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Synthesis and stimulation of seed germination of γ-aminopropyl silatrane derivatives

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γ-Aminopropyl silatranes have generated wider interests due to their plant growth-regulating activity, safe and demonstrate potential to be plant growth regulators. Therefore, its N-substituted derivatives were synthesized and confirmed by IR, ^{1}H NMR, ESI-MS, and elemental analysis. The seed germination test showed that most compounds possessed better activities of regulating the roots growth on corn (monocotyledon) and radish (dicotyledon) than 1-chloromethylsilatrane (CMS). Moreover, the effect of these compounds was higher at around 10^{-7} mol/l, while 10^{-6} mol/l CMS was most efficient.

1. Introduction

Silatranes, hetero-tricyclic compounds having an intramolecular transannular dative Si←N bond, are of high interest both from their structural and applications point of view (Chuit et al., 1993; Puri et al., 2011). Due to their demonstrated antiviral, anti-inflammatory, anticancer and antitumor activities, as well as to their seed germination effects and applications in agriculture, silatranes were widely used in biological systems (Puri et al., 2011; Voronkov and Baryshok, 2004; Lin et al., 2011). Broad-spectrum plant growth regulators with silatranes as a main component have been produced in batches as agricultural chemicals, and are well known for their characteristics of high performance resulting in increased production, low toxicity, easy biodegradation, and environmental friendliness (Lin et al., 2011). A previous report showed that, treatment of tomatoes at flowering time with aqueous solution of 1-chloromethylsilatrane (CMS) considerably increased the amount of fruits and shortened ripening time (Voronkov, 1979). It was preliminarily confirmed that silatrane derivatives resistant to indoleacetic acid (IAA) oxidase, the auxin-inactivating system (Galston and Dalberg, 1954), were practically as active as auxins themselves. The presence of the silatrane group prolonged the effect or facilitated the auxin transport through the biomembranes (Voronkov, 1979).

The particular biological activities of silatrane derivatives, are likely to be bonds the silicon atom forms with adjacent atoms and the presence of some functional groups (Loginov et al., 2011). Moreover, the toxicity of silatranes varied greatly and was mainly dependent on the nature of substitution at the silicon atom. Most 1-arylsilatranes, with high and specific physiological activity (Voronkov, 1968), were almost twice as toxic as such well-known poisons as strychnine and hydrocyanic acid (Voronkov, 1979). However, 1-alkyl- and 1-alkoxy-silatranes were almost non-toxic (Voronkov and Lukevics, 1969). In particular, CMS (belonging to 1-alkyl-silatranes) first synthesized at Favorsky Irkutsk Institute of Chemistry, was efficient, essentially nontoxic, and ecologically safe stimulators of crop growth and productivity (Khankhodzhaeva and Voronkov, 1993). Furthermore, the compound not only increased seed germinability but also enhanced its germination at the optimum concentration (10^{-7} M to 10^{-5} M) (Voronkov et al., 2005; Burlakova et al., 1996). Nevertheless, organosilicon compounds containing a nitrogen atom in the γ- or δ-position in relation to the silicon atom may be of high physiological activity and lower toxicity (Voronkov and Lukevics, 1969; Voronkov et al., 1968), such as γ-aminopropyl silatrane (as shown in Fig. 1). Some of its derivatives are known to possess various activities in biology, physiology, pharmacology, medicine, etc. (Voronkov et al., 1982). Apart from these, they might be very promising in plant industry.

It was of interest to determine whether this type of silatranes possess the fairly or higher stimulatory effect on seed germination than CMS, under the same concentration (10^{-7} M to 10^{-5} M). Additionally, this was a necessity for investigation of the effects of

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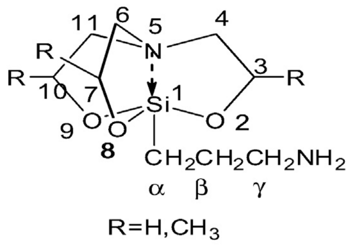

Fig. 1. The structure of γ-aminopropyl silatrane.

its micromolar and appropriate concentration range. This knowledge allowed us to synthesize a series of derivatives of γ-aminopropyl silatrane, and to conduct botanical experiments, making it possible to obtain new compounds, efficient, practical and safe for ecologically pure chemicals used in agriculture.

