

Preliminary studies on Insecticidal activities of 5-substituted pyrazole oxime ether derivatives

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Abstract : The pyrazole family was discovered as effective insecticides/acaricides, and fenpyroximate and tebufenpyrad were introduced in the market. In this study, new series of pyrazole oxime ethers were designed and synthesized. The pyrazolone 1 was prepared by the condensation of ethyl acetoacetate with methylhydrazine, and then subsequently subjected to the Vilsmeier-Haack chloroformylation yielding 4-formyl-5-chloro-pyrazole 2. The nucleophilic aromatic substitution of 2 generally allowed the introduction of a wide range of heterocycles into the pyrazole ring. The resulting pyrazole aldehydes 3a-i were readily converted to the corresponding pyrazole oxime derivatives 4a-i, and subsequently to 5-substituted pyrazole oxime ether derivatives 6a-i. The screening assay results clearly show that the activities of 6d were comparable to those of fenpyroximate (6a) against BPH, DBM, and TSSM. It indicates that 6d has a potential to be developed as an insecticidal agent. (Received November 4, 2004; accepted December 20, 2004)

Introduction

Proton-translocating NADH:ubiquinone oxidoreductase (complex I) was found to be the molecular target of new and structurally diverse insecticides of natural and synthetic origin. Further enzymatic analysis indicated that they specially blocked ubiquinone-dependent NADH oxidation with high efficacy (Vinogradov, 1998). From this point, extended structural and mechanistic knowledge of the complex I spurred into the identification of new efficient inhibitors for agrochemical application. During the past two decades, many of new potent insecticides/acaricides had been synthesized for inhibition of mitochondrial electron transport at complex I (Lümmen, 1998). The synthetic insecticides/acaricides could be grouped by two main classes: the pyrazoles as one and substituted pyrimidines, pyridines and quinazolines as the other class.

Members of the substituted pyrazole family were

discovered as effective insecticides/acaricides and among them fenpyroximate and tebufenpyrad were introduced in the market. The structural feature of pyrazole-based insecticides suggests that they have notably both a nitrogen-containing heterocyclic moiety and a hydrophobic tail structure.

The primary role of the tail moiety might be kinetically favorable to partitioning into the hydrophobic environment of the ubiquinone catalytic site, which is believed to be part of the membrane-embedded segment of complex I (Miyoshi, 1998).

Fenpyroximate was introduced in Japan by Nihon Nohyaku in 1991, and enjoyed initial rapid growth on sales at about the \$ 20 million level. It was launched in most European countries from 1995 and now registered in approximately 30 countries. We are interested in nucleophilic aromatic substitution in the pyrazole skeleton, which would produce fenpyroximate analogs with structural diversity.

Here, we would like to report the synthesis and insecticidal activities of 5-substituted pyrazole oxime ether derivatives.

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Materials and Methods

Materials

Unless noted otherwise, all starting materials were obtained from commercial suppliers and were used without further purification. All solvents used for reactions were reagent grade and distilled as indicated below. Anhydrous methanol and ethanol were purchased and used directly in the reactions. *N,N*-Dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) were distilled under reduced pressure from calcium hydride and stored over 4 Å molecular sieves under nitrogen. 1,2-Dichloroethane was freshly distilled from calcium hydride. All solvents used for routine work-up and chromatographic isolation were reagent grade and used without purification. Thin-layer chromatography and flash chromatography were performed using 0.25 mm silica gel plates (Merck) and silica gel 60 (230-400 mesh, Merck), respectively. ¹H NMR spectra were recorded on Bruker DRX-300 FT-NMR Spectrometer as solutions in chloroform-*d* (CDCl₃). Chemical shifts are expressed in part per millions (ppm, δ) downfield from tetramethylsilane and are referenced to CDCl₃. ¹H NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), and number of protons.

Bioassay

In the primary insecticidal activity test, all compounds were prepared at the rate of 500 ppm. Proper amount of test compound was measured with an analytical balance and dissolved in 5 mL of acetone, and mixed with 45 mL of 100 ppm aqueous solution of Triton-X-100. The compounds showing significant insecticidal activity at the primary test were prepared to serially diluted test solutions of various concentrations, and bioassay was performed against relevant insect species.

