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Synthesis, characterization and bioactivity of Fipronil derivatives as a lead for new insecticide

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Fipronil is the first phenylpyrazole insecticide introduced for pest control. In order to further study fipronil derivatives of better bioactivity and systemic property derived from the pyrazole-5-amine by addition reaction/elimination/substitution reaction, ten modified compounds were prepared. Their insecticidal bioactivities against the 3rd instar larvae of *Plutella xylostella* were determined. The data suggested that bioactivities of most of the compounds are higher than that of fipronil, while some of them showed only modest activity as compared with fipronil.

Keywords: Fipronil derivatives, Insecticidal activity, *Plutella xylostella*

Fipronil research led to commercial operations firstly by French Phone Poulenc Company registered in China in 1992. It was the first phenylpyrazole insecticide introduced for pest control. This second generation insecticide acting on the GABA receptor, interfering with the nerve system of insect by blocking the chloride channel led to insect death in a certain dose[1]. Internationally, fipronil is considered as the new generation, high-tech pesticide for its novel structure, unique action mechanism and high activity. This was followed by its derivatives some of which have been commercialized[2,3]. In 2004, Mitsubishi Chemical Co., Japan reported 2 new fipronil derivatives, pyrafluprole (V3039) and pyriprole (V3086) with improved synthesis method. These derivatives exhibited better biological activity than fipronil. In the mean time, RAJ Dalian Co., Ltd. developed the pesticide fipronil butane which showed good control against the pest on riceand vegetable[4,5].

Uptake and xylem transport of fipronil in sunflower have been proved, but so far no research indicatd that fipronil has the phloem mobility in plant[7]. *Wu et al*.[8] succeeded to synthesize a fipronil derivative by substituting trifluoromethylsulfinyl on phenylpyrazole of fipronil with ethylsulfinyl, and the new compound showed lower toxicity to mammals, its better uptake and systemic ability. In this article, structures of 10 fipronil derivatives synthesized by modifying the amino group on pyrazole of fipronil, and their activities against diamondback moth *Plutella xylostella* are reported.

Experimental Procedure

Instruments and Reagents

Heidolph LABOROTA4001-Rotary Evaporator, Germany; Melting point instrument MP-500; Nuclear Magnetic Resonance Apparatus, Bruker AC-P400Q; Mass Spectrometer VG-7070E; TLC silica gel GF-254 (Qingdao Ocean Chemical Co., Ltd.) were used. All reagents used were analytically pure.

Synthesis and characterization of fipronil derivatives

1-(2,6-Dichloro-4 -(trifluoromethyl)phenyl)- 4-(trifluoromethyl-sulfinyl)-1H-pyrazole-3-carbonitrile (1)

Fipronil (98%, 4.4 g, 0.01 mol) was dissolved in acetonitrile (20 mL), to which isoamyl nitrite (1.8 g, 0.015 mol) was added and stirred at 60ºC for 2 h. The reaction mixture was evaporated in vacuo to give a dense yellow liquid. The resulting concentrate was stirred with phosphorous acid (1.3 g) at ambient temperature. To it, dichloromethane (20 mL) was added and washed first with 1 M HCl (10 mL) and then with water (2×20 mL). The organic extracts were dried with anhydrous sodium sulphate and then evaporated in vacuo. The solid residue was recrystallized by petroleum ether and ethyl acetate to give colourless crystals (yield: 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (s, H, pyrazole-H), 7.80 (s, 2H, C6H2); ESIMS m/z (%): 422(%)[M + H]+, 424[M + H + 2]+.

2-Chloro – N - (3-cyano-1-(2, 6 - dichloro -4- (trifluoromethyl)-phenyl)-4-(trifluoromethylsulfinyl)-1H-pyrazol-5-yl) acetamide (2)

Fipronil (98%, 4.4 g, 0.01 mol) was dissolved in tetrahydrofuran (30 mL), to which sodium hydride (0.8 g of an 36% oil dispersion) was added in batches on ice bath under nitrogen and stirred below 10ºC for 1 h. To this mixture chloroacetic chloride (1.2 g) was added and refluxed for 24 h. The reaction mixture was cooled, poured onto ice water and extracted with ether (3×40 mL). The ether extracts were dried with anhydrous magnesium sulphate, filtered and then evaporated in vacuo. The solid residue was purified by chromatography eluting with petroleum ether and ethyl acetate to give clear colourless crystals (m.p.175-177ºC, yield: 23%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (s, 2H, C_6H_2), 4.24 (s, 2H, CH_2), 3.31 (s, H, NH).

