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Design, synthesis, and herbicidal activity of *sec-p*-menthane-7-amine derivatives as botanical herbicides†

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In this study, a series of novel *p*-menthane type secondary amines (*sec-p*-menthane-7-amine derivatives **3a–3y**) were synthesized and then characterized by FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS. The post-emergence herbicidal activities of these amines against barnyard grass and rape were evaluated by the culture dish method. Most of the *sec-p*-menthane-7-amine derivatives showed excellent herbicidal activities equivalent to or even higher than either diuron or glyphosate. The alkyl-substituted derivatives were more active than the phenyl-substituted derivatives. The herbicidal activities of compounds **3p**, **3r**, **3u**, and **3w** against the root growth of barnyard grass were 404% higher, respectively, than those of glyphosate. The herbicidal activities of compounds **3q**, **3v**, **3w**, and **3x** against the root growth of rape were 561%, 494%, 491%, and 544% higher, respectively, than those of diuron, and 484%, 760%, 423%, and 665% higher respectively, than those of diuron against shoot growth of rape. In addition, compounds **3p**, **3u**, and **3v** are almost harmless to rice, wheat, sorghum, maize, and peanuts at a concentration of 100 mg L^{-1}. Most of the compounds are nontoxic to HUVEC-C and BALB/c 3T3 cells. It is indicated that the title compounds could be utilized as botanical herbicides for future weed control.

1 Introduction

With the increase in world population, it has become urgent to improve agricultural production to solve the problem of grain shortage.[1] Weeds can compete with crops for nutrients, moisture, and light and bring pests and diseases, which cause the reduction of grain yields.[2,3] In recent decades, herbicides have provided an effective approach for crop protection. However, traditional herbicides are chemically synthesized and their excessive and continual use can lead to drug residues, environmental pollution, herbicidal resistance, and other serious problems.[4–6] Given the defects of synthetic herbicides, there is an urgent need to develop eco-friendly and sustainable herbicides with high activity and low toxicity such as botanical herbicides.[7,8]

Essential oils possess potential inhibitory properties against weed growth and seed germination. This phenomenon is called allelopathy.[9] Many monoterpenes in the essential oils such as α-pinene, β-pinene, 1,8-cineole, carvacrol, thymol, and limonene exhibit allelopathic properties, which are widely distributed in many plants.[9–15] Aside from their direct utilization, the chemically modified derivatives of essential oils including amides, amines, Schiff bases, thioureas, and esters have also attracted much attention due to their excellent herbicidal activities. The compounds derived from turpentine have been reported to exhibit a wide range of biological activities and have become the main source of botanical herbicides.[16–20]

In our previous studies, a series of herbicidal active substances with a *p*-menthane skeleton were synthesized from turpentine. Among them, *p*-menth-3-enylamine and *cis*-1,8-*p*-menthane-diamine type Schiff base compounds showed herbicidal activities against ryegrass and barnyard grass superior to those of commercial herbicide glyphosate.[21–23] However, the imine group (C=N) of Schiff base compounds is usually unstable in storage and herbicidal assays because it can be easily oxidized by air, hydrolyzed in an acidic environment, and subjected to transamination by nucleophiles.[24,25] Therefore, it is necessary to convert the Schiff bases into a more stable secondary amines using the reduction. It has been proven that secondary amines such as *sec-p*-menth-3-enylamines and *cis*-

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1,8-*p*-menthane-di-*sec*-amines exhibit higher herbicidal activities than that of Schiff bases, and they show low toxicity to common crops and mammal cells.[26,27]

Perillaldehyde is a natural monoterpene of the *p*-menthane group originating from plant *Perilla frutescens*. It was reported that perillaldehyde displayed various bioactivities such as antidepression and antibacterial activity,[28,29] but the herbicidal activity is rarely reported. In order to study the herbicidal activities of perillaldehyde-derived secondary amines with the *p*-menthane skeleton and find new herbicidal active substances, a variety of *sec-p*-menthane-7-amine derivatives were designed and synthesized using perillaldehyde as raw material. These derivatives were evaluated for their herbicidal activities against barnyard grass (*Echinochloa crus-galli*) and rape (*Brassica napus*) as well as with regard to crop safety and cytotoxicity to normal mammal cells.

