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Development of a novel acaricide, pyflubumide

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Pyflubumide is a novel carboxanilide acaricide discovered and developed by Nihon Nohyaku Co., Ltd., that exhibits excellent acaricidal activity against *Tetranychus* and *Panonychus* species, including strains that have developed resistance to conventional acaricides. Its safety profile against non-target arthropods is suitable for integrated pest management (IPM) programs. Pyflubumide was registered and launched in Japan in 2015 and Korea in 2017. This paper describes pyflubumide's invention history, synthesis, exploratory synthesis, biological activity, toxicological profile, and mode of action.

Keywords: pyflubumide, carboxanilide, acaricide, mitochondrial complex II, IPM.

Introduction

Pyflubumide, discovered and developed by Nihon Nohyaku Co., Ltd., is a novel carboxanilide acaricide that has a unique methoxyhexafluoroisopropyl substituent on the 4′-position (Fig. 1). It is the only acaricide with a carboxanilide structure, and it is classified in the new subgroup 25B (carboxanilide) in the IRAC (Insecticide Resistance Action Committee) MoA classification as a novel inhibitor of mitochondrial complex II on the respiratory chain. It shows a high control efficacy against phytophagous mites, including populations of spider mites that have developed resistance to conventional acaricides. Furthermore, pyflubumide is harmless to non-target arthropods, including beneficial insects and natural enemies, suggesting that it could be favorable for IPM programs.

Here, we describe pyflubumide's invention history, synthesis, discovery, biological activity, toxicological profile, and mode of action.

1. History of invention

1.1. Discovery of the lead compound

Pyflubumide was discovered from the research of carboxamide fungicides that inhibit mitochondrial complex II (Scheme 1). Since carboxin first appeared on the market in 1966, a number of mitochondrial complex II-inhibiting carboxamides have been developed. In particular, many broad-spectrum carboxamides **2** with bulky substituents on the 2′-position, *e.g.*, boscalid and penthiopyrad, have been launched successively.[1)]

We were quite interested in the development of carboxamides with bulky substituent on the 2′-position in the late 1990s. Meanwhile, flubendiamide[2)] and pyrifluquinazon,[3)] novel insecticides having unique heptafluoroisopropyl group, were discovered by our colleagues in 1998 and 1999, respectively.

Continuous research with the goal of discovering a new pesticide by synthesizing compounds with this unique haloalkyl group has led to our synthesis of the 4′-heptafluoroisopropyl-substituted carboxamide **3**. The compound only showed low fungicidal activity. This low fungicidal activity seemed to be mainly attributed to its high lipophilicity; hence, we designed the less lipophilic derivative **4**. Although the fungicidal activity of the hexafluoroalkyl derivative **4** did not improve, it did show low acaricidal activity.

This unexpected acaricidal activity drew our strong attention because mites are quite problematic due to its rapid develop-

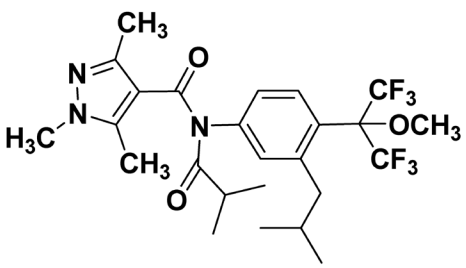

Fig. 1. Chemical structure of pyflubumide.

