1,2,4-オキサジアゾールと1,2,4-チアジアゾール誘導体の合成と殺虫活性

誌名	Journal of pesticide science
ISSN	1348589X
著者名	鈴木,潤 岡村,大悟 具志川,徹 平井,憲次 安藤,哲
発行元	日本農薬学会
巻/号	36巻3号
掲載ページ	p. 392-401
発行年月	2011年8月

J. Pestic. Sci., **36**(3), 392–401 (2011)
DOI: 10.1584/jpestics.G11-28

Original Article

Synthesis and insecticidal activity of 1,2,4-oxadiazole and 1,2,4-thiadiazole derivatives

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(Received April 14, 2011; Accepted May 24, 2011)

A series of 5-substituted 1,2,4-oxadiazoles and 1,2,4-thiadiazoles were prepared as muscarinic acetylcholine receptor agonists and evaluated for their insecticidal activity. These derivatives were synthesized through sequential reactions consisting of the condensation of pyridinecarboamides or pyridinecarbothioamides with *N,N*-dimethylacetamide dimethyl acetal, cyclization with hydroxylamine, quaternization by alkyl halide and reaction with sodium borohydride. All 1,2,4-oxadiazoles and 1,2,4-thiadiazoles synthesized were evaluated for insecticidal activity and their structure-activity relationships are discussed. It was discovered that many compounds were active against representative insects such as *Nilaparvata lugens*, *Nephotettix cincticeps* and *Aphis craccivora*. In particular, it was also found that 3-pyridyl-substituted derivatives of 1,2,4-oxadiazole and 1,2,4-thiadiazole exhibited good insecticidal activity against all the insects tested. Among the compounds evaluated, 3-methyl-5-(1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-thiadiazole (**9m-2**) showed the highest activity against *Nilaparvata lugens*, and provided a high level of activity against the imidacloprid-resistant strain. Based on the preliminary binding assay using the binding inhibition of mAChR antagonist [^{3}H]NMS to the nerve-cord membranes as an index of the binding activity, **9m-2** exhibited a range of potencies for the insect muscarinic receptor.

Keywords: oxadiazole, thiadiazole, tetrahydropyridine, insecticidal activity, muscarinic acetylcholine receptor.

Introduction

The acetylcholine receptor (AChR) combines binding sites for the neurotransmitter acetylcholine (ACh) and a cationic transmembrane ion channel. Although all AChRs, by definition, are activated by ACh, they respond to other molecules, such as nicotine and muscarine. Nicotinic acetylcholine receptors (nAChRs) are particularly responsive to nicotine and are known as ionotropic acetylcholine receptors (ligand-gated ion channels). In contrast, muscarinic acetylcholine receptors (mAChRs) are particularly responsive to muscarine and are known as metabotropic acetylcholine receptors (non-ion channels). nAChRs and mAChRs are the two main types of cholinergic receptors.

Neonicotinoid insecticides such as imidacloprid and acetamiprid act as nAChR agonists by selectively perturbing neuronal signal transduction (Fig. 1A).[1–4] These insecticides are active against a wide range of insects, such as *Nilaparvata lugens*, *Nephotettix cincticeps* and *Myzus persicae* and show particularly excellent activity against hemipteran insects. Although several kinds of neonicotinoids have been launched and used worldwide, no insecticide regulating insect mAChR function has been found until now. Therefore, mAChR in the cholinergic system could be an alternative target for discovering novel insecticides with no target site cross-resistance against existing insecticides.

On the other hand, recent research efforts have focused on the development of centrally acting muscarinic agonists as a potential remedy for Alzheimer's disease.[5,6] Among them, methyl *N*-methyl-1,2,5,6-tetrahydropyridine-4-carboxylate (arecoline), which is a naturally occurring alkaloid (Fig. 1B),[7] is a well known classical muscarinic agonist; however, clinical evaluation of arecoline in patients suffering from Alzheimer's disease produced small improvements in perception. This unfavorable performance may be caused by arecoline's short du-

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Published online July 16, 2011

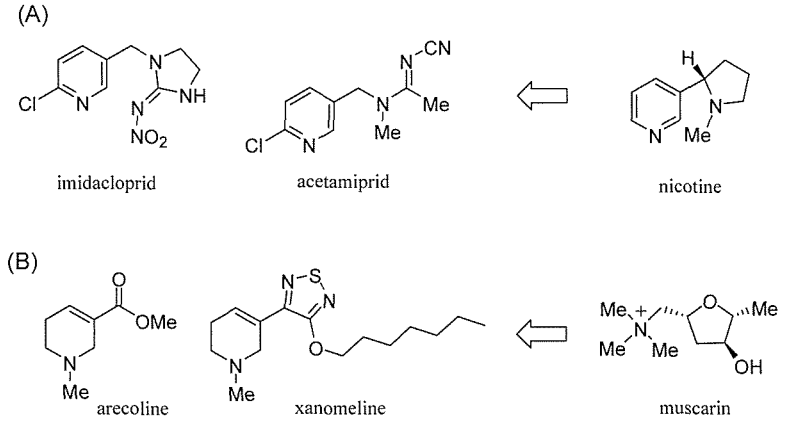


Fig. 1. Existing insecticides targeting nAChR (A) and representative pharmaceuticals targeting mAChR (B).

ration of action owing to the lability of its methyl ester moiety toward metabolic hydrolysis. Another mAChR agonist, xanomeline (Fig. 1B),[8] the 1,2,5-thiadiazole ring of which could be a bioisoster of the ester functionality of arecoline, has a clinically more useful profile.

It is interesting that several mAChR agonists such as arecoline and xanomeline have a 1,2,5,6-tetrahydropyridine ring as a common substituent. It is probable that an acid-base interaction between the acidic part of mAChR and the basic nitrogen atom of the 1,2,5,6-tetrahydropyridine ring plays an important role in inhibiting activity. Furthermore, the 1,2,5-thiadiazole ring of xanomeline may be effective for the interaction with mAChR, although xanomeline does not show marked insecticidal activity. On the other hand, among related studies including patents, insecticidally active compounds possessing a 1,2,4-oxadiazole or 1,2,4-thiadiazole ring, which is a five-membered heterocycle similar to the 1,2,5-thiadiazole ring of xanomeline, have been reported.[9,10] Therefore, we synthesized structurally new 1,2,4-oxadiazole and 1,2,4-thiadiazole derivatives by introducing the 1,2,5,6-tetrahydropyridine ring, which could enable them to interact with mAChR, and evaluated their insecticidal activity. In the present paper, we report on the synthesis and insecticidal activity of 1,2,4-oxadiazoles and 1,2,4-thiadiazoles connected to the 3-position (*meta*) of a 1,2,5,6-tetrahydropyridine ring (Fig. 2) such as **5m-2** and **9m-1**, and the regioisomers connected to the 4-position (*para*) such as **5p-1** and **9p-1**, and to the 2-position (*ortho*) such as **3o-1** and **7o-1**, as depicted in Figs. 4–6. Based on the lethal effect against some pest insects, we discuss their structure-activity relationships.