2 Experimental methods

2.1 Materials

NMR spectra were recorded on an Avance III 500 MHz spectrometer (Bruker, Switzerland) using $CDCl_3$ as solvent and TMS as the internal reference. FT-IR spectra were recorded using a Nicolet IS10 IR instrument (Thermo, USA) connected to an OMNIC operating system. HRMS analyses were performed using a XEVO G2-XS mass spectrometer (Waters, USA) under electron spray ionization. GC analysis was carried out using a GC-2014AF (Shimadzu, Japan) with a HP-5 quartz capillary column (30 m × 0.25 mm, df 0.25 μm). Herbicidal activities were carried out in a ZRG-1000B-L artificial climatic incubator from Shanghai Binglin Electronic Technology Co. Ltd (Shanghai, China). Cytotoxicity assays were completed in a Winooski EL-X800 microplate reader (BioTech Instrument, USA). Column chromatography was carried out on a 300 mm × 40 mm column of silica gel (200–300 meshes). Other reagents were analytically pure and obtained commercially from Shanghai Aladdin Chemistry Co. Ltd (Shanghai, China).

2.2 Synthesis of *p*-menthane-7-aldehyde

To start, 50 g of perillaldehyde (**1**) and 2.5 g of Pd/C were added to a 100 mL micro autoclave with magnetic stirring. Then, the autoclave was filled with hydrogen for 5 MPa, and reacted at 120 °C for 15 h. After the reaction system was cooled to room temperature, the Pd/C was removed by filtration, and the crude product was recovered by rectification to obtain *p*-menthane-7-aldehyde (**2**, GC 98%, a mixture consisted of *cis*- and *trans*-isomer, $n_{cis\text{-}} : n_{trans\text{-}} = 1 : 1$).

2.3 Synthesis of *sec-p*-menthane-7-amine derivatives

Firstly, 20 mmol *p*-menthane-7-aldehyde and 20.2 mmol amine were stirred in a 250 mL round-bottom flask containing 50 mL of methanol for 48 h. The system was cooled down to 0 °C in an ice bath, and then 60 mmol $NaBH_4$ total was added three times over the course of 30 min and reacted at 0 °C for another 0.5–1 h. After stirring, 20 mL of cold distilled water was added to quench excess $NaBH_4$. The mixture was extracted with CH_2Cl_2 and washed with distilled water three times. The organic phase was collected and dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure to yield thick oil as a crude product. Finally, the product was purified by recrystallization or column chromatography eluted by petroleum ether and ethyl acetate (Scheme 1).

2.4 Herbicidal activity evaluation

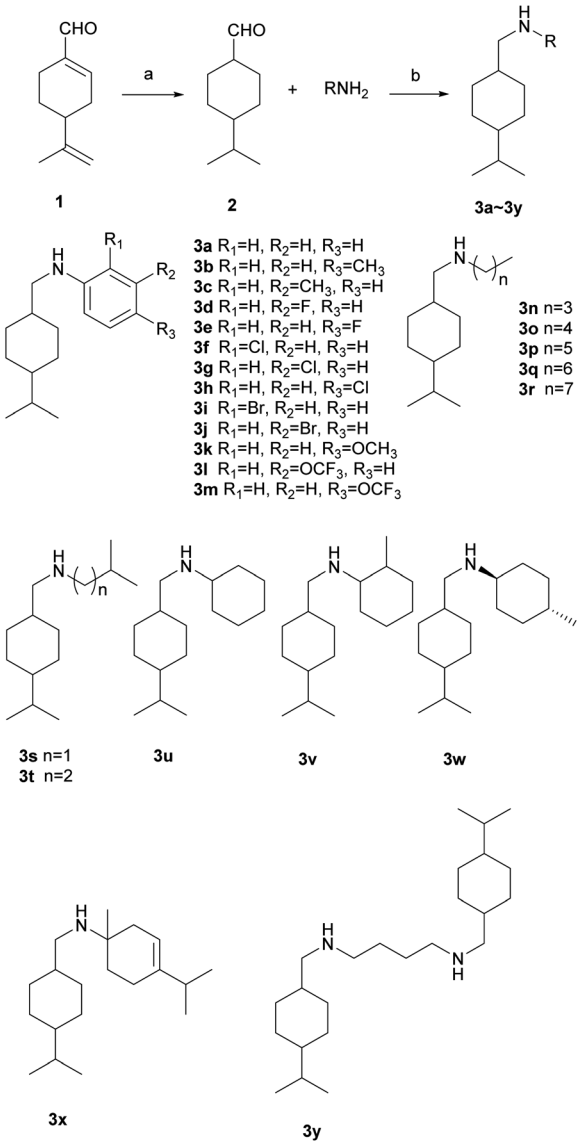
The monocotyledon barnyard grass (*Echinochloa crus-galli*) and dicotyledon rape (*Brassica napus*) were chosen as the test plants for primary herbicide bioassay tests. First, 1 mmol of each compound was dissolved in 1 mL DMF in a 100 mL volumetric flask; then, 0.1 g Tween-80 was added as the emulsification reagent to the volume flask and diluted with distilled water to 10 mmol L^{-1}. Then, the concentration was diluted with the control solution (1% DMF and 0.1% Tween-80 in distilled water) to 5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, 0.0391, 0.0195, 0.0098, and 0.0049 mmol L^{-1} in gradual succession. The seeds were soaked in warm water (28 °C) for 12 h, then filtered out with distilled water and incubated at 28 °C for another 24 h before use. Then, the test compound solution (10 mL for barnyard grass, 6 mL for rape) of each concentration and 10 mL of the control solution were added to the corresponding Petri dishes (9 cm in diameter) lined with two layers of filter paper (9 cm in diameter). Then, 10 seeds were added to each Petri dish (at each concentration and for the controls, with three replicates) and cultivated under artificial climate conditions: the seeds were incubated at 28 °C, illuminated at 5000 lx, photoperiod 16 : 8 (day/night), and kept at a relative humidity 70–80% for 96 h. The inhibition rate (%) of the root or shoot growth was calculated according to eqn (1).