2. Results and discussion

2.1. Chemistry

The structure and general synthesis of analogs designed are illustrated and outlined in Fig. 2. Upon mixing of γ-aminopropyl triethoxysilane, and triisopropanolamine or triethanolamine, this mixture was stirred at 150 °C for 2 h for compounds **1a** and **1b**, respectively. Thereafter, they were obtained by vacuum distillation in high yields (**1a**: 74.96%; **1b**: 76.83%). And the results were consistent with the data in this literature (Dumitriu et al., 2012), established by IR, ^{1}H NMR as well as elemental analysis. The synthesis of **2** was accomplished by sulfonylation reaction of **1b** with tosyl chloride in 53.7% yields. All these compounds (**3a**–**f** and **4a**–**d**) were obtained from N-acylation of **1a**–**b** with different substituted benzoyl chlorides (yields: **3a**–**f**: 38.4–66.7%, **4a**–**d**: 51.2–67.9%).

The IR spectrum of substituted aminopropyl silatranes showed that there was a peak between 570 and 580 cm^{-1} corresponding to the characteristic absorption of N→Si. And the shift of H-α in the HNMR was under 0.5 ppm, attributing to the electrophobic effect of silicon atom. In addition, the fragment peaks of silatrane or 3,7,10-trimethyl silatrane can be observed normally in MS. The above phenomena corresponded to prediction.

2.2. Seed germination test results

All tested compounds (**1a**–**b**, **2**, **3a**–**f** and **4a**–**d**) significantly enhanced seed germination. The maximal effect of them on germination of both corn and radish seeds was observed at 10^{-7} mol/l, and their activity trends are presented in Fig. 3 (the length of roots was converted into efficacy using an empirical formula shown in this figure).

According to the above figure, at 10^{-7} mol/l, efficacy of **3f** on corn was best. Meanwhile, **4d** performed the strongest stimulatory effect on radish seedlings. In order to clarify the relationship between concentration and bioactivity, compounds **3f** and **4d** were selected as representatives in comparison to CMS as shown in Fig. 4.

These above figures showed that both **3f** (10^{-7} mol/l) and **4d** (10^{-7} mol/l) increased seed germinability most efficiently, and likewise with other aminopropyl derivatives, while the optimum concentration of CMS was higher (10^{-6} mol/l). It was of interest that the effect of these compounds did not show a dose-dependent relationship but an optimum concentration, consistent with the results reported in many literatures (Voronkov, 1979; Voronkov et al., 2005). It might have been supposed that the aminopropyl silatrane derivatives inhibit IAA-oxidase more efficient and at lower concentration than CMS.

In addition, most compounds possess higher activity than CMS, especially at 10^{-7} mol/l. In generally, N-substituted derivatives were more active than aminopropyl silatranes (**1a**–**b**). And the compounds adorned with methyl groups (**3a**–**f**) had significant loss of efficacy in comparison with the unadorned (**4a**–**d**). Besides that, introduction of substitution such as chloro or methyl on the phenyl connected with aminopropyl groups to form the compounds **3b**, **3e**, **3f** or **4b**–**d** resulted in stronger efficacy compared to the compound **3a** or **4a**, both of which had no substitution.

In conclusion, based on the discussion, the aminopropyl silatrane derivatives containing nitrogen atom in 1-substitute group worked on seed germination better than the common 1-alkyl and 1-alkoxy silatranes. These derivatives could potentially be used as plant growth regulators at appropriate concentrations. Nevertheless, the mechanism of enhancement of seed germination by these compounds still needs to be further investigated.

