESI calculated for $C_{14}H_{17}Cl_3N_3O_2S$ $[M+H]^+$ was 396.0108, and we found 396.0107.

3.23. 4-Bromo-2,5-difluoro-N-*methyl*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C23**)

Yield: 5.4%, m.p. 121–122°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.19 (d, J=2.3 Hz, 3H), 2.24 (d, J=2.3 Hz, 3H), 2.63 (s, 3H), 3.75 (d, J=2.3 Hz, 3H), 4.01 (s, 2H), 7.48–7.52 (m, 1H), 7.64–7.68 (m, 1H). The HRMS-ESI calculated for $C_{14}H_{16}F_2BrN_3O_2SNa$ $[M+Na]^+$ was 430.0012, and we found 430.0013.

3.24. N-*Methyl-4-phenylazo*-N-[(*1,3,5-trimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C24**)

Yield: 6.6%, m.p. 74–75°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.20 (s, 3H), 2.27 (s, 3H), 2.55 (s, 3H), 3.78 (s, 3H), 3.96 (s, 2H), 6.97–6.99 (m, 1H), 7.55–7.59 (m, 3H), 7.97–7.99 (m, 4H), 8.07–8.09 (m, 2H). The HRMS-ESI calculated for $C_{20}H_{23}N_5O_2SNa$ $[M+Na]^+$ was 420.1470, and we found 420.1469.

3.25. [(*3,5-dimethyl-1-phenyl-1*H-*pyrazol-4-yl*)*methyl*]*methylamine* (**B1**)

B1 was prepared using a method previously described.[21] Methylamine–HCl (25 mmol) and paraformaldehyde (30 mM, 6 eq) were combined in absolute EtOH (0.5 M) and was heated for 2 hr at 60°C. At this point, the 3,5-dimethyl-1-phenylpyrazole (1.15 g, 5 mmol) was added, and the reaction was heated to 75°C and stirred for 10 hr. Then, the reaction was cooled to room temperature, and the solvent was removed under reduced pressure. The residue mixture was solved in 50 mL of $CHCl_3$ and washed with a saturated aqueous solution of $NaHCO_3$ (1×20 mL). The aqueous layer was then extracted with $CHCl_3$ (3×30 mL) and dried over with Na_2SO_4; the solvent was removed under reduced pressure. The resulting oil was purified *via* flash chromatography with EtOAc : hexanes = 1 : 1 (v : v) to yield the target compound as an oil (0.94 g, yield: 82%); ^{1}H NMR ($CDCl_3$); δ 2.1 (s, 3H), 2.24 (s, 3H), 2.85 (s, 1H), 3.26 (s, 2H), 5.20 (s, 2H), 7.03 (dd, J=1.2, 7.6 Hz, 2H), 7.40–7.14 (m, 3H). The HRMS-ESI calculated for $C_{14}H_{20}N_3O_3$ (M+H+) was 230.1652, and we found 230.1656.

3.26. 3,4-dichloro-N-*methyl*-N-[(*3,5-dimethyl-1-phenyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C25**)

Preparation of **C25** was carried out by reacting **B1** with 3,4-dichlorobenzenesulfonyl chloride by a method similar to that used to prepare compound **C1**, as described above. Yield: 12%, m.p. 116–117°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.26 (s, 3H), 2.28 (s, 3H), 2.60 (s, 3H), 4.01 (s, 2H), 7.35–7.39 (m, 3H), 7.44–7.48 (m, 2H), 7.66 (s, 2H), 7.93 (s, 1H). The HRMS-ESI calculated for $C_{16}H_{19}Cl_2N_3O_2SNa$ $[M+Na]^+$ was 410.0473, and we found 410.0427.