$$y = \frac{x_0 - x_i}{x_0} \quad (1)$$

where y is the inhibition rate of the root or shoot growth, x_0 is the root or shoot length of the blank control group, and x_i is the root or shoot length of the group treated with the test compounds.

2.5 Crop safety

The crop safety assays for seven crops (rice, wheat, sorghum, maize, peanut, cucumber, and radish) were tested. To start, 100 mg of each compound was dissolved in 10 mL DMF in a 1 L volumetric flask; then 1 g Tween-80 was added as the emulsification reagent to the volumetric flask and diluted with distilled water to 100 mg L^{-1}. Crop seeds were soaked in warm water for 15 h in a 28 °C incubator before use. Next, 8 mL of the test compound solution of 100 mg L^{-1} and 8 mL of the control solution were added to the corresponding Petri dishes (9 cm in diameter) lined with two layers of filter paper (9 cm in diameter) and 10 seeds of each crop were added to each Petri dish, with three replicates, which were then cultivated in the dark at 28 °C for 120 h. The crop safety was evaluated visually and rated with scores between 0 and 100, where complete control of the crop is 100 and no control is 0.



Scheme 1 Synthetic route for the *sec-p*-menthane-7-amine derivatives (a) substrate 50 g, Pd/C 2.5 g, 5 MPa, 120 °C, 15 h; (b) substrate 20 mmol, RNH_2 20.2 mmol, methanol, room temperature (25 °C), 24 h, $NaBH_4$ 2 eq., ice-bath.

2.6 Cytotoxicity assays

The BALB/c 3T3 cell line, which is recommended for cytotoxicity evaluation tests by the European Union,[30] was chosen as a target for cytotoxicity assay measurements by CCK-8 method.[31] The HUVEC-C cell line was commonly used in pesticide safety analyses, so it was also selected.[32] First, the test samples were dissolved in DMSO and diluted with the culture medium (DMEM containing 10% FBS and 1% mixture of penicillin and streptomycin for BALB/c 3T3 cells, and Kaighn's Modification of Ham's F-12 Medium containing 15% FBS for HUVEC-C cells) at 10 mmol L^{-1} concentration (DMSO was less than 0.1%). Next, a cryopreservation tube containing the BALB/c 3T3 or HUVEC-C cell line was removed from the liquid nitrogen tank and quickly unfrozen in a water bath at 37 °C. Then, the cellular suspension was transferred to a culture flask containing the corresponding complete medium. The cultures were incubated under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. After 1-2 generations of reproduction, the cells were cultivated in a 96-well plate at a density of 4×10^3 cells per 100 μL of the corresponding complete medium in each well for 24 h (culture conditions: 5% CO_2 95% air at 37 °C). Then, 100 μL of each solution of the test compound (10 μmol L^{-1}) and were added to a separate 96-well plate, respectively, and 100 μL of medium was added as a negative control. Then, the cultures were incubated at 37 °C. After 48 h of incubation, 10 μL of CCK-8 solution was added to each well and was allowed to incubate for 4 h. Later, the 96-well plate was shaken slightly to eliminate air bubbles; an absorbance at a wavelength of 450 nm was recorded. The inhibition rate (%) of cell growth was calculated according to eqn (2).

$$m = \frac{n_0 - n_i}{n_0} \quad (2)$$

where m is the inhibition rate of cell growth, n_0 is the OD value of the blank control group, and n_i is the OD value of the experimental group.