5-Bromo-1-(2,6–dichloro-4-(trifluoromethyl)phenyl)-4-(trifluoro methylsulfinyl)-1H-pyrazole-3-carbonitrile (3)

Acetonitrile (20 mL) containing 98%fipronil (4.4 g, 0.01 mol) was dropped to a mixture of KBr (1.2 g) and isoamyl nitrite (1.8 g, 0.015 mol), on ice bath and stirred for 1 h. Then the mixture was refluxed for 3 h. Then isoamyl nitrite (1.2 g, 0.01 mol) was added into the reaction system on ice bath and refluxed for further 5 h with stirring. The reaction mixture was evaporated in vacuo to give a yellow solid residue, which was recrystallized by toluene and *n*-hexane to give clear colourless crystals (m.p. 146-148ºC, yield: 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80-7.79 (s, 2H, C_6H_2).

1-(2, 6- Dichloro- 4- trifluoromethyl)phenyl) -5-hydroxyl- 4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile (4)

Fipronil (98%, 4.4 g, 0.01 mol) was dissolved in 98% H_2SO_4 (10 mL), to which sodium nitrite (1.7 g) was added in batches while stirring below 20ºC. Then the reaction mixture was refluxed for 15 min. Later water (5 mL) was added and refluxed for about 1 h. The reaction mixture was cooled, poured onto water (100 mL) and precipitated immediately. The precipitate was filtered and the filtrate was dissolved in ethyl acetate, dried with anhydrous sodium sulfate, evaporated in vacuo and purified by chromatography, eluting with petroleum and ethyl acetate to give clear colourless crystals (yield: 54%).^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80-7.79 (s, 2H, C_6H_2), 4.66 (s, H, OH).

5-(3 Cyano- 1- (2, 6- dichloro-4- (trifluoromethyl)phenyl)- 4-(trifluoromethylsulfinyl)-1H-pyrazol-5-yl)acetamide (5)

The synthesis was identical to that of compound 2. Recrystallization of the resultant product from petroleum ether gave a clear colourless crystalline solid (m.p. 183-185ºC, yield: 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.23 (s, H, NH), 8.41-8.35 (s, 2H, C_6H_2), 3.35 (s, 3H, CH_3).

1-(3-Cyano-1-(2, 6- dichloro-4- (trifluoromethyl)phenyl) -4- (trifluoromethylsulfinyl)-1H-pyrazol-5-yl)-3-methylurea (6)

Fipronil (98%, 8.8 g, 0.02 mol) was dissolved in 20 mL chloroform, to which triethylamine (2.7 g) and 4-*N,N*–dimethylaminopyridine (0.25 g) were added and stirred for a while. Then *N*-dimethylformamide (2.4 g) was dropped slowly into the reaction system under ice bath, then at room temperature for 30 min. Finally the mixture was refluxed overnight. The reaction mixture was cooled to room temperature, washed by 1% HCL (2×40 mL), then washed by water (2×60 mL) and extracted by ethyl acetate. The extracts were dried over anhydrous sodium sulfate, evaporated in vacuo and recrystallized using petroleum ether and ethyl acetate to give clear colourless crystals (yield: 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.59-8.55 (s, 2H, 2NH), 7.77-7.76 (s, 2H, C_6H_2), 3.32 (s, 3H, CH_3), 12.59 (s, $2NH_2$); ESIMS *m/z*(%): 494(%)$[M+H]^+$, 496$[M+H+2]^+$.

