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Synthesis and Antifungal Activities of Alkyl N-(1,2,3-Thiadiazole-4-Carbonyl) Carbamates and S-Alkyl N-(1,2,3-Thiadiazole-4-Carbonyl) Carbamothioates

ZAIFENG LI,*,† ZENGRU WU,‡ AND FUYING LUO†

Center of Nature Science and Technology, Zhanjiang Normal College, Zhanjiang, Guangdong 524048, China, and Division of Pharmaceutics and Pharmaceutical Chemistry, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210

A series of alkyl N-(1,2,3-thiadiazole-4-carbonyl) carbamates and S-alkyl N-(1,2,3-thiadiazole-4-carbonyl) carbamothioates with unsubstituted or monobrominated straight chain alkyl groups were synthesized and evaluated as fungistatic agents against *Gibberella zeae* and *Alternaria kikuchiana*. These compounds showed variable antifungal activities at concentrations of 5 and 50 μg/mL. The results showed that antifungal activities depended on the length of the alkyl chain with the optimal chain length of 6−11 carbons. Carbamic acid, (1,2,3-thiadiazole-4-ylcarbonyl)-, hexyl ester (**4**) showed a strong fungistatic activity against *A. kikuchiana* at both concentrations, with 90.7 and 54% growth inhibition at 50 and 5 μg/mL, respectively. Carbamic acid, (1,2,3-thiadiazole-4-ylcarbonyl)-, heptyl ester (**5**); Carbamic acid, (1,2,3-thiadiazole-4-ylcarbonyl)-, octyl ester (**6**); and Carbamic acid, (1,2,3-thiadiazole-4-ylcarbonyl)-, undecyl ester (**9**) showed strong fungistatic activity against *G. zeae* at both concentrations. Their growth inhibitions against *G. zeae* at the concentration of 5 μg/mL were 78, 63, and 59%, respectively.

KEYWORDS: Alkyl N-(1,2,3-thiadiazole-4-carbonyl) carbamates; S-alkyl N-(1,2,3-thiadiazole-4-carbonyl) carbamothioates; fungistatic; *Gibberella zeae*; *Alternaria kikuchiana*

INTRODUCTION

Carbamate compounds represent early organic antifungal agents that act as protectants on the plant surface. They are multisite inhibitors that react with thiol groups that are present in the enzymes of fungi (*1*). Some heterocyclic compounds, such as triazoles, are also widely used as fungicides. The main mechanism of action of triazoles is the inhibition of the cytochrome P450-dependent demethylation of an intermediate in the sterol biosynthesis pathway in fungi (*2*). Triazoles not only act as surface protectants but also enter plant tissues as systemic fungicides. Because triazoles are site specific inhibitors, it is easier for fungi to develop resistance (*1*). However, resistance to nonsystemic fungicides is rare because they are mostly multisite inhibitors. Alternating the use of fungicide with a different mode of action is one of the methods used to hinder the development of resistance (*1*).

The length of carbon chains also showed great impacts on bioactivities of parent compounds (*3*). Gershon and Shanks (*4*) reported that the fungitoxicities of *n*-alkanol, α,ω-*n*-alkanediols, and ω-chloro-α-alkanols were influenced by chain length. Kochansky and Wright (*5*, *6*) studied a series of aliphatic carbamates and thiocarbamates and showed that the length of aliphatic chains determined the toxicities of these compounds to scabies mites.

We herein designed a novel series of compounds containing a carbamate group and a heterocyclic ring, to find potent systemic antifungal agents that have a broad antifungal spectrum but with less potential to develop resistance. In this series of compounds, we tested the effect of different alkyl chains and O, S, and Br substitutions on their antifungal activities. The structures of this series of compounds are shown in **Scheme 1**.

MATERIALS AND METHODS

Materials. *Alternaria kikuchiana* and *Gibberella zeae* were provided through the courtesy of South China Agriculture University. The solvents and reagents were reagent grade or better and were used as received. Melting points were determined on a Yanagimoto MFG. Co. apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet DXV_4-56 apparatus, solid compounds using the pressed disk (KBr) method and liquid compounds using the pressed film method, and the ν values were recorded in wavenumber (cm^{-1}). ^1H NMR spectra were obtained on a Bruker DPX-300 MHz spectrometer, and the chemical shift values were recorded in parts per million (ppm) on the δ scale with tetramethylsilane as the internal reference (deuterioacetone as solvent). The mass spectra were obtained on a Finnigan Mat.4510 GC/MS/DS spectrometer (70 eV). Elemental analyses were performed with a Carlo Eber-1 elemental analyzer. The general synthesis of target compounds is shown in **Scheme 1**.