3.27. 3,4-Dichloro-N-*methyl*-N-[(*1-Allyl-3,5-dimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C26**)

Yield: 70%, m.p. 90–91°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 2.18 (d, J=2.1 Hz, 3H), 2.20 (d, J=2.1 Hz, 3H), 2.53 (d, J=2.3 Hz, 3H), 3.94 (s, 2H), 4.62–4.64 (m, 2H), 4.95 (d, J=16.9 Hz, 1H), 5.19 (d, 10.3 Hz, 1H), 5.88–5.97 (m, 1H), 7.65 (s, 2H), 7.92 (s, 1H). The HRMS-ESI calculated for $C_{16}H_{19}Cl_2N_3O_2SNa$ $[M+Na]^+$ was 410.0473, and we found 410.0427.

3.28. 3,4-Dichloro-N-*methyl*-N-[(*1*-tert-*butyl-3,5-dimethyl-1*H-*pyrazol-4-yl*)*methyl*]*benzenesulfonamide* (**C27**)

Yield: 25%, m.p. 131–132°C, ^{1}H NMR (400 MHz, $CDCl_3$); δ: 1.58–1.62 (m, 9H), 2.14 (s, 3H), 2.40 (s, 3H), 2.52 (s, 3H), 3.91 (s, 2H), 7.65 (s, 2H), 7.92 (s, 1H). The HRMS-ESI calculated for $C_{17}H_{23}Cl_2N_3O_2SNa$ $[M+Na]^+$ was 426.0786, and we found 426.0786.

4. Plant materials, growth conditions, and triple response assay

Seeds of *Arabidopsis* (ecotype Columbia) were purchased from Lehle Seeds (Round Rock, TX, USA). Seeds used for the assay were sterilized in 1% NaOCl for 20 min and washed with sterile distilled water. Seeds were sown on a 1% solidified agar medium containing 1/2 Murashige and Skoog (MS) salt added to 24-well plates (Fukae Kasei Co., Ltd., Kobe, Japan) with or without chemicals. Plants were grown under dark conditions in a growth chamber with or without chemicals. The biological activities of the test compounds were measured 5 days after the seeds were sown. Stock solutions of all of the chemicals were dissolved in DMSO in designed growth media at 0.1% (v/v), as described previously.[22]

A triple response assay was performed in a 24-well plate. A solution of 1 μL of the compound and 1 mL of the plant growth media containing 1/2 MS salt and 1% solidified agar was added to each well. *Arabidopsis* seedlings were germinated and grown in the dark, as described above. The biological activities of the test compounds were examined visually by measuring the length of the hypocotyls. Observing the angle of the apical hook and the length of the root was done as we described previously.[22]

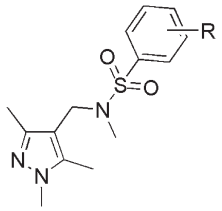
Results

1. Effect of substituents on phenyl moiety on induction of triple response in Arabidopsis *seedlings*

To further determine the structure–activity relationship of **EH-1** derivatives, 24 analogues with different substituents on a phenyl moiety were synthesized and subjected to biological studies. In the present work, ethephon was used as a positive control, and **EH-8** {3,4-Dichloro-*N*-methyl-*N*-[(1,3,5-trimethyl-1*H*-pyrazol-4-yl)methyl]benzene-sulfonamide}, which displayed the most potent activity among the previously reported compounds for inducing the triple response in *Arabidopsis* seedlings,[19] was used as a reference for structure–activity relationship discussions.

We first determined the effect of the synthesized compounds on apical hook development of *Arabidopsis* seedlings. As shown in Table 1, the curvature of the hook of the non-chemically treated control was found to be approximately 38±9 degrees,

Table 1. Effect of phenyl substituents on apical hook development of *Arabidopsis* seedlings