3 Results and discussion

3.1 Synthesis and characterization of *sec-p*-menthane-7-amines derivatives

The *sec-p*-menthane-7-amine derivatives which were mixture of *cis*- and *trans*-isomers, were synthesized using perillaldehyde and amines as raw materials, $NaBH_4$ as a reductant, were obtained in yields that ranged between 40–98%. The crude products were purified by silica gel column chromatography using petroleum ether and ethyl acetate as eluents. These compounds were confirmed by FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopy as well as HRMS. In the FTIR spectra, the peak at 3436–3406 cm^{-1} was characteristic of the N–H stretching vibration band in secondary amines. The peaks in the ranges of 2960–2840 cm^{-1}, 1650–1450 cm^{-1}, and 1390–1300 cm^{-1} represented the stretching vibration band of C–H alkane groups, the stretching vibration band of the C=C bonds of an aromatic ring, and the stretching vibration band of C–N bonds, respectively. In the ^{1}H NMR spectra, a singlet with δ 5.34 ppm belonged to the amino proton in compounds **3a–3m**, and a singlet of δ 1.95 ppm belonged to the amino proton in compounds **3n–3y**. Peaks ranging from δ 7.13–6.49 ppm belonged to the aromatic ring. A doublet with δ 3.15–2.38 ppm was assigned to the signals of H-7. Other peaks with δ 2.00–0.85 ppm belonged to the hydrogen proton of *p*-menthane. In the ^{13}C NMR spectra, peaks ranging from δ 165.21–103.27 ppm were assigned to the phenyl. The peak with δ 50.50 ppm was attributed to C-7, and peaks with δ 44.18–19.80 ppm were assigned to the *p*-menthane. The total number of hydrogen and carbon atoms is consistent with that of the corresponding compounds, and it was further confirmed by HRMS that compounds **3a–3y** are *sec-p*-menthane-7-amine derivatives.

3.2 Herbicidal activities against barnyard grass and rape

The post-emergence herbicidal activities of newly synthesized compounds **3a–3y** against barnyard grass and rape were

Table 1 Inhibition rates of *sec-p*-menthane-7-amine derivatives against root growth of barnyard grass

	Inhibition rates against root growth of barnyard grass									
Compd	2.5[a]	1.25	0.625	0.313	0.156	0.078	0.039	0.0195	0.0098	0.0049
1	81.2[b]	54.3	53.4	48.5	21.5	20.2	[c]	[c]	[c]	[c]
2	45.4[b]	3.3	5.0	9.6	7.2	0.1	[c]	[c]	[c]	[c]
3n	100	100	98.7	88.5	66.9	59.6	54.0	49.6	42.1	32.1
3o	100	100	100	95.2	74.7	67.1	59.3	55.3	47.9	37.6
3p	100	100	100	97.2	88.5	79.1	68.7	60.8	55.0	46.0
3q	100	100	100	97.5	88.4	75.1	66.5	57.8	49.0	40.8
3r	100	100	100	95.6	91.0	83.1	72.7	66.9	60.2	52.3
3s	100	100	99.7	91.7	74.0	67.3	58.4	53.2	47.8	22.4
3t	100	100	99.6	92.5	79.4	69.4	62.4	50.3	46.4	36.5
3u	100	100	100	95.4	81.5	72.6	66.5	60.3	53.6	46.9
3v	100	100	100	98.3	90.0	70.3	64.2	56.8	50.6	44.6
3w	100	100	100	95.2	86.6	75.7	68.7	60.5	53.9	45.2
3x	100	100	99.8	96.1	87.7	82.0	73.1	62.2	52.6	33.5
3y	100	100	100	95.9	88.8	57.1	34.8	16.4	[c]	[c]
Diuron	97.0	95.4	94.7	93.6	89.9	53.1	29.6	17.6	15.5	10.6
Glyphosate	100	99.8	92.3	83.9	79.6	69.5	43.9	24.9	16.6	8.9

[a] The data in this line are the concentrations of different *sec-p*-menthane-7-amine derivatives solutions (mmol L^{-1}). [b] The inhibition rates of compounds **1** and **2** at 5 mmol L^{-1} against root growth of barnyard grass were 100% and 82.2%, against shoot growth were 100% and 77.6%. [c] Have no inhibition activity at this concentration.

evaluated. The toxicity regression equations were calculated by DPS v17.10 software. The commercial herbicides glyphosate and diuron were chosen as positive controls. The results were listed in Tables 1–4 and Fig. 1–2; the inhibition rates of compounds **3a–3m** against barnyard grass growth can be seen in the ESI,† Tables S3 and S4,† and the toxicity regression equations, IC_{50} values and IC_{90} values were shown in Tables S5 and S6.† When treated with **3a–3y** solutions, barnyard grass and rape exhibited different symptoms: the barnyard grass emerged with shoot and root flavescence, with some even nigrescence, and the rape seeds rotted and stunk in the solutions. As is demonstrated, most of the *sec-p*-menthane-7-amine derivatives showed remarkable herbicidal activities against barnyard grass and rape. For the barnyard grass, compounds **3a–3m** showed very weak activity: the inhibition rates for root growth were less than 30% when treated at 5 mmol L^{-1}, and even exhibited negative inhibition.