For the primary test, all compounds were evaluated against 5 insect species that reared in Korea Research Institute of Chemical Technology (KRICT) insectarium for 15 years or more, and selected compounds from the primary test were evaluated against relevant insect

species with 3 replications, respectively.

Brown planthopper (BPH, *Nilaparvata lugens*) has been reared with rice seedling mat. Five rice seedlings grown for 7-8 days after seeding was placed in a test tube (diameter 3 cm x height 15 cm) with a water-soaked cotton roll. Fifteen of 3rd instar nymphs of BPH were introduced into the test tube with an aspirator connected to a diaphragm pump. The test solution was directly sprayed for 3 times into the inside of the test tube with a hand microsyringe (approximate spraying volume of 100 µL/spray). After the treatment, the test tube was capped with a lid covered with a stainless steel wire screen (100 mesh), and preserved at 25°C, 60% RH, and 16:8 (light:dark) condition. The mortality of BPH was evaluated at 24, 48 hours after treatment.

Diamond-back moth (DBM, *Plutella xylostella*) has been reared with radish seedling pot. Leaf-disk (diameter 5 cm) of cabbage was dipped into the test solution for 30 seconds, and dried well in a ventilated hood. The dried leaf-disk was placed into a disposable plastic petridish (diameter 5 cm), and ten of 3rd instar larvae of DBM were inoculated with a fine paintbrush. After the inoculation, the petridish were capped with a lid, and preserved at 25°C, 60% RH, and 16:8 (light:dark) condition. The mortality of DBM was evaluated at 24, 48 hours after treatment.

Green peach aphid (GPA, *Myzus persicae*) has been reared with tobacco pot. Leaf-disk (diameter 5.5 cm) of tobacco was dipped into the test solution for 30 seconds, and dried in a ventilated hood. The dried leaf-disk was placed into a disposable plastic petridish (diameter 5.5 cm), and ten of 5-day-old nymphs of GPA were inoculated with a fine paintbrush. After the inoculation, the petridish were capped with a lid, and preserved at 25°C, 60% RH, and 16:8 (light:dark) condition. The mortality of GPA was evaluated at 24, 48 hours after treatment.

Tobacco cutworm (TCW, *Spodoptera litura*) has been reared with cabbage leaf and caterpillar artificial diet (developed in KRICT insectarium). Leaf-disk (diameter 5.5 cm) of cabbage was dipped into the test solution for 30 seconds, and dried well in a ventilated hood. The

dried leaf-disk was placed into a disposable plastic petridish (diameter 5.5 cm), and ten of 3rd instar larvae of TCW were inoculated with a fine tweezers. After the inoculation, the petridish was capped with a lid, and preserved at 25°C, 60% RH, and 16:8 (light:dark) condition. The mortality of TCW was evaluated at 24, 48 hours after treatment.

Two-spotted spidermite (TSSM, *Tetranychus urticae*) has been reared with kidney bean pot. Leaf-disk (diameter 5.5 cm) of kidney bean was placed over a water-soaked cotton disk (diameter 4 cm) in a disposable plastic petridish (diameter 5.5 cm). 30 female adults of TSSM were inoculated with a fine paintbrush. After the inoculation, the test solution was directly sprayed into the TSSM-inoculated petridish with a hand microsyringe (approximate spraying volume of 150 μ L/spray) till the leaf-disk fully soaked with the solution (15~20 times of spray). After the spray, the petridish was dried in a ventilated hood and capped with a lid covered with a stainless steel wire screen (100 mesh). The petridish was preserved at 25°C, 60% RH, and 16:8 (light:dark) condition. The mortality of TSSM was evaluated at 24, 48 hours after treatment.