Preparation of 1,2,3-Thiadiazole-4-carbonyl Chloride. A mixture of 1,2,3-thiadiazole-4-carboxylic acid (0.65 g, 5 mmol) and excess

* To whom correspondence should be addressed. Tel: 86-759-318-3061. Fax: 86-759-334-1440. E-mail: lizaifeng_zjnu@163.com.
† Zhanjiang Normal College.
‡ The Ohio State University.

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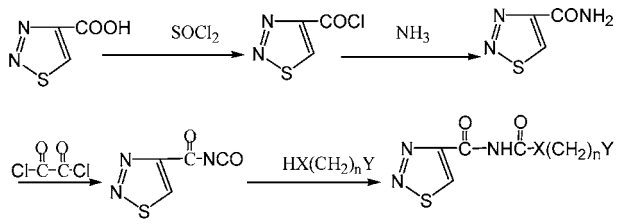
Scheme 1



1 X=O n=4 Y=H	**8** X=O n=10 Y=H	**15** X=S n=3 Y=H
2 X=O n=5 Y=H	**9** X=O n=11 Y=H	**16** X=S n=4 Y=H
3 X=O n=2 Y=$CH(CH_3)_2$	**10** X=O n=0 Y=C_6H_{11}	**17** X=S n=6 Y=H
4 X=O n=6 Y=H	**11** X=O n=14 Y=H	**18** X=S n=7 Y=H
5 X=O n=7 Y=H	**12** X=O n=16 Y=H	**19** X=S n=8 Y=H
6 X=O n=8 Y=H	**13** X=O n=10 Y=Br	
7 X=O n=9 Y=H	**14** X=O n=12 Y=Br	

thionyl chloride was refluxed and stirred for 7 h until hydrochloric acid gas evolution ceased forming. The unreacted thionyl chloride was removed under reduced pressure to give a black thick residue, which was then poured into anhydrous light petroleum ether (bp 34 °C). After it was dried, the 1,2,3-thiadiazole-4-carbonyl chloride was obtained as a colorless sheet of crystalline solid (0.58 g, 78.4%).

Preparation of 1,2,3-Thiadiazole-4-carboxamide. Dry ammonia gas was bubbled though a solution of 1,2,3-thiadiazole-4-carbonyl chloride (0.5 g, 3.4 mmol) in anhydrous petroleum ether (60 mL) for about 0.5 h under an ice bath and stirring conditions. A large amount of precipitate was produced. After filtration, the precipitate was washed with petroleum ether to give a white solid. Recrystallization from 95% ethanol gave 1,2,3-thiadiazole-4-carboxamide as white flake crystals (0.31 g, 70%) with a mp of 218−220 °C (ref *7*: mp 220−222 °C).

Preparation of 1,2,3-Thiadiazole-4-carbonyl Isocyanate. A solution of 1,2,3-thiadiazole-4-carboxamide (0.8 g, 6.2 mmol) in anhydrous 1,2-dichloroethane (25 mL) was refluxed and stirred under anhydrous conditions. After the mixture was cooled, oxalyl chloride (1.5 mL, 17 mmol) was added dropwise into the reaction system within 0.5 h and the reaction fluid became clear gradually. The reaction mixture was refluxed for 12 h, and the unreacted oxalyl chloride and solvent were then removed under reduced pressure. The crude intermediate can be directly used for next step reaction. The reaction residue was distilled under reduced pressure, and a fraction with a bp of 58 °C/33.33kPa was collected, which was 1,2,3-thiadiazole-4-carbonyl isocyanate (0.79 g, 83%). ^{1}H NMR: δ 8.40 (s, 1H, HetH). IR (KBr): 3028 (C−H), 2220 (N═C═O), 1720 (CO), 1650 (CO), 1500, 1305 (CN).

Preparation of Bromoalkanol. A solution of the corresponding dibasic alcohol $HO(CH)_nOH$, n = 10, 12, and 47% hydrobromic acid (the molar ratio of dibasic alcohol and hydrobromic acid was 1:1) in *n*-heptane (50 mL) was heated under reflux for 12−24 h. The suspension was transferred into separation funnel at warm conditions (about 50 °C) after the reaction was completed. The aqueous layer was separated from the organic layer and was then extracted twice with ether. The ether extracts were combined with the above-mentioned organic layer and then washed with 5% aqueous sodium carbonate. The organic phase was dried with sodium sulfate overnight. The ether and *n*-heptane were removed by distilling. The residues were bromoalkanols: $HO(CH_2)_{10}Br$ (white solid) and $HO(CH_2)_{12}Br$ (white solid), respectively.