3. Experimental

3.1. General comments

Uncorrected melting points (mp) were determined using an XRC-1 micro-melting point apparatus. Thin-layer chromatography (TLC, R_f values) was performed on F_{254} or silica gel plates F_{254} (0.2 mm thick), and all the synthesized compounds were visualized under ultraviolet light. ^{1}H NMR spectra were recorded on a Brucker Avance-3600 Spectrometer (600 MHz). Samples were dissolved in $CDCl_3$ while TMS was used as internal standard.

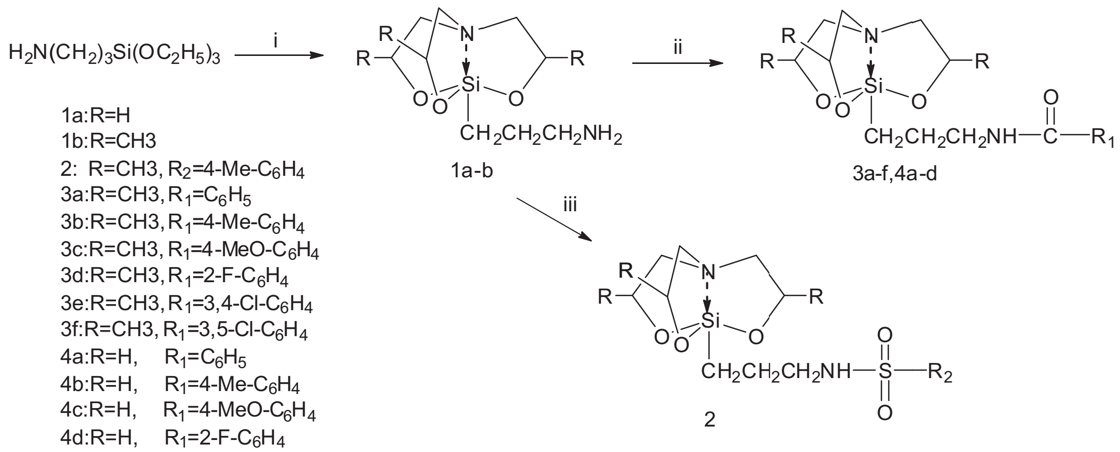

Fig. 2. Synthesis of γ-aminopropyl silatrane derivatives. Regents and conditions: (i) γ-aminopropyl triethoxysilane, triisopropanolamine or triethanolamine; reflux, 2 h; (ii) chloroform, different substituted benzoyl chlorides, triethylamine; 25 °C, 2 h; (iii) tosyl chloride, triethylamine, acetone; 25 °C, 2 h.

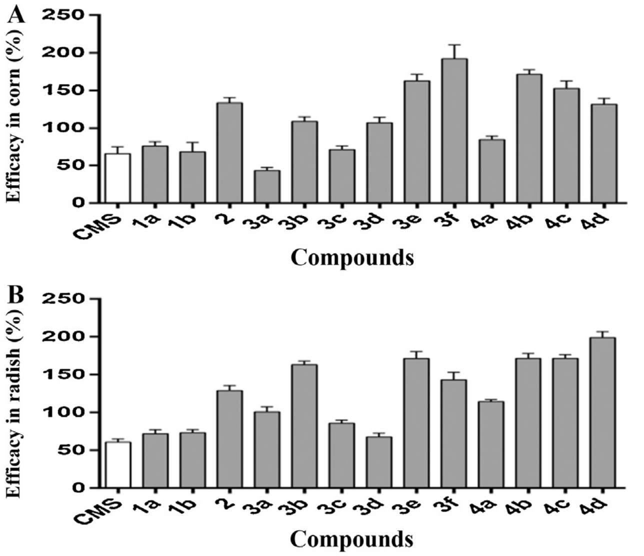

Fig. 3. Effect of all the compounds tested on corn and radish germination efficacy. Efficacy = (the average length of sample roots − the average length of blank roots)/ the average length of blank roots × 100%. (A) Efficacy in corn (%) at 10^{-7} mol/l. (B) Efficacy in radish (%) at 10^{-7} mol/l. Values are mean ± S.D. $n = 3$, $p < 0.05$, ANOVA, Duncan's multiple-range test.