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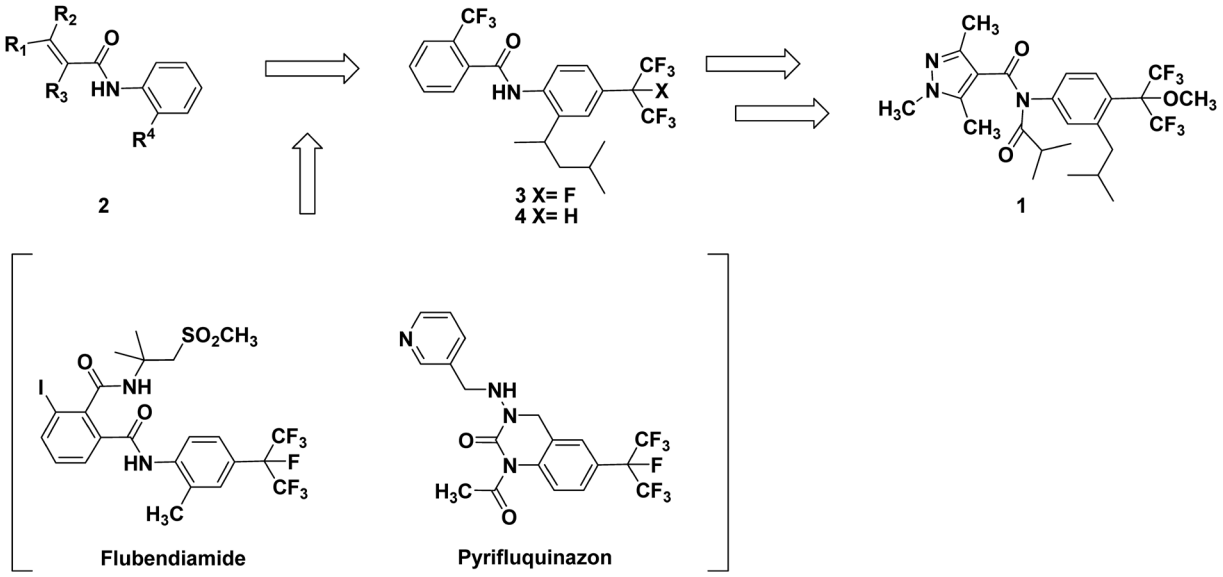


Scheme 1. Discovery of pyflubumide.

ment of resistance to agrochemicals; therefore, novel acaricides have been highly and constantly demanded. Thus, we decided to start researching a new acaricide.

1.2. Optimization of the lead compound and the structure-activity relationship

Lead compound **4** was divided into four parts; acid moiety, aniline moiety, fluoroalkyl substituent, and amide modification for its optimization. The summary of the SAR is shown in Table 1.

1.2.1. Optimization of the acid moiety

The acid moiety was examined by introducing the same acid moiety as that of mitochondrial complex II-inhibiting carboxamides, and we found that the furametpyr derivative showed good acaricidal activity. Further examination of the pyrazole substituents revealed that the 1,3,5-trimethyl pyrazole derivative showed excellent acaricidal activity.[6)]

1.2.2. Optimization of the anilino moiety

First, the effect of the substituent on the 2′-position was examined and the 1,3-dimethyl butyl group, the same substituent as that of penthiopyrad, was found to be the best substituent for the 2′-position.

The compound with the same substituent as that of mitochondrial complex II-inhibiting carboxamide fungicides showed excellent activity, and accordingly, we then examined the 3′-position because 3′-substituted carboxamide fungicides such as flutolanil also existed. The 3′-isopropyroxy derivative with the same substituent as that of flutolanil was examined. As expected, the flutolanil derivative showed acaricidal activity, although its activity level was moderate. We further investigated 3′-alkyl derivatives. As a result, we found that the 3′-isobutyl derivative showed activity comparable to that of the 2′-(1,3-dimethylbutyl) derivative.[7)]

From a manufacturing point of view, 3′-isobutyl derivatives are more advantageous because 3-isobutylaniline, the intermediate of the derivatives, can be easily prepared from less expensive materials and it has no chiral center. Accordingly, we then focused on optimizing 3′-isobutyl derivatives.

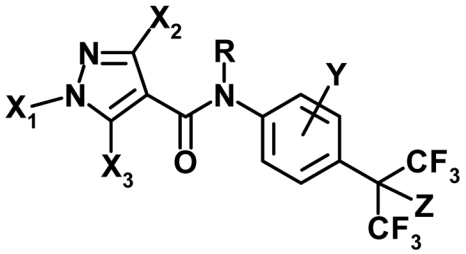
1.2.3. Optimization of the fluoroalkyl group

The optimization of the fluoroalkyl group revealed that hydrogen or a methoxy group was the best substituent, probably due to their favorable lipophilicity.

