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Article

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Identification, Synthesis and Safety Assessment of Thidiazuron (1-phenyl-3-(1,2,3-thidiazol-5-yl)urea) and Its Metabolites in Kiwifruits

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■**ABSTRACT**

Quality of kiwifruit became worse due to the abuse of plant growth regulators (PGRs). The safety of the fruits treated with PGRs are also worried by consumers. Therefore, the present study analysed the structure of thidiazuron (TDZ, (1-phenyl-3-(1,2,3-thidiazol-5-yl)urea)) (1) and its metabolites of bio-transformation in kiwifruits by using liquid chromatography hybrid ion trap time-of-flight mass spectrometry (LC-IT-TOF-MS). Standard compounds were also synthesized and used for structural identification of those metabolites. In addition, cytotoxicity of thidiazuron and its metabolites were tested through Sulforhodamine B assays against normal Chinese hamster ovary (CHO) cells. Four metabolites were identified. They were 4-hydroxy-thidiazuron (2), 3-hydroxy-thidiazuron (3), thidiazuron -4-*O*-β-D-glucoside (4), and thidiazuron-3-*O*-β-D-glucoside (5). Values of IC_{50} of compound 1, 2, and 3 to CHO cells were 18.3±1.8 μM, 37.56±1.5 μM, and 23.36±1.59 μM, respectively. Compound 4 and 5 had no effect on CHO cells.

KEYWORDS: Thidiazuron, Metabolites, LCMS-IT-TOF, Safety Assessment

■INTRODUCTION

Kiwifruit is rich in vitamin C and a famous fruit all over the world with its special sweet, sour taste and exquisite flavor. China is the largest producer of kiwifruit in the world, which occupies about 38 % of the total world amount[1]. In recent years, how to produce more safe and healthy kiwifruit has been raised more attention of people because of pesticide residues pollution. For example, the phenylurea herbicides and their metabolites have been studied, such as: diuron in surface water and ground water[2], bio-transformed derivatives of chlortoluron and isoproturon[3], degradation products of chlortoluron[4], degradation products of forchlorfenuron[5] and results of phenylurea herbicide photochemical behavior[6].

Thidiazuron (1-phenyl-3-(1,2,3-thidiazol-5-yl)urea, TDZ) (Figure 1), a plant growth regulator of phenylurea with the ability to enhance plant cell division and cell expansion[7], is applied to increase fruit size and weight[8-12]. This low dose (0-10 ppm) of TDZ increased kiwifruit size without affecting fruit soluble solid content, flesh firmness or concentration of nonstructural carbohydrates[13, 14]. In recent years, presence of TDZ residues and their safety in kiwifruit and the environment have increasingly caused concern[15-17]. Toxic properties may persist in metabolites, which may even exhibit increased toxicity[3]. Therefore, understanding their degradation and metabolism is a most interesting goal and challenge in kiwifruit.

In recent years, time-of-flight (TOF) mass spectrometry has been applied successfully to the global identification of target and non-target components in herbal medicine, component analysis, drug metabolite identification and degradation

studies[18-22]. LCMS-IT-TOF (Shimadzu) is a type of mass spectrometer that combines ion trap and TOF (time-of-flight) technologies. The instrument possesses some advantages and functions, such as high speed，high accuracy MS^n，formula predictions software and so on. To check types of formed metabolites, plus detect both expected and unexpected metabolites, MetID Solution can be used to compare both blank and treated samples[23-25].

In this study, we used LCMS-IT-TOF to discover the main metabolites of TDZ in kiwifruit, and in turn, to identify these metabolites by the synthesis standard. Then, cytotoxicity of TDZ and its metabolites were also evaluated..

■ MATERIAL AND METHODS

General. Thidiazuron, **1**, (purity>99%, Figure 1), high performance liquid chromatography (HPLC) grade methanol and acetonitrile were provided by Sigma-Aldrich (St Louis, Missouri). Formic acid and ethyl acetate used were of analytical grade (Sigma-Aldrich, St Louis, Missouri). HPLC-grade water was obtained by Milli-Q-Plus ultrapure water system from Millipore (Milford, MA). Nuclear magnetic resonance spectra were recorded on an AVANCE III, 500 MHz spectrometer (Bruker, Fällanden, Switzerland). Optical density (OD) was read on iMark Microplate Reader (Bio-Rad, Richmond, CA). Commercial solvents and reagents were of analytical grade Sigma-Aldrich, St Louis, Missouri).

Plant material. The trial was carried out between 2014-2015, in Xi'an city, Shaanxi province, China, in a 7-year-old kiwifruit orchard of Hayward from Shaanxi Bairui Kiwi Fruit Research Institute Co. Ltd($34^o03'$ N, $108^o25'$ E). The vines were

trained to the pergola system, with 4 m between vines within the row and 5 m between rows. These experiments were designed according to the standard operating procedures on pesticide registration residue field trials, issued by the Institute for the Control of Agrochemicals, Ministry of Agriculture, China[26]. Fifteen kiwifruit vines were selected, with uniform vegetative and reproductive characteristics. Fifteen days after full bloom, all initial fruits of fifteen vines were dipped for 5 s in aqueous TDZ solution (60 mg/kg), while the fruit of the other five vines were dipped in water only, as a control set. After ninety days, representative treated and the control samples were randomly were stored in a refrigerated room under normal atmosphere (T, -0.5±0.5 ^{o}C) until used.

Sample preparation. Briefly, 200 g of chopped and thoroughly-homogenized sample was extracted ultrasonically with a solvent containing ethyl acetate (200 mL), anhydrous magnesium sulfate (80 g) , and anhydrous sodium acetate (20 g) for 30 min. The supernatant of the mixture was paper-filtered and the solid residue was washed twice with 60 mL of ethyl acetate. Solution were collected and concentrate to less than 2 mL on a rotary evaporator (40 ^{o}C and 250 mbar). Then the concentrate was reconstituted in 2 mL of methanol, filtered through a microporous membrane (0.22 μm), and stored at −40 ^{o}C. The sample was prepared according to the method described in a previous paper[20].