Materials and Methods

1. *Synthesis of chemicals*

1.1. *General procedure*

The synthesis route of 5-(1,2,5,6-tetrahydropyridyl)-1,2,4-oxadiazole (**5m-2**) is depicted in Fig. 3. Nicotinamide (**1m**) was reacted with dimethyl acetal of *N*,*N*-dimethylformamide (DMF) or *N*,*N*-dimethylacetamide (DMAc) to give *N*-(3-pyridylcarbonyl)amidines (**2m-1**, **2**), which were cyclized with hydroxylamine-*O*-sulfonic acid (HOSA),[11] giving 5-(3-pyridyl)-1,2,4-oxadiazoles (**3m-1**, **2**) in low yield. In contrast, the cyclization reaction using hydroxylamine instead of HOSA gave the desired products (**3m-1**, **2**) in more than 78% yield.[12] Thus, the obtained pyridine derivatives (**3m-1**, **2**) were reacted with methyl iodide to give the corresponding pyridinium salts (**4m-1**, **2**). A low-field chemical shift of the pyridine ring protons in ^{1}H NMR spectra certainly suggested the formation of pyridinium salts (**4m-1**, **2**). Pyridinium salt (**4m-2**) was subsequently converted to 5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-oxadiazole (**5m-2**) in moderate

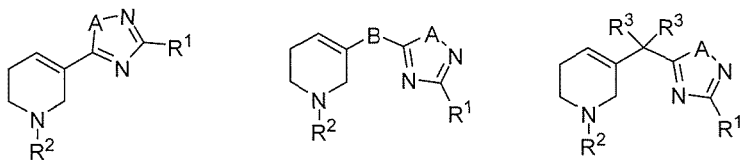


Fig. 2. Target compounds in the present study.

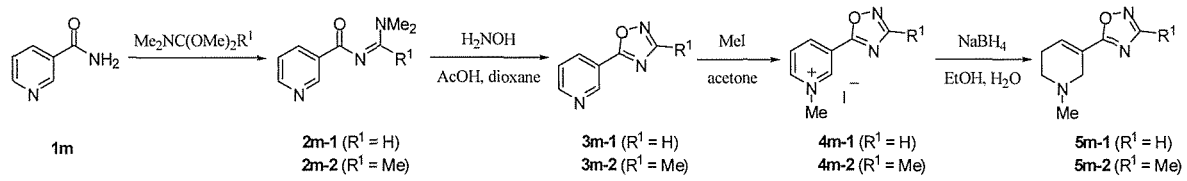


Fig. 3. Synthesis route of 3-methyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-oxadiazole (**5m-2**).

yield by reducing it with sodium borohydride.

In the present text,the character of **m**, **p**, or **o** in the compound's number means that the substituent on the pyridine or tetrahydropyridine ring is located at the 3-, 4-, or 2-position, namely, at the *meta*, *para*, or *ortho* position. Therefore, for example, nicotinamide, whose amide group is substituted at the 3-position of the pyridine ring, is named **1m** while isonicotinamide and 2-picolinamide are named **1p** and **1o**, respectively.

When isonicotinamide (**1p**) was used as a starting material instead of nicotinamide (**1m**), the sequential reactions with *N,N*-dimethylacetamide dimethyl acetal, hydroxylamine, methyl iodide and sodium borohydride provided 3-methyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-4-yl)-1,2,4-oxadiazole (**5p-1**), as shown in Fig. 4. Using 2-picolinamide (**1o**) as a starting material, however, tetrahydropyridine (**5o-1**) was not obtained due to unsuccessful reduction of the corresponding pyridinium salt (**4o-1**).

Fig. 5 shows the synthesis route of 5-(1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-thiadiazoles (**9m-1–10**) and their intermediates (**7m**, **8m**). 5-(3-Pyridyl)-1,2,4-thiadiazoles (**7m-1**, **2**) were synthesized by an oxidative cyclization reaction[11] of HOSA with *N*-(3-pyridylthiocarbonyl)amidines, which were easily prepared by condensation of thionicotinamide (**6m**) with dimethyl acetal of DMF or DMAc. Quaternization of **7m** with several alkyl halides followed by reduction with sodium borohydride gave the desired 5-(1,2,5,6-tetrahydro-1-alkylpyridin-3-yl)-1,2,4-thiadiazoles (**9m-1–10**). Further reduction of the tetrahydropyridine ring of **9m** in the presence of Adam's catalyst under 0.3 MPa of hydrogen gas afforded 5-(1-methylpiperidin-3-yl)-1,2,4-thiadiazole (**10m-2**) in good yield.

Similarly, 5-(pyridin-4-yl)-1,2,4-thiadiazole (**7p-1**), 5-(1,2,5,6-tetrahydropyridin-4-yl)-1,2,4-thiadiazole (**9p-1**) and 5-(piperidin-4-yl)-1,2,4-thiadiazole (**10p-1**) were synthesized from thioisonicotinamide (**6p**), as shown in Fig. 6. In the reaction of 2-thiopicolinamide (**6o**), however, the pyridinium salt (**8o-1**) obtained could not be reduced to 5-(1,4,5,6-tetrahydropyridin-2-yl)-1,2,4-thiadiazole (**9o-1**) under similar conditions using sodium borohydride.

1.2. Typical procedure

General synthesis methods of the representative compounds are described below. Chemical structures of all compounds were confirmed by ^{1}H NMR spectra, which were recorded on Bruker DPX-250 (250 MHz) and AV-400 (400 MHz) spectrometers using tetramethylsilane (TMS) as an internal standard. All melting points (mp) were measured by a Büchi-530 micro melting point apparatus and are uncorrected.

