Note

Fungicidal Activity of 2-Aminochromone-3-carboxamides

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INTRODUCTION

In the previous papers,¹⁻³⁾ we have reported the fungicidal efficacy of 2-pyrimidinylhydrazones. In our continuous random screening program to elicit pesticide potentialities of various chemicals, we again found a new class of fungicidal 2-aminochromone-3-carboxamide. This lead compound (1)⁴⁾ had been originally prepared in the course of a search for new antiallergic agents exemplified by amlexanox⁵⁾ in our company and left without any further examination in the laboratory.

Compound 1 is associated with the chromone (4-oxo-4H-1-benzopyran) family and structurally unrelated to any other class of fungicides known to us up until now. The first member (1) of this class displayed promising activity *in vitro* and *in vivo* against rice plant pathogenic fungi such as *Pyricularia oryzae*, *Rhizoctonia solani*, without being phytotoxic to the host plant. Because of the structural novelty and new fungicidal profile, this compound 1 was investigated as a clue to develop potential fungicides. In this report, synthetic variations of individual structural elements in the compound are evaluated to gain a better understanding of structure-activity rela-

tionships.

MATERIALS AND METHODS

1. Synthesis of Compounds

Twenty-six chromone derivatives (1-26) were synthesized according to the following four synthetic routes: I) condensation reaction of acetylsalicyl chlorides with malononitrile in the presence of aqueous alkali and hydration of the resulting 2-aminochromone-3-carbonitriles in 80% sulfuric acid,⁶⁾ II) reaction of chromone-3-carbonitriles with 2 mol of hydroxylamine in an aqueous medium,^{4,7)} III) acylation or alkylation of 2-aminochromone-3-carboxamides (4, 9) in the usual manner and IV) ammonolysis of 2-methylchromone-3-carboxylic acid methyl ester^{8,9)} in the usual manner.

2-Methylchromone-3-carboxamide (2): A mixture of methyl 2-methylchromone-3-carboxylate (2.18 g)^{8,9)} and 28% ammonium hydroxide (5 ml) in methanol (10 ml) was stirred for a while at ambient temperature. The reaction mixture was concentrated *in vacuo* to give a solid residue, which was recrystallized from ethanol into colorless needles, $Y=1.96$ g (96%), mp 240°C. Anal. Found: C, 65.52; H, 4.59; N, 6.86. Calcd. for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89%.

4,5-Dioxo-3H-[1]benzopyrano[2,3-d]pyrimidine (20): A solution of 2-aminochromone-3-carboxamide (1: 0.51 g)^{6,7)} in formic acid (10 ml) was boiled under reflux for 16 hr. The reaction mixture was concentrated *in vacuo* to afford a solid residue, which was recrystallized from a mixture of formic acid and water into colorless crystals, $Y=0.40$ g (75%), mp >340°C. Anal. Found: C, 61.63; H, 2.64; N, 13.22. Calcd. for $C_{11}H_6N_2O_8$: C, 61.68; H, 2.81; N, 13.08%.

2-Methylamino-6-*t*-butylchromone-3-carboxamide (26): Iodomethane (1.60 g) was added dropwise to a mixture of 2-amino-6-*t*-butylchromone-3-carboxamide (9: 1.30 g) and potassium *t*-butoxide (0.66 g) in anhydrous tetrahydrofuran (25 ml) under stirring below 10°C. After boiling for 17 hr under reflux, the reaction mixture was concentrated *in vacuo* to yield a solid residue, which was extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate and condensed *in vacuo*. The residue was chromatographed on a silica gel column (eluent; $CHCl_3$: $AcOEt=20:1$) to give 26 as colorless crystals in 38% yield (0.60 g), mp 237-240°C. Anal.

Fig. 1 Amlexanox (Takeda, antiallergic, left) and the lead compound (1).

Table 1 Chemical structures and fungicidal activities of chromones.