Chromatographic conditions. Liquid chromatography consisted of an LC-20AD binary pump, DGU-14A degasser, SIL-20AD auto-sampler, and CTO-20AC column oven (Shimadzu, Kyoto, Japan). The analytes were separated on a Shim-pack XR ODS column (2.2 μm 3.0 mm×75 mm Shimadzu). The flow rate, injection volume,

and column temperature were set to 0.2 mL/min, 20 μL, 35°C, respectively. The mobile phase consisted of solvent A (acetonitrile) and B (0.1% formic acid in water). A linear mobile phase gradient started at 90–30% B (0–4min), 30–0% B (4–6 min), retained for 1 min, then quickly returned to initial 90% B, and maintained 3 min for column equilibration.

MS^n analyses were conducted on an LC-IT-TOF-MS (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization (ESI) source, under the following optimized operating conditions: positive ion mode, detector voltage at 1.6 kV, curved desolvation line (CDL) temperature at 200 °C, heat block temperature at 200 °C, nebulizing gas flow of 1.5 L/min, drying gas (N_2) pressure of 100 kPa, and scan ranges of *m/z* 100–1000 for MS^1, *m/z* 100–800 for MS^2 and *m/z* 100–800 for MS^3. Metabolite analyses were performed by MetID solution, ver. 1.1. Finally, the Composition Formula Predictor software (Shimadzu) was used to provide chemical formulae for TDZ and its metabolites.[5]

Cytotoxicity Assay. TDZ and four metabolites were evaluated for reproductive cytotoxicity by sulforhodamine B (SRB) assay, using Chinese hamster ovary (CHO)[27]. CHO cells were cultured in high-glucose DMEM medium, containing 10% (v/v) fetal bovine serum (FBS), penicillin (100 KU/L) and streptomycin (100 μg/mL), at 37 ℃, in a 5% CO_2 atmosphere with 95% (v/v) humidity. TDZ and its metabolites were dissolved in DMSO, then diluted in culture media to the required concentration. The DMSO content of the final concentration was below 0.1 %(v/v). Cytotoxicity of CHO cells were determined by the SRB assay performed as described by Zhang Z.[5]

Synthesis of the reference standards. The general synthetic routes of the reference standards (4-hydroxy-thidiazuron, 3-hydroxy-thidiazuron,

thidiazuron-4-*O*-β-D-glucoside, thidiazuron-3-*O*-β-D-glucoside) are outlined in Figure 2. Detail synthetic and purification procedures are the same as described by Zhang Z[5], characterization data are given in the supporting information. The NMR data are showed as follows:

4-hydroxy-thidiazuron (**2**) was a colorless solid, yield 71%. ^{1}H NMR (500 MHz, MeOD) δ 8.37 (s, 1H, -CHN=N), 7.15 (d, J = 8.8 Hz, 2H, Ar-H), 6.73 – 6.61 (m, 2H, Ar-H). ^{13}C NMR (125 MHz, MeOD) δ, 155.882（Ar-C）, δ 155.43（-C=O）, 154.42（N-C-S）, 134.68（-CN=N）, 130.94（Ar-C）, 124.15（Ar-C）, 116.67（Ar-C）.

3-hydroxy-thidiazuron (**3**) was a colorless solid, yield 75%. ^{1}H NMR (500 MHz, MeOD) δ, 8.42 (s, 1H, -CHN=N), 7.11 (t, J = 8.1 Hz, 1H, Ar-H), 7.01 (t, J = 2.2 Hz, 1H, Ar-H), 6.88 (ddd, J = 8.0, 2.0, 0.8 Hz, 1H, Ar-H), 6.55 (ddd, J = 8.1, 2.3, 0.7 Hz, 1H, Ar-H). ^{13}C NMR (125 MHz, MeOD) δ, 158.45（Ar-C）, 154.40（-C=O）, 152.97（N-C-S）, 139.70（Ar-C）, 134.24（-CN=N）, 130.55（Ar-C）, 111.85（Ar-C）, 111.70（Ar-C）, 107.62（Ar-C）.

Thidiazuron-4-*O*-β-D-glucoside (**4**) was a colorless solid, yield 65%. ^{1}H NMR (500 MHz, MeOD) δ, 8.51 (s, 1H, -CHN=N), 7.41 (d, J = 9.0 Hz, 2H, Ar-H), 7.12 (d, J = 9.0 Hz, 2H, Ar-H), 4.88 (s, 1H, O-CH-O), 3.92 (dd, J = 12.1, 2.1 Hz, 1H, -CH_2OH), 3.73 (dd, J = 12.0, 5.5 Hz, 1H, -CH_2OH), 3.52 – 3.39 (m, 4H , -CH_2C<u>H</u>OH-, - CH_2CHC<u>H</u>OH, -CH_2CHCHC<u>H</u>OH, -OOHCC<u>H</u>OH). ^{13}C NMR (125 MHz, MeOD) δ, 155.96（Ar-C）, 155.36（-C=O）, 154.13（N-C-S）, 134.77（-CN=N）, 134.06（Ar-C）, 122.92（Ar-C）, 118.51（Ar-C）, 102.89（O-C-O）, 78.31 (-CH_2<u>C</u>HOH-), 78.13 (-CH_2CH<u>C</u>HOH), 75.07 (-CH_2CHCH<u>C</u>HOH), 71.54 (-OOHC<u>C</u>HOH), 62.68

160 （CH_2）.