*1.2.1. 3-Methyl-5-(3-pyridyl)-1,2,4-oxadiazole (**3m-2**)*

A mixture of nicotinamide (3.00 g, 24.6 mmol) and *N,N*-dimethylacetamide dimethyl acetal (DMAc-DMA; 5 mL) was stirred under reflux for 1.5 hr and evaporated to remove an excess amount of DMAc-DMA. To the residue obtained, a 1,4-dioxane (30 mL) solution of an aqueous solution of hydroxylamine (50 ww%, 2.20 mL, 37.0 mmol) and acetic acid (35 mL) was added and heated at 90°C for 1 hr while stirring. The resulting mixture was evaporated, treated with an aqueous $NaHCO_3$ solution, and extracted three times with $CHCl_3$. The combined organic layers were dried over anhydrous magnesium sulfate and then evaporated. The residue was purified by column chromatography on silica gel using ethyl acetate/hexane (1 : 1) to give 3-methyl-5-(3-pyridyl)-1,2,4-oxadiazole (3.10 g, 19.2 mmol) in 78% yield as a white solid. mp: 95–97°C; ^{1}H NMR δ_H ($CDCl_3$, TMS): 2.50 (3H, s, CH_3), 7.49 (1H, dd, J=8.0, 4.8 Hz, 5-pyridine), 8.38 (1H, d, J=8.0 Hz, 4-pyridine), 8.82 (1H, d, J=4.8 Hz, 6-pyridine), 9.35

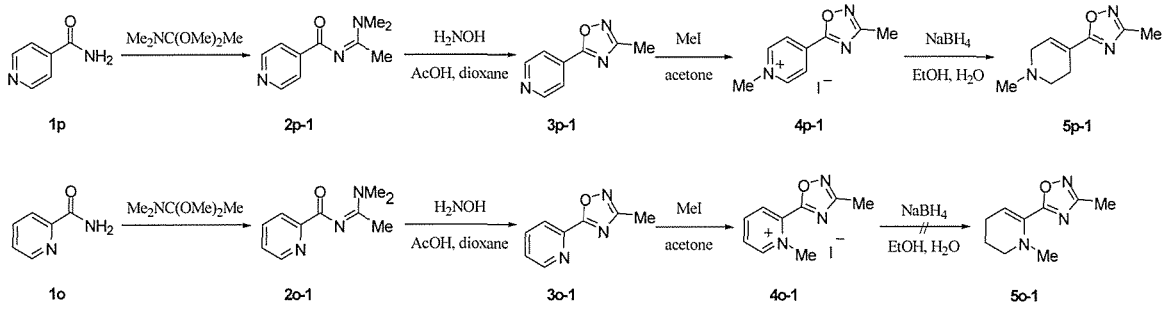


Fig. 4. Synthesis routes of 3-methyl-5-(4-pyridyl)-1,2,4-oxadiazole (**3p-1**), 3-methyl-5-(2-pyridyl)-1,2,4-oxadiazole (**3o-1**) and their derivatives.

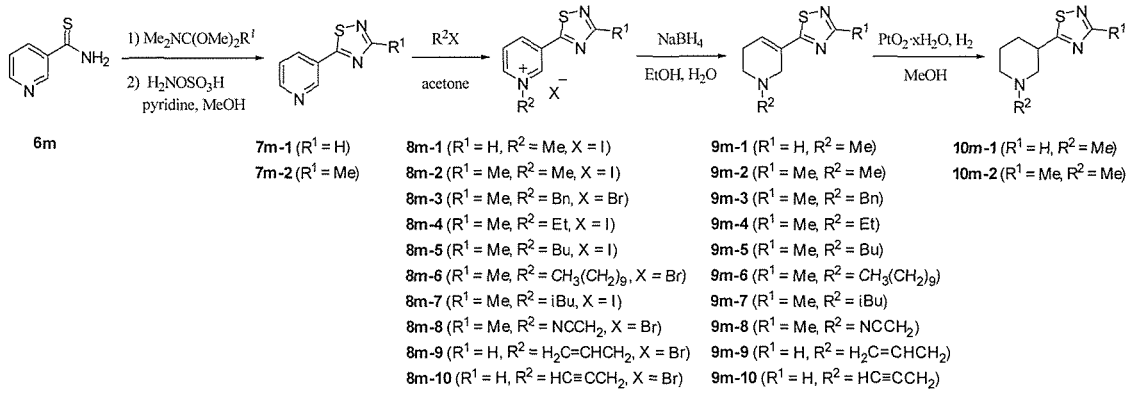


Fig. 5. Synthesis route of 5-(1,2,4,5-tetrahydropyridin-3-yl)-1,2,4-thiadiazole (**9m-1–10**).

(1H, s, 2-pyridine). ^{13}C NMR δ_C ($CDCl_3$, TMS): 11.7, 120.7, 123.8, 135.1, 149.1, 153.2, 168.0, 173.4.

*1.2.2. 3-(3-Methyl-1,2,4-oxadiazol-5-yl)pyridinium methiodide (**4m-2**)*

A solution of 3-methyl-5-(3-pyridyl)-1,2,4-oxadiazole (605 mg, 3.75 mmol) and methyl iodide (1.60 g, 11.3 mmol) in acetone (6 mL) was stirred at room temperature for 18 hr. The reaction mixture was filtrated to obtain 3-(3-methyl-1,2,4-oxadizazol-5-yl)pyridinium methiodide (1.11 g, 3.66 mmol) in 98% yield as a yellow solid. mp: 155–157°C; ^{1}H NMR δ_H (DMSO-d_6): 2.51 (3H, s, CH_3), 4.81 (3H, s, NCH_3), 8.36 (1H, dd, J=8.3, 6.0 Hz, 5-pyridine), 9.16 (1H, d, J=8.3 Hz, 4-pyridine), 9.23 (1H, d, J=6.0 Hz, 6-pyridine), 9.83 (1H, s, 2-pyridine). ^{13}C NMR δ_C (DMSO-d_6): 11.2, 48.5, 123.4, 128.4, 143.1, 145.3, 148.6, 168.3, 170.3.