No.	Compounds ^{a)}						<i>in vitro</i> ^{d)}					<i>in vivo</i> ^{e)}	
	R ₁	R ₂	R ₃	Md ^{b)}	mp (°C) ^{e)}	Po	Ho	Hs	Rs	Sb			
2	H	Me	CONH ₂	IV	240	>100	>100	>100	>100	0			
3	H	NH ₂	CN	I	330 ^{6,7,10)}	>100	>100	>100	>100	0			
4	H	NH ₂	CONH ₂	I	269–271 ^{6,7)}	25	>100	12.5	12.5	100			
5	6-Me	NH ₂	CONH ₂	II	293–295(d.) ⁷⁾	6.25	25	6.25	6.25	100			
1	6-Et	NH ₂	CONH ₂	II	273–274(d.) ^{4,7)}	0.78	25	— ^{f)}	3.25	90			
6	6- <i>i</i> -Pr	NH ₂	CONH ₂	II	200–202	1.56	100	3.12	100	85			
7	6- <i>n</i> -Bu	NH ₂	CONH ₂	II	209–211	50	>100	50	>100	90			
8	6- <i>s</i> -Bu	NH ₂	CONH ₂	II	193–195	0.78	25	0.78	6.25	79			
9	6- <i>t</i> -Bu	NH ₂	CONH ₂	II	245–247	0.78	6.25	0.78	0.78	100			
10	6-EtMe ₂ C	NH ₂	CONH ₂	II	264–266	3.12	50	6.25	>100	100			
11	6-MeEt ₂ C	NH ₂	CONH ₂	II	278–280	1.56	>100	3.12	>100	100			
12	6- <i>n</i> -PrMe ₂ C	NH ₂	CONH ₂	II	244–246	1.56	>100	6.25	>100	100			
13	6- <i>i</i> -PrMe ₂ C	NH ₂	CONH ₂	II	289–290	3.12	>100	6.25	>100	100			
14	6-F	NH ₂	CONH ₂	I	ca. 289	25	>100	25	12.5	100			
15	6-Cl	NH ₂	CONH ₂	I	>300 ^{6,7)}	3.12	>100	12.5	6.25	96			
16	6-Br	NH ₂	CONH ₂	I	>300 ¹¹⁾	1.56	>100	1.56	3.12	100			
17	6-I	NH ₂	CONH ₂	I	>300	3.12	12.5	3.12	12.5	100			
18	6,8-Cl ₂	NH ₂	CONH ₂	I	>300	>100	>100	>100	100	100			
19	6,8-Br ₂	NH ₂	CONH ₂	I	>300	>100	>100	>100	>100	76			
20	H	N=CH-NH-CO ^{g)}	CONH ₂	III	>340	>100	>100	>100	>100	0			
21	H	NHAc	CONH ₂	III	360(d.)	50	>100	100	25	90			
22	6- <i>t</i> -Bu	N=CH-NH-CO ^{g)}	CONH ₂	III	>300	>100	>100	>100	>100	0			
23	6- <i>t</i> -Bu	NHAc	CONH ₂	III	189–190	3.12	100	6.25	6.25	100			
24	6- <i>t</i> -Bu	NHCO ₂ Et	CONH ₂	III	194–196	25	>100	25	50	100			
25	6- <i>t</i> -Bu	NHCONHMe	CONH ₂	III	>300 ^{b)}	0.78	100	0.78	>100	100			
26	6- <i>t</i> -Bu	NHMe	CONH ₂	III	237–240	1.56	25	1.56	25	100			
St ^{d)}		fertimzone				3.12	25	1.56	50	100			

^{a)} All new compounds were satisfactory in elemental analysis.^{b)} General synthetic routes (I–IV). See experimental section for No. 2, 20, 26.^{c)} Not corrected.^{d)} MIC (μg/ml) against *Pyricularia oryzae* (Po), *Helminthosporium oryzae* (Ho), *Helminthosporium sigmoideum* (Hs) and *Rhizoctonia solani* (Rs) by the agar dilution method.^{e)} Control value (%) against sheath blight (Sb) in the rice seedling test by curative foliar treatment at a concentration of 250 ppm for No. 1, 4, 5, 15 or at 500 ppm for the others. ^{f)} Not tested. ^{g)} R₂ + R₃. ^{h)} Crystals contain 1-mol methanol. ⁱ⁾ Reference standard.

Table 2 Fungicidal activities of Nos. 6, 8 and 9.

Compounds No.	<i>in vitro</i>	<i>in vivo</i>		
	Gf ^{a)}	Bd ^{b)}	Cu ^{c)}	Pr ^{c)}
6	>100	6.0	94	74
8	>100	6.5	96	19
9	12.5	3.0	96	81
Ferimzone	>100	— ^{d)}	100	99
Untreated	—	94.0	0	0

^{a)} MIC ($\mu\text{g/ml}$) against *Gibberella fujikuroi* (Gf) by the agar dilution method.

^{b)} Ratio (%) of infected rice seedlings in seed disinfection against bakanae disease (Bd) by the soaking method at a concentration of 1000 ppm.

^{c)} Control value (%) against blast in the rice seedling test by curative (Cu) and preventive (Pr) foliar treatments at a concentration of 500 ppm.

^{d)} Not tested.