161 Thidiazuron-3-*O*-β-D-glucoside (**5**) was a colorless solid, yield 63%. ^{1}H NMR
162 (500 MHz, DMSO) δ, 9.54 (s, 1H, -NH), 9.21 (s, 1H, -NH), 8.91 (s, 1H, -CHN=N),
163 7.27 (s, 1H, Ar-H), 7.04 – 6.93 (m, 2H, Ar-H), 6.33 (d, J = 6.8 Hz, 1H, Ar-H), 5.65
164 (dd, J = 10.2, 7.4 Hz, 2H, O-CH-O, OH), 5.39 (d, J = 5.0 Hz, 1H, OH), 5.26 (d, J =
165 5.5 Hz, 1H, OH), 4.73 (t, J = 5.6 Hz, 1H, OH), 3.80 (dt, J = 14.8, 7.4 Hz, 1H, -CHOH),
166 3.74 (dd, J = 9.7, 5.9 Hz, 1H, -CH_2OH), 3.50 (q, J = 5.5 Hz, 2H, - CH_2C<u>H</u>OH-, -
167 CH_2CHC<u>H</u>OH), 3.43 – 3.38 (m, 1H, - CH_2CHCHC<u>H</u>OH), 3.29 (dt, J = 14.7, 7.3 Hz,
168 1H, -OOHCC<u>H</u>OH). ^{13}C NMR (125 MHz, DMSO) δ, 170.64（Ar-C）, 163.18
169 （-C=O）, 157.61（N-C-S）, 141.75（Ar-C）, 129.14（-CN=N）, 125.09（Ar-C）,
170 108.88（Ar-C）, 108.61（Ar-C）, 104.95（Ar-C）, 94.33（O-C-O）, 80.64（-CH_2<u>C</u>HOH-）,
171 76.45 (-CH_2CH<u>C</u>HOH）, 72.37（-CH_2CHCH<u>C</u>HOH）, 69.29（-OOHC<u>C</u>HOH）, 60.69
172 （CH_2）.

173 **■RESULTS AND DISCUSSION**

174 Based on the published findings[5], the proposed strategy for identifying and
175 assessing the safety of TDZ metabolites involves four procedural steps: TDZ
176 metabolites detected by total ion current (TIC) were recorded, searching for evident
177 differences between peaks of the blanks and those of treated samples. Obtained MS^1
178 and MS^n spectra of differences in peaks were used to deduce TDZ metabolites. The
179 standard substances were synthesized, and comparison between synthetic standard
180 substances and TDZ metabolites was performed. Safety of TDZ and its metabolites
181 were assessed.

Fragmentations of TDZ. For the fragmentation pattern study, 200 μg/mL TDZ (**1**) and four synthetic standard substances of its metabolites (**2**, **3**, **4**, **5**), prepared in methanol, were used. Extract-ion chromatograms of 20 μg/mL TDZ and four synthetic standard substances (**2**, **3**, **4**, **5**) appear in Figure 3. The proposed fragmentation patterns are illustrated in Figure 4. Figure S9 presents accurate mass measurements of the protonated molecules and fragment ions of TDZ and four synthetic metabolites. Table 1 shows data for TDZ and synthetic metabolites by LC-ESI-IT/TOF-MS analysis in positive ion mode.

Analysis of metabolites. The treated sample and the control sample were analyzed by LC-ESI-IT-TOF/MS. In a search of possible metabolites, targeted data analysis was carried out with the aid of MetID solution software. Extract-ion chromatograms of the treated sample and the control sample are depicted in Figure 5, while Figure 6 shows mass spectra of **6-10** in positive ion mode. MS^n of metabolite data from TDZ in positive ion mode, by LC-ESI-IT-TOF/MS analysis, are presented in Table 2.

Metabolite 6 (Figure 6a, Table 2，Table 2) eluting at 5.562 min, shows the predominant protonated molecule ion $[M+H]^+$ at *m/z* 221.0493 (error, 0.45ppm). **6** yields two main MS^2 ions at *m/z* 127.9922 (error, 7.03 ppm), 102.0127 (error, 6.86 ppm). **6** calculates as $C_9H_8N_4OS$ according to the accurate mass by Formula Predictor software. The difference of retention time between 1 and 6 was 0.009 min. So, metabolite 6 was identified as 1.

Metabolite 7 (Figure 6b, Table 2) eluting at 4.242 min, shows the predominant

protonated molecule ion $[M+H]^+$ at *m/z* 237.0441 (error, 0 ppm). **7** yields three main MS^2 ions at *m/z* 127.9922 (error, 7.03 ppm), 110.0609 (error, 8.18 ppm), 102.0127 (error, 6.86 ppm). **7** calculates as $C_9H_8N_4O_2S$ according to the accurate mass by Formula Predictor software. The protonated molecule at *m/z* 237.0441 is 15.9949 Da higher than the protonated molecule of TDZ (*m/z* 221.0492) corresponding to addition of oxygen. In order to deduce the site of hydroxylation on **7** (*m/z* 221.0492), we compared the MS^n data of **7** with the corresponding data for TDZ. The MS^2 ions of **7** at *m/z* 127.9922 and 102.0127 form through neutral loss of C_6H_7N and $C_7H_5NO_2$; therefore, the most likely hydroxylation position is located on the benzene ring. Based on the results of the above analysis, standards (**2**, **3**) of possible metabolites were synthesized. As can be seen in Figure S9 (Figure 6b), **7** shares with **2** the common MS^1 ion at *m/z* 237.0441, MS^2 ions at *m/z* 127.9922/102.0127 and *m/z* 110.0609 (error, 8.18 ppm). We conclude that **7** and **2** share the same fragment pathway. The retention time difference for **7** and **2** is 0.002 min. Therefore, **7** is identified as **2** (4-hydroxy-thidiazuron).