Chemical shifts were recorded in (ppm) values relative to TMS and J values are expressed in Hertz. MS were recorded using an Agilent-1100 LC–MS Spectrometer. IR spectra (KBr) were recorded on a Bruker Vector 22 spectrometer. All reagents were of analytical-reagent grade and were used without further purification.

*3.2. General procedure for syntheses of the compound **2***

The compound **1b** (0.35 mmol) and a catalytic amount of triethylamine were dissolved in 7 ml of acetone. At room temperature, in a period of 20–30 min, the equimolar tosyl chloride was added dropwise with stirring. The reaction mixture was stirred at room temperature for 2 h, and then the precipitate (triethylamine hydrochloride) formed was discarded. The filtrate, concentrated under reduced pressure, was poured into diethyl ether, and a yellow precipitate formed. The target product was purified by column chromatography on silica gel using petroleum ether and ethyl acetate as eluent.

*3.2.1. γ-(4-Methybenzenesulfonamide) propyl-3,7,10-trimethyl silatrane (**2**)*

mp: 125–126 °C. IR (KBr) ν_{max}: 584 (N→Si), 1325 ($-SO^{2-}$) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ: 0.23–0.40 (m, 2H, H-α), 1.09–1.42 (m, 9H, CH_3-3,7,10), 1.54–1.75 (m, 2H, H-β), 2.15–2.35 (m, 2H, H-γ), 2.39 (s, 3H, Ph–CH_3), 2.67–3.20 (m, 6H, H-4,6,11), 3.77–4.15 (m, 3H, H-3,7,10), 5.84 (br, 1H, NH), 7.26 (d, 2H, J = 7.8 Hz, Ph–H), 7.72 (d, 2H, J = 7.8 Hz, Ph–H); ESI-MS m/z 429 $[M+H]^+$; found: C, 53.44; H, 7.82; N, 6.74; Si, 6.51. Calc. for $C_{19}H_{32}N_2O_5SSi$: C, 53.24; H, 7.53; N, 6.54; Si, 6.55%.

*3.3. General procedure for syntheses of the compounds **3a–f** and **4a–d***

To a mixture of γ-aminopropyl silatranes **1a–b** (10 mmol) in 20 mL of chloroform and 2 mL of triethylamine was added different substituted benzoyl chlorides (10 mmol) by dripping slowly. The mixture was refluxed in a three-necked round bottom flask fitted with a magnetic stirrer for 2 h. The crude product was filtered to remove the precipitate. Finally, the compounds **3a–f** and **4a–d** were purified as white solids by column chromatography, using an eluent of petroleum ether and ethyl acetate.

*3.3.1. γ-Benzamidepropyl-3,7,10-trimethyl silatrane (**3a**)*

mp: 143–144 °C. IR (KBr) ν_{max}: 576 (N→Si), 1627 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ: 0.25–0.48 (m, 2H, H-α), 1.08–1.49 (m, 9H, CH_3-3,7,10), 1.56–1.78 (m, 2H, H-β), 2.05–2.35 (m, 2H, H-γ), 2.61–3.29 (m, 6H, H-4,6,11), 3.79–4.20 (m, 3H, H-3,7,10), 6.55 (br, 1H, NH), 7.21–7.78 (m, 5H, Ph–H); ESI-MS m/z 379 $[M+H]^+$; Found: C, 60.40; H, 7.87; N, 7.32; Si, 7.69. Calc. for $C_{19}H_{30}N_2O_4Si$: C, 60.29; H, 7.99; N, 7.40; Si, 7.42%.