Preparation of Alkanethiol. The corresponding 1-bromo alkyl compounds $CH_3(CH_2)_nBr$ (50 mmol) and thiourea (52 mmol) were dissolved in 95% ethanol (30 mL), and the reaction mixture was refluxed and stirred for 3 h. A solution of 2.5 mol/L sodium hydroxide (30 mL) was then added into the reaction mixture and continued to reflux for another 2 h. The aqueous layer was separated and acidified (pH 1) by the addition of diluted hydrochloric acid. The acidic solution was then extracted twice with petroleum ether. The organic phase was combined and washed with brine. After the organic phase was dried over sodium sulfate, the petroleum ether solvent was removed by distilling. The residue was identified as alkanethiol: $CH_3(CH_2)_2SH$ (colorless liquid, bp 67−69 °C, 86%), $CH_3(CH_2)_3SH$ (colorless liquid, bp 97−99 °C, 72%), $CH_3(CH_2)_5SH$ (colorless liquid, bp 150−152 °C, 64%), $CH_3(CH_2)_6SH$ (colorless liquid, bp 173−175 °C, 62%), and $CH_3(CH_2)_7SH$ (colorless liquid, bp 196−198 °C, 58%), respectively.

Synthesis of the Target Compounds. A mixture of 1,2,3-thiadiazole-4-carbonyl isocyanate (0.79 g, 5 mmol), a corresponding alcohol, bromoalkanol or alkanethiol (5.2 mmol), and a small amount of triethylamine (about 30 mg) was dissolved in anhydrous dichloroethane (30 mL). The reaction mixture was refluxed for 5−10 h. The completeness of the reaction was determined by the disappearance of 1,2,3-thiadiazole-4-carbonyl isocyanate as monitored by thin-layer chromatography. The reaction mixture was evaporated to dryness under reduced pressure. The off-white solid residue was separated on silica gel G column adsorption chromatography. Recrystallization from a mixture of ethyl acetate and petroleum ether (1:1) gave the corresponding target compounds.

Bioassays of Fungistatic Activities. The fungistatic activity measurement method was adopted from the method described by Molina-Torres et al. (*8*). The synthesized target compounds were dissolved in acetone to the concentrations of 500 and 50 μg/mL, respectively. The solutions (1 mL) were mixed rapidly with thawed potato dextrose agar culture medium (9 mL) under 50 °C, respectively. The mixtures were poured into Petri dishes. After the dishes were cooled, the solidified plates were inoculated with 4 mm mycelium disk, inverted, and incubated at 28 °C for 72 h. Water was used as the negative control. Each concentration and control were repeated three times. The mycelial elongation radius (mm) of fungi settlements was measured after 72 h of culture. The growth inhibition rates were calculated with the following equation: $I = [(C - T)/C] \times 100\%$. Here, I is the growth inhibition rate (%), C is the control settlement radius (mm), and T is the treatment group fungi settlement radius (mm).