Metabolite **8** (Figure 6c, Table 2) eluting at 4.735 min, shows the predominant protonated molecule ion $[M+H]^+$ at *m/z* 237.0428 (error, -5.48 ppm). **8** yields three main MS^2 ions at *m/z* 127.9903 (error, -7.81 ppm), 110.0607 (error, 6.36 ppm), 102.0115 (error, -4.90 ppm). Using the accurate mass by the Formula Predictor software, **8** calculates as $C_9H_8N_4O_2S$. The protonated molecule at *m/z* 237.0428 is 15.9936 Da higher than the protonated molecule of TDZ (*m/z* 221.0492). Based on the analysis result for **7**, the most likely hydroxylation position for **8** is at the benzene ring.

The retention time difference for **8** and **3** is 0.005 min. Therefore, **8** is identified as **3** (3-hydroxy-thidiazuron).

Metabolite **9** (Figure 6d, Table 2) shows the protonated molecular ion $[M+H]^+$ at *m/z* 399.0969 with mass error of 0 ppm, retention time 2.892 min. **9** yields four main MS^2 ions at *m/z* 237.0423 (error, -7.59 ppm), 127.9914 (error, 0.78 ppm), 110.0608 (error, 7.27 ppm), 102.0127 (error, 6.86 ppm). The fragment ion at *m/z* 237.0423 leads to a MS^3 product ions at *m/z* 102.0117 (error, -2.94 ppm). In comparison with the protonated molecule of **2** (**3**), **9** represents an increase of 162.0528 Da ($C_6H_{10}O_5$). **9** calculates as $C_{15}H_{18}N_4O_7S$, according to the accurate mass by Formula Predictor software. Thus, we deduce that **9** is a glycosylated metabolite of **2** (**3**). Comparing the data of **9** and the standard substances (**4**, **5**), the retention time difference for **9** and **4** is 0.009 min. Therefore, **9** is identified as **4** (thidiazuron-4-*O*-β-D-glucoside).

Metabolite **10** (Figure 6e, Table 2), shows the protonated molecular ion $[M+H]^+$ at *m/z* 399.0967 with mass error of -0.5 ppm, and representing an increase of 162.0537 Da ($C_6H_{10}O_5$), compared to the spectra of MS^2 at *m/z* 237.0430. The fragment ion at *m/z* 237.0430 (error, -4.64 ppm) leads to three MS^3 product ions at *m/z* 127.9908 (error, -3.91 ppm), 110.0604 (error, 3.63 ppm), 102.0112 (error, -7.84 ppm). **10** calculates to be $C_{15}H_{18}N_4O_7S$, and thus the glycosylated metabolite of **2**, **3**. As can be seen in Table 1, Table 2, the retention time error is 0.008 min between **5** and **10**. Therefore, **10** is identified as **5** (thidiazuron-3-*O*-β-D-glucoside).

Except for four metabolites of TDZ (4-hydroxy-thidiazuron(2), 3-hydroxy-thidiazuron(3), thidiazuron-4-O-β-D-glucoside(4), and thidiazuron-3-O-β-D-glucoside(5) were synthesized, which are meta or para

derivatives, we have tried our best and used different methods to find out and synthesize ortho substituted derivatives, but no results are available until now. These derivatives may be discovered and synthesized by other researchers in future studies.

Cytotoxicity. Cytotoxic action is expressed as the value of 50% inhibition concentration. Cytotoxicity to CHO cells from high to low was: 1 (IC_{50}=18.3±1.8 μM), 3(IC_{50}=23.36±1.59 μM), 2(IC_{50}=37.56±1.5 μM), while **4** and **5** exhibit no activity in CHO. Hydroxylation reduced cytotoxicity of TDZ, whereas glycosylation resulted in loss of cytotoxicity. TDZ improve fruit size and weight, increase farmer income, but produce some hazards to food safety. As a researcher, we ought to breed new varieties instead of spraying in solution of plant growth regulator.

Supporting Information Available:

^{1}H, ^{13}C NMR spectra of 2, 3, 4, 5 are provided in electronic supporting information.

This information is available free of charge via the Internet.

The supporting information consists of the following information:

Figure S1. ^{1}H NMR spectra of 4-hydroxy-thidiazuron(2)

Figure S2. ^{13}C NMR spectra of 4-hydroxy-thidiazuron(2)

Figure S3. ^{1}H NMR spectra of 3-hydroxy-thidiazuron (3)

Figure S4. ^{13}C NMR spectra of 3-hydroxy-thidiazuron (3)

Figure S5. ^{1}H NMR spectra of thidiazuron -4-*O*-β-D-glucoside (4)

Figure S6. ^{13}C NMR spectra of thidiazuron -4-*O*-β-D-glucoside (4)

Figure S7. ^{1}H NMR spectra of thidiazuron-3-*O*-β-D-glucoside(5)

Figure S8. ^{13}C NMR spectra of thidiazuron-3-*O*-β-D-glucoside(5)

Figure S9. Mass spectra of the synthetic standards in positive ion mode

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441 **FIGURE CAPTIONS**

442 **Figure 1.** Structures of thidiazuron and synthetically prepared metabolites of
443 thidiazuron (numbers of the compounds refer to Table 1).

444 **Figure 2.** Synthetic reagents, conditions, and route of thidiazuron metabolites (a).
445 80 ℃, 8 h, (b) BBr_3, CH_2Cl_2, -30 ℃, 12 h, (c) TBAB, K_2CO_3, $CHCl_3$, 50 ℃, 10 h, (d)
446 $NH_3 \cdot H_2O$, CH_3OH, rt. ,8 h.

447 **Figure 3.** Extracted ion chromatograms of the synthetic standards of thidiazuron
448 metabolites (numbers of the compounds refer to Table 1).