*3.3.2. γ-(4-Methybenzamide) propyl-3,7,10-trimethyl silatrane (**3b**)*

White solid; mp: 139–140 °C. IR (KBr) ν_{max}: 569 (N→Si), 1633 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ: 0.22–0.49 (m, 2H, H-α), 1.05–1.50 (m, 9H, CH_3-3,7,10), 1.51–1.82 (m, 2H, H-β), 2.02–2.33 (m, 2H, H-γ), 2.35 (s, 3H, Ph–CH_3), 2.62–3.28 (m, 6H, H-4,6,11), 3.85–4.20 (m, 3H, H-3,7,10), 6.69 (br, 1H, NH), 7.32 (d, 2H, J = 8.4 Hz, Ph–H), 7.84 (d, 2H, J = 8.4 Hz, Ph–H); ESI-MS m/z 393 $[M+H]^+$; found: C, 61.08; H, 8.21; N, 7.15; Si, 7.19. $C_{20}H_{32}N_2O_4Si$: C, 61.19; H, 8.22; N, 7.14; Si, 7.15%.

*3.3.3. γ-(4-Methoxybenzamide) propyl-3,7,10-trimethyl silatrane (**3c**)*

mp: 140–141 °C. IR (KBr) ν_{max}: 572 (N→Si), 1685 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ: 0.28–0.53 (m, 2H, H-α), 1.07–1.49 (m, 9H, CH_3-3,7,10), 1.53–1.83 (m, 2H, H-β), 2.05–2.38 (m, 2H, H-γ), 2.65–3.42 (m, 6H, H-4,6,11), 3.82 (s, 3H, $-OCH_3$), 3.85–4.13 (m, 3H, H-3,7,10), 6.71 (br, 1H, NH), 6.87 (d, 2H, J = 8.4 Hz, Ph–H), 7.73 (d, 2H, J = 8.4 Hz, Ph–H); ESI-MS m/z 409 $[M+H]^+$; found: C, 59.12; H, 8.20; N, 7.24; Si, 6.93. Calc. for $C_{20}H_{32}N_2O_5Si$: C, 58.79; H, 7.89, N, 6.86; Si, 6.87%.

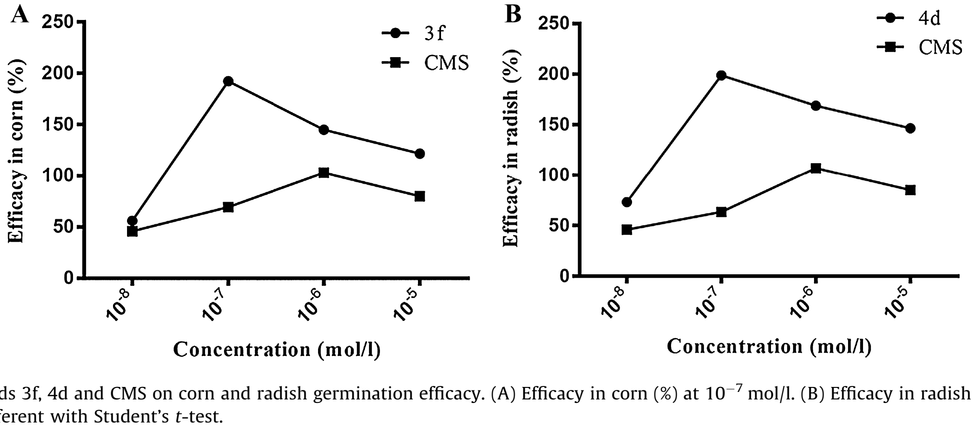

Fig. 4. Effect of compounds 3f, 4d and CMS on corn and radish germination efficacy. (A) Efficacy in corn (%) at 10^{-7} mol/l. (B) Efficacy in radish (%) at 10^{-7} mol/l. Values $p < 0.01$, significantly different with Student's t-test.

