Development of Pesticides Based on Phytoalexins. Part 1: Design and Synthesis of Flavanone Analogues via Bioisosterism Substitution

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In order to search for novel fungicides with high activity, a series of 2-heteroaryl-4-chromanones were designed and synthesized via bioisosterism substitution. The structures of all new compounds were confirmed by spectroscopic methods and microanalyses. Preliminary bioassays indicated that some compounds showed fungicidal activity against *Rhizoctonia solani*, *Fusarium oxysporum*, *Physalospora piricola* and *Cercospora beticola*.

Keywords 2-Heteroaryl-4-chromanones, phytoalexins, bioisosterism substitution, fungicidal activity

Introduction

Recently, the discovery of plant activator of benzothiadiazoles may be regarded as an important milestone in the history of fungicidal development due to the revolutionary change of fungicidal action model. Studies on the mechanism of action of these compounds have implicated the immune system as the site of antifungal action. These compounds embody fungicidal activity by activating plant immune system rather than killing fungal directly. Since then, development of novel environmentally-kind fungicides targeted immune system of plants has become a very hot research field.[1-3] Now, there are two ways to develop novel fungicides targeted plant immune system. One is activator of immune system; the other is structural modification of plant immune substance. This paper belongs to the second situation.

Phytoalexins are low molecular weight chemical that immune system of plants produce and accumulate in response to infection especially of fungal origin. However, in comparison with modern synthetic fungicides, phytoalexins are poorly active *in vitro* (ED_{50}: 10^{-4}—10^{-5} M) and almost inactive in *in vivo* tests. Therefore, much attention has been paid to the structural modification of some phytoalexins with the aim of obtaining more active compounds in the past two decades, but so far no commercial fungicide has been obtained.[4-7]

Flavanone is a kind of important phytoalexin inhibiting several biological properties. A drawback of these phytoalexins is that they are not easily translocated in the plant tissues due to the existence of polyhydroxyl groups in their molecular structure. Many studies have been done on the structural modification and structure-activity relationships of flavanone derivatives in the past decades.[8-10] But all of the structural modifications are only concerned with the substituents on benzene rings rather than the skeletal structure and all of structure-activity relationships are qualitative results. In addition, bioisosterism represents one important approach used by the medicinal and pesticidal chemist for the rational modification of lead compounds into more effective

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Received October 23, 2000; revised and accepted January 5, 2001.
Project supported by the National Natural Science Foundation of China (No. 29802002), the Natural Science Foundation of Hubei Province, the Dawn Plan of Science and Technology for Young Scientists of Wuhan City and Foundation for University Key Teacher by the Ministry of Education of China.

agents.[11] We have paid attention to the successful uses of the classical bioisosteres benzene, thiophene, furane and pyridine in literatures. To develop novel fungicidal structures and also to improve the hydrophobicity of flavonane phytoalexins, we have modified the skeletal structure of flavonane in view of bioisosterism and designed the title compounds, 2-heteroaryl-4-chromanones **4** by replacing the benzene ring with thiophene, furane or pyridine ring. Herein, we describe the synthesis and fungicidal activity of the designed title compounds.

Experimental

Melting points were determined with a Yanaco MT-500 apparatus without correction. ^{1}H NMR spectra were taken on a Bruker AC-P200 spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The nuclei that are deshielded relative to their respective standards are assigned a positive chemical shift. Mass spectra were recorded on a Hewlett-Packard 5988 instrument. Elemental analyses were carried out on a Yana MT-3 instrument. All solvents and materials were of reagent grade and purified as required. The intermediate **2** was prepared according to the reported method.[12] The purity of these products was tested by TLC and their characterization data are recorded in Tables 1 and 2, respectively.

Table 1 Physical constants of compounds **4**

No.	Het[a]	R	Reaction time (h)[b]	Eluting solvent[c]	mp (℃)	Yields (%)[d]	Elemental analysis (Calcd/Found, %)		
							C	H	N
4a	A	6-Br	20	PE:E=4:1	79—80	25.0	50.48/50.67	2.91/3.14	—
4b	A	7-CH_3	24	PE:E=4:1	72—73	26.5	68.85/68.57	4.92/5.13	—
4c	A	7-CH_3O	42	PE:E=4:1	88—89	37.5	64.62/64.77	4.62/4.43	—
4d	A	H	14	PE:E=4:1	82—83	31.5	67.83/67.56	4.35/4.55	—
4e	B	6-Br	22	PE:E=4:1	92—94	25.0	53.24/53.51	3.07/2.86	—
4f	B	H	24	PE:E=4:1	92—94	31.0	72.89/73.11	4.67/4.49	—
4g	B	7-CH_3O	40	PE:E=4:1	85—86	20.0	68.85/68.57	4.92/4.76	—
4h	B	7-CH_3	22	PE:E=4:1	96—97	25.0	73.68/73.89	.26/5.53	—
4i	C	7-CH_3O	36	PE:E=4:1	92—93	43.0	70.59/70.81	5.09/4.86	5.49/5.23
4j	C	6-Cl	16	PE:E=4:1	96—97	45.0	64.73/65.01	3.85/3.54	5.39/5.62
4k	C	6-CH_3	40	PE:E=4:1	81—82	41.0	75.31/75.59	5.43/5.71	5.85/6.17
4l	C	H	20	PE:E=4:1	38—39	47.0	74.66/74.88	4.88/4.57	6.22/6.43
4m	C	6-Br	12	PE:E=4:1	106—108	44.0	55.26/55.60	3.28/3.53	4.60/4.74
4n	D	H	12	PE:A=3:1	—	30.0	74.66/74.39	4.88/5.06	6.22/5.97
4o	D	6-Br	25	PE:A=3:1	93—94	51.0	55.26/54.99	3.28/3.51	4.60/4.83
4p	D	6-CH_3	24	PE:A=3:1	87—88	50.0	75.31/75.67	5.43/5.19	5.85/6.11
4q	D	6-Cl	21	PE:A=3:1	94—95	43.7	64.73/64.48	3.85/3.58	5.39/5.04
4r	D	7-CH_3	16	PE:A=3:1	66—67	45.7	75.31/75.60	5.49/5.28	5.85/6.00
4s	D	7-CH_3O	15	PE:A=3:1	66—68	40.0	70.59/70.26	5.09/5.27	5.49/5.81