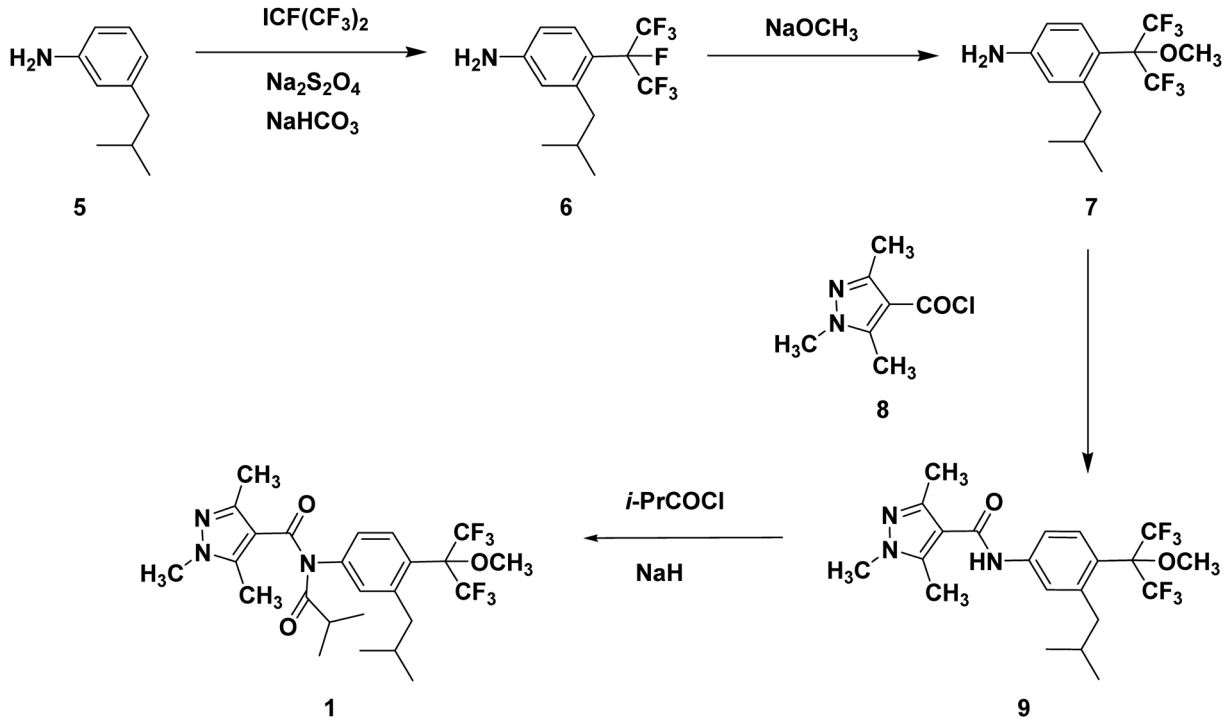
1.2.4. Optimization of the amide moiety

As a result of the amide modification, acyl derivatives showed excellent activity, and lower alkyl carbonyl derivatives showed especially superior activity. These results could be explained by considering acyl-substituted derivatives as propesticides. In agrochemistry, several compounds, including acaricides, have been already known to work as propesticides.[8)]

Table 1. Summary of structure-activity relationship of carboxamides against *Tetranychus urticae*



Substituents	Structure-activity relationship
X_1	CH_3>*n*-Pr>H
X_2	CH_3>Cl, H
X_3	CH_3>Cl>H
Y	2-(1,3-Me_2-Bu), 3-*i*-Bu>2-(3-Me-Bu), 2-(1,3-Me_2-Pentyl), 3-(2-Me-Bu)>3-O-*i*-Pr, 3-*n*-Pr≫2-*i*-Bu, H
Z	H, OCH_3>OEt>O-*n*-Pr≫F
R	Alkyl carbonyl>H



Scheme 2. Synthetic pathway of pyflubumide.