RESULTS AND DISCUSSION

The target compounds were characterized as follows.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Butyl Ester (1). Yield, 88%; white powder solid; mp 86−88 °C. ^{1}H NMR: δ 0.92 (t, 3H, CH_3), 1.08−1.80 (m, 4H, CH_2), 4.16 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.88 (s, 1H, Het−H), 11.25 (s, 1H, NH). IR (KBr): 3380 (NH), 3100, 2950, 2850 (C−H), 1780 (CO), 1700 (CO), 1500, 1460 (CH_2), 1400, 1220 (CN), 1150, 940, 880, 770 cm^{-1}. Anal. calcd for $C_8H_{11}N_3O_3S$: C, 41.91; H, 4.84; N, 18.33. Found: C, 41.51; H, 4.68; N, 18.22.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Pentyl Ester (2). Yield, 80%; white columnar crystal; mp 73−75 °C. ^{1}H NMR: δ 0.92 (t, 3H, CH_3), 1.12−2.00 (m, 6H, CH_2), 4.26 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.44 (br, 2H, Het−H, NH). IR (KBr): 3300 (NH), 3120, 2950 (C−H), 1780 (CO), 1685 (CO), 1500, 1400 (CH_2), 1230 (CN), 1155, 950, 900, 770 cm^{-1}. Anal. calcd for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27. Found: C, 44.65; H, 5.56; N, 17.41.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, *iso*-Pentyl Ester (3). Yield, 89%; white flake crystal; mp 89−91 °C. ^{1}H NMR: δ 0.92 (d, 6H, CH_3, ^3J = 7.2 Hz), 1.36−1.84 (m, 3H, CH_2CH), 4.32 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.40 (br, 2H, Het−H, NH). IR (KBr): 3380 (NH), 3100, 2950, 2850 (C−H), 1780 (CO), 1705 (CO), 1500, 1460 (CH_2), 1375, 1230 (CN), 1150, 940, 870 cm^{-1}. Anal. calcd for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27. Found: C, 44.75; H, 5.60; N, 17.36.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Hexyl Ester (4). Yield, 66%; white needle crystal; mp 86−87 °C. ^{1}H NMR: δ 0.88 (t, 3H, CH_3), 1.12−1.88 (m, 8H, CH_2), 4.26 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.40 (s, 1H, Het−H), 9.46 (1H, NH). IR (KBr): 3380 (NH), 3200, 2950, 2900, 2850 (C−H), 1775 (CO), 1700 (CO), 1500, 1460 (CH_2), 1400, 1225 (CN), 1170, 770 cm^{-1}. Anal. calcd for $C_{10}H_{15}N_3O_3S$: C, 46.68; H, 5.88; N, 16.33. Found: C, 46.75; H, 5.70; N, 16.34.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Heptyl Ester (5). Yield, 48%; white needle crystal; mp 88−90 °C. ^{1}H NMR: δ 0.96 (t, 3H, CH_3), 1.12−1.92 (m, 10H, CH_2), 4.24 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.92 (s, 1H, Het−H), 11.28 (1H, NH). IR (KBr): 3320 (NH), 3105, 2900, 2850 (C−H), 1770 (CO), 1700 (CO), 1510, 1465 (CH_2), 1400, 1230 (CN), 1180, 765 cm^{-1}. Anal. calcd for $C_{11}H_{17}N_3O_3S$: C, 48.69; H, 6.32; N, 15.49. Found: C, 48.85; H, 6.37; N, 16.57.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Octyl Ester (6). Yield, 48%; white powder crystal; mp 95−97 °C. ^{1}H NMR: δ 0.84 (t, 3H, CH_3, ^3J = 7.2 Hz), 1.04−1.76 (m, 12H, CH_2), 4.12 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.84 (s, 1H, Het−H), 11.08 (1H, NH). IR (KBr): 3380 (NH), 3120, 2930, 2850 (C−H), 1775 (CO), 1700 (CO), 1510, 1465 (CH_2), 1400, 1230 (CN), 1155, 940, 775 cm^{-1}. Anal. calcd for $C_{12}H_{19}N_3O_3S$: C, 50.51; H, 6.71; N, 14.73. Found: C, 50.78; H, 6.56; N, 14.72.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Nonyl Ester (7). Yield, 69%; white flake crystal; mp 96−98 °C. ^{1}H NMR: δ 0.88 (t, 3H, CH_3, ^3J = 7.2 Hz), 1.12−1.86 (m, 14H, CH_2), 4.28 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.40 (br, 2H, Het−H, NH). IR (KBr): 3320 (NH), 3110, 2950, 2850 (C−H), 1770 (CO), 1700 (CO), 1510, 1465 (CH_2), 1400, 1230 (CN), 1175, 950, 770 cm^{-1}. Anal. calcd for $C_{13}H_{21}N_3O_3S$: C, 52.15; H, 7.07; N, 14.04. Found: C, 52.18; H, 6.76; N, 14.05.