3.3.4. γ-(2-Fluorobenzamide) propyl-3,7,10-trimethyl silatrane (**3d**)

White solid; mp: 152–153 °C. IR (KBr) ν_{max}: 578 (N→Si), 1640 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.20–0.55 (m, 2H, H-α), 1.00–1.55 (m, 9H, CH_3-3,7,10), 1.60–1.87 (m, 2H, H-β), 2.23–2.55 (m, 2H, H-γ), 2.75–3.45 (m, 6H, H-4,6,11), 3.85–4.19 (m, 3H, H-3,7,10), 7.04 (br, 1H, NH), 7.47–7.86 (m, 4H, Ph–H); ESI-MS *m/z* 397 [M+H]$^+$; found: C, 56.99; H, 7.35; N, 7.01; Si, 6.98. $C_{19}H_{29}N_2O_4SiF$: C, 57.55; H, 7.37; N, 7.06; Si, 7.06%.

3.3.5. γ-(3,4-Dichlorobenzamide) propyl-3,7,10-trimethyl silatrane (**3e**)

mp: 116–118 °C. IR (KBr) ν_{max}: 570 (N→Si), 1637 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.26–0.53 (m, 2H, H-α), 1.08–1.48 (m, 9H, CH_3-3,7,10), 1.64–1.84 (m, 2H, H-β), 2.18–2.48 (m, 2H, H-γ), 2.58–3.09 (m, 6H, H-4,6,11), 3.79–4.20 (m, 3H, H-3,7,10), 7.04 (br, 1H, NH), 7.47–7.86 (m, 3H, Ph–H); ESI-MS *m/z* 447 [M+H]$^+$; found: C, 51.20; H, 6.51; N, 6.25; Si, 6.31. Calc. for $C_{19}H_{28}N_2O_4SiCl_2$: C, 51.00; H, 6.31; N, 6.26; Si, 6.28%.

3.3.6. γ-(3,5-Dichlorobenzamide) propyl-3,7,10-trimethyl silatrane (**3f**)

mp: 130–132 °C. IR (KBr) ν_{max}: 579 (N→Si), 1627 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.27–0.55 (m, 2H, H-α), 1.10–1.45 (m, 9H, CH_3-3,7,10), 1.66–1.87 (m, 2 H, H-β), 2.20–2.42 (m, 2H, H-γ), 2.75–3.45 (m, 6H, H-4,6,11), 3.85–4.19 (m, 3H, H-3,7,10), 7.13 (br, 1H, NH) 7.56–7.78 (m, 3H, Ph–H); ESI-MS *m/z* 447 [M+H]$^+$; found: C, 51.21; H, 6.51; N, 6.06; Si, 6.39. Calc. for $C_{19}H_{28}N_2O_4SiCl_2$: C, 51.00; H, 6.31; N, 6.26; Si, 6.28%.

3.3.7. γ-Benzamidepropyl silatrane (**4a**)

mp: 177–178 °C. IR (KBr) ν_{max}: 572 (N→Si), 1685 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.22–0.42 (m, 2H, H-α), 1.54–1.76 (m, 2H, H-β), 2.13–2.36 (m, 2H, H-γ), 2.61–3.26 (m, 6H, H-4,6,11), 3.80–4.19 (m, 6H, H-3,7,10), 5.28 (br, 1H, NH), 7.28–7.80 (m, 5H, Ph–H); ESI-MS *m/z* 337 [M+H]$^+$; found: C, 57.30; H, 6.89; N, 8.03; Si, 8.33. Calc. for $C_{16}H_{24}N_2O_4Si$: C, 57.12; H, 7.19; N, 8.33; Si, 8.35%.