Among acyl derivatives, pyflubumide (**1**) was finally selected as the compound to develop.

2. Synthesis

3-Isobutylaniline (**5**) was reacted with heptafluoroisopropyliodine under radical conditions to produce heptafluoroisopropyl derivative **6**. The fluorine atom at the benzylic position of **6** was converted to the methoxy group by sodium methoxide to give aniline **7**.[4] The amidation of **7** with acid chloride **8** gave carboxanilide **9**, followed by acylation to give pyflubumide (**1**, Scheme 2).

3. Physical and chemical properties

Common Name: Pyflubumide
Experimental code: NNI-0711
CAS registry No.: 926914-55-8
Chemical name: 3′-isobutyl-*N*-isobutyryl-1,3,5-trimethyl-4′-[2,2,2-trifluoro-1-methoxy-1-(trifluoromethyl)ethyl]pyrazole-4-carboxanilide
Molecular formula: $C_{25}H_{31}F_6N_3O_3$
Molecular weight: 535.52
Appearance: White powder
Melting point: 86°C
Solubility in water: 0.27 mg/L (20°C)
Partition coefficient: $\log P_{o/w}=5.34$ (25°C)

4. Biological activity

4.1. Insecticidal and acaricidal spectrum of pyflubumide

The insecticidal and acaricidal spectrum of pyflubumide is shown in Table 2. Pyflubumide was specifically and highly active against all stages of agriculturally important spider mites, such as two-spotted spider mite, kanzawa spider mite and citrus red mite. It showed, in contrast, low activity against other pests, including tarsonemid and eriophyid mites and a broad range of insect species.

4.2. Effects of pyflubumide on beneficial arthropods and natural enemies

As shown in Table 3, pyflubumide exhibited quite low toxicity against beneficial arthropods such as pollinators and natural en-

Table 2. Insecticidal and acaricidal spectrum of pyflubumide

Order	Common name	Test stages	LC_{50} (mg a.i./L)
Acarina	Two-spotted spider mite	Adult	1.2
	Kanzawa spider mite	Adult	1.9
	Citrus red mite	Adult	1.3
	European red mite	Adult	2.2
	Pink citrus rust mite	Adult, Larva	>100
	Tea rust mite	Adult, Larva	>100
	Broad mite	Adult, Larva	>100
Hemiptera	Green peach aphid	Adult, Larva	>500
	Cotton whitefly	Egg	>100
Thysanoptera	Western flower thrips	1st Larva	>500
Diptera	Tomato leaf miner	Egg	>500
Coleoptera	Rice weevil	Adult	>500
Lepidoptera	Common cutworm	3rd Larva	>500

Table 3. Effects of pyflubumide on beneficial arthropods and natural enemies

Common name	Scientific name	Test stage	LC_{30} (mg a.i./L)
Silkworm	*Bombyx mori*	4th Larva	>100
Honey bee	*Apis mellifera*	Adult	>200
Hornfaced bee	*Osmia cornifrons*	Adult	>100
Predatory mite	*Phytoseiulus persimilis*	Egg	>200
	Amblyseius californicus	Adult	>100
	Amblyseius swirskii	Egg	>200
Lady beetle	*Harmonia axyridis*	Adult	>100
Predatory midge	*Aphidoletes aphidimyza*	Larva	>100
Parasite wasp	*Apanteles glomeratus*	Pupa	>200
	Encarsia formosa	Pupa	>200
Predatory bug	*Orius strigicollis*	Adult	>100
Wolf spider	*Pardosa pseudoannulata*	Larva	>200

emies, including predatory mites and parasitoids that are commercially available and artificially introduced into various cultivation programs. Those results indicated that this carboxamide could be an excellent control agent against spider mites in horticulture, with high suitability to IPM programs.[9)]

5. Toxicological properties

Pyflubumide showed quite low acute oral toxicity against mammals and was not classified as poisonous or deleterious compound. In addition, pyflubumide TGAI and its 20% SC showed no critical eye and skin irritation nor skin sensitization. Therefore, they were evaluated as "not classified" in GHS system. Various studies regarding carcinogenicity, mutagenicity, teratogenicity, reproduction, and so on confirmed that the compound would not cause human health concerns. Pyflubumide exhibited rather low toxicity toward aquatics, ready biodegradability in the environment, and low bioconcentration in fish, suggesting that the compound would have low environmental impact under GAP and administration for use.