449 **Figure 4.** The fragmentation pattern of (A) 1, (B) 2 and 3, (C) 4 and 5 (numbers of
450 the compounds refer to Table 1).

451 **Figure 5.** Extracted ion chromatograms of thidiazuron and its metabolites in the
452 treated and control kiwifruit samples (numbers of the compounds refer to Table 2).

453 **Figure 6.** Mass spectra analysis of thidiazuron and its metabolites in kiwifruits in
454 positive ion mode (a, 6; b, 7; c, 8; d, 9; e, 10; numbers of the compounds refer to
455 Table 2).

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463 **Table 1 Composition, retention rime and mass spectral fragmentation data[a] of**

464 **synthetically prepared metabolites of thidiazuron**

NO.	RT[b] (min)	Theoretical Value (*m/z*)	Elemental compositions	MS^n	$[M+H]^+$ (*m/z*)	Error (ppm)
1[c]	5.553	221.0492	$C_9H_8N_4OS$	**1**-MS^1	221.0488	-1.81
		127.9913	$C_3H_3N_3OS$	**1**-MS^2	127.9919	4.69
		102.0120	$C_2H_3N_3S$	**1**-MS^2	102.0115	-4.90
		94.0651	C_6H_7N	**1**-MS^2	94.0657	6.38
2	4.240	237.0441	$C_6H_8N_4O_2S$	**2** MS^1	237.0426	-6.33
		127.9913	$C_3H_3N_3OS$	**2** MS^2	127.9905	-6.25
		110.0600	C_6H_7NO	**2** MS^2	110.0606	5.45
		102.0120	$C_2H_3N_3S$	**2** MS^2	102.0127	6.86
3	4.740	237.0441	$C_6H_8N_4O_2S$	**3** MS^1	237.0425	-6.75
		127.9913	$C_3H_3N_3OS$	**3** MS^2	127.9919	4.69
		110.0600	C_6H_7NO	**3** MS^2	110.0596	-3.63
		102.0120	$C_2H_3N_3S$	**3** MS^2	102.0114	-5.88
4	2.883	399.0969	$C_{15}H_{18}N_4O_7S$	**4** MS^1	399.0961	-2.00
		237.0441	$C_6H_8N_4O_2S$	**4** MS^2	237.0431	-4.22
		127.9913	$C_3H_3N_3OS$	**4** MS^2	127.9917	3.13
		110.0600	C_6H_7NO	**4** MS^2	110.0603	2.73
		102.0120	$C_2H_3N_3S$	**4** MS^2	102.0123	2.94
		127.9913	$C_3H_3N_3OS$	**4** MS^3	127.9916	2.34
		110.0600	C_6H_7NO	**4** MS^3	110.0597	-2.73
		102.0120	$C_2H_3N_3S$	**4** MS^3	102.0113	-6.86
5	3.325	399.0969	$C_{15}H_{18}N_4O_7S$	**5** MS^1	399.0952	-4.26
		237.0441	$C_6H_8N_4O_2S$	**5** MS^2	237.0435	-2.53
		127.9913	$C_3H_3N_3OS$	**5** MS^2	127.9914	0.78
		110.0600	C_6H_7NO	**5** MS^2	110.0608	7.29
		102.0120	$C_2H_3N_3S$	**5** MS^2	102.0127	6.86
		127.9913	$C_3H_3N_3OS$	**5** MS^3	127.9908	-3.91
		110.0600	C_6H_7NO	**5** MS^3	110.0605	4.54
		102.0120	$C_2H_3N_3S$	**5** MS^3	102.0126	5.88

465 [a] The instrumentation: LCMS-IT-TOF, conditions: positive ion mode; [b] RT = retention time; [c] 1,
466 thidiazuron; 2, 4-hydroxy-thidiazuron; 3, 3-hydroxy-thidiazuron; 4,
467 thidiazuron-4-*O*-β-D-glucoside; 5, thidiazuron-3-*O*-β-D-glucoside.

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Table 2 Composition, retention rime and mass spectral fragmentation data[a] of thidiazuron metabolites in kiwifruits

NO.	RT[b] (min)	Theoretical Value (*m/z*)	Elemental compositions	MS^n	$[M+H]^+$ (*m/z*)	Error (ppm)
6[c]	5.562	221.0492	$C_9H_8N_4OS$	**6**-MS^1	221.0493	0.45
		127.9913	$C_3H_3N_3OS$	**6**-MS^2	127.9922	7.03
		102.0120	$C_2H_3N_3S$	**6**-MS^2	102.0127	6.86
7	4.242	237.0441	$C_6H_8N_4O_2S$	**7** MS^1	237.0441	0
		127.9913	$C_3H_3N_3OS$	**7**MS^2	127.9922	7.03
		110.0600	C_6H_7NO	**7** MS^2	110.0609	8.18
		102.0120	$C_2H_3N_3S$	**7** MS^2	102.0127	6.86
8	4.735	237.0441	$C_6H_8N_4O_2S$	**8** MS^1	237.0428	-5.48
		127.9913	$C_3H_3N_3OS$	**8** MS^2	127.9903	-7.81
		110.0600	C_6H_7NO	**8** MS^2	110.0607	6.36
		102.0120	$C_2H_3N_3S$	**8** MS^2	102.0115	-4.90
9	2.892	399.0969	$C_{15}H_{18}N_4O_7S$	**9** MS^1	399.0969	0
		237.0441	$C_6H_8N_4O_2S$	**9** MS^2	237.0423	-7.59
		127.9913	$C_3H_3N_3OS$	**9** MS^2	127.9914	0.78
		110.0600	C_6H_7NO	**9**MS^2	110.0608	7.27
		102.0120	$C_2H_3N_3S$	**9** MS^2	102.0127	6.86
		102.0120	$C_2H_3N_3S$	**9**MS^3	102.0117	-2.94
10	3.317	399.0969	$C_{15}H_{18}N_4O_7S$	**10** MS^1	399.0967	-0.50
		237.0441	$C_6H_8N_4O_2S$	**10** MS^2	237.0430	-4.64
		127.9913	$C_3H_3N_3OS$	**10** MS^2	127.9918	3.91
		110.0600	C_6H_7NO	**10** MS^2	110.0603	2.73
		102.0120	$C_2H_3N_3S$	**10** MS^2	102.0121	0.98
		127.9913	$C_3H_3N_3OS$	**10** MS^3	127.9908	-3.91
		110.0600	C_6H_7NO	**10** MS^3	110.0604	3.63
		102.0120	$C_2H_3N_3S$	**10** MS^3	102.0112	-7.84