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, and Decayl Ester (8). Yield, 45%; white needle crystal; mp 100−101 °C. ^{1}H NMR: δ 0.88 (t, 3H, CH_3), 1.16−1.92 (m, 16H, CH_2), 4.28 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.38 (br, 2H, Het−H, NH). IR (KBr): 3320 (NH), 3105, 2950, 2850 (C−H), 1775 (CO), 1700 (CO), 1510, 1470 (CH_2), 1400, 1230 (CN), 1180, 770, 640 cm^{-1}. MS *m*/*z* (relative intensity): 314 (M + 1, 5), 286 (1), 242 (1), 214 (1), 174 (28), 130 (30), 113 (6), 85 (17), 69 (23), 57 (74), 43 (100). Anal. calcd for $C_{14}H_{23}N_3O_3S$: C, 53.65; H, 7.40; N, 13.41. Found: C, 53.64; H, 7.27; N, 13.43.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Undecyl Ester (9). Yield, 42%; white powder crystal; mp 102−103 °C. ^{1}H NMR: δ 0.86 (t, 3H, CH_3), 1.12−1.80 (m, 18H, CH_2), 4.16 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.88 (s, 1H, Het−H), 11.20 (s, 1H, NH). IR (KBr): 3320 (NH), 3105, 2900, 2850 (C−H), 1770 (CO), 1700 (CO), 1510, 1465 (CH_2), 1400, 1230 (CN), 1180, 950, 770 cm^{-1}. Anal. calcd for $C_{15}H_{25}N_3O_3S$: C, 55.02; H, 7.70; N, 12.84. Found: C, 54.96; H, 7.66; N, 12.88.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Cyclohexyl Ester (10). Yield, 56%; white powder crystal; mp 143−145 °C. ^{1}H NMR: δ 1.12−2.16 (m, 10H, CH_2), 4.56−4.96 (m, 1H, CH), 9.92 (s, 1H, Het−H), 11.20 (s, 1H, NH). IR (KBr): 3380 (NH), 3100, 2900, 2850 (C−H), 1775 (CO), 1710 (CO), 1400, 1230 (CN), 1155, 770 cm^{-1}. Anal. calcd for $C_{10}H_{13}N_3O_3S$: C, 47.05; H, 5.14; N, 16.4. Found: C, 47.58; H, 5.34; N, 16.45.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Tetradecyl Ester (11). Yield, 41%; white powder crystal; mp 109−110 °C. ^{1}H NMR: δ 0.88 (t, 3H, CH_3), 1.16−1.80 (m, 24H, CH_2), 4.16 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.88 (s, 1H, Het−H), 11.19 (s, 1H, NH). IR (KBr): 3320 (NH), 3110, 2900, 2850 (C−H), 1775 (CO), 1700 (CO), 1510, 1460 (CH_2), 1400, 1230 (CN), 1185, 955, 770 cm^{-1}. Anal. calcd for $C_{18}H_{31}N_3O_3S$: C, 58.51; H, 8.46; N, 11.37. Found: C, 58.50: H, 8.26; N, 11.06.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, Hexadecyl Ester (12). Yield, 37%; white powder crystal; mp 107−108 °C. ^{1}H NMR: δ 0.86 (t, 3H, CH_3), 1.08−1.80 (m, 28H, CH_2), 4.26 (t, 2H, OCH_2, ^3J = 7.2 Hz), 9.37 (br, s, 2H, Het−H, NH). IR (KBr): 3320 (NH), 3100, 2900, 2850 (C−H), 1770 (CO), 1700 (CO), 1510, 1465 (CH_2), 1400, 1230 (CN), 1180, 950, 770 cm^{-1}. MS *m*/*z* (relative intensity): 398 (M + 1, 1), 370 (1), 336 (1), 275 (1), 174 (53), 148 (11), 130 (4), 113 (8), 97 (12), 85 (23), 69 (40), 57 (100), 43 (96). Anal. calcd for $C_{20}H_{35}N_3O_3S$: C, 60.42; H, 8.87; N, 10.57. Found: C, 60.44; H, 8.76; N, 10.52.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, ω-Bromo-decyl Ester (13). Yield, 42%; white flake crystal; mp 88−89 °C. ^{1}H NMR: δ 1.20−2.00 (m, 16H, CH_2), 3.40 (t, 2H, CH_2Br, J = 7.2 Hz), 4.28 (t, 2H, OCH_2, J = 7.2 Hz), 9.38 (s, 1H, Het−H), 9.46 (s, 1H, NH). IR (KBr): 3350 (NH), 3100, 2900, 2850 (C−H), 1770 (CO), 1700 (CO), 1510, 1465 (CH_2), 1400, 1230 (CN), 1180, 950, 770 cm^{-1}. Anal. calcd for $C_{14}H_{22}N_3O_3SBr$: C, 42.86; H, 5.65; N, 10.71. Found: C, 42.98; H, 5.76; N, 10.64.

