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Synthesis and anti-TMV activity of novel *N*-(3-alkyl-1*H*-pyrazol-4-yl)-3-alkyl-4-substituted-1*H*-pyrazole-5-carboxamides

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Abstract

In order to investigate the biological activity of novel bis-pyrazole compounds, a series of *N*-(3-alkyl-5-(*N*-methylcarbamyl)-1*H*-pyrazol-4-yl)-3-alkyl-4-substituted-1*H*-pyrazole-5-carboxamides were designed and synthesized with ethyl 3-alkyl-1*H*-pyrazole-5-carboxylate **1** as starting materials. *N*-Methyl-3-alkyl-4-amino-1*H*-pyrazole-5-carboxamides **6** were obtained from **1** *via* 5 steps. 3-Alkyl-4-substitued-1*H*-pyrazole-5-carboxyl chlorides **4a**, **4b**, **11a**, **11b**, **11c** or **12** were also obtained from **1** *via* several steps. Target compounds **7a**–**7g** were obtained after the reaction of **6** with the above 1*H*-pyrazole-5-carboxyl chlorides. Preliminary bioassay showed some compounds possessing good inactivation effect against TMV (tobacco mosaic virus). Compound **7a** showed higher activity superior to ningnanmycin at a concentration of 5.0×10^{-4} g/mL and equal activity at 1.0×10^{-4} g/mL; **7b** and **7c** showed equal activity to virazole both at concentrations of 5.0×10^{-4} g/mL and 1.0×10^{-4} g/mL.

Keywords: Bis-pyrazole compounds; 3-Alkyl-4-amino-1*H*-pyrazole-5-carboxamides; 3-Alkyl-1*H*-pyrazole-5-carboxyl chlorides; Inactivation effect; TMV

Pyrazole compounds play important roles in pesticide science and some of them have been used as herbicides, insecticides and fungicides world widely, such as fipronil and chlorantraniliprole, azimsulfuron and pyrazoxyfen, furametpyr and penthiolpyrad, which are the representatives of green pesticides for their low toxicity, high selectivity and environmental kindness [1–6]. Great efforts are still focused on the design and synthesis of pyrazole compounds in order to find novel leading structures for pesticides innovation [7–14]. There are many novel pyrazole structures which have been reported recently nearly in every aspect of pesticide research, and that some di- or bis-pyrazole derivatives were found possessing herbicidal, fungicidal or viralcidal activities [15–18]. Using ethyl 3-alkyl-1*H*-pyrazole-5-carboxylates **1** as starting material, we designed and synthesized a series of bis-pyrazole compounds, in which two substituted pyrazole subunits were attached to each other with an amide bond. All the seven target compounds were reported for the first time.

The synthetic route of the compounds was outlined in Scheme 1. Starting materials **1** were obtained by literal methods [19]. Intermediates **6** were obtained in high yields from **1** *via* hydrolysis, nitration, chlorination, ammoniation

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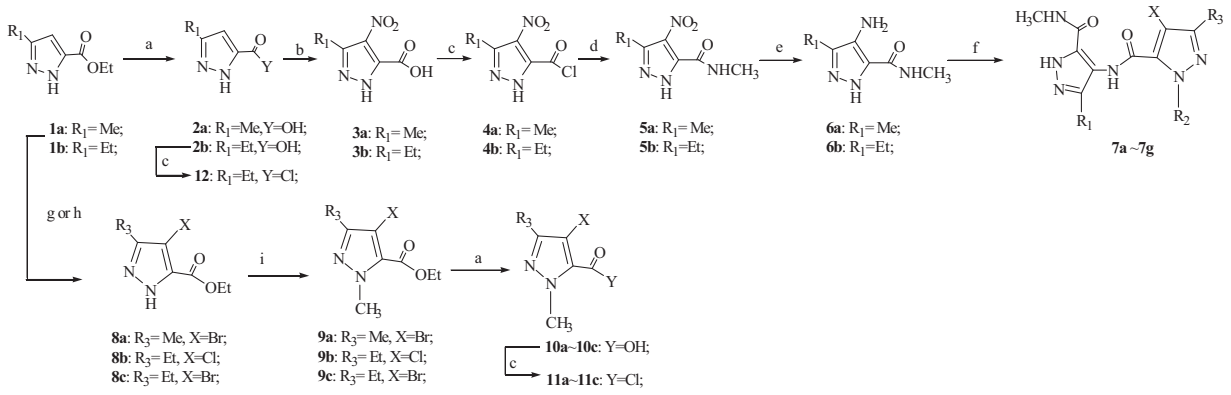