Results and Discussion

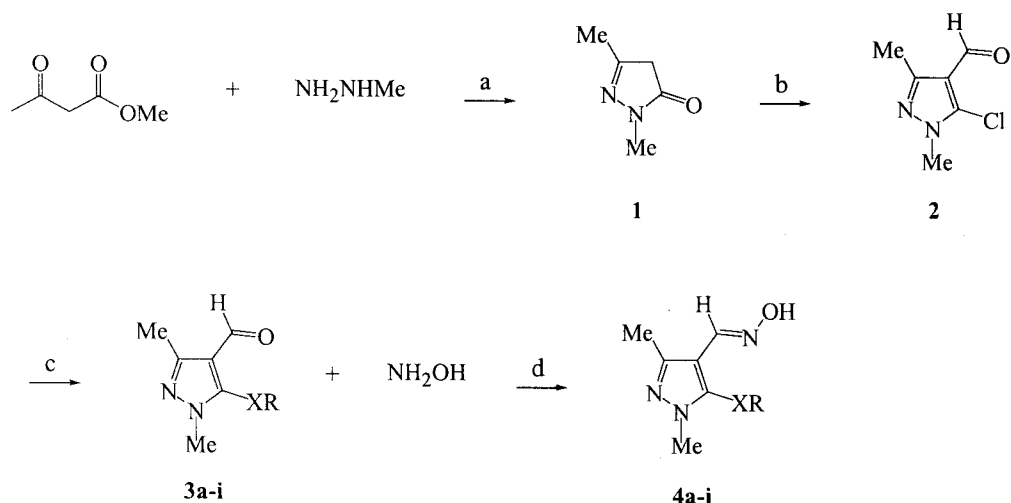
As part of our study, we are interested in nucleophilic aromatic substitution in the pyrazole skeleton, which would produce structures with constraint to a limited number of conformations by the heterocyclic core and hindered rotation by the substituent. Such a displacement will be more facilitated by the introduction of electron-withdrawing formyl group in a conjugated position of the ring. In the previous report, we carried out the nucleophilic aromatic substitution of 4-formyl-5-chloro-pyrazole **2** to yield various 5-substituted 4-formyl-pyrazoles **3a-i**. Thus, it was envisioned to utilize the pyrazole aldehydes **3a-i** as starting materials to offer a simple route to pyrazole oxime ethers **6a-i**, analogs of fenpyroximate. Here, we would like to report the synthesis and insecticidal activities of 5-substituted pyrazoles of oxime ether

derivatives **6a-i**.

The starting pyrazole **2** was prepared according to the well-known procedure, in which the required pyrazolone **1** was easily prepared by the condensation of ethyl acetoacetate with methylhydrazine. The pyrazolone **1** was then subjected to the Vilsmeier-Haack chloroformylation using DMF and an excess POCl₃ to yield 4-formyl-5-chloro-pyrazole **2** (Porai-Koshits *et al.*, 1970), as shown in Scheme 1. The nucleophilic aromatic substitution was conveniently carried out by heating (ca. 120 °C) a mixture of **2** with phenol (3 equiv), and powdered KOH (1.5 equiv) in DMSO. Thus, we extended the nucleophilic aromatic substitution with readily available N-containing nucleophiles such as pyrrole, imidazole, pyrazole, indole, pyrrolidine, piperazine, and dimethylamine to give the corresponding 5-substituted pyrazoles **3a-i** in 56-91% yield (Park *et al.*, 2004). The reaction generally allowed the introduction of a wide range of heterocycles into the pyrazole ring. Then, pyrazole aldehydes **3a-i** were readily converted to the corresponding pyrazole oxime derivatives **4a-i** in 58-87% yield, by the treatment with hydroxylamine hydrochloride and NaOH in MeOH.