2 - Bromo -N- (3-cyano - 1- (2 ,6- dichloro- 4- (trifluoromethyl)-phenyl)-4-(trifluoromethylsulfinyl)-1H-pyrazol-5-yl) acetamide (7)

Fipronil (98%, 4.4 g, 0.01 mol) was dissolved in tetrahydrofuran (40 mL), to which sodium hydride (0.8 g of an 36% oil dispersion) was added three times below 0ºC under nitrogen and stirred below 10ºC. After 3 h, bromoacetyl bromide (7 mL) was added and stirred at room temperature for another 3 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo. The solid residue was purified by chromatography eluting with petroleum ether/ethyl acetate (3:1) to give clear colourless crystals (m.p.105-107ºC, yield: 72%). ^{1}H NMR($CDCl_3$, 600 MHz) δ: 9.22(s, 1H, NH), 7.73-7.80 (s, 2H, ArH), 4.11-4.12 (s, 2H, CH_2); ^{13}C NMR($CDCl_3$, 150 MHZ) δ: 26.63, 107.90, 109.64, 126.18, 126.57, 126.59, 134.36, 134.57, 134.80, 135.58, 136.03, 141.38, 164.07, 171.37; ESIMS m/z(%):559 [M+H]+, 581[M+Na]+ ,597 [M+K]+.

2-Azido-N-(3-cyano-1-(2, 6-dichloro-4-(trifluoromethyl)phenyl)-4-(trifluoromethylsulfinyl)-1H-pyrazol-5-yl) acetamide (8)

Compound 7 (2 g, 0.0035 mol) was dissolved in acetone (12 mL), then NaN_3 (0.26 g, 0.004 mol) in 8 mL water was added into the solution below 0ºC under nitrogen and stirred under ice bath for 1 h. The reaction mixture was filtered and the filtrate was

evaporated in vacuo. The concentrate was eluted with ethyl acetate and water, dried with anhydrous sodium sulphate, evaporated in vacuo and recrystallized with petroleum ether and ethyl acetate to give clear colourless crystals (m.p. 167-169ºC, yield: 98%). ^{1}H NMR($CDCl_3$, 600 MHz) δ: 9.23(s, 1H, NH), 7.73-7.80 (s, 2H, ArH), 1.59-2.13 (s, 2H, CH_2); ^{13}C NMR($CDCl_3$,150 MHZ) δ: 52.05, 106.56, 109.47, 120.98, 122.80, 122.80, 124.03, 133.97, 133.97, 134.63, 135.47, 136.83, 142.01, 164.57; ESIMS *m/z*(%):520 $[M]^+$,522$[M+2]^+$, 560 $[M+K+1]^+$.

N-(3-Cyano- 1-(2, 6-dichloro- 4-(trifluoromethyl)phenyl)- 4- (trifluoromethylsulfinyl)-1H-pyrazol-5-yl)-2-thiocyanatoacetamide (9)

Actonitrile (50 mL), 4ÅMS (20 g), tetrabutyl ammonium bromide (2.5 g) and potassium thiocyanate (1 g, 0.01 mol) were stirred for 1 h at room temperature. To it, compound 7 (3.0 g, 0.0053 mol) was added and refluxed for about 10 h. The reaction mixture was filtered, the filtrate evaporated in vacuo, the residue eluted with ethyl acetate and water, and dried over anhydrous sodium sulphate. It was evaporated in vacuo and recrystallized with petroleum ether and ethyl acetate to give clear colourless crystals (m.p.181-183ºC, yield: 24%). ^{1}H NMR($CDCl_3$, 600 MHz) δ: 7.76 (s, 1H, NH), 7.26 (m, 2H, ArH), 3.85-3.89 (d, 2H, CH_2); ^{13}C NMR($CDCl_3$, 150 MHZ) δ: 109.13, 111.30, 111.93, 120.93, 121.16, 121.61, 122.97, 123.84, 124.78, 133.32, 133.83, 134.06, 134.29, 160.43, 168.51.