*1.2.3. 3-Methyl-5-(1-methyl-1,2,5,6-tetrahydopyridin-3-yl)-1,2,4-oxadiazole (**5m-2**)*

A solution of 3-(3-methyl-1,2,4-oxadizazol-5-yl)pyridinium methiodide (700 mg, 2.31 mmol) and sodium borohydride (170 mg, 4.49 mmol) in EtOH (3 mL) and H_2O (3 mL) was stirred for 1 hr in an ice-cooled water bath. The resulting mixture was extracted three times with $CHCl_3$, and the organic layers combined were dried over anhydrous magnesium sulfate then evaporated. The residue was purified by column chromatography on silica gel using EtOAc/MeOH (4 : 1) to give 3-methyl-5-(1-methyl-1,2,5,6-tetrahydopyridin-3-yl)-1,2,4-oxadiazole (101 mg, 0.563 mmol) in 24% yield as a yellow oil. ^{1}H NMR δ_H ($CDCl_3$, TMS): 2.37–2.43 (2H, m, 5-CH_2), 2.40 (3H, s, CH_3), 2.46 (3H, s, NCH_3), 2.60 (2H, t, J=5.8 Hz, 6-CH_2), 3.34–3.39 (2H, m, 2-CH_2), 7.05–7.10 (1H, m, 4-CH). ^{13}C NMR δ_C ($CDCl_3$, TMS): 11.6, 26.7, 45.6, 50.8, 53.2, 122.8, 135.5, 167.2, 174.1.

*1.2.4. 3-Methyl-5-(3-pyridyl)-1,2,4-thiadiazole (**7m-2**)*

A mixture of thionicotinamide (3.00 g, 21.7 mmol) and DMAc-DMA (5 mL) was stirred for 2 hr at room temperature. The resulting mixture was evaporated to remove an excess amount of DMAc-DMA. The residue was dissolved in MeOH (30 mL) and reacted with hydroxylamine-*O*-sulfonic acid (HOSA; 2.70 g, 23.9 mmol) for 2 hr in the presence pyridine (3.43 g, 43.4 mmol). The reaction mixture was evaporated, treated with an aqueous $NaHCO_3$ solution, and extracted three times with $CHCl_3$. The combined organic layers were dried over anhydrous magnesium sulfate and then evaporated. The residue was purified by column chromatography on silica gel using EtOAc/hexane (1 : 1) to give 3-methyl-5-(3-pyridyl)-1,2,4-thiadiazole (1.84 g, 10.4 mmol) in 48% yield as a white solid. mp: 101–103°C; ^{1}H NMR δ_H ($CDCl_3$, TMS): 2.76 (3H, s, CH_3), 7.46 (1H, dd, J=8.3, 5.0 Hz, 5-pyridine), 8.24 (1H, d, J=8.3 Hz, 4-pyridine), 8.75 (1H, d, J=5.0 Hz, 6-pyridine), 9.17 (1H, s, 2-pyridine). ^{13}C NMR δ_C ($CDCl_3$, TMS): 18.9, 124.0, 126.8, 134.4, 148.4, 152.5, 174.5, 184.7.

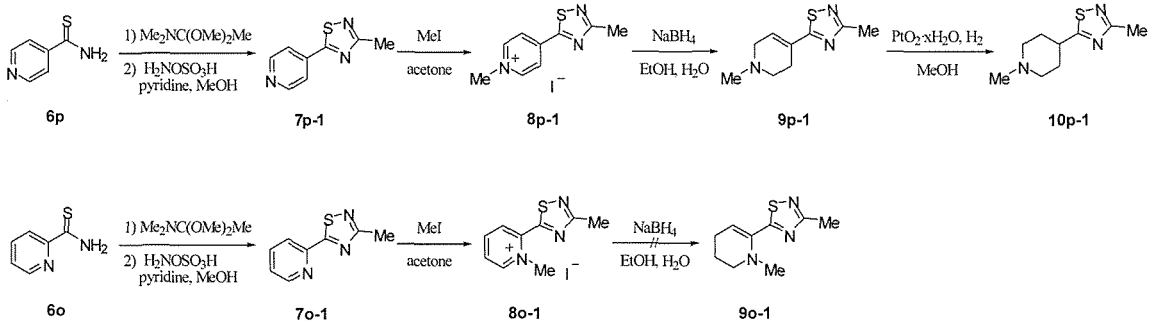


Fig. 6. Synthesis routes of 5-(4-pyridyl)-1,2,4-thiadiazole (**7p-1**), 3-methyl-5-(2-pyridyl)-1,2,4-thiadiazole (**7o-1**) and their derivatives.

*1.2.5. 3-(3-Methyl-1,2,4-thiadiazol-5-yl)pyridinium methiodide (**8m-2**)*

A solution of 3-methyl-5-(3-pyridyl)-1,2,4-thiadiazole (1.41 g, 7.96 mmol) and methyl iodide (3.40 g, 24.0 mmol) in acetone (14 mL) was stirred at room temperature for 18 hr. The reaction mixture was filtrated to obtain 3-(3-methyl-1,2,4-thiadiazol-5-yl)pyridinium methiodide (2.40 g, 7.52 mmol) in 94% yield as a yellow solid. mp: 161-163°C; ^{1}H NMR δ_H (DMSO-d_6): 2.73 (3H, s, CH_3), 4.45 (3H, s, NCH_3), 8.30 (1H, dd, J=8.0, 6.3 Hz, 5-pyridine), 9.15 (1H, d, J=8.0 Hz, 4-pyridine), 9.18 (1H, d, J=6.3 Hz, 6-pyridine), 9.73 (1H, s, 2-pyridine). ^{13}C NMR δ_C (DMSO-d_6): 18.5, 48.3, 128.2, 129.1, 142.9, 144.5, 147.4, 174.0, 180.9.

*1.2.6. 3-Methyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-thiadiazole (**9m-2**)*

A mixture of 3-(3-methyl-1,2,4-thiadiazol-5-yl)pyridinium methiodide (2.40 g, 7.52 mmol) and sodium borohydride (569 mg, 15.0 mmol) in EtOH (12 mL) and H_2O (12 mL) was stirred for 1 hr in an ice-cooled water bath. The resulting mixture was extracted three times with $CHCl_3$, and the combined organic layers were dried over anhydrous magnesium sulfate and evaporated. The residue obtained was purified by column chromatography on silica gel using EtOAc/MeOH (5 : 1) to give 3-methyl-5-(1-methyl-1,2,5,6-tetraydropyridin-3-yl)-1,2,4-thiadiazole (760 mg, 3.89 mmol) in 52% yield as a yellow oil. ^{1}H NMR δ_H ($CDCl_3$, TMS): 2.42-2.51 (2H, m, 5-CH_2), 2.48 (3H, s, NCH_3), 2.62 (2H, t, J=5.5 Hz, 6-CH_2), 2.65 (3H, s, CH_3), 3.39–3.44 (2H, m, 2-CH_2), 6.77–6.82 (1H, m, 4-CH). ^{13}C NMR δ_C ($CDCl_3$, TMS): 18.9, 26.8, 45.7, 51.1, 54.8, 129.3, 132.2, 173.4, 187.1.