[a] The instrumentation: LCMS-IT-TOF, conditions: positive ion mode; [b] RT = retention time;

[c] 6, thidiazuron; 7, 4-hydroxy-thidiazuron; 8, 3-hydroxy-thidiazuron; 9, thidiazuron-4-*O*-β-D-glucoside; 10, thidiazuron-3-*O*-β-D-glucoside.

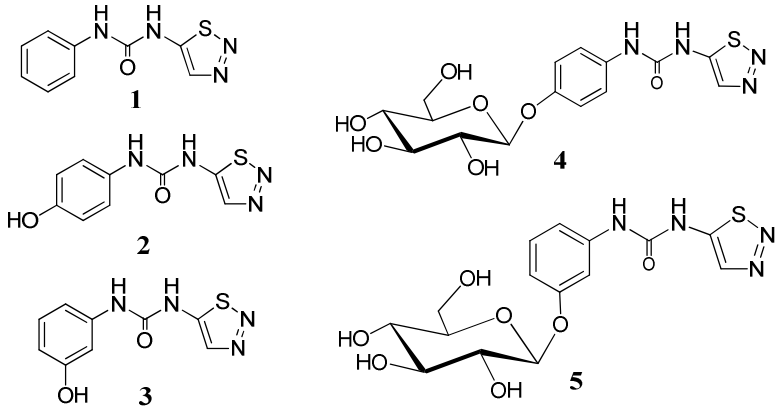

Figure 1. Structures of thidiazuron and synthetically prepared metabolites of thidiazuron (numbers of the compounds refer to Table 1).

1, thidiazuron (TDZ, (1-phenyl-3-(1,2,3-thidiazol-5-yl)urea)); 2, 4-hydroxy-thidiazuron; 3, 3-hydroxy-thidiazuron; 4, thidiazuron -4-*O*-β-D-glucoside; 5, thidiazuron-3-*O*-β-D-glucoside.

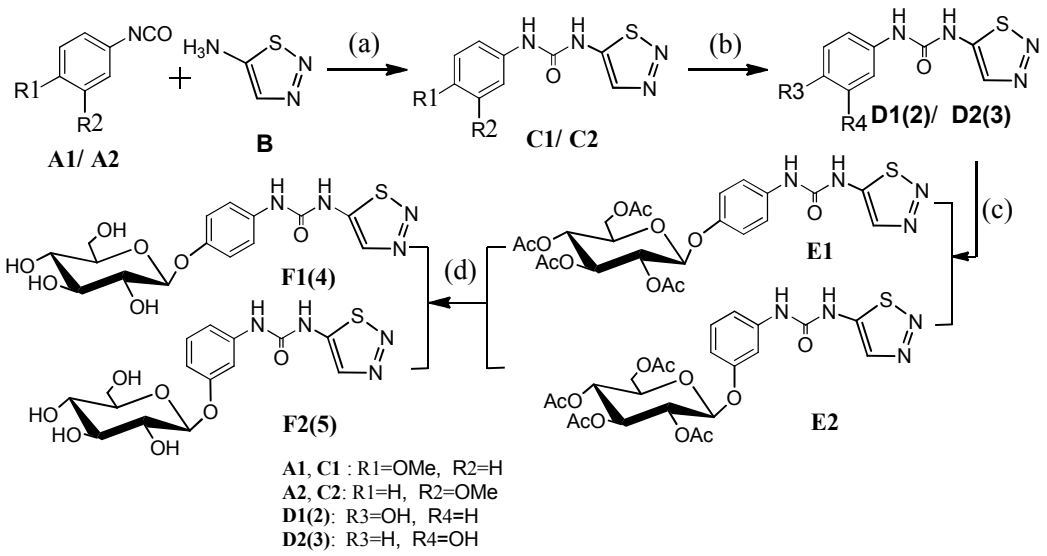

Figure 2. Synthetic reagents, conditions, and route of thidiazuron metabolites: (a) 80 ℃, 8 h, (b) BBr_3, CH_2Cl_2, -30 ℃, 12 h, (c) TBAB, K_2CO_3, $CHCl_3$, 50 ℃, 10 h, (d) $NH_3 \cdot H_2O$, CH_3OH, rt. ,8 h.