Data for Carbamic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, ω-Bromo-dodecyl Ester (14). Yield, 35%; white flake crystal; mp 89−91 °C. ^{1}H NMR: δ 1.20−2.00 (m, 20H, CH_2), 3.40 (t, 2H, CH_2Br, J = 7.2 Hz), 4.28 (t, 2H, OCH_2, J = 7.2 Hz), 9.38 (s, 1H, Het−H), 9.46 (s, 1H, NH). IR (KBr): 3340 (NH), 3100, 2900, 2850 (C−H), 1770 (CO), 1700 (CO), 1510, 1460 (CH_2), 1400, 1270 (CN), 1230, 1175, 950, 770 cm^{-1}. Anal. calcd for $C_{16}H_{26}N_3O_3SBr$: C, 45.72; H, 6.23; N, 10.00. Found: C, 45.92; H, 6.30; N, 9.96.

Data for Carbamothioic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, S-Propyl Ester (15). Yield, 62%; white needle crystal; mp 101−103 °C. ^{1}H NMR: δ 1.00 (t, 3H, CH_3, ^3J = 7.2 Hz), 1.44−1.92 (m, 2H, CH_2), 2.96 (t, 2H, SCH_2, J = 7.2 Hz), 9.36 (s, 1H, Het−H), 9.88 (s, 1H, NH). IR (KBr): 3380 (NH), 3200, 3080, 2950, 2850 (C−H), 1705 (CO), 1650 (CO), 1500, 1400, 1300 (CN), 1260, 855, 800, 745 cm^{-1}. MS *m*/*z* (relative intensity): 231 (M^+, 2), 189 (1), 156 (34), 130 (4), 113 (50), 90 (4), 85 (26), 70 (9), 57 (58), 43 (100). Anal. calcd for $C_7H_9N_3O_2S_2$: C, 36.35; H, 3.92; N, 18.17. Found: C, 36.20; H, 3.86; N, 18.12.

Data for Carbamothioic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, S-Butyl Ester (16). Yield, 38%; white flake crystal; mp 80−82 °C. ^{1}H NMR: δ 0.96 (t, 3H, CH_3, ^3J = 7.2 Hz), 1.12−1.92 (m, 4H, CH_2), 3.00 (t, 2H, SCH_2, J = 7.2 Hz), 9.40 (s, 1H, Het−H), 9.92 (s, 1H, NH). IR (KBr): 3400 (NH), 3200, 2900, 2850 (C−H), 1705 (CO), 1650 (CO), 1490, 1400, 1300 (CN), 1260, 860, 750 cm^{-1}. Anal. calcd for $C_8H_{11}N_3O_2S_2$: C, 39.17; H, 4.52; N, 17.13. Found: C, 39.61; H, 4.62; N, 16.98.

Data for Carbamothioic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, S-Hexyl Ester (17). Yield, 68%; white needle crystal; mp 78−80 °C. ^{1}H NMR: δ 0.88 (t, 3H, CH_3, ^3J = 7.2 Hz), 1.04−1.84 (m, 8H, CH_2), 2.96 (t, 2H, SCH_2, J = 7.2 Hz), 9.36 (s, 1H, Het−H), 9.92 (s, 1H, NH). IR (KBr): 3400 (NH), 3200, 3100, 2950, 2850 (C−H), 1720 (CO), 1650 (CO), 1500, 1440, 1260, 850, 750 cm^{-1}. Anal. calcd for $C_{10}H_{15}N_3O_2S_2$: C, 43.94; H, 5.53; N, 15.37. Found: C, 44.05; H, 5.44; N, 15.45.

Data for Carbamothioic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, S-Heptyl Ester (18). Yield, 52%; white flake crystal, mp 88−90 °C. ^{1}H NMR: δ 0.84 (t, 3H, CH_3), 1.04−1.84 (m, 10H, CH_2), 2.96 (t, 2H, SCH_2, J = 7.2 Hz), 9.36 (s, 1H, Het−H), 9.88 (s, 1H, NH). IR (KBr): 3400 (NH), 3200, 3080, 2900, 2850 (C−H), 1705 (CO), 1645 (CO), 1500, 1440, 1300 (CN), 1260, 860, 750 cm^{-1}. Anal. calcd for $C_{11}H_{17}N_3O_2S_2$: C, 45.97; H, 5.96; N, 14.62. Found: C, 46.12; H, 5.98; N, 14.18.

Data for Carbamothioic Acid, (1,2,3-Thiadiazole-4-ylcarbonyl)-, S-Octyl Ester (19). Yield, 58%; white flake crystal; mp 75−77 °C. ^{1}H NMR: δ 0.88 (t, 3H, CH_3), 1.12−1.84 (m,

12H, CH_2), 3.00 (t, 2H, SCH_2, $J = 7.2$ Hz), 9.40 (s, 1H, Het−H), 9.92 (s, 1H, NH). IR (KBr): 3400 (NH), 3200, 3100, 2900, 2850 (C−H), 1705 (CO), 1655 (CO), 1500, 1440, 1305 (CN), 1265, 860, 740 cm^{-1}. Anal. calcd for $C_{12}H_{19}N_3O_2S_2$: C, 47.82; H, 6.35; N, 13.94. Found: C, 47.92; H, 6.23; N, 13.66.