Table 2 Inhibition rates of *sec-p*-menthane-7-amine derivatives against shoot growth of barnyard grass

Compd	2.5[a]	1.25	0.625	0.313	0.156	0.078	0.039	0.0195	0.0098[d]
1	76.4[b]	59.5	32.1	25.1	20.1	17.5	[c]	[c]	[c]
2	38.8[b]	13.6	6.8	1.7	2.7	3.3	[c]	[c]	[c]
3n	100	100	92.0	56.5	35.8	30.9	17.2	11.6	[c]
3o	100	100	99.5	70.9	45.7	33.7	24.7	17.1	[c]
3p	100	100	96.6	72.5	56.1	46.1	29.5	9.8	[c]
3q	100	100	96.5	66.9	52.3	34.9	28.6	12.4	[c]
3r	100	100	96.9	62.0	51.3	40.8	21.5	10.5	[c]
3s	100	100	97.5	72.4	45.9	30.4	21.2	15.2	[c]
3t	100	100	90.1	67.2	46.0	36.1	27.9	12.6	[c]
3u	100	100	95.5	76.4	54.1	50.3	35.5	26.2	19.3
3v	100	100	100	87.3	71.0	50.7	40.5	34.6	23.4
3w	100	100	93.6	77.2	59.0	46.3	30.7	23.2	10.3
3x	100	100	91.5	68.4	58.7	52.0	39.9	26.9	19.0
3y	100	81.4	43.2	26.7	15.6	[c]	[c]	[c]	[c]
Diuron	27.3	26.3	20.8	24.9	14.0	12.3	13.4	10.5	[c]
Glyphosate	97.5	91.9	77.2	64.0	54.1	38.5	12.2	[c]	[c]

[a] The data in this line are the concentrations of different *sec-p*-menthane-7-amine derivatives solutions (mmol L^{-1}). [b] The inhibition rates of compounds **1** and **2** at 5 mmol L^{-1} against root growth of barnyard grass were 100% and 82.2%, against shoot growth were 100% and 77.6%. [c] Have no inhibition activity at this concentration. [d] There were no inhibition activities of compounds against shoot growth of barnyard grass at 0.0049 mmol L^{-1}.

Table 3 Inhibition rates of *sec-p*-menthane-7-amine derivatives against root growth of rape

	Inhibition rates against root growth of rape								
Compd	2.5[a]	1.25	0.625	0.313	0.156	0.078	0.039	0.0195	0.0098[b]
1	97.0	91.0	69.1	33.2	23.9	11.9	[c]	[c]	[c]
2	98.4	81.2	59.7	44.9	38.7	23.0	12.9	[c]	[c]
3n	100	100	98.9	95.6	82.6	60.3	53.4	33.9	23.4
3o	100	100	100	98.0	87.0	56.6	40.1	28.1	17.6
3p	100	100	99.8	97.9	92.4	65.5	50.2	29.5	15.8
3q	100	100	100	98.5	94.4	79.5	50.2	46.4	33.4
3r	100	100	100	98.2	85.0	55.9	38.5	20.6	15.0
3s	100	100	93.8	89.5	68.3	48.9	35.2	13.4	[c]
3t	100	100	99.2	94.9	82.0	54.4	40.3	33.5	20.5
3u	100	100	99.4	97.9	87.2	63.2	50.3	33.4	15.9
3v	100	100	100	98.7	91.7	70.7	60.7	45.3	25.4
3w	100	100	100	98.4	90.5	77.3	59.6	38.1	28.2
3x	100	100	99.4	96.0	91.0	74.2	57.0	44.5	34.3
3y	100	100	100	97.2	92.5	71.6	45.4	34.9	16.4
Diuron	85.0	80.4	78.1	69.5	52.8	36.2	26.4	16.4	[c]
Glyphosate	97.9	90.9	88.5	81.4	77.7	69.7	62.8	52.0	28.1

[a] The data in this line are the concentrations of different *sec-p*-menthane-7-amine derivatives solutions (mmol L^{-1}). [b] The inhibition rates at 0.0049 mmol L^{-1} to root and shoot growths of rape were not determined. [c] Have no inhibition activity at this concentration.