Scheme 1. Reagents and conditions: (a) (i) aqueous NaOH (4 equiv.), methanol, reflux, 4 h; (ii) H^+, pH = 2; (b) HNO_3 (98%)/$H_2SO_4{\cdot}xSO_3$ (1:1.2, v/v), −5 °C for 1 h, 60 °C for 18 h; (c) $SOCl_2$, reflux, 4 h; (d) aqueous CH_3NH_2 (35%) (25 equiv.), Et_2O, 0 °C for 3 h, r.t. for 5 h; (e) H_2 (8 atm), Pd/C (0.5% equiv.), methanol, r.t., 24 h; (f) **4a**, **4b**, **11a**, **11b**, **11c** or **12** (1.2 equiv.), Et_3N (1.5 equiv.), CH_2Cl_2 or THF, 0 °C for 3 h, r.t. for 5 h; (g) SO_2Cl_2 (1.3 equiv.), $CHCl_3$, 0 °C, reflux for 4 h; (h) NBS (1.2 equiv.), $CHCl_3$, r.t., 12 h; (i) CH_3I (1.5 equiv.), K_2CO_3 (1.0 equiv.), and acetone, 50 °C, 10 h.

and reduction successively and used for the next step directly [20–22]. During the preparation of **6**, intermediates **2**, **3**, **5** were purified by recrystallization in methanol, and **4** could be used directly without further purification. 3-Alkyl-1,4-disubstituted-1*H*-pyrazole-5-carboxyl chlorides **4a**, **4b**, **11a**–**11c**, and **12** were prepared from **1a** or **1b** *via* two to four steps [23–25]. Intermediates **11** were also obtained from **1** *via* halogenation, methylation, hydrolysis and chlorination successively and used directly to react with **6**. During the preparation, compounds **8** and **9** were purified by column chromatography. And **12** could be easily obtained from **2b**. Target compounds **7a**–**7g** were obtained as white solids in moderate to high yields after the two sub-structures reacted in dichloromethane, which could be further purified by recrystallization in methanol. Structural and some physical data of **7a**–**7g** were listed in Table 1 and ^{1}H NMR data of them were illustrated in Table 2.

Inactivation effect of compounds **7a**–**7g** against TMV *in vivo* was evaluated on *N. tabacum L.* leaves [26]. The healthy fresh leaves growing at 6 leaves age were selected for the test. The TMV virus with a concentration of 5.88×10^{-6} g/mL was inhibited by mixing with the target compound solution at the same volume for 30 min. Then the mixture was inoculated on the upper three leaves using the conventional juice robbing method, and the solvent was smeared on the lower three leaves as a control. The local lesion numbers were recorded 2–3 days after inoculation. For each compound, three repetitions were conducted. All compounds for testing were conducted at concentrations of 5.0×10^{-4} g/mL and 1.0×10^{-4} g/mL respectively. Virazole and ningnanmycin were used as positive control at the same time. The activity data of inactivation effect against TMV was calculated by the following equation:

$$\text{Inhibition}(\%) = \frac{\text{CK} - T}{\text{CK}} \times 100\%$$

Table 1
Structures and some physical data of compounds **7a**–**7f**.

Compd.	X	R_1	R_2	R_3	mp (°C)	Yields (%)[†]	MS (*m/z*, ESI)	
							Calcd.	Found
7a	NO_2	Et	H	Et	154–155	78	335.13	358.12 ($M+Na^+$)
7b	NO_2	Et	H	Me	212–214	69	321.12	344.11 ($M+Na^+$)
7c	NO_2	Me	H	Me	270–272	88	307.27	330.09 ($M+Na^+$)
7d	H	Et	H	Et	213–214	53	290.15	313.14 ($M+Na^+$)
7e	Cl	Et	Me	Et	184–186	78	338.13	361.12 ($M+Na^+$)
7f	Br	Et	Me	Et	171–172	56	382.08	405.07 ($M+Na^+$)
7g	Br	Et	Me	Me	221–222	73	368.06	391.05($M+Na^+$)

[†] Yields of the last step.