(Z)-1-(2,6-Dichloro- 4- (trifluoromethyl)phenyl)- 5- (2-hydroxybenzylideneamino)-4-(trifluoromethylsulfinyl)-1H -pyrazole-3-carbonitrile (10)

Fipronil (98%, 4.4 g, 0.01 mol), 2-hydroxybenzaldehyde (1.8 g, 0.015 mol), resin 732 (8 g), 4Å MS (10 g), and tolune (20 mL) were refluxed for 5 days. After cooling to room temperature, the reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was purified by chromatography to give yellow crystal (m.p. 135-137ºC, yield: 40%). ^{1}H NMR($CDCl_3$, 600 MHz) δ: 10.75(s, 1H, OH), 9.17 (s, 1H, HC=N), 6.99-7.86 (s, 6H, ArH); ^{13}C NMR($CDCl_3$, 150 MHZ) δ: 97.60, 113.08, 116.30, 117.65, 118.06, 120.08, 122.13, 126.03, 126.05, 127.95, 128.41, 133.62, 133.62, 135.69, 135.85, 136.42, 150.10, 161.27, 166.42; ESIMS *m/z*(%):540 $[M+H]^+$, 563$[M+Na]^+$.

Bioactivity assay against *Plutella xylostella*

Rearing of Plutella xylostella

The healthy larvae of *Plutella xylostella* were collected from the experimental farm of Dongguan Agricultural Science Research Center, Guangdong, China and reared on Chinese cabbage (*Brassica rapa*) under the cage conditions for 3 generations. Feeding conditions are as follows: temperature 24-29ºC, 70-80% RH and photoperiod 16:8 h light:dark.

Assessment of bioactivity on Plutella xylostella

The bioactivities of fipronil derivatives and fipronil against the 3rd instar larvae of *P. xylostella* were determined by the leaf disc-dipping assay. Leaves of Chinese cabbage grown in the greenhouse were collected, and discs (5.5 cm diameter) were punched from each leaf. The compounds were dissolved in acetone and suspended in distilled water containing Triton X-100. Leaf discs were dipped in each test solution for 30 s and allowed to dry for 2 h. The treated leaf discs were placed into Petri dishes (9 cm diameter). Then, ten *P. xylostella* larvae were introduced into each dish. Distilled water containing acetone-Triton X-100 solution (not the tested compound) was used as the control. Petri dishes were kept in incubator at 26ºC and 85% relative humidity under a photoperiod of 16:8 h light:dark. All treatments were replicated five times. Mortalities were determined 24 h and 48 h after treatment. The death rate of each treatment group was confirmed. LC_{50} value was calculated by the SPSS.

Results and Discussion

Structural modification of fipronil on amino group substituted by acetyl group, pyrrole heterocycle or halogen, such as bromine or iodine have been attepmpted and many fipronil derivatives have shown good bioactivities against pest, which indicated that the pyrazole-5-amine of fipronil is a good reaction site for further study[8-17]. Here, the synthesis of 10 compounds by attacking the pyrazole-5-amine of fipronil thorough addition/elimination/sub stitution reaction has been successfully achieved, 5 of which are novel (compounds 6, 7, 8, 9 and 10) (Figs 1 and 2). Bioactivity results (Table 1) showed that the activities of compounds 1, 2, 3, 4 and 10 against *Plutella xylostella* after 24 h are 12.41, 10.26, 18.93 and 11.33 $\mu g \bullet mL^{-1}$ respectively, better than that of fipronil. Compound 8 showed the activity similar to fipronil while compound 5 and 7 showed lower activities than that of fipronil. Compound 6 and 9 totally lost their activities against *Plutella xylostella*.

The activities of fipronil derivatives mostly relied on their binding capability to r-GABA receptor. The binding capability mainly lied on the steric hindrance

of the structure of the compound and electro-negativity of relative groups. Compounds 1, 3 and 4 showed good activities against *Plutella xylostella*, because their structures are similar with fipronil that may help to reduce the steric hindrance due to which they can easily bind with r-GABA receptor of the insect. Compound 2 and 6 also possessed excellent activities, because compound 2 has chlorine on the substitution group on the pyrazole-5-amine that may increase the activity, and compound 10 is a schiff's base of which the large π bond formed in condensation reaction may enhance the activity.