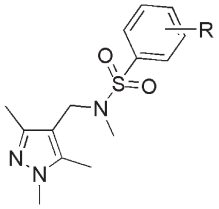
Comp. No.	R	Curvature of the hook (Degree)	
		10 (μM)	100 (μM)
C1	2-Fluoro	42±2	72±10
C2	3-Fluoro	53±10	85±19
C3	4-Fluoro	46±9	95±21
C4	2,4-Difluoro	42±6	59±13
C5	3,4-Difluoro	39±14	123±21
C6	2,4,5-Trifluoro	60±21	84±16
C7	3-Chloro-2-fluoro	54±8	106±32
C8	3-Chloro-4-fluoro	58±7	223±35
C9	4-Chloro-3-trifluoromethyl	83±22	203±22
C10	5-Chloro-2-methoxy	68±10	142±36
C11	4-Methyl	75±8	115±26
C12	4-Ethyl	153±17	184±26
C13	4-Propyl	153±10	199±39
C14	4-*tert*-Butyl	70±8	220±16
C15	4-Phenyl	53±7	193±25
C16	2,5-Dimethyl	68±16	108±17
C17	2,4,6-Trimethyl	115±19	135±33
C18	4-Methoxy	72±7	120±28
C19	4-Isopropoxy	77±8	179±30
C20	4-Trifluoromethyl	74±21	195±52
C21	2-Trifluoromethyl	92±17	190±25
C22	2,4,5-Trichloro	95±14	223±20
C23	4-Bromo-2,5-difluoro	106±35	213±18
C24	4-Phenylazo	124±15	166±20
EH-8	3,4-Dichloro	185±14	275±32
Ethephone		128±10	209±16
Control		38±9	

Data are the means±S.E. obtained from 11 to 15 plants. All the experiments were done three times to establish the repeatability.

while the curvature of the hooks of ethephon-treated *Arabidopsis* seedlings were found to be approximately 128±10 degrees (10 μM) and 209±16 degree (100 μM). These results indicate that ethephon induced an exaggerated apical hook in *Arabidopsis* seedlings in our assay system. In contrast, the reference compound of **EH-8** displayed potent activity, inducing an exaggerated apical hook approximately 185±14 degrees (10 μM) and 275±32 degrees (100 μM).

For the test compounds synthesized in the present work with fluorine atom(s) substitutions (**C1**–**C6**), we found that the curvature of the hook of the *Arabidopsis* seedlings were from approximately 39±14 to 60±21 degrees (at a concentration of 10 μM). Compared with non-chemically treated and **EH-8**-treated *Arabidopsis* seedlings, data obtained indicated that the fluorine atom(s) substitutions on the phenyl moiety have a negative effect on the enhancement of biological activity. Other compounds (**C7**–**C24**), despite a variety of chemical substituents, have been introduced on the phenyl moiety, including different lengths of the alkyl chain (**C11**–**C17**) or the alkyloxy chain (**C18**–**C19**) and other substituents (**C7**–**C10, C20**–**C24**); none of these compounds promoted biological activity.

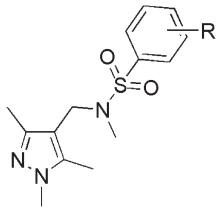
Table 2. Effect of phenyl substituents on stem elongation of *Arabidopsis* seedlings