Table 4. Inhibitory activities of pyflubumide (**1**) and its NH-form (**9**) against mitochondrial complex II of a variety of species

Order	Common name	IC_{50} (μM)	
		Compounds No.	
		1	**9**
Acarina	Two-spotted spider mite	>10	0.025
Hymenoptera	Honey bee	NT	>1
Lepidoptera	Common cutworm	NT	>10
Diptera	Northern blowfly	NT	>10
Salmoniformes	Rainbow trout	>10	>10
Rodentia	Norway rat	>1	>10

6. Mode of action

Based on its structural similarity with the carboxamide fungicides, pyflubumide's mode of action was expected to inhibit mitochondrial complex II. Thus, we investigated the inhibitory effects of pyflubumide on mitochondrial complex II. Pyflubumide did not show remarkable inhibitory activity; however, *N*-deisobutylated compound **9**, *i.e.*, the NH-form of pyflubumide, provided potent inhibitory activity against mitochondrial complex II from spider mites.[10)] Additionally, analytical chemical research revealed that pyflubumide can be metabolized rapidly to its NH-form in the spider mite homogenate. These results suggest that pyflubumide could be a propesticide, expressing its acaricidal activity through its metabolism to the active form in the spider mite's body. On the other hand, it showed no inhibition against mitochondria from organisms other than spider mites (Table 4). Accordingly, inhibitory selectivity *in vitro* could account for the selective toxicity *in vivo*.

It has already been reported that *beta*-ketonitrile acaricides also inhibit mitochondrial complex II.[11,12)] We investigated the biochemical difference between pyflubumide and a *beta*-ketonitrile derivative by using a double-inhibitor titration assay. The inhibition of the NH-form of pyflubumide and the OH-form of cyenopyrafen were not additive (Fig. 2A), which is in contrast

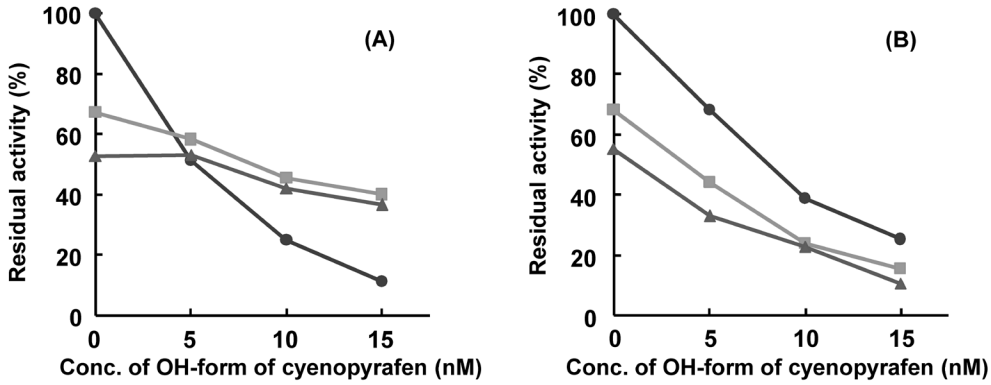


Fig. 2. The inhibition of complex II of spider mite mitochondria by the OH-form of cyenopyrafen in the presence of the NH-form of pyflubumide (A) or the OH-form of cyenopyrafen (B); (A) titration by the OH-form of cyenopyrafen in the presence of 0 (●), 20 (■), or 35 nM (▲) of the NH-form of pyflubumide; (B) titration by the OH-form of cyenopyrafen in the presence of 0 (●), 5 (■), or 6.5 nM (▲) of the OH-form of cyenopyrafen.

to the case using the same compound (the OH-form of cyenopyrafen *vs.* the OH-form of cyenopyrafen), in which almost additive inhibition was attained (Fig. 2B).