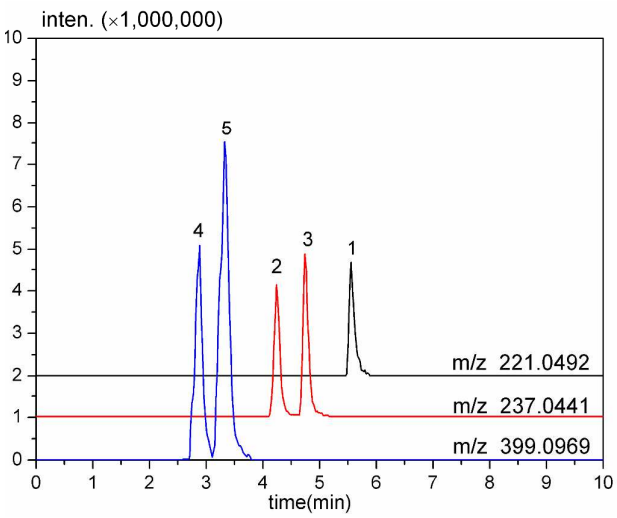

Figure 3. Extracted ion chromatograms of the synthetic standards of thidiazuron metabolites (numbers of the compounds refer to Table 1)

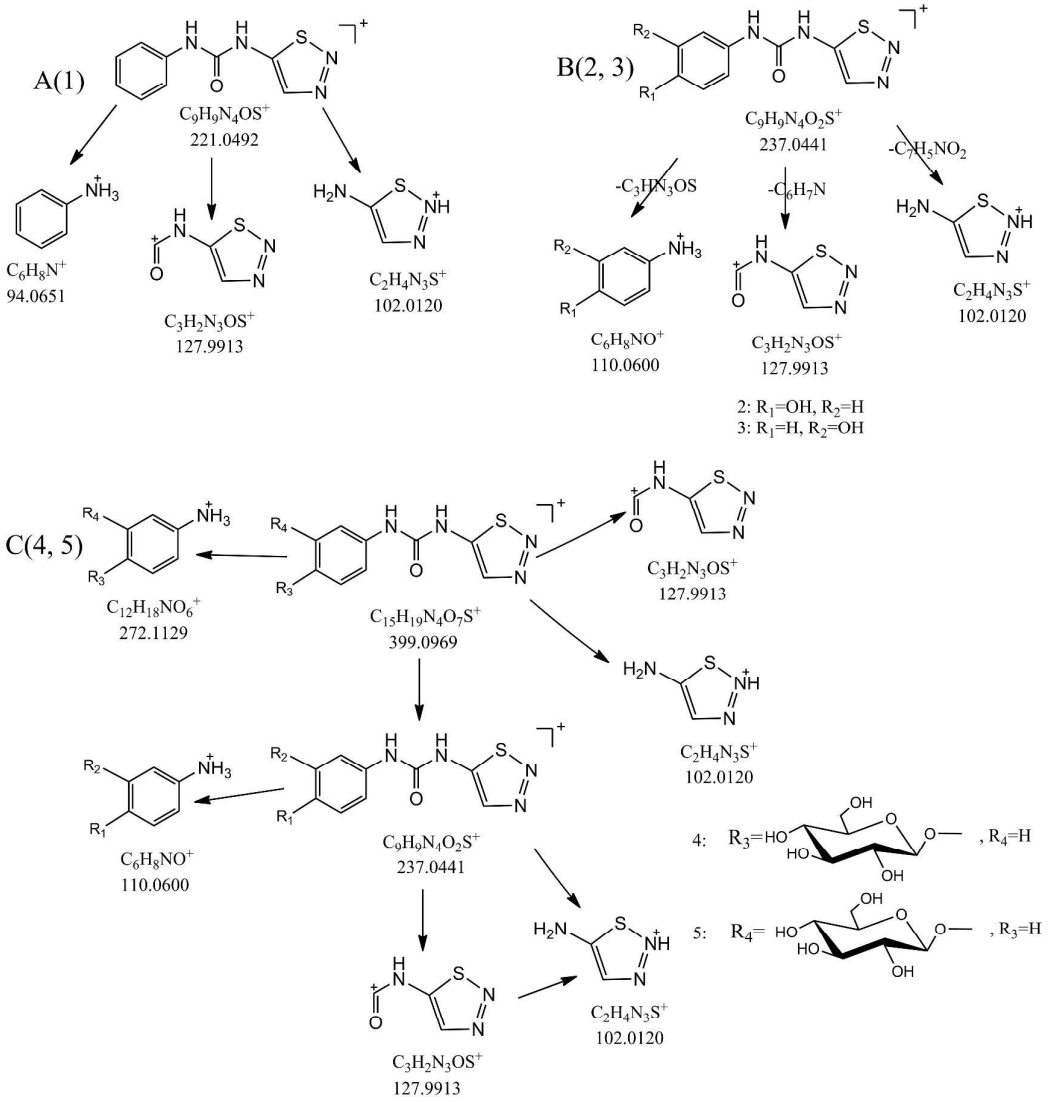

Figure 4. The fragmentation pattern of (A) 1, (B) 2 and 3, (C) 4 and 5 (numbers of the compounds refer to Table 1)

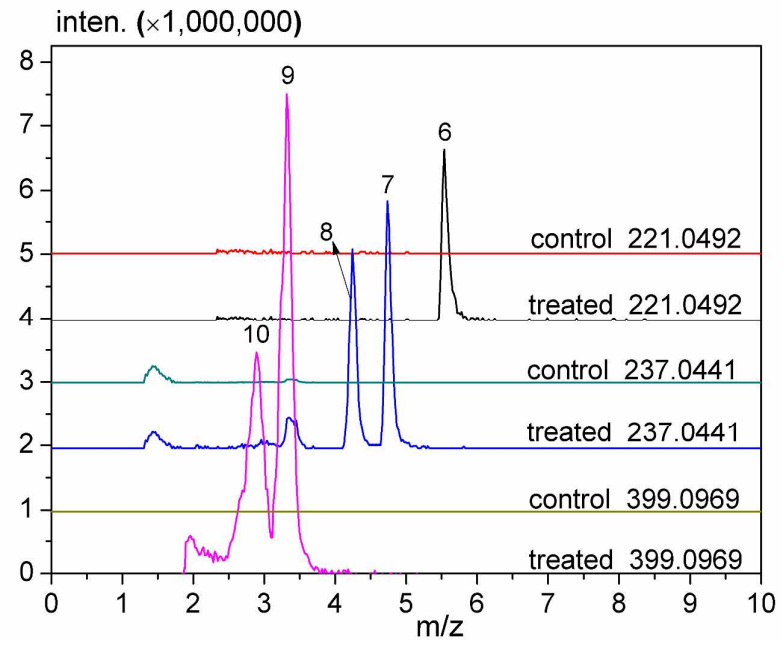

Figure 5. Extracted ion chromatograms of thidiazuron and its metabolites in the treated and control kiwifruit samples (numbers of the compounds refer to Table 2).