The substituted pyrazole-5-amine group of compound 6 possessed high steric hindrance and of compound 9 high electro-negativity, which may be repulsed by r-GABA receptor and hence showed no activities. The structures of compound 2, 7, 8, and 9 are similar and their activities lie on the extent of electro-negativity of the substitution groups when compared to each other. Since the electro-negativity of compound 2, 7 and 8 are comparable, it is not surprising to find out their similar activities against *Plutella xylostella,* while compound 9 totally lost its activity for its strong electro-negativity.

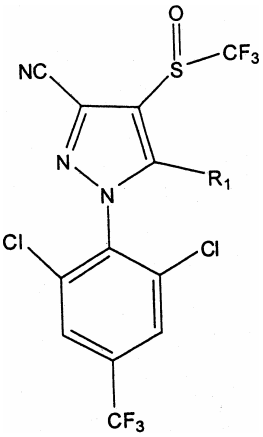

Fig. 1 — General structure of fipronil derivatives.

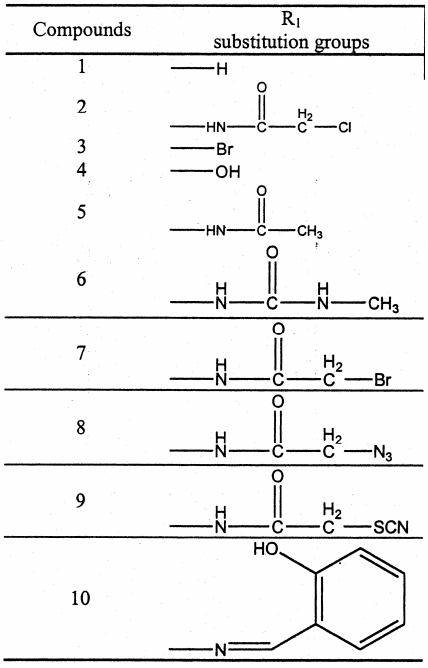

Fig. 2 —Substituent groups of fipronil derivatives.

Table 1 — The effect of fipronil derivatives against 3st larvae of Diamondback moth (*Plutella xyllostella* Linn)

Sample	Time (h)	Linear regression equation (y= a+bx)	Correlation coefficient(r)	LC_{50} ($\mu g \cdot mL^{-1}$)	95%Confidence interval of LC_{50} ($\mu g \cdot mL^{-1}$)
1	24	y=3.2494+1.6005x	0.9995	12.41	8.22-18.74
	48	y=3.6323+1.6768x	0.9955	6.54	4.10-10.45
2	24	y=3.2449+1.7355x	0.9630	10.26	7.02-15.01
3	24	y=2.4661+1.9839x	0.9945	18.93	11.49-31.21
	48	y=2.6061+2.1133a	0.9797	13.58	9.63-19.13
4	24	y=2.7872+1.7501x	0.9945	18.38	12.87-26.26
	48	y=3.0544+1.8450x	0.9541	11.34	6.88-18.68
5	48	y=1.1883+2.0321x	0.9939	75.11	28.19-200.11
6	24	——	——	——	——
	48	——	——	——	——
7	24	y=1.7754+1.7751x	0.9983	65.5466	52.50-88.31
	48	y=2.2974+1.6048x	0.9677	48.3186	39.66-61.98
8	24	y=3.6022+0.8488x	0.9961	44.3369	32.26-69.40
	48	y=3.2661+1.370x	0.9724	18.4318	12.12-27.34
9	24	——	——	——	——
	48	——	——	——	——
10	24	y=2.9830+1.9126x	0.9689	11.3386	9.57-13.95
	48	y=2.64341+2.7185x	0.9772	7.3598	5.65-9.98
fipronil	24	y=2.9391+1.4679x	0.9668	25.35	10.86-59.19
	48	y=3.2688+1.5864x	0.9423	12.34	8.14-18.70

Conclusion

It is important to study fipronil by modifying its structure or synthesize fipronil derivatives possessing excellent bioactivities according to the structure-activity relationship. The results in this paper indicated a definite structure-activity relationship of fipronil derivatives which needs to be explored further. Fipronil derivatives of high efficiency and low toxicity, also having the phloem mobility, were expected to be screened out for further development to control pests with piercing sucking mouth parts.

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