*1.2.7. 3-Methyl-5-(1-methylpiperidin-3-yl)-1,2,4-thiadiazole (**10m-2**)*

A methanol (2 mL) solution of 3-methyl-5-(1-methyl-1,2,5,6-tetraydropyridin-3-yl)-1,2,4-thiadiazole (200 mg, 1.02 mmol) in the presence of $PtO_2 \cdot xH_2O$ (10 mg) was stirred at room temperature for 18 hr in a stainless steel autoclave under 0.3 MPa of hydrogen gas for 18 hr. After the reaction was complete, the catalyst was filtered off and the filtrate was evaporated. The residue obtained was purified by column chromatography on silica gel using EtOAc/MeOH (3:1) to give 3-methyl-5-(1-methylpiperidin-3-yl)-1,2,4-thiadiazole (180 mg, 0.912 mmol) in 89% yield as a brown oil. ^{1}H NMR δ_H ($CDCl_3$, TMS): 1.62–1.80 (3H, m, 3-CH and 4-CH, 5-CH), 1.90–1.95 (1H, m, 5-CH), 2.34 (3H, s, NCH_3), 2.39–2.55 (3H, m, 4-CH and 6-CH_2), 2.64 (3H, s, CH_3), 2.71–2.77 (1H, m, 2-CH), 3.43–3.51 (1H, m, 2-CH). ^{13}C NMR δ_C ($CDCl_3$, TMS): 18.8, 23.1, 29.3, 39.4, 46.3, 55.4, 59.7, 172.1, 193.7.

2. Insecticidal assays

Test solutions containing 3.2 g/L of each test compound in 75% (v/v) acetone and 25% (v/v) xylene with an appropriate volume of emulsifier (Sorpol) were prepared. The test solution was diluted with water at various concentrations before being used for evaluations. The biological test described below was repeated twice for each concentration.

2.1. Insecticidal assay for brown rice planthopper (Nilaparvata lugens) *and green rice leafhopper* (Nephotettix cincticeps)

Rice seedlings (*Oryza sativa* L.) were sprayed with the test solution described above and allowed to dry. The treated seedlings were placed in a plastic container with a nylon net, and 10 fourth-instar larvae of *N. lugens* or *N. cincticeps* were released into the container. The larvae were kept under a 16 hr light–8 hr dark cycle at 25°C. Mortality was checked 7 days after treatment.

2.2. Insecticidal assay for cowpea aphid (Aphis craccivora)

Three apterous adults of *A. craccivora* were released onto each broad bean seedling (*Vicia faba*). After 1 day, bean seedlings were sprayed with the test solution described above and allowed to dry. The aphids were kept under a 16 hr light—8 hr dark cycle at 25°C. Mortality was checked 4 days after each treatment.

Results and Discussion

1. Synthesis

The desired 5-(1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-oxadiazole (**5m-2**) was synthesized through successive reactions consisting of the formation of 1,2,4-oxadiazole (**3m-2**), quaternization of the pyridine ring of **3m** and reduction of the pyridinium salt (**4m-2**) as shown in Fig. 3. In the cyclization reaction of *N*-(3-pyridylcarbonyl)amidines (**2m-1**, **2**) with HOSA or hydroxylamine leading to 5-(3-pyridyl)-1,2,4-oxadiazoles (**3m-1**, **2**). Next, we attempted to convert **3m** into the reduced products but failed to reduce them by sodium borohydride. Generally, pyridines are not reduced by borohydrides unless they have electron-withdrawing groups at the 3- and 5-positions. On the other hand, it is well known that pyridinium quaternary salts are easily reduced by alkaline borohydrides in alcoholic media; in fact, **4m** prepared were readily reduced by sodium borohydride in aqueous alcoholic solution, leading thus to **5m** in moderate yields. The position of C–C double bonds in the tetrahydropyridine ring of **5m** was determined to be located at the 3-position by ^{1}H NMR analysis, that is, the olefinic proton signal was observed as a triplet-doublet at around 6.84–7.20 ppm. It is reported that if the pyridinium salt bears a substituent at the 3-position, one of the regioisomers is selectively formed.[13] Incidentally, in the reduction of **4m**, other regioisomers whose C–C double bonds were located at the 2- or 4-position of the tetrahydropyridine ring were not obtained.

1,2,4-Oxadiazole (**5p-1**), bearing the 1,2,5,6-tetrahydropyridin-4-yl group at the 5-position, was also similarly synthesized by the methods used for **5m**. In the reduction of **4p-1**, only one regioisomer (**5p-1**) yielded as much as **5m**; however, 5-(1,4,5,6-tetrahydropyridin-2-yl)-1,2,4-oxadiazole (**5o-1**) was not obtained because the pyridinium salt (**4o-1**) was converted to complex mixtures by the reaction with sodium boro-

hydride under usual conditions. These results are usual in the reduction of *N*-acylpyridinium salts, whose substituents on the pyridine-nitrogen atom are different from that of the pyridinium salts in this study. To obtain the desired products, more appropriate methodology using specific reducing reagents should be applied but further modifications of the reaction conditions were not examined because 5-(2-pyridyl)-1,2,4-oxadiazole (**3o-1**) and its pyridinium salt (**4o-1**) exhibited poor insecticidal activity, as mentioned below (Fig. 4).

Following the synthesis methods for 1,2,4-oxadiazoles (**5m**), 5-(1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-thiadiazoles (**9m-1–10**) were also synthesized, provided 5-(3-pyridyl)-1,2,4-thiadiazoles (**7m-1**, **2**) prepared by an oxidative cyclization reaction[11] of *N*-(3-pyridylthiocarbonyl)amidines with HOSA (Fig. 5). When dimethyl acetals of DMF or DMAc were used instead of HOSA, large amounts of 5-(3-pyridyl)-1,2,4-oxadiazoles (**3m**) were generated as by-products and the desired 1,2,4-thiadiazoles (**7m**) were not obtained in a pure form. The quaternization of **7m** with several alkyl halides and the subsequent reduction with sodium borohydride were successfully advanced to give **9m**. As mentioned later, 1,2,4-thiadiazoles (**7m**) were insecticidally more active against almost all insects tested than 1,2,4-oxadiazoles (**3m**); therefore, many kinds of **7m** with various substituents on the pyridine-nitrogen atom were synthesized. In order to elucidate the significance of the olefin part, the tetrahydropyridine ring of **9m** was hydrogenated by Adam's catalyst. The thiadiazoles (**9m**), in spite of having a sulfur atom, were easily reduced to give 5-(piperidin-3-yl)-1,2,4-thiadiazoles (**10m**). The corresponding 5-(piperidin-4-yl)-1,2,4-thiadiazole (**10p-1**) could be similarly produced but the reduction of 2-(3-methyl-1,2,4-thiadiazol-5-yl)pyridinium methiodide (**8o-1**) was unsuccessful, as was the corresponding 1,2,4-oxadiazole-substituted pyridinium salt (**4o-1**), as shown in Fig. 6.