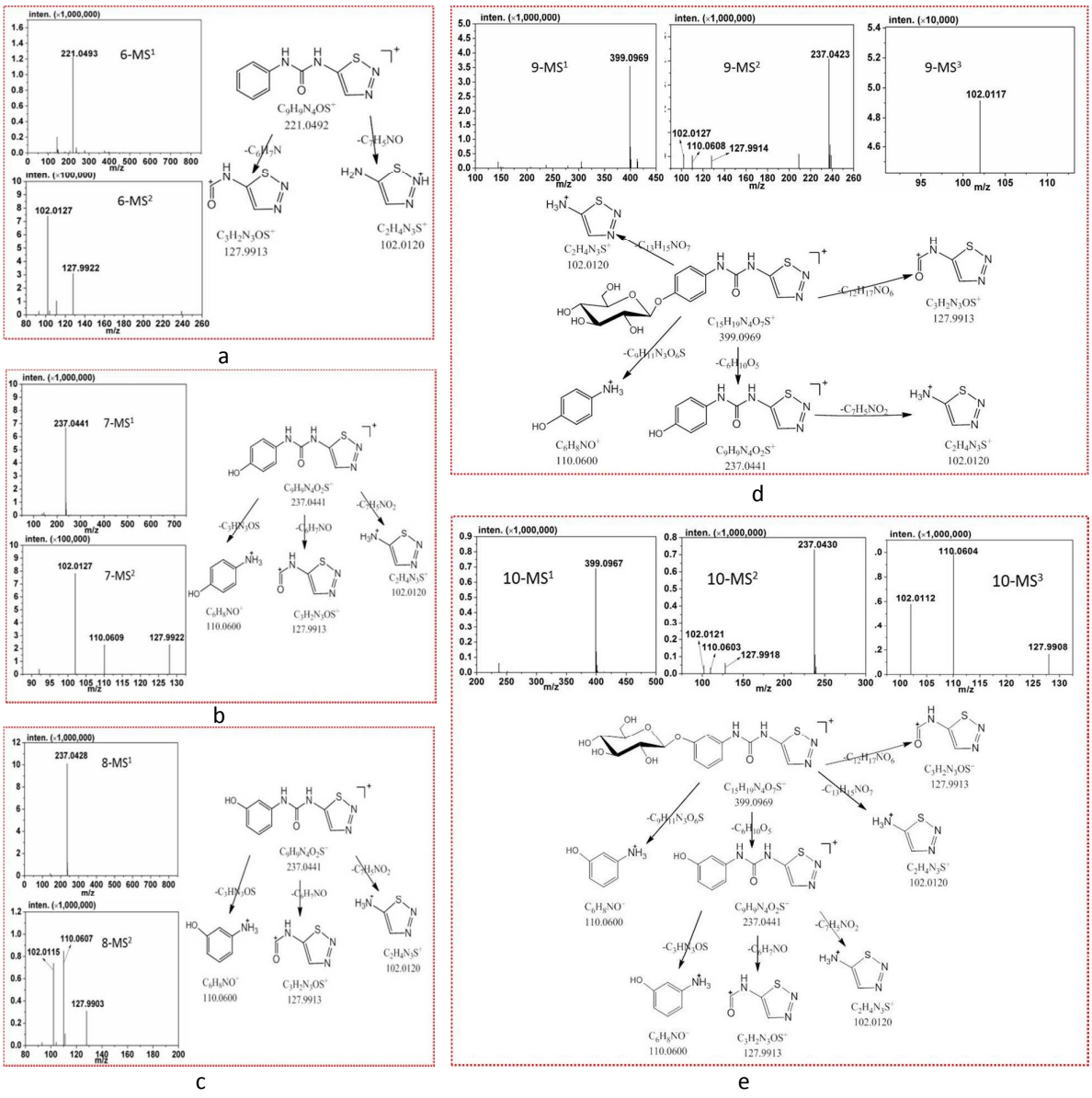

Figure 6. Mass spectra analysis of thidiazuron and its metabolites in kiwifruits in positive ion mode（a, 6; b, 7; c, 8; d, 9; e, 10; numbers of the compounds refer to Table 2）.

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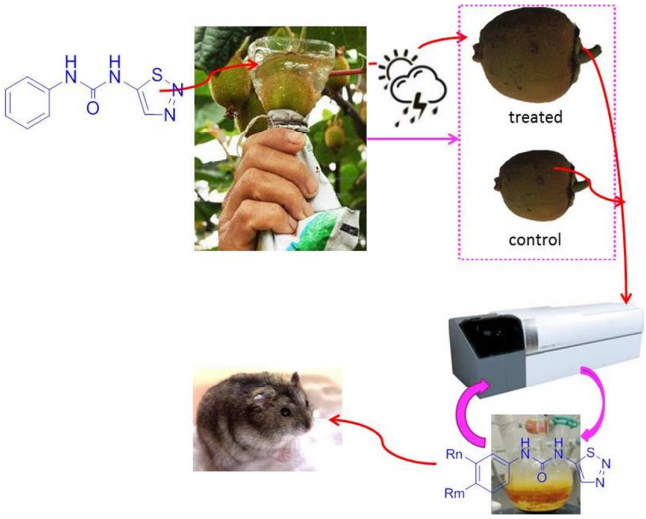
587 TOC Graphic



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