Preparation of 1,2,3-Thiadiazole-4-carbonyl Isocyanate. The synthesis of 1,2,3-thiadiazole-4-carboxamide was based on the method published by Pain and Slack (*7*) and Looker and Wilson (*9*). In their reports, 1,2,3-thiadiazole-4-carboxylic acid and excess thionyl chloride were refluxed for 4.5 h, and then, the unreacted thionyl chloride was removed by reduced pressure. The residue was poured into a large amount of petroleum ether (bp 40−60 °C), and 1,2,3-thiadiazole-4-carbonyl chloride was obtained as a colorless sheet of crystalline solid. 1,2,3-Thiadiazole-4-carbonyl chloride was then poured directly into ice-cold ammonium hydroxide, and 1,2,3-thiodiazole-4-carboxamide separated out. However, during our experiments, we found that the reaction time should be no less than 6.5 h. Furthermore, to get a colorless sheet of crystalline solid of 1,2,3-thiadiazole-4-carbonyl chloride, the black thick residue should be poured into anhydrous light petroleum ether (bp 34 °C) instead of petroleum ether (bp 40−60 °C). We also modified the method of preparing 1,2,3-thiodiazole-4-carboxamide from 1,2,3-thiadiazole-4-carbonyl chloride, since we only got a black thick solution when we followed their procedure. Dry ammonia gas was bubbled through 1,2,3-thiadiazole-4-carbonyl chloride in anhydrous petroleum ether solution under an ice bath and stirring conditions. By doing this, the yield of 1,2,3-thiadiazole-4-carboxamide was increased from 65 to 76%.

There are many methods to synthesize heterocyclic isocyanates from heterocyclic carboxamides. Among those, reacting heterocyclic carboxamides with phosgene or oxalyl chloride is a commonly used and low cost method. The synthetic pathway of isocyanate from carboxamide and oxalyl chloride was reported to have good yield (*10*). Speziale et al. (*11*) reported that aryl amide or aryl carboxamide reacting with oxalyl chloride might be used as the preparation for the corresponding aryl isocyanate. Bai and Wang (*12*) reported the synthesis of a heterocyclic isocyanate from 2-amino-4,6-dichloro-1,3,5-triazine and oxalyl chloride (molar ratio 1:4) with the yield of 79%. However, few reports are about the synthesis of heterocyclic carbonyl isocyanates by reaction of heterocyclic carboxamide and oxalyl chloride. In this study, oxalyl chloride was slowly dropped into the reaction system of 1,2,3-thiadiazole-4-carboxamide (1,2-dichloroethane as solvent) under strictly controlled anhydrous conditions (the molar ratio of 1,2,3-thiadiazole-4-carboxamide and oxalyl chloride additions was controlled as 1:35). By doing this, the key intermediate 1,2,3-thiadiazole-4-carbonyl isocyanate was synthesized successfully.

Synthesis of the Target Compounds. The target compounds were synthesized by the reaction of 1,2,3-thiadiazole-4-carbonyl isocyanate with alcohol, bromoalkanol, or alkanethiol, respectively. The possible reaction mechanism for isocyanates with alcohols had been reported (*13*, *14*). The reaction undergoes a four-membered ring transition state and can be accelerated by the electron-donating effect of alcohol; on the other hand, the electron-withdrawing group and the steric hindrance effect of the alcohol molecule, which influences the nucleophilicity of the alcohol, have a negative effect on the reaction. The reaction can be catalyzed by triethylamine, pyridine, and organotin compounds (*15*). The steric hindrance of the alcohol had a great influence on the reaction, and the reaction rate slowed with the increasing steric hindrance of the alcohols. The reaction rates of primary alcohol, secondary alcohol, and tertiary alcohol with isocyanate are about 1.0, 0.3, and 0.003−0.007, respectively (*15*). In the present study, the yields of target compounds were higher when the steric hindrance effect of the alcohols was reduced. In addition, the isocyanates are easily polymerized by themselves (*12*). It is impossible to avoid the isocyanates polymerization by themselves under the condition of long reacting time and high reacting temperature. Therefore, 1,2-dichloroethane and petroleum ether were chosen as solvent, and triethylamine was used as the catalyst. The reaction time was set at about 10 h for the synthesis of the target compounds.