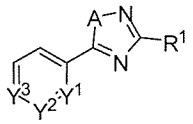
2. *Insecticidal activity*

Insecticidal activity against *N. lugens*, *N. cincticeps* and *A. craccivora* was evaluated for 7 types of 1,2,4-oxadiazoles and 25 types of 1,2,4-thiadiazoles and their results are summarized in Tables 1–3. Insecticidal activity was graded as follows: 10: mortality 100%, 9: 99–90%, 8: 89–80%, 7: 79–70%, 6: 69–60%, 5: 59–50%, 4: 49–40%, 3: 39–30%, 2: 29–20%, 1: 19–10%, 0: 9–0%.

2.1. *Insecticidal activity of pyridine-substituted 1,2,4-oxadiazoles and 1,2,4-thiadiazoles*

Table 1 shows the influence of the nitrogen position of the pyridine ring on insecticidal activity in the 1,2,4-oxadiazoles (**3o-1**, **3m-1**, **3m-2**, **3p-1**) and 1,2,4-thiadiazoles (**7o-1**, **7m-1**,

Table 1. Insecticidal activity of pyridine substituted 1,2,4-oxadiazoles (**3o-1**, **3m-1**, **3m-2**, **3p-1**) and 1,2,4-thiadiazoles (**7o-1**, **7m-1**, **7m-2**, **7p-1**)



No.	R^1	A	Y^1	Y^2	Y^3	mp (°C)	Concentration (ppm)	Insecticidal activity		
								Nl[a]	Nc[b]	Ac[c]
3o-1	Me	O	N	CH	CH	66–68	200	1	—[d]	0
3m-1	H	O	CH	N	CH	80–82	200	5	—	2
3m-2	Me	O	CH	N	CH	95–97	200	9	—	3
							100	6	10	—
3p-1	Me	O	CH	CH	N	85–87	200	1	—	0
7o-1	Me	S	N	CH	CH	79–81	200	4	—	3
7m-1	H	S	CH	N	CH	83–85	200	10	—	10
							100	10	7	—
							10	6	4	—
7m-2	Me	S	CH	N	CH	101–103	200	10	—	10
							100	10	8	—
							10	8	3	—
7p-1	Me	S	CH	CH	N	71–73	200	10	—	4
							100	10	4	—
							10	2	2	—

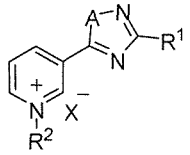
[a] Nl: *Nilaparvata lugens*. [b] Nc: *Nephotettix cincticeps*. [c] Ac: *Aphis craccivora*. [d] —: Not tested.

7m-2, **7p-1**) series substituted with the 2-pyridyl, 3-pyridyl or 4-pyridyl group. In both series, 3-pyridyl-substituted derivatives (**3m-1**, **3m-2**, **7m-1**, **7m-2**) had the tendency to exhibit good insecticidal activity against all the insects tested; however, 5-(4-pyridyl)-1,2,4-thiadiazole (**7p-1**) showed exceptional tolerable activity against *N. lugens*. Among the 3-pyridyl derivatives of both heterocycles, 1,2,4-thiadiazoles (**7m-1**, **2**) exhibited excellent activity against *N. lugens* and *A. craccivora* at the rate of 200 ppm and fully controlled *N. lugens* even at 100 ppm. In addition, introduction of a methyl group at the 3-position of the 1,2,4-oxadiazole ring increased activity and 1,2,4-oxadiazole (**3m-2**) was particularly active against only *N. lugens* compared with **3m-1**, while no considerable influence was recognized in the relation between 1,2,4-thiadiazole (**7m-1**) and its methyl derivative (**7m-2**), provided that they were both highly active.

2.2. *Insecticidal activity of pyridinium salts substituted with the 1,2,4-oxadiazole or 1,2,4-thiadiazole ring*

Table 2 summarizes the insecticidal activity of the pyridinium salts (**4m-1–2**, **8m-1–10**) substituted with the 1,2,4-oxadiazole or 1,2,4-thiadiazole ring at the 3-position of the pyridine ring. Other compounds (**4p-1**, **4o-1**, **8p-1**, **8o-1**) whose 1,2,4-oxadiazole or 1,2,4-thiadiazole ring is substituted at the 2-position or 4-position of the pyridine ring are not shown in Table 2 because of poor insecticidal activity. The thiadiazole-substituted *N*-methyl pyridinium salts (**8m-1–2**) were slightly more active than the oxadiazole-substituted salts (**4m-1–2**), but showed poor activity against *N. lugens* and *A. craccivora* in comparison with the corresponding pyridine derivatives (**3m-1–2**, **7m-1–2**). Regarding other pyridinium salts whose nitrogen atoms were substituted with various alkyl groups besides the methyl group, pyridinium salts (**8m-4–6**) having straight-chain alkyl groups, regardless of their carbon chain length, exhibited low activity against *N. lugens* and *A. craccivora*, although *N*-isobutyl pyridinium salt (**8m-7**) showed more than 90% mortality against *N. lugens* even at a low concentration, *i.e.*, 10 ppm. We thus assumed that the branched alkyl group on the nitrogen atom would be effective for higher insecticidal activity. Furthermore, a marked increase in the activity of **8m-8** and **8m-10** obviously indicated that the cyanomethyl and

Table 2. Insecticidal activity of pyridinium salts (**4m-1–2**, **8m-1–10**) substituted with 1,2,4-oxadiazole or 1,2,4-thiadiazole ring