Table 4 Inhibition rates of *sec-p*-menthane-7-amine derivatives against shoot growth of rape

	Inhibition rates against shoot growth of rape								
Compd	2.5[a]	1.25	0.625	0.313	0.156	0.078	0.039	0.0195	0.0098[b]
1	99.4	71.6	45.2	29.0	14.1	[c]	[c]	[c]	[c]
2	87.1	53.3	41.7	30.9	31.0	22.5	14.5	[c]	[c]
3n	100	100	95.5	67.0	47.0	38.6	25.1	17.0	[c]
3o	100	100	100	76.0	37.8	21.1	[c]	[c]	[c]
3p	100	100	96.1	72.4	38.4	25.0	13.9	[c]	[c]
3q	100	100	91.0	76.1	42.7	34.2	22.6	14.9	[c]
3r	100	100	94.7	73.9	36.2	27.4	17.4	[c]	[c]
3s	100	100	88.9	62.0	46.2	31.5	21.2	[c]	[c]
3t	100	100	92.1	48.7	23.0	12.8	[c]	[c]	[c]
3u	100	100	96.1	76.0	48.2	31.3	22.3	12.2	[c]
3v	100	100	100	88.3	61.2	48.5	27.9	15.8	[c]
3w	100	100	98.7	77.5	35.3	26.1	14.2	[c]	[c]
3x	98.6	96.2	87.7	73.2	51.8	36.3	30.2	21.6	11.9
3y	100	98.5	93.8	60.4	42.9	22.1	15.2	[c]	[c]
Diuron	69.0	57.0	50.5	39.1	27.8	18.0	[c]	[c]	[c]
Glyphosate	79.6	61.0	45.8	37.3	26.3	16.0	[c]	[c]	[c]

[a] The data in this line are the concentrations of different *sec-p*-menthane-7-amine derivatives solutions (mmol L^{-1}). [b] The inhibition rates at 0.0049 mmol L^{-1} to root and shoot growths of rape were not determined. [c] Have no inhibition activity at this concentration.

Compounds **3n–3y** displayed significant inhibition efficacy for both barnyard grass root and shoot. The inhibition rates of **3n–3y** are higher than that of **1** and **2**, indicating that the introduction of a secondary amino improves herbicidal activities. Root and shoot growth were completely inhibited at the concentrations of 2.5 mmol L^{-1} and 1.25 mmol L^{-1}. When the treatment concentration was as low as 0.0049 mmol L^{-1}, the inhibition rates of compounds **3p**, **3r**, **3u**, **3v** and **3w** were 46.0%, 52.3%, 46.9%, 44.6%, 45.2%, respectively. The inhibition rates of **3n–3y** for root growth were higher than that for shoot growth, at the concentration of 0.0098 mmol L^{-1}, the inhibition rates against shoot growth of **3u**, **3v** and **3x** were 19.3%, 23.4%, and 19.0%, respectively, but against root growth were higher than 50%. There are two N–H groups in the structure of compound **3y**, but its inhibition rates are less than that of **3n–3x**, indicating the increasing number of N–H groups cannot enhance the herbicidal activity.

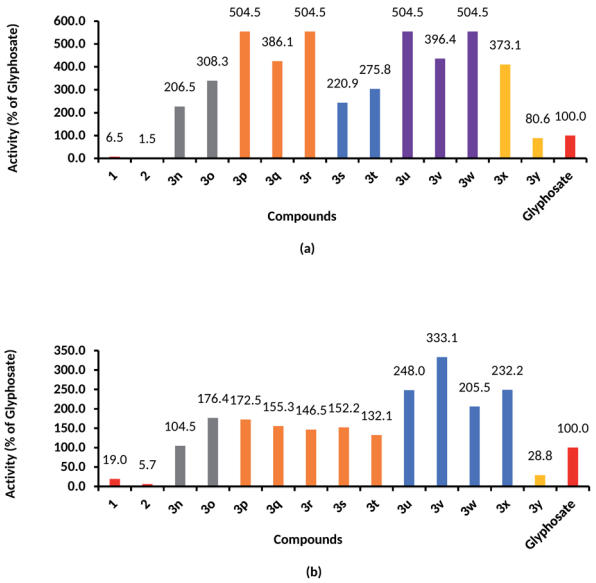


Fig. 1 Herbicidal activities of *sec-p*-menthane-7-amine derivatives against barnyard grass root growth (a) and shoot growth (b) comparing with glyphosate (IC_{50} of glyphosate/IC_{50} of test compounds).

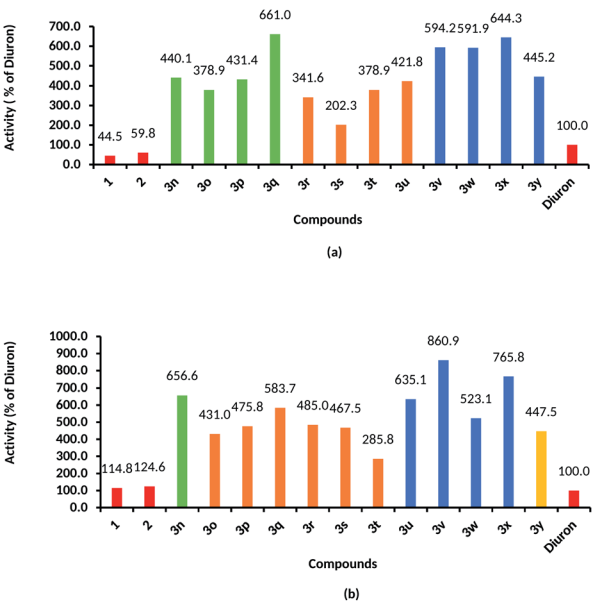


Fig. 2 Herbicidal activities of *sec-p*-menthane-7-amine derivatives against rape root growth (a) and shoot growth (b) comparing with diuron (IC_{50} of diuron/IC_{50} of test compounds).