Table 2
^{1}H NMR data of compounds **7a–7f**.

Compd.	Solvent	^{1}H NMR (300 MHz, TMS): δ
7a	DMSO-d_6	13.94 (s, 1H), 13.00 (s, 1H), 9.88 (s, 1H), 8.06 (d, 1H), 2.95 (q, 2H, J = 7.5 Hz), 2.72 (s, 3H), 2.71 (q, 2H, J = 7.6 Hz), 1.27 (t, 3H, J = 7.5 Hz), 1.18 (t, 3H, J = 7.6 Hz)
7b	DMSO-d_6	13.95 (s, 1H), 13.01 (s, 1H), 9.89 (s, 1H), 8.07 (d, 1H), 2.72 (s, 3H), 2.71 (q, 2H, J = 7.6 Hz), 2.53 (s, 3H), 1.18 (t, 3H, J = 7.6 Hz)
7c	DMSO-d_6	13.96 (s, 1H), 13.02 (s, 1H), 9.93 (s, 1H), 7.78 (s, 1H), 2.72 (d, 3H, J = 4.5Hz), 2.54 (s, 3H), 2.27 (s, 3H)
7d	DMSO-d_6	13.04 (s, 1H), 12.91 (s, 1H), 9.90 (s, 1H), 8.12 (d, 1H), 6.48 (s, 1H), 2.77 (q, 2H, J = 7.5 Hz), 2.66 (q, 2H, J = 7.6 Hz), 1.21 (t, 3H, J = 7.6 Hz), 1.14 (t, 3H, J = 7.5 Hz)
7e	$CDCl_3$	10.43 (s, 1H), 9.57 (s, 1H), 6.91 (d, 1H), 4.11 (s, 3H), 2.95 (d, 3H), 2.89 (q, 2H, J = 7.5Hz), 2.66 (q, 2H, J = 7.5 Hz), 1.26 (t, 3H, J = 7.5 Hz), 1.25 (t, 3H, J = 7.5 Hz)
7f	$CDCl_3$	9.57 (s, 1H), 6.97 (d, 1H), 5.20(s, 3H), 4.11 (s, 3H), 2.97 (d, 3H), 2.89 (q, 2H, J = 6.9 Hz), 2.62 (q, 2H, J = 6.9 Hz)
7g	DMSO-d_6	13.05 (s, 1H), 9.79 (s, 1H), 8.10 (d, 1H), 3.96 (s, 1H), 2.71 (s, 3H), 2.67 (q, 2H, J = 7.5 Hz), 2.17 (s, 3H), 1.17 (t, 3H, J = 7.5 Hz)

Table 3
Anti-TMV activity of compounds **7a–7f**.

Compd.		**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	Virazole	Ningnanmycin
Inhibit. (%)	5.0×10^{-4} g/mL	62.02	37.21	32.56	32.56	10.08	13.18	12.40	34.11	46.51
	1.0×10^{-4} g/mL	37.21	27.13	27.13	4.65	8.53	10.85	3.88	23.26	39.53

where CK was the average number of viral inflammations on the control leaves *in vivo*, *T* was the average number of viral inflammations on the target compound treated leaves *in vivo*. The inhibition rates of the title compounds were listed in Table 3.

From the data, we could find that compounds **7a**, **7b**, **7c** and **7d** possessing good anti-TMV activity at a concentration of 5.0×10^{-4} g/mL. After the concentration was reduced to 2.0×10^{-4} g/mL, the activity of **7a**, **7b** and **7c** was still higher than that of virazole, but the activity of **7d** decreased sharply. Compounds **7e**, **7f** and **7g** showed very poor activity at both concentration. After a structural comparison, it was very clear that X group at C4 of the pyrazole-5-carboyl part was very important to the antivirus activity of compounds **7a–7g**. When X group was NO_2, title compounds, such as **7a–7c**, showed high anti-TMV activity both at high or low concentration, but the activity disappeared when X was changed to a Cl or Br atom and N1 position was attached with a methyl group. This work provides us with useful information in further research for novel anti-viral leading compounds.

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