Comp. No.	R	Hypocotyl length (cm)	
		10 (μM)	100 (μM)
C1	2-Fluoro	1.14±0.03	1.01±0.01
C2	3-Fluoro	1.10±0.02	0.98±0.03
C3	4 Fluoro	1.07±0.06	0.84±0.03
C4	2,4-Difluoro	1.12±0.01	1.03±0.08
C5	3,4-Difluoro	1.10±0.05	0.79±0.08
C6	2,4,5-Trifluoro	1.12±0.05	1.04±0.01
C7	3-Chloro-2-fluoro	1.14±0.01	0.92±0.06
C8	3-Chloro-4-fluoro	1.15±0.05	0.40±0.03
C9	4-Chloro-3-trifluoromethyl	1.00±0.03	0.33±0.02
C10	5-Chloro-2-methoxy	1.14±0.09	0.73±0.04
C11	4-Methyl	1.02±0.04	0.80±0.05
C12	4-Ethyl	0.62±0.03	0.53±0.04
C13	4-Propyl	0.80±0.05	0.45±0.02
C14	4-*tert*-Butyl	0.96±0.02	0.26±0.01
C15	4-Phenyl	1.08±0.04	0.60±0.03
C16	2,5-Dimethyl	1.12±0.05	0.97±0.05
C17	2,4,6-Trimethyl	0.79±0.03	0.49±0.04
C18	4-Methoxy	1.03±0.04	0.64±0.03
C19	4-Isopropoxy	1.13±0.03	0.54±0.03
C20	4-Trifluoromethyl	0.82±0.06	0.37±0.03
C21	2-Trifluoromethyl	1.06±0.03	0.45±0.02
C22	2,4,5-Trichloro	0.90±0.04	0.55±0.03
C23	4-Bromo-2,5-difluoro	0.92±0.03	0.62±0.04
C24	4-Phenylazo	0.88±0.05	0.72±0.01
EH-8	3,4-Dichloro	0.63±0.03	0.48±0.02
Ethephone		0.69±0.06	0.41±0.01
Control		1.15±0.01	

Data are the means±S.E. obtained from 11 to 15 plants. All the experiments were done three times to establish the repeatability.

Table 3. Effect of phenyl substituents on root growth of *Arabidopsis* seedlings



Comp. No.	R	Root length (mm)	
		10 (μM)	100 (μM)
C1	2-Fluoro	2.5±0.1	2.5±0.1
C2	3-Fluoro	2.5±0.1	2.4±0.2
C3	4 Fluoro	2.5±0.3	2.1±0.3
C4	2,4-Difluoro	2.6±0.1	2.4±0.2
C5	3,4-Difluoro	2.3±0.2	2.4±0.1
C6	2,4,5-Trifluoro	2.5±0.3	2.6±0.3
C7	3-Chloro-2-fluoro	2.6±0.2	2.5±0.3
C8	3-Chloro-4-fluoro	2.5±0.1	2.3±0.3
C9	4-Chloro-3-trifluoromethyl	2.6±0.1	2.4±0.2
C10	5-Chloro-2-methoxy	2.8±0.2	3.5±0.2
C11	4-Methyl	2.4±0.1	2.4±0.3
C12	4-Ethyl	2.4±0.1	2.1±0.2
C13	4-Propyl	2.4±0.1	1.7±0.3
C14	4-*tert*-Butyl	2.6±0.2	1.3±0.1
C15	4-Phenyl	2.3±0.1	2.3±0.2
C16	2,5-Dimethyl	2.3±0.3	2.7±0.2
C17	2,4,6-Trimethyl	3.7±0.5	2.8±0.2
C18	4-Methoxy	2.1±0.1	1.6±0.2
C19	4-Isopropoxy	2.8±0.2	2.3±0.2
C20	4-Trifluoromethyl	2.5±0.2	2.5±0.2
C21	2-Trifluoromethyl	1.8±0.2	1.0±0.2
C22	2,4,5-Trichloro	1.6±0.3	1.9±0.2
C23	4-Bromo-2,5-difluoro	1.9±0.2	2.1±0.2
C24	4-Phenylazo	1.8±0.1	1.9±0.3
EH-8	3,4-Dichloro	1.9±0.3	1.5±0.4
Ethephone		1.0±0.1	0.7±0.1
Control		2.3±0.1	

Data are the means±S.E. obtained from 11 to 15 plants. All the experiments were done three times to establish the repeatability.