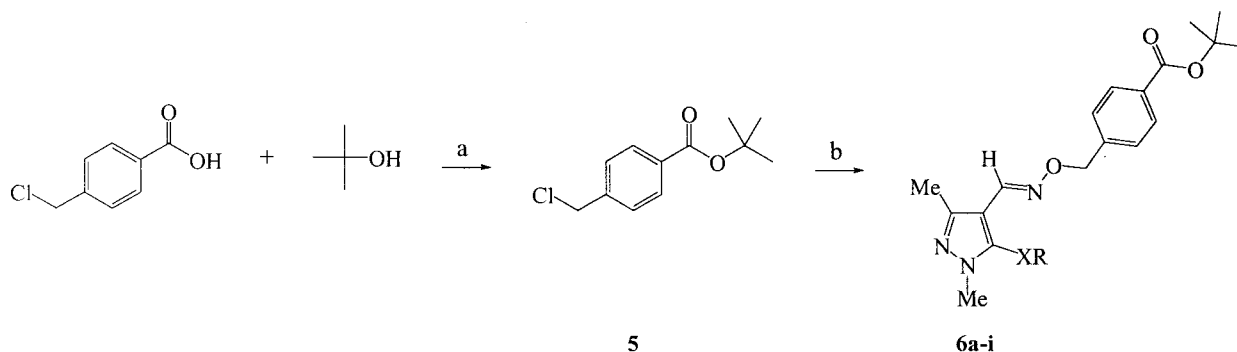
With the pyrazole oxime derivatives **4a-i** ready, we tried to prepare *p*-chloromethyl benzoic acid *t*-butyl ester **5** but encountered a problem in the synthesis. It has been known that an efficient method for the synthesis of *t*-butyl esters of aromatic and hindered tertiary carboxylic acids still remains a challenging problem. Literatures showed that *t*-butyl *p*-toluate was synthesized by the acylation from *p*-toluoyl chloride and *t*-BuOH in pyridine (Rosowsky *et al.*, 1989), or *p*-toluoyl chloride and lithium *t*-butoxide (Crowther *et al.*, 1971). Later, *t*-butyl *p*-toluate was converted to *t*-butyl *p*-bromomethyl benzoate by benzylic bromination, in the presence of NBS and Bz₂O₂ in CCl₄ (Rosowsky *et al.*, 1989). However, in both acylation reactions, we commonly observed the side reaction to give *p*-toluic anhydride in a considerable amount.

After several trials, we found that *t*-butyl *p*-chloromethyl benzoate **5** could be prepared from 4-chloromethyl benzoic acid by the acid-catalyzed reaction



a) EtOH, 90°C, 2 hr, 97%; b) POCl₃, DMF, 120°C, 4 hr, 76%; c) H-XR, KOH, DMSO, 120°C, 2~22 hr, 91-56%; d) NaOH, MeOH, 60°C, 2 hr, 87~58%.

Scheme 1. Preparation of 5-substituted pyrazole oxime derivatives.



a) MgSO₄, H₂SO₄, (CH₂Cl₂)₂, rt, 18 hr, 78%, b) 4a-i, KOH, DMSO, 50~60°C, 2 hr, 73-43%.

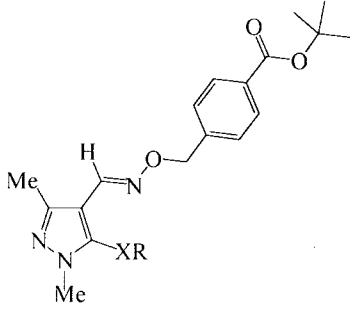
Scheme 2. Preparation of 5-substituted pyrazole oxime ether derivatives

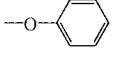



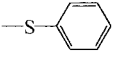



with isobutene via in situ generation from *t*-butanol (Wright *et al.*, 1997), as shown in Scheme 2. In a typical experiment, concentrated sulfuric acid (4 equiv) was added to a vigorously stirred suspension of anhydrous magnesium sulfate (4 equiv) in dichloroethane and the mixture was stirred for 15 min. After 4-chloromethyl benzoic acid (1 equiv) and *t*-butanol (5 equiv) were added, the reaction mixture was then closed tightly with a rubber septum and stirred for 18 hr at room temperature, providing the desired *t*-butyl ester **5** in 78% yield.

5-Substituted pyrazole oxime ethers **6a-i** were prepared

by the Williamson ether (Blass, 2002) synthesis using pyrazole oximes **4a-i** and *t*-butyl *p*-chloromethyl benzoate **5** in 73-43% yield, in the presence of KOH in DMSO. The structures and ¹H NMR spectra of the prepared pyrazole oxime ether derivatives **6a-i** are summarized in Table 1.