No.	R^1	R^2	X	A	mp (°C)	Concentration (ppm)	Insecticidal activity		
							Nl[a]	Nc[b]	Ac[c]
4m-1	H	Me	I	O	148–150	200	6	—[d]	2
4m-2	Me	Me	I	O	155–157	200	1	—	1
8m-1	H	Me	I	S	153–155	200	8	—	3
8m-2	Me	Me	I	S	161–163	200	4	—	6
8m-3	Me	Bn	Br	S	198–200	200	2	—	1
8m-4	Me	Et	I	S	167–169	200	2	—	2
8m-5	Me	Bu	I	S	155–157	200	5	—	1
8m-6	Me	$H_3C(CH_2)_9$	Br	S	138–140	200	0	—	6
8m-7	Me	iBu	I	S	157–159	200	10	—	2
						100	9	5	—
						10	9	1	—
8m-8	Me	$NCCH_2$	Br	S	181–188	200	9	—	7
						100	8	4	—
						10	5	1	—
8m-9	Me	$CH_2{=}CHCH_2$	Br	S	161–163	200	4	—	1
8m-10	Me	$CH{\equiv}CCH_2$	Br	S	183–185	200	9	—	7
						100	8	5	—
						10	4	1	—

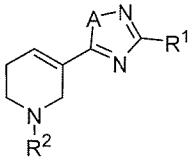
[a] Nl: *Nilaparvata lugens*. [b] Nc: *Nephotettix cincticeps*. [c] Ac: *Aphis craccivora*. [d] —: Not tested.

propargyl groups are also effective for eliciting good efficacy against *N. lugens*. These findings suggest that C–N and C–C triple bonds might be of importance for the interaction with mAChR. On the other hand, both *N*-benzyl (**8m-3**) and *N*-allyl (**8m-9**) salts were almost inactive.

2.3. *Insecticidal activity of tetrahydropyridine-substituted 1,2,4-oxadiazoles and 1,2,4-thiadiazoles*

Table 3 shows the insecticidal activity of 1,2,4-oxadiazole (**5m-2**) and 1,2,4-thiadiazoles (**9m-1–10**), whose 3-positions were substituted with the 1,2,5,6-tetrahydropyridin-3-yl group. The insecticidal activity of other compounds (**5p-1**,

Table 3. Insecticidal activity of tetrahydropyridine substituted 1,2,4-oxadiazole (**5m-2**) and 1,2,4-thiadiazoles (**9m-1–10**, **10m-2**)



No.	R^1	R^2	A	mp (°C)	Concentration (ppm)	Insecticidal activity		
						Nl[a]	Nc[b]	Ac[c]
5m-2	Me	Me	O	oil	200	10	—[e]	0
					100	9	9	—
					10	7	5	—
9m-1	H	Me	S	oil	200	6	—	3
9m-2	Me	Me	S	oil	200	10	—	4
					100	10	3	—
					10	8	0	—
9m-3	Me	Bn	S	oil	200	10	—	1
					100	10	3	—
					10	1	0	—
9m-4	Me	Et	S	oil	200	10	—	4
					100	10	5	—
					10	4	1	—
9m-5	Me	Bu	S	oil	200	10	—	3
					100	10	2	—
					10	3	1	—
9m-6	Me	$H_3C(CH_2)_9$	S	oil	200	3	—	6
9m-7	Me	iBu	S	oil	200	10	—	5
					100	10	1	—
					10	7	0	—
9m-8	Me	$NCCH_2$	S	oil	200	10	—	5
					100	10	5	—
					10	1	0	—
9m-9	Me	$CH_2{=}CHCH_2$	S	oil	200	10	—	4
					100	10	6	—
					10	6	1	—
9m-10	Me	$CH{\equiv}CCH_2$	S	oil	200	10	—	3
					100	10	6	—
					10	3	2	—
10m-2[d]	Me	Me	S	oil	200	3	—	0

[a] Nl: *Nilaparvata lugens*. [b] Nc: *Nephotettix cincticeps*. [c] Ac: *Aphis craccivora*. [d] This compound (3-methyl-5-(1-methylpiperidin-3-yl)-1,2,4-thiadiazole) was synthesized by reduction of **9m-2**. The chemical structure of **10m-2** is depicted in Fig. 5. [e] —: Not tested.

9p-1, **10p-1**) with a 1,2,4-oxadiazole or 1,2,4-thiadiazole ring at the 2- or 4-position of the tetrahydropyridine ring is omitted because of poor insecticidal activity. Compared with other 5-(1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-oxadiazole derivatives (no data), 3-(1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-oxadiazole (**5m-2**) was the most active compound and showed 90% mortality against *N. lugens* and *N. cincticeps* at 100 ppm but was inactive against *A. craccivora*. In contrast, many 1,2,4-thiadiazoles (**9m-2–5**, **9m-7–10**), except for **9m-1** (R^1=H) and **9m-6** (R^2=$H_3C(CH_2)_9$), perfectly controlled *N. lugens* at 100 ppm; **9m-2** was most active among them. These results clearly indicate that the methyl group at the 3-position of the 1,2,4-thiadiazole ring markedly increased insecticidal activity against *N. lugens*. A variety of R^2 substituents on the tetrahydropyridine ring did not significantly change the activity against *N. lugens* at 100 ppm; however, a long-chained alkyl group such as the decyl group of **9m-6** extremely reduced mortality as well as **8m-6**. Against *N. cincticeps* and *A. craccivora*, 1,2,4-thiadiazole derivatives (**9m-1–10**) showed no noteworthy activity at less than 100 ppm. On the other hand, the piperidine analog (**10m-2**), which was synthesized by further reduction of the tetrahydropyridine precursor (**9m-2**), showed extremely low activity against *N. lugens* and *A. craccivora* at the rate of 200 ppm. It is obvious that the double bond on the tetrahydropyridine ring is essential for providing good efficacy. We thus assume that the double bond may regulate the relative configuration between the thiadiazole ring and the nitrogen atom of the tetrahydropyridine ring.

Consequently, in the series of 1,2,4-ocadiazole derivatives, 3-methyl-5-(3-pyridyl)-1,2,4-oxadiazole (**3m-2**) and 3-methyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-oxadiazole (**5m-2**) were found to be especially active against *N. lugens* and *N. cincticeps*. In the series of 1,2,4-thiadiazole derivatives, many compounds exhibited potent activity against *N. lugens*. In particular, 5-(3-pyridyl)-1,2,4-thiadiazole (**7m-1**), 3-methyl-5-(3-pyridyl)-1,2,4-thiadiazole (**7m-2**), 3-(3-methyl-1,2,4-thiadiazol-5-yl)pyridinium isobutiodide (**8m-7**) and 3-methyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-thiadiazole (**9m-2**) were very active against *N. lugens*. **7m-1** and **7m-2** were also active against *A. craccivora*.