Next, we determined the biological activity of the synthesized compounds on inhibiting the stem elongation of *Arabidopsis* seedlings grown in the dark. As shown in Table 2, the hypocotyl length of the non-chemically treated control was approximately 1.15±0.01 cm, while the hypocotyl length of ethephon-treated *Arabidopsis* seedlings was approximately 0.41±0.01 cm (at a concentration of 100 μM). This result indicated that ethephon inhibits the stem elongation of *Arabidopsis* seedlings in our assay system. **EH-8** displayed biological activity that reduced stem elongation at a degree of 0.48±0.02 cm (at a concentration of 100 μM), indicating that **EH-8** displayed promise for reducing stem elongation in *Arabidopsis* seedlings. As shown in Table 2, none of the compounds synthesized in the present work with different substituents on phenyl moieties (**C1**–**C24**) significantly enhanced biological activity.

Finally, we determine the effect of the test compounds on the inhibition of root elongation. As shown in Table 3, the root length of non-chemically treated *Arabidopsis* was found to be 2.3±0.1 mm, while *Arabidopsis* seedlings treated with ethephon displayed short roots—approximately 1.0±0.1 at 10 μM and 0.7±0.1 mm at 100 μM. This result indicated that ethephon reduced the root elongation of *Arabidopsis* seedlings in our assay system. As shown in Table 3, none of the compounds significantly reduced root elongation at a concentration of 100 μM except compound **C14**, which is an analogue with 4-*tert*-butyl

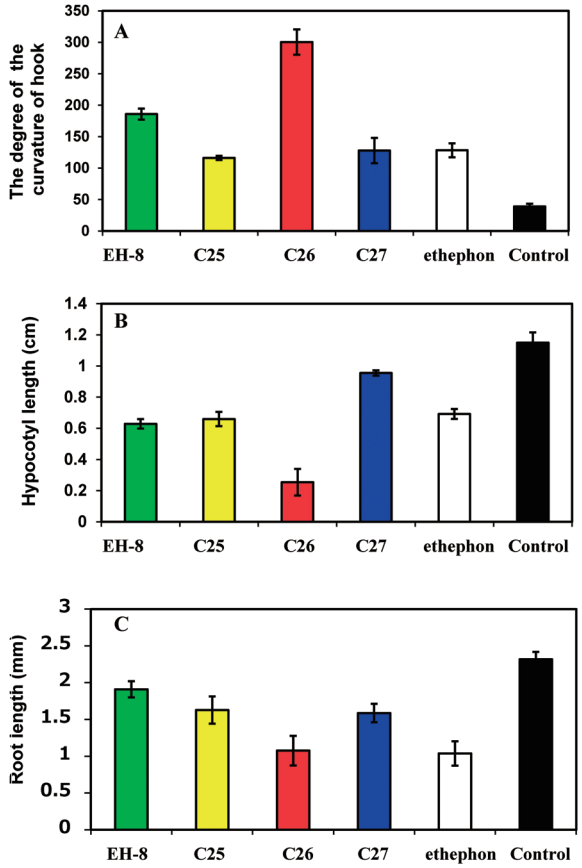
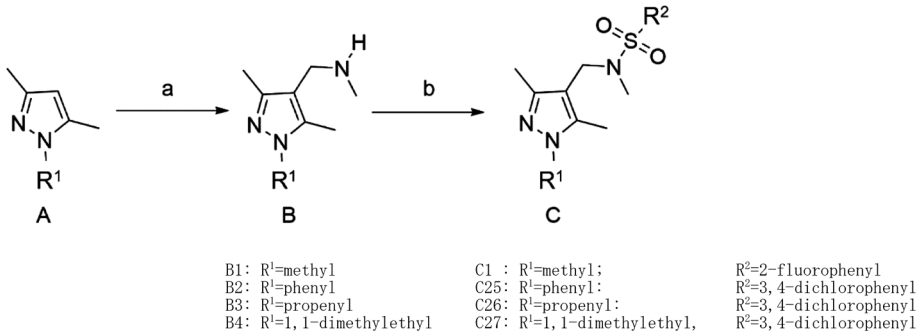