Table 6 Cytotoxicity of *sec-p*-menthane-7-amine derivatives at 10 μmol L^{-1}

Compd	Inhibition rate (%) to BALB/c 3T3	Inhibition rate (%) to HUVEC-C
2	1.58	1.64
3n	2.00	−0.41[a]
3o	2.61	2.85
3p	0.17	−0.14[a]
3q	1.56	−0.54[a]
3r	2.35	−0.14
3s	2.52	1.90
3t	4.34	2.31
3u	2.09	3.40
3v	2.09	1.09
3w	0.35	3.26
3x	1.13	2.05
3y	85.40	86.01

[a] When the growth inhibition rate of the compound against the cells is negative (the absolute value is higher than 10%), it is generally considered that the compound promotes the proliferation of the cells.

The IC_{50} values of **3n–3x** against barnyard grass root and shoot growth were 0.0088–0.0215 mmol L^{-1} and 0.0493–0.1571 mmol L^{-1}, respectively, lower than that of diuron (IC_{50} values of root and shoot growth were 0.0529 mmol L^{-1} and >10 mmol L^{-1}, respectively), were comparable to that of glyphosate (IC_{50} values of root and shoot growth were 0.0444 mmol L^{-1} and 0.1642 mmol L^{-1}, respectively). By comparison of the IC_{50} values of glyphosate with the IC_{50} values of *sec-p*-menthane-7-amine derivatives in Fig. 1, it was found that the herbicidal activities of compounds **3p**, **3r**, **3u**, and **3w** against barnyard grass root growth were 404% higher than that of glyphosate, respectively. Compounds **3u**, **3v**, **3w**, and **3x** exhibited 148%, 233%, 105%, 132% higher herbicidal activities against shoot growth than those of glyphosate, respectively.

Compounds **3n–3y** also exhibited good herbicidal activities against rape growth. The inhibition rates to rape root growth were higher than that of shoot growth. Most of the compounds showed over 50% control against the root growth of rape at a concentration of 0.078 mmol L^{-1}: when the concentration was 0.0098 mmol L^{-1}, many compounds still showed inhibition efficacy. The IC_{50} values of **3n–3y** against root growth and shoot growth of rape were 0.0231–0.0755 mmol L^{-1} and 0.0813–0.2449 mmol L^{-1}, respectively, lower than that of diuron (IC_{50} value of diuron against root and shoot growth of rape were 0.1527 mmol L^{-1} and 0.6999 mmol L^{-1}, respectively), indicating that the herbicidal activities of *sec-p*-menthane-7-amines were

Table 5 Crop safety of compounds **3p**, **3u**, **3v** at 100 mg L^{-1}

	Injury rate (%)						
Compd	Wheat	Rice	Sorghum	Maize	Peanut	Cucumber	Radish
3p	30	20	10	10	0	80	80
3u	20	20	10	15	0	90	75
3v	20	30	10	10	5	90	80
Glyphosate	90	40	90	95	95	90	60

higher than that of diuron. The IC_{50} values of **3q**, **3v**, **3w**, and **3x** against root growth of rape were 0.0231, 0.0257, 0.0258, 0.0237 mmol L^{-1}, respectively, equivalent to that of glyphosate (IC_{50} value of glyphosate against root growth of rape was 0.0249 mmol L^{-1}). The IC_{50} values of **3n–3y** against shoot growth of rape were lower than that of glyphosate (IC50 value of glyphosate against root growth of rape was 0.6078 mmol L^{-1}). It can be seen from Fig. 2 that the herbicidal activities of compounds **3q**, **3v**, **3w**, and **3x** against root growth of rape were 561%, 494%, 491%, 544% higher, respectively, than those of diuron, and 484%, 760%, 423%, 665%, respectively, higher than those of diuron against shoot growth of rape.