2.4. *Insecticidal activity against imidacloprid-resistant* N. lugens

One of the purposes of the present study was the development of insecticidally active compounds that can effectively control existing insecticide-resistant insects. This property could be a major advantage for the development of new practical insecticides; therefore, we evaluated insecticidal activity against imidacloprid-resistant and imidacloprid-susceptible *N. lugens* using highly active 1,2,4-thiadiazole derivatives (**7m-2**, **9m-2**). As shown in Table 4, against the resistant strain, both **7m-2** and **9m-2** exhibited almost the same activity levels as against the susceptible strain, although their activity at a low concentration was lower than that of imidacloprid. These results suggest that the target site of **7m-2** and **9m-2** may be different from nAChR.

Table 4. Insecticidal activity of 3-methyl-5-(3-pyridyl)-1,2,4-thiadiazole (**7m-2**) and 3-methyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-thiadiazole (**9m-2**) against imidacloprid-susceptible and imidacloprid-resistant *N. lugens*

Compounds	Concentration (ppm)	Insecticidal activity against *N. lugens*	
		Susceptible strain	Resistant strain
7m-2	100	10	10
	10	9	9
	1	6	5
9m-2	100	10	10
	10	8	9
	1	3	5
Imidacloprid	10	10	10
	1	10	8
	0.1	9	5

3. *Evaluation of binding to housefly mAChR*

Next, we preliminarily estimated the binding activity of two insecticidally active compounds (**7m-2**, **9m-2**) to mAChR in the head of an adult housefly (*Musca domestica* L.)[14] Nerve-cord membranes of the housefly, including mAChR, were easily prepared by modifying the method of Ozoe *et al.*[15] Binding activity was evaluated as binding inhibition of mAChR antagonist [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) or nAChR agonist [^{3}H]epibatidine ([^{3}H]EPI) to the nerve-cord membranes by **7m-2** and **9m-2** according to the method of Ozoe *et al.*[16] The general procedure is as follows. A mixture of nerve-cord membranes [200 μg protein in 0.9 mL buffer containing 10 mM sodium phosphate containing 300 mM sodium chloride (pH 7.5)], 10 nM of [^{3}H]NMS or [^{3}H]EPI in 0.1 mL buffer and 2.5 mM of the test compound in 4 μL dimethyl sulfoxide (DMSO) was incubated at 21°C for 60 min. After the incubation was terminated by rapid filtration through Whatman GF/B filter mats followed by washing with cold binding buffer, the membrane-bound radioactivity on the filters was counted in a liquid scintillation counter. Then, the binding activity of the test compound was calculated as the inhibition percent of [^{3}H]NMS and [^{3}H]EPI binding to the membrane according to Eq. 1. A nonspecific binding assay was performed in the presence of 2.5 mM unlabeled NMS or EPI instead of the test compound, and a total binding assay was carried out in the absence of the test compound and unlabeled NMS or EPI. All experiments were conducted in triplicate.

Table 5. Inhibitions of [^{3}H]NMS and [^{3}H]EPI binding to nerve-cord membranes prepared from housefly by 3-methyl-5-(3-pyridyl)-1,2,4-thiadiazole (**7m-2**) and 3-methyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-thiadiazole (**9m-2**)

Compounds	Inhibition (%)[a]	
	[^{3}H]NMS binding	[^{3}H]EPI binding
7m-2	12±6	4±3
9m-2	62±7	6±4

[a] Incubation at 21°C for 60 min (3 rep.). Concentration of radioligand: 1 nM. Concentration of test compound: 10 mM.

$$\text{Binding activity (\%)} = (\text{Total binding} - \text{Test compound assay}) / (\text{Total binding} - \text{Nonspecific binding}) \times 100 \quad \text{(Eq. 1)}$$

As a result, significantly lower levels of inhibition of [^{3}H]EPI binding to the membranes were detected by both 1,2,4-thiadiazole derivatives (**7m-2**, **9m-2**). Specifically, the binding activity of **7m-2** and **9m-2** to nAChR was less than 10%. These results reasonably explain that these compounds exhibit almost the same levels of insecticidal activity against imidacloprid-resistant *N. lugens* as against the susceptible strain, as discussed in the previous section. On the other hand, 3-pyridylthiazole (**7m-2**) slightly inhibited [^{3}H]NMS binding; however, 3-tetrahydropyridylthiadiazole (**9m-2**) induced significantly inhibition of [^{3}H]NMS binding. According to Eq. 1, the binding activity of **7m-2** and **9m-2** to mAChR was calculated as 12% and 62%, respectively. Based on these results, we assume there is a possibility that at least **9m-2** exerts its insecticidal activity by interacting with mAChR. Taking into account the differences in the chemical structures of **7m-2** and **9m-2**, the tertahydropyridine ring might be important for binding with mAChR. To elucidate the precise mode of action of the compounds discussed in this paper, further evaluations are required and will be the subject of a future manuscript.

In conclusion, our findings indicate that 3-methyl-5-(3-pyridyl)-1,2,4-thiadiazole (**7m-2**) and 3-methyl-5-(1-methyl-1,2,5,6-tetrahydropyridin-3-yl)-1,2,4-thiadiazole (**9m-2**) controlled *N. lugens* when applied at 100 ppm and no cross resistance to the existing neonicotinoid insecticides was found for these compounds. Further investigations of their particular mode of action, including binding inhibitory activity, are now in progress.

Acknowledgments

The authors gratefully acknowledge Professor Dr. Yoshihisa Ozoe of Shimane University for providing valuable suggestions concerning the binding assay using housefly mAChR.

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ムスカリン性アセチルコリン受容体アゴニストとして作用することが期待される1,2,4-オキサジアゾール誘導体や1,2,4-チアジアゾール誘導体を合成し，殺虫活性を評価した．これらの化合物は，ピリジンカルボアミドやピリジンカルボチオアミドと*N,N*-ジメチルアセトアミドジメチルアセタールとの縮合，ヒドロキシルアミンとの環化，アルキルハライドによる4級化，水素化ホウ素ナトリウムによる還元等の一連の反応により合成した．これら合成した化合物の多くがトビイロウンカやツマグロヨコバイ，マメアブラムシのような代表的な害虫に強い殺虫活性を示した．中でも，3-メチル-5-(1,2,5,6-テトラヒドロピリジン-3-イル)-1,2,4-チアジアゾール（化合物 **9m-2**）がトビイロウンカに対して最も高い殺虫活性を示し，イミダクロプリド抵抗性種に対しても有効な活性を示した．

1,2,4-オキサジアゾールと1,2,4-チアジアゾール誘導体の合成と殺虫活性

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