The prepared pyrazole oxime ethers **6a-i** was conducted to primary insecticidal activity assay against 5 major plant insects, brown planthopper (BPH), green peach aphid (GPA), diamondback moth (DBM), tobacco cutworm (TCW), and two-spotted spidermite (TSSM). The primary activity assay data are summarized in Table

Table 1. The structures and ^1H NMR spectra of the prepared pyrazole oxime ether derivatives **6a-i**.


Compounds	-XR	^1H NMR (300 MHz, CDCl_3)
6a		7.90-7.93(d, 2H), 7.82(s, 1H), 7.26-7.33(m, 4H), 7.09-7.13(t, 1H), 6.86-6.88(d, 2H), 5.03(s, 2H), 3.59(s, 3H), 2.34(s, 3H), 1.59(s, 9H).
6b		7.95-7.99 (d, 2H), 7.79 (s, 1H), 7.38-7.42 (d, 2H), 6.72-6.74 (t, 2H), 6.37-6.40 (t, 2H), 5.13 (s, 2H), 3.61 (s, 3H), 2.41 (s, 3H), 1.60 (s, 9H)
6c		7.92-7.96 (d, 2H), 7.75 (s, 1H), 7.66 (s, 1H), 7.32-7.36 (d, 2H), 7.26 (s, 1H), 7.02 (s, 1H), 5.06 (s, 2H), 3.59 (s, 3H), 2.34 (s, 3H), 1.57 (s, 9H)
6d		8.14 (s, 1H), 7.96-7.99 (d, 2H), 7.41-7.44 (d, 2H), 5.15 (s, 2H), 3.65 (s, 3H), 3.13-3.18 (t, 2H), 2.26 (s, 3H), 1.87-1.91 (t, 2H), 1.60 (s, 9H)
6e		8.13 (s, 1H), 7.87-7.90 (d, 2H), 7.32-7.35 (d, 2H), 7.09-7.18 (m, 3H), 6.89-6.92 (t, 2H), 5.09 (s, 2H), 3.70 (s, 3H), 2.35 (s, 3H), 1.51 (s, 9H)
6f		7.92-7.95 (d, 2H), 7.70-7.73 (d, 1H), 7.71 (s, 1H), 7.29-7.32 (d, 2H), 7.03-7.28 (m, 4H), 6.76-6.77 (d-1H), 5.06 (s, 2H), 3.55 (s, 3H), 2.47 (s, 3H), 1.61 (s, 9H)
6g		7.96-7.97 (d, 1H), 7.94-7.97 (d, 2H), 7.79-7.84 (dd, 1H), 7.84 (s, 1H), 7.60-7.61 (d, 1H), 7.36-7.40 (d, 2H), 5.11 (s, 2H), 3.68 (s, 3H), 2.38 (s, 3H), 1.59 (s, 9H)
6h	$-\text{NMe}_2$	8.15 (s, 1H), 7.94-7.96 (d, 2H), 7.41-7.43 (d, 2H), 5.15 (s, 2H), 3.61 (s, 3H), 2.71 (s, 6H), 2.23 (s, 3H), 1.57 (s, 9H)
6i		8.06 (s, 1H), 8.01 (s, 1H), 7.94-7.96 (d, 2H), 7.37-7.40 (d, 2H), 5.13 (s, 2H), 3.70 (s, 3H), 3.59-3.62 (m, 2H), 3.33-3.66 (m, 2H), 2.91-2.99 (m, 4H), 2.23 (s, 3H), 1.58 (s, 9H)

2. The insecticidal activities of fenpyroximate (**6a**) are well confirmed with the results previously reported. Although the compound **6b** shows high activity only against TSSM, the compound **6d** shows a high degree of activities against BPH, DBM, and TSSM.

With the promising results from the primary activity assay data, **6a**, **6b**, and **6d** were selected to assay for comparison against BPH, DBM, and TSSM at lower concentrations. The comparison activity data are summarized in Table 3. The compound **6d** shows better systematic control activity than **6a**. The data show that the insecticidal activities of **6d** are comparable to those

of fenpyroximate (**6a**) in biological spectrum. It indicates that **6d** has a potential to be developed as an insecticidal agent since it shows a high control activity against various insects.