Fig. 2. Effect of EH-8 and its analogues on inducing the morphology typical of the triple response in *Arabidopsis* seedlings. The triple response of *Arabidopsis* seedlings was measured by determining the hypocotyl length, root length, and curvature of the hook. Approximately 50 seeds/well of *Arabidopsis* were grown for five days in the dark in a 24-well plate on medium containing 1/2 MS, and the concentration of the test compounds, including ethephon and the reference compound **EH-8**, was set at 10 μM. Ethephon was used as a positive control, while DMSO mock treatment was used as a control. (A) Effect of pyrazole derivatives on inducing exaggerated apical hooks of *Arabidopsis* seedlings; (B) effect of pyrazole derivatives on hypocotyl elongation; (C) effect of pyrazole derivatives on root growth. All the experiments in this work were conducted by measuring 11 seeds. Data are the means±S.E. obtained from 11 to 15 plants. All experiments were done three times to establish repeatability.



Scheme 1 Synthetic route for the preparation of target compounds. a: Methylamine–HCl (25 mmol), paraformaldehyde (30 mmol), at 60°C (2 hr). Then pyrazole (5 mmol) was added and warmed at 75°C (10 hr).[21] b: Benzenesulfonyl chloride was added to a methylene chloride solution of trimethylamine, and compound B (1 eq) was added and stirred at room temperature for 12 hr.[19]

substitution on the phenyl moiety. The degree of reduced roots of *Arabidopsis* seedlings was found to be approximately 1.3±0.1 mm. This result indicated that the compounds prepared in the present work display weakened activity for reducing root elongation of *Arabidopsis* seedlings.

3.2. *Effect of 1*-N-*alkyl substitution of pyrazoles on the induction of a triple response*

Through analyzing the structure–activity relationship of 24 analogues with different chemical substituents on a phenyl moiety **(C1–C24)**, we found that **EH-8** is the most potent compound in this synthetic series. Thus, we next carried out further structure–activity relationship studies by fixing the phenyl moiety as 3,4-dichlorophenyl, and the chemical structure of 3,5-dimethylpyrazole was modified by introducing phenyl, allyl, and *tert*-butyl to position 1 of the 3,5-dimethylpyrazole moiety **(C25–C27)**.

We used **EH-8** as a reference and ethephon as a positive control for further structure–activity relationship studies. First, we determined the effect of the compounds on apical hook development. As shown in Fig. 2A, the curvature of the hook of the non-chemically treated control was approximately 58±16 degrees (the black bar), while the curvature of the hook of ethephon-treated (10 μM) *Arabidopsis* seedlings was approximately 128±19 degrees (the white bar). This result indicates that ethephon induced an exaggerated apical hook in *Arabidopsis* seedlings. In terms of the biological activity of **EH-8** and analogues synthesized in the present work, we found that the curvature of the hook of the *Arabidopsis* seedlings treated with **EH-8** (10 μM) was 185±14 degrees (the green bar). This result indicated that **EH-8** exhibits potent activity in inducing an exaggerated apical hook in *Arabidopsis* seedlings. However, when introducing a phenyl ring instead of the methyl moiety at position 1 of the pyrazole moiety (**C25**), we found that the curvature of the hook of the *Arabidopsis* seedlings was approximately 116±12 degrees (the yellow bar). This result indicates that a phenyl moiety at this position had a negative effect on enhancing biological activity. Introducing an allyl group (**C26**) significantly enhanced the biological activity that induced an exaggerated apical hook of *Arabidopsis* seedlings. The apical hooks were approximately 300±23 degrees (the red bar). The *tert*-butyl analogue (**C27**) also induced an exaggerated apical hook of *Arabidopsis* seedlings with approximately 128±26 degrees (the blue bar). This result indicated that all of the test compounds for biological studies in the present work displayed potent activity that induced exaggerated apical hooks in *Arabidopsis* seedlings, while the allyl analogue **(C26)** is the most potent compound.