The structure–activity relationship was preliminarily obtained based on the herbicidal activities of *sec-p*-menthane-7-amine derivatives. The presence of electron-donating substituents on the phenyl ring was beneficial to herbicidal activities, compounds **3b**, **3c** (substituent CH_3) and **3k** (substituent OCH_3) showed higher inhibition rates than compounds with electron-withdrawing substituents (halogen atom and OCF_3 group). Compared with phenyl-substituted compounds, alkyl-substituted compounds showed better herbicidal activities, for example, compounds **3n**-**3y** exhibited higher inhibition rates and lower IC_{50} values than those of compounds **3n–3y**. Among the alkyl-substituted *sec-p*-menthane-7-amine derivatives, compounds with the alkyl-substituted part containing a cyclohexyl (**3u**, **3v**, **3w**) showed higher herbicidal activities than those containing a linear chain alkyl (**3n**, **3o**, **3q**, **3r**), and compounds containing a branched alkyl (**3s**, **3t**) showed weaker herbicidal activities. Compound **3x** with two *p*-menthane skeletons in its structure showed great herbicidal activity, which meets the superposition principle. Compound **3y**, with two secondary amino groups in the structure, showed weaker herbicidal activity than compounds with only one, indicating that the number of secondary amino groups key to herbicidal activities and that the increase of secondary amino groups may not necessarily enhance herbicidal activities. The possible reason is steric hindrance: **3y** cannot easily to go through the cell wall to dock with the active site.

3.3 Crop safety

An eligible herbicide must be sensitive to weeds and highly safe to crops. Compounds **3p**, **3u**, and **3v** were selected for the crop safety test targeting seven crops: rice, wheat, sorghum, maize, peanut, cucumber, and radish, to which glyphosate was used as a positive control. The results were listed in Table 5, and it can be found that these compounds were almost harmless (≤30%) to rice, wheat, sorghum, maize, peanut at the concentration of 100 mg L^{-1}, while glyphosate caused over 80% injury to these crops. The cucumber and radish sustained critical injuries (>80%) with compounds **3p**, **3u**, and **3v**, indicating that *sec-p*-menthane-7-amine derivatives could be developed as a potential herbicide for weed control in rice, wheat, sorghum, maize, and peanut fields.

3.4 Cytotoxicity analysis

Agrochemicals must be nontoxic or pose low toxicity to humans and other organisms. The cytotoxicity of compounds **3n–3y** against BALB/c 3T3 and HUVEC-C cell lines were evaluated using the CCK-8 method. As showed in Table 6, when treated with a 10 μmol L^{-1} concentration of compounds **3n–3x**, the inhibition rates against the growth of BALB/c 3T3 and HUVEC-C cells were 0.17% to 4.34% and −0.54% to 3.26%, respectively, meaning that these compounds exhibited nearly no toxicity to BALB/c 3T3 and HUVEC-C cells. Nevertheless, compound **3y** showed severe toxicity to BALB/c 3T3 and HUVEC-C cells, where inhibition rates were 85.40% and 86.01%, respectively.

4 Conclusions

In summary, a series of novel *sec-p*-menthane-7-amine derivatives were designed and synthesized using perillaldehyde as raw material. The results indicated that some of these compounds exhibited better herbicidal activities than commercial herbicides diuron and glyphosate. The IC_{50} values of **3n–3y** against root and shoot growth of barnyard grass were 0.0088–0.0551 mmol L^{-1} and 0.0493–0.5711 mmol L^{-1}, respectively. The herbicidal activities of compounds **3p**, **3r**, **3u** and **3w** against root growth of barnyard grass were 404% higher, respectively, than those of glyphosate. The herbicidal activities of compounds **3u**, **3v**, **3w**, and **3x** against shoot growth of barnyard grass were 148%, 233%, 105%, 132% higher, respectively, than those of glyphosate. The IC_{50} values of **3n–3y** against root and shoot growth of rape were 0.0231–0.0755 mmol L^{-1} and 0.0813–0.2449 mmol L^{-1}, respectively. The herbicidal activities of *sec-p*-menthane-7-amine derivatives against root and shoot growth of rape were higher than those of diuron. The structure–activity relationship studies showed that the alkyl-substituted *sec-p*-menthane-7-amine derivatives exhibited higher herbicidal activities than those of phenyl-substituted derivatives, and compound **3u** with an alkyl-substituted part containing a cyclohexyl, showed the best herbicidal activities: the IC_{50} value against barnyard grass root growth was 0.0088 mmol L^{-1}. Furthermore, compounds **3p**, **3u**, and **3v** were found to show good safety to rice, wheat, sorghum, maize and peanut. Compounds **3n–3x** have been verified to be nontoxic for BALB/c 3T3 and HUVEC-C cells. In conclusion, compound **3u** was expected to be the most promising botanical herbicide for future weed control.

Author contributions

Synthesis and characterization, writing original draft, H. M. Z.; writing, review, and editing, Y. X. C., S. C. X., J. W., H. H. D., Z. D. Z. and J. X. J. All authors have read and agree to the published version of the manuscript.

Conflicts of interest

The authors declare no competing financial interest.

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