3.3.8. γ-(4-Methybenzamide) propyl silatrane (**4b**)

White solid; mp: 168–169 °C. IR (KBr) ν_{max}: 570 (N→Si), 1637 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.23–0.45 (m, 2H, H-α), 1.65–1.85 (m, 2H, H-β), 2.47–2.69 (m, 2H, H-γ), 2.52 (s, 3H, $PhCH_3$), 2.65–3.40 (m, 6H, H-4,6,11), 3.75–4.13 (m, 6H, H-3,7,10), 6.71 (br, 1H, NH), 7.30 (d, 2H, J = 8.4 Hz, Ph–H), 7.82 (d, 2H, J = 8.4 Hz, Ph–H); ESI-MS *m/z* 351 [M+H]$^+$; found: C, 58.11; H, 7.50; N, 7.81; Si, 7.80. $C_{17}H_{26}N_2O_4Si$: C, 58.26; H, 7.48; N, 7.99; Si, 8.01%.

3.3.9. γ-(4-Methoxybenzamide) propyl silatrane (**4c**)

mp: 161–162 °C. IR (KBr) ν_{max}: 578 (N→Si), 1627 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.21–0.45 (m, 2H, H-α), 1.60–1.89 (m, 2H, H-β), 2.08–2.41 (m, 2H, H-γ), 2.65–3.40 (m, 6H, H-4,6,11), 3.77 (s, 3H, $-OCH_3$), 3.75–3.81 (m, 6H, H-3,7,10), 6.81 (br, 1H, NH), 7.02 (d, 2H, J = 8.12 Hz, Ph–H), 7.82 (d, 2H, J = 8.32 Hz, Ph–H); ESI-MS *m/z* 367 [M+H]$^+$; found C, 55.23; H, 7.23; N, 7.69; Si, 7.71. Calc. for $C_{17}H_{26}N_2O_5Si$: C, 55.71; H, 7.15; N, 7.64; Si, 7.66%.

3.3.10. γ-(2-Fluorobenzoyl)-aminopropyl silatrane (**4d**)

White solid; mp: 170–171 °C. IR (KBr) ν_{max}: 581 (N→Si), 1642 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 0.23–0.50 (m, 2H, H-α), 1.45–1.87 (m, 2H, H-β), 2.52–2.65 (m, 2H, H-γ), 2.65–3.38 (m, 6H, H-4,6,11), 3.72–3.87 (m, 6H, H-3,7,10), 6.96 (br, 1H, NH), 7.30–7.76 (m, 4H, Ph–H); ESI-MS *m/z* 355 [M+H]$^+$; found: C, 54.89; H, 6.77; N, 7.80; Si, 7.50. $C_{16}H_{23}N_2O_4SiF$: C, 54.22; H, 6.54; N, 7.90; Si, 7.92%.

3.4. Germination test

The experiment was carried out according to the standard operation procedure of bioassay established by Ministry of Efficacy of National Pesticide Research & Development South Center (Shanghai) (Li et al., 2007). Corn and radish seeds produced in 2011 were purchased from Shandong Academy of Agricultural Sciences (their germinability was evaluated before sowing). Both kinds of seeds were respectively sterilized with 1% $KMnO_4$ and kept in water for 12 h, which is a common agricultural practice. Each treatment was performed with 30 seeds. Then the seeds were placed in large Petri dishes on several layers of filter papers moistened with distilled water (blank control) or aqueous solutions of compounds tested, prepared by successive tenfold dilution from 10^{-8} to 10^{-5} mol/l for the compounds synthesized or CMS (positive control). And the seeds were left at 25 °C and dim light during the day time in an artificial climate chamber (Climacell RXZ-0450) for 7 days. The filter papers were daily wetted with the above solutions. Preliminary experiments demonstrated that such procedures were optimal, and there were no signs of fermentation during seed soaking (Voronkov et al., 2005). The length of roots was measured on the 7th day in comparison with the no dose group. All experiments were repeated three times.

3.5. Statistical analysis

Results were mean ± S.D. of three parallel measurements. All statistical comparisons were determined by Student's *t*-test and one-way ANOVA followed by Duncan's multiple-range test, *p*-values < 0.05 were regarded as statistically significant.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2013.12.011.

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