Fenpyroximate inhibits mitochondrial NADH-coenzyme Q reductase on the electron transport chain in *Tetranychus urticae* (two-spotted spider mite) and in rats (Motoba *et al.*, 1992). Fenpyroximate is classified as a phenoxypyrazole acaricide used for the control against phytophagous mites, relatively less active against predacious mites, and inactive against animal parasitic and soil mites. The only insect against which it is

Table 2. Primary insecticidal activity assay

Entry	Conc. (ppm)	Mortality (%)				
		BPH ^{a)}	GPA	DBM	TCW	TSSM
6a	500	90	40	88	0	100
6b	500	15	0	0	0	100
6c	500	5	0	0	0	0
6d	500	75	0	100	0	100
6e	500	0	0	0	0	0
6f	500	0	0	0	0	0
6g	500	0	0	0	0	0
6h	500	25	0	50	0	0
6i	500	0	0	0	0	0

^{a)}BPH : brown planthopper, GPA : green peach aphid, DBM : diamondback moth, TCW : tobacco cutworm, TSSM : two spotted spider mite.

Table 3. Comparison assay for the selected compounds

Conc. (ppm)	BPH		DBM		TSSM		
	6a	6d	6a	6d	6a	6b	6d
500	90 ^{a)}	75	87.5	100	100	100	100
250	60	60	85	85	100	84.4	98.8
125	52.5	48.5	40	65	100	38.5	98.8
62.5	27.5	22.5	25	30	100	40	98.8
31.25	17.5	12.5	10	10	100	15.5	98.8
15.63	12.5	5	10	5	100	13.3	96.6

^{a)}Mortality (%).

active is *Empoasca onukii* (tea green leafhopper) (Taninaka, 1993). From our results, there is a strong possibility that the chemical derivation of phenoxy pyrazole compound could broaden the activity spectrum among insect species. The insecticidal activity resulted from BPH and DBM in Table 3 would be a promising evidence for this possibility even though the activity from BPH and DBM is still less than that from TSSM.

Further research should be focused on the more detailed and variable substitution in the active site based on the QSAR to discover more potent and reliable compounds. Inhibition analysis and comparison of mitochondrial NADH-coenzyme Q reductase among various insect species would be helpful for the development of new control agent against pest insects in the point of view relating the assessment between toxicity and activity.

Acknowledgement

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새로운 pyrazole oxime ether 유도체의 살충활성 연구

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요약 : 효과적인 살충제로 pyrazole 계통의 화합물이 널리 알려졌으며, 이중에 fenpyroximate와 tebufenpyrad는 시판되고 있다. 본 연구에서는 fenpyroximate의 새로운 유도체로써 5-번 위치가 치환된 pyrazole oxime ether를 합성하였다. 본 연구의 핵심 중간체인 4-formyl-5-chloro-pyrazole **2**는 ethyl acetoacetate와 methylhydrazine의 축합반응으로 얻은 pyrazolone **1**을 Vilsmeier-Haack chloroformylation을 통하여 합성하였다. **2**에 nucleophilic aromatic substitution 반응을 하여 질소고리화합물이 5-번 위치에 도입된 pyrazole aldehyde **3a-i**를 만든 후, 차례로 oxime 화합물 **4a-i** 그리고 oxime ether 화합물 **6a-i**를 각각 합성하였다. 합성된 화합물 중에서 **6d**는 벼멸구 (Brown Planthopper, BPH), 배추좀나방 (Diamondback Moth, DBM), 점박이응애 (Two Spotted Spider Mite, TSSM)에서 높은 활성을 보여주고 있으며, 이는 fenpyroximate의 살충활성과 비슷하였다. 본 결과는 **6d**가 다양한 곤충 및 응애에 대하여 효과가 우월하여 살충, 살비제로써 개발 가능성을 보여주고 있다.

Key words : 5-Substituted pyrazole, *t*-butyl ester, pyrazole oxime ether, fenpyroximate, insecticide.

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