Next, we determined the effect of **EH-8** and the analogues synthesized in the present work on the stem elongation of *Arabidopsis* seedlings grown in the dark. As shown in Fig. 2B, the hypocotyl length of the non-chemically treated control was approximately 1.15±0.01 cm (the black bar), while the hypocotyl length of ethephon (10 μM)-treated *Arabidopsis* seedlings was approximately 0.69±0.07 mm (the filled black bar). This result indicates that ethylene inhibits the stem elongation of *Arabidopsis* seedlings in our assay system. In terms of the biological activity of the synthesized **EH-8**, we found that **EH-8** (10 μM) reduced the hypocotyl length of *Arabidopsis* seedlings from 1.15±0.01 mm (the black bar) to 0.63±0.05 cm (the green bar). This result indicates that the inhibitory potency of **EH-8** on stem elongation of *Arabidopsis* seedlings is stronger than that of ethephon. Introducing a phenyl ring instead of a methyl group (**C25**) slightly weakened the inhibitory potency with the hypocotyl length of *Arabidopsis* seedlings to approximately 0.66±0.08 cm (the yellow bar). This result indicates that introducing a phenyl ring at this position has a negative effect on promoting the inhibitory activity of the stem elongation of *Arabidopsis* seedlings. Introducing an allyl group (**C26**) enhanced the inhibitory activity as compared with **EH-8**, with hypocotyl length of approximately 0.25±0.02 cm. The introduction of a *tert*-butyl group (**C27**) significantly weakened the potency of the inhibition of stem elongation of *Arabidopsis* seedlings with a degree of approximately 0.95±0.02 cm (the blue bar). Among the compounds prepared in the present work, we found that the compound with the allyl group **(C26)** displayed the most potent inhibitory activity.

Finally, we determined the effect of **EH-8** and its analogues on the inhibition of root elongation. As shown in Fig. 2C, the root length of the non-chemically treated control was approximately

2.3±0.1 mm (the black bar), while the root length of ethephon-treated (10 μM) *Arabidopsis* seedlings was found to be approximately 1.0±0.1 mm (the white bar). This result indicated that ethylene inhibits the root elongation of *Arabidopsis* seedlings in our assay system. Regarding the biological activity of the chemicals prepared in the present work, we found that **EH-8** (10 μM) reduced the root length of *Arabidopsis* seedlings from 2.3±0.1 to 1.9±0.2 mm (the green bar). This result indicates that the inhibitory potency of **EH-8** on the root elongation of *Arabidopsis* seedlings is weaker than that of ethephon. However, when a phenyl ring was introduced instead of a methyl moiety **(C25)**, we found that the root length of *Arabidopsis* seedlings was approximately 1.6±0.2 mm. This result indicates that the analogue with a phenyl ring at this position enhanced the inhibitory activity on root elongation. Introducing an allyl group at position 1 of the 3,5-dimethylpyrazole moiety **(C26)** enhanced the inhibitory activity on root elongation with root lengths of approximately 1.1±0.1 mm, which are similar to those of ethephon. The introduction of a *tert*-butyl group to position 1 of the 3,5-dimethylpyrazole moiety slightly reduced the biological activity in comparison to that of **C26**, with root lengths approximately 1.6±0.2 mm **(C27)**. Among all of the test compounds, **C26** displayed the most potent activity on reducing the root elongation of *Arabidopsis* seedlings.

Discussion

In order to develop potent compounds that induce the triple response in *Arabidopsis* seedlings, we carried out structure–activity relationship studies using 27 newly synthesized analogues. Twenty-four analogues with different substituents on the phenyl moiety provided important information regarding the biological activity of this synthetic series. Data obtained indicated that introducing fluorine atom(s) to the phenyl moiety significantly reduced the activity **(C1–C6)**. These results suggest that strong electron withdraw group like fluorine atom on the phenyl moiety have a negative effect. Introducing different lengths of alkyl or alkoxy groups to position 4 of the phenyl moiety had different effects (**C11–C15, C18–C20**). Obtained data suggested that an alkyl group with a long length has a positive effect **(C13, C14)** on inducing the triple response in *Arabidopsis* seedlings. Among all test compounds with different substitutions on phenyl moiety, **EH-8** is the most potent for inducing the triple response in *Arabidopsis* seedlings.