Table 1. Fungistatic Activities of Target Compounds[a]

	A. kikuchiana		*G. zeae*	
compds	50 μg/mL	5 μg/mL	50 μg/mL	5 μg/mL
1	50.0 ± 1.5	23.0 ± 0.9	11.0 ± 0.5	6.0 ± 0.5
2	45.0 ± 1.8	36.0 ± 0.9	42.0 ± 1.5	4.0 ± 0.2
3	50.0 ± 2.3	25.0 ± 0.5	100.0 ± 0.9	15.0 ± 0.5
4	90.7 ± 1.5	54.0 ± 0.5	92.0 ± 0.5	23.0 ± 0.6
5	50.0 ± 2.3	20.0 ± 1.0	92.0 ± 0.9	78.0 ± 1.2
6	50.0 ± 1.5	20.0 ± 0.5	81.0 ± 0.6	63.0 ± 1.8
7	50.0 ± 2.8	27.0 ± 1.8	60.0 ± 0.5	23.0 ± 0.2
8	54.0 ± 1.3	54.0 ± 1.8	75.0 ± 0.2	42.0 ± 0.5
9	40.0 ± 1.8	10.0 ± 1.0	78.0 ± 1.5	59.0 ± 0.9
10	59.0 ± 1.8	32.0 ± 1.0	17.0 ± 1.0	0
11	50.0 ± 0.9	15.0 ± 0.5	100.0 ± 0.5	0
12	27.0 ± 0.5	14.0 ± 0.9	67.0 ± 0.9	0
13	41.0 ± 0.9	0	42.0 ± 0.9	25.0 ± 0.5
14	36.0 ± 1.3	13.0 ± 0.6	21.0 ± 1.0	6.0 ± 1.3
15	36.0 ± 2.0	9.0 ± 0.9	54.0 ± 0.5	27.0 ± 0.4
16	36.0 ± 1.5	32.0 ± 0.5	10.0 ± 0.2	0
17	27.0 ± 2.6	4.0 ± 0.5	75.0 ± 1.3	25.0 ± 0.5
18	32.0 ± 0.5	4.0 ± 0.3	35.0 ± 1.3	12.0 ± 0.9
19	18.0 ± 0.8	0	31.0 ± 1.1	10.0 ± 0.6
control	0	0	0	0

[a] Growth inhibition expressed as a percentage of the control (mean ± SD, $n = 3$).

Fungicidal Activities of the Target Compounds. *G. zeae* (causal agent of fusarium head blight of wheat) and *A. kikuchiana* (causal agent of black spot of Japanese pear) were selected to test the fungistatic activity of these series of compounds. Mycelial growth inhibitions after 3 days of culture with the synthetic compounds were tested, and the results are shown in **Table 1**. All 19 compounds showed variable degrees of fungistatic activity to *G. zeae* and *A. kikuchiana*. Among the compounds tested, **4** showed the best fungistatic ratio (90.7%) to *A. kikuchiana* at the concentration of 50 μg/mL, while most of the other compounds showed much lower antifungal activity with a fungistatic ratio around or less than 50%. Although both **4** and **8** showed similar activity (54%) at 5 μg/mL, **8** did not show any dose-dependent increase in activity as that observed in **4**. In comparison, more compounds showed fungistatic activities to *G. zeae*; compounds **3**−**6**, **8**, **9**, **11**, and **17** showed fungistasis ratios higher than 70% at 50 μg/mL concentration. At a lower concentration (5 μg/mL), compounds **5**, **6**, and **9** showed relatively higher fungistatic activities (growth inhibition ratio >50%).

Comparing the structure and fungicidal activity of these compounds, it is clear that for *A. kikuchiana*, both the linkage atom and the length of the alkyl chain are very critical. The oxygen linkage seemed to be preferred to the sulfur linkage, since none of the sulfur linkage compounds (**15**−**19**) showed good fungistatic activities. Furthermore, for the oxygen-linked compounds, six carbon chains (**4**) are optimal for the fungicidal activity. However, for *G. zeae*, the fungicidal activity seemed to be less sensitive to the length of the alkyl chain, and oxygen-linked compounds showed better activity overall as compared

to the sulfur-linked compounds. Compounds with more than six carbon atoms showed similar fungicidal activities at 50 μg/mL, and compounds with 6−11 carbon atoms (**4**−**9**) seemed to be more potent at 5 μg/mL.

In summary, alkyl N-(1,2,3-thiadiazole-4-carbonyl) carbamates and S-alkyl N-(1,2,3-thiadiazole-4-carbonyl) carbamothioates are new classes of lead compounds to control plant fungal diseases. Their activities depend on the length of the alkyl chain with the optimal length of 6−11 carbons. The oxygen linkage seems to be purely more favorable than sulfur, but bromo substitution did not show any improvement in activity. Further evaluations are necessary to determine the antifungal spectrum of these compounds and their effects on plant protection in the field.

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