From the viewpoint of the above-mentioned results regarding the mode of action and the *in vivo* evaluation of its cross-resistance, mitochondrial complex II inhibitors recently launched in the market are categorized into the following separate subgroups: 25A (*beta*-ketonitrile derivatives; cyenopyrafen, cyflumetofen) and 25B (carboxanilides; pyflubumide) in classifying the mode of action by the Insecticide Resistance Action Committee (IRAC).[13)]

Conclusion

The usage of mitochondrial complex II-inhibiting carboxamides had been primarily limited to disease control. However, we have found a novel acaricidal activity by introducing unique fluoroalkyl groups, which finally led to the discovery of pyflubumide.

As of May 2017, pyflubumide was registered and commercialized in Japan/Korea, under the trade names of Dani-Kong, Noblesse® (single agent) and Double-Face (mixture with fenpyroximate). The registration of pyflubumide has been scheduled for approval as a pesticide in additional countries in the near future. It is also expected that pyflubumide, an excellent acaricide with unique characteristics, will be widely used to contribute to the success of agriculture in the world.

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References

1) Y. Yanase, H. Katsuta, K. Tomiya, M. Enomoto and O. Sakamoto: *J. Pestic. Sci.* **38**, 120–129 (2013), in Japanese.
2) M. Tohnishi, H. Nakao, T. Furuya, T. Shimizu, A. Seo, K. Sakata, S. Fujioka, H. Kodama, T. Hirooka and T. Nishimatsu: *J. Pestic. Sci.* **30**, 354–360 (2005).
3) M. Uehara, M. Watanabe, M. Kimura, M. Morimoto and M. Yoshida (Nihon Nohyaku Co., Ltd.); *Jpn Tokkyo Koho JP* 4099622B (in Japanese).
4) T. Furuya, K. Machiya, A. Suwa and S. Fujioka (Nihon Nohyaku Co., Ltd.): *PCT Int. Appl.* WO2005/115994 (2005).
5) T. Furuya, H. Kanno, K. Machiya, A. Suwa, N. Yasokawa and S. Fujioka (Nihon Nohyaku Co., Ltd.): *PCT Int. Appl.* WO2007/020986 (2007).
6) T. Furuya, M. Yamaguchi, M. Tohnishi, A. Seo, M. Morimoto, T. Takemoto and S. Fujioka (Nihon Nohyaku Co., Ltd.): *Pct. Int. Appl.* WO2002/96882 (2002).
7) T. Furuya, A. Suwa, M. Nakano, S. Fujioka, N. Yasokawa and K. Machiya: *J. Pestic. Sci.* **40**, 38–43 (2015).
8) P. Jeschke: *Pest Manag. Sci.* **72**, 210–225 (2016).
9) T. Furuya, M. Nakano, A. Suwa, N. Yasokawa, S. Fujioka and K. Machiya: "Discovery and Synthesis of Crop Protection Products," ACS symposium series, vol. 1204, eds. by P. Maienfisch, P. and T. M. Stevenson), American Chemical Society, Washington, DC, pp. 379–389 (2015).
10) M. Nakano, N. Yasokawa, A. Suwa, S. Fujioka, T. Furuya and K. Sakata: *J. Pestic. Sci.* **40**, 15–24 (2015).
11) K. Nakahira, S. Takii and H. Murakami: Abstracts of papers, 11th IUPAC International Congress of Pesticide Chemistry, August, Kobe, Book of Abstract (2). II-1-i-23B (2006).
12) N. Hayashi, Y. Sasama, N. Takahashi and N. Ikemi: *Pest Manag. Sci.* **69**, 1080–1084 (2013).
13) http://www.irac-online.org/documents/moa-structures-poster-english/?ext=pdf (Accessed Mar 26, 2017)