To further determine the structure–activity relationships of this synthetic series, we prepared three analogues with different alkyl substitutions at position 1 of the 3,5-dimethyl-1*H*-pyrazol moiety (**C25–C27**). Data obtained from biological studies indicated that 3,4-dichloro-*N*-methyl-*N*-[(1-allyl-3,5-dimethyl-1*H*-pyrazol-4-yl) methyl]benzenesulfonamide **(C26)** displayed the most potent activity for inducing the triple response in *Arabidopsis* seedlings. At a concentration of 10 μM, **C26** induced exaggerated apical hooks in *Arabidopsis* seedlings. The curvature of the hooks of the *Arabidopsis* seedlings was found to be approximately 300±23 degrees, while those of ethephon-treated (10 μM) and the non-chemically treated control were found to be approximately 128±19 and 58±16 degrees, respectively. This result indicates that the potency of **C26** for inducing exaggerated apical hooks is greater than that of ethephon. **C26** also displayed potent activity for inhibiting stem elongation. The hypocotyl length of *Arabidopsis* seedlings treated with **C26** (10 μM) was found to be approximately 0.25±0.02 cm, while those of ethephon-treated (10 μM) and non-chemically treated were found to be approximately 0.69±0.06 and 1.15±0.01 cm, respectively. This result indicates that the biological activity of **C26** for inhibiting the stem elongation of *Arabidopsis* seedlings is greater than that of ethephon. Data obtained from the experiment regarding effects on root growth inhibition indicated that **C26** displayed potency similar to that of ethephon. As shown in Fig. 2C, the average root length of *Arabidopsis* seedlings treated with **C26** was found to be approximately 1.1±0.1 mm, while those of ethephon-treated (10 μM) and the non-chemically treated control were found to be approximately 1.1±0.1 and 2.3±0.1 mm, respectively.

Thus, we have discovered a new compound, **C26**, that displays promising activity for inducing the triple response in *Arabidopsis* seedlings grown in the dark. The biological activity of **C26** for inhibiting stem elongation and inducing exaggerated apical hooks in *Arabidopsis* seedlings is greater than that of ethephon. However, **C26** displayed activity for inhibiting root growth similar to that of ethephon. Data obtained from the present work indicate that the pyrazole moiety is of significant importance for promoting the biological activity of this synthetic series. The size of the substituents dramatically affects their biological activity. As shown in Fig. 2, the analogue with the phenyl substituent (**C25**) displayed weakened biological activity, while the analogue with a propenyl substituent (**C26**) displayed potent activity. This result implies that the pyrazole moiety may bind to the binding site of the target protein. Based on this observation, the effect of the chemical structure on the pyrazole moiety needs to be further determined. Another chemical structure that needs to be determined is the *N*-methylsulfonamide moiety. We expect that the further chemical optimization of this synthetic series will lead to the finding of more potent compounds that induce the triple response in plants.

Ethylene is a key hormone involved in the morphogenesis of dark-grown plant seedlings. When dark-grown seedlings are exposed to ethylene, plant seedlings display a thickened hypocotyl and an exaggerated apical hook. These morphological characteristics are of significant importance in the process of dark-growing plant seedlings. Thus, chemicals that display biological activity that induces short stems and exaggerated apical hooks in dark-grown seedlings are candidates for growth regulators that can be used to modify morphological changes of young dark-grown plants. Moreover, we have previously shown that this synthetic series triggers ethylene responses but displays different transcriptional changes as compared to the ethylene biosynthesis precursor of ACC.[19] Thus, studies on the mode of action of our synthetic series may provide new information on ethylene

signal transduction. We expect that further studies on the use of this synthetic series may lead to developing a new type of plant growth regulator.

Acknowledgements

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