Found: C, 65.58; H, 6.33; N, 9.94. Calcd. for $\text{C}_{15}\text{H}_{18}\text{N}_2\text{O}_3$: C, 65.67, H, 6.61; N, 10.21%. ^1H NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$ ppm: 10.30, 11.30 (2H, br, CONH_2), 8.30–7.10 (3H, m, ArH), 5.67 (1H, br, NH), 3.15 (3H, d, N-Me), 1.37 (9H, s, C-Me).

Other compounds obtained *via* general synthetic routes I, II, III are tabulated in Table 1 along with their fungicidal activities.

2. Fungicidal Activity

Test results are shown in Tables 1 and 2 along with the test methods in notes. The reference standard was ferimzone, a new fungicide derived from 2-pyrimidinylhydrazones¹⁻³⁾.

RESULTS AND DISCUSSION

The examination of original active lead compound 1 led us to prepare a simpler 2-methyl analog (2) easily accessible for the determination of basic structural requirements for the activity. The bioassay revealed that the amino group in the 2-position cannot be replaced by a methyl group. A 3-carbonitrile analog (3), the synthetic intermediate of 4, was found to be devoid of the activity against the fungi in screening, while the potency of its hydrated product 4 was very encouraging. This finding implied that both 2-amino and 3-carboxamide moieties in the chromone skeleton are minimum requisites to exert the fungicidal activity.

Positive contribution of the 6-ethyl group in

the lead 1 to the activity as compared with compound 4 prompted us to prepare some homologs (6–9). Among them a 6-*t*-butyl congener (9) achieved a remarkable improvement. The enhancement led us to elaborate some more homologs (10–13) having a tertiary alkyl group (C_3 , C_6) in the 6-position on the chromone ring. They all did not seem to surpass a *t*-butyl congener (9), however, although correlations between *in vitro* and *in vivo* activities were by no means strictly quantitative and no simple terms could explain the activity discrepancy found. Halogeno derivatives (14–17) also showed comparably high activity. But an additional introduction of a halogen atom into the benzene ring (18, 19) was not advantageous to the activity.

In addition, the 2-amino group in chromone(s) 4 and/or 9 was acylated and methylated to clarify the scope and limitations of modification. Formylation using formic acid attenuated the intrinsic activity and produced inert fused products (20, 22), in which 2-amino and 3-carboxamide groups were intramolecularly annelated to form a pyrimidine ring. The acylation and methylation of compound(s) 4 and/or 9 provided the corresponding active products (21, 23–26). The acylation or methylation of the amino group did not result in a remarkable reduction of the fungicidal activity.

In conclusion, chromone 9 was the most active of all compounds examined against major fungal diseases of rice plant such as sheath blight (Table 1), rice blast and bakanae disease (Table 2), curatively rather than preventively, in the greenhouse test. Based on the results of the present study, compound 9 was chosen for field evaluation and found to be quite comparable or relatively superior to the reference standard, ferimzone, in efficacy against sheath blight (data not presented). Chromone 9 paved the way for the development of a whole new series of pesticidally useful compounds, but gave way to ferimzone in commercialization from the viewpoints of integrated evaluation and marketability. Nevertheless, in conjugation with the studies on 2-pyrimidinylhydrazones in the previous papers,¹⁻³⁾ the performance described in this report well stressed the availability of a random screening program in which serendipity plays a prominent role. Now pesticide chemistry is moving towards rational molecular designing based on QSAR and CAMD, but random screening abreast with analog syntheses, including isosteric substitution in a traditional manner, will still predominate and be rewarding for the time being.

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要 約

2-アミノクロモン-3-カルボキサミド類の殺菌活性

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既存農薬の化学構造には見られない新規な基本骨格を有する新農薬活性化化合物創製の手掛かりを得る目的で初めたランダム・スクリーニング試験を続行し, 先に報告した 2-ピリミジニルヒドラゾン系化合物に次いでクロモン系化合物の一種がイネ紋枯病菌などに顕著な殺菌活性を有することを見いだした。この手掛かりを農薬用新殺菌剤創製に向けて発展させるべく, 化学構造と殺菌活性の相関性に配慮しつつ, 新たに一連の関連化合物を合成し試験した。その結果, 分子内のアミノ基とカルボキサミド基が活性発現に必須の要素であり, したがって構造を修飾しうる範囲は狭く, わずかにアミノ基のアシル化とアルキル化およびベンゼン環への各種置換基の導入に留まった。しかし, 置換基としての 6-位の分枝状アルキル基, とくに第3級ブチル基が活性にとって好ましく, 対照化合物の 2-ピリミジニルヒドラゾン系化合物, ferimzone に比肩しうるまたはこれを陵駕する活性化化合物 (9) を得ることができた。