[a] A: 2-thienyl; B: 2-furanyl; C: 2-pyridinyl; D: 3-pyridinyl. [b] Refers to the reaction time of cyclization of chalones **3** to **4**. [c] PE: petroleum ether (60—900℃); E: ethyl ether; A: acetone. [d] Yield of cyclization of chalones **3** to **4** determined by column chromatography isolation.

General procedure for the preparation of chalcones **3**

To a vigorously stirred solution of acetophenone **2** (5 mmol) and heteroaryl aldehyde (5 mmol) in 40 mL of absolute ethanol, 3 g of sodium hydroxide was added at 55℃ (or at room temperature for pyrimidinyl aldehyde). TLC recorded the resultant reaction mixture. After the reaction finished, the whole mixture was poured into ice-H_2O, acidified to pH = 6—7 with 1 M HCl and filtered off to give the chalcone **3**, which was used for the next reaction without further purification.

Table 2 Spectral and analytical data of compounds **4**

No.	^{1}H NMR (TMS, $CDCl_3$, δ)	MS (m/z, %)
4a	2.84—3.35(m, 2H, CH_2), 5.68(dd, $^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.82—7.98(m, 6H, C_6H_3 + C_4H_3S).	309(M^+, 15.41), 307(M^+-2, 14.10), 199(5.00), 197(4.43), 110(100), 63(56.53).
4b	2.32(s, 3H, CH_3), 2.82—3.30(m, 2H, CH_2), 5.65(dd, $^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.68—7.68(m, 6H, C_6H_3 + C_4H_3S).	244(M^+, 50.30), 134(45.46), 110(100), 77(53.81), 39(38.83).
4c	2.80—3.32(m, 2H, CH_2), 3.80(s, 3H, OCH_3), 5.68 (dd, $^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.42—7.85(m, 6H, C_6H_3 + C_4H_3S).	260(M^+, 71.06), 149(40.91), 122(41.95), 110(100), 79(49.10), 51(48.36).
4d	2.85—3.26(m, 2H, CH_2), 5.70(dd, $^3J_{2,3a}$ = 9.0 Hz, 1H, CH, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.90—7.92 (m, 7H, C_6H_4 + C_4H_3S).	230(M^+, 28.26), 110(100), 92(55.54), 65(31.31), 64(39.51), 63(47.03), 39(39.98).
4e	2.80—3.40(m, 2H, CH_2), 5.48(dd, $^3J_{2,3a}$ = 9.9 Hz, $^3J_{2,3e}$ = 4.5 Hz, 1H, CH), 6.28—7.98(m, 6H, C_6H_3 + C_4H_3O).	293(M^+, 14.03), 94(100), 66(31.98), 63(48.50), 39(30.33).
4f	2.79—3.40(m, 2H, CH_2), 5.48(dd, $^3J_{2,3a}$ = 9.9 Hz, $^3J_{2,3e}$ = 4.5 Hz, 1H, CH), 6.32—7.92(m, 7H, C_6H_4 + C_4H_3O).	214(M^+, 17.40), 120(21.31), 94(100), 92(51.19), 65(44.03), 64(37.56), 63(47.21), 39(63.00).
4g	2.71—3.40(m, 2H, CH_2), 3.78(s, 3H, OCH), 5.48(dd, $^3J_{2,3a}$ = 9.9 Hz, $^3J_{2,3e}$ = 4.5 Hz, 1H, CH), 6.26—7.82(m, 6H, C_6H_3 + C_4H_3O).	244(M^+, 51.95), 150(21.61), 122(34.52), 107(33.1), 94(100), 79(38.67), 65(43.64), 39(48.13).
4h	2.32(s, 3H, CH_3), 2.76—3.36(m, 2H, CH_2), 5.50 (dd, $^3J_{2,3a}$ = 9.9 Hz, $^3J_{2,3e}$ = 4.5 Hz, 1H, CH), 6.30—7.80(m, 6H, C_6H_3 + C_4H_3O).	228(M^+, 31.93), 134(28.28), 94(100), 78(44.92), 77(29.39), 65(39.00), 51(31.38), 39(57.45).
4i	2.85—3.28(m, 2H, CH_2), 3.78(s, 3H, OCH), 5.50 (dd, $^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.44—8.56(m, 7H, C_6H_3 + C_5H_4N).	255(M^+, 8.10), 150(27.72), 122(46.90), 107(47.17), 79(100), 78(37.38), 63(41.59), 51(89.12), 39(21.19).
4j	2.96—3.38(m, 2H, CH_2), 5.52(dd, $^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.92—8.58 (m, 7H, C_6H_3 + C_5H_4N).	260(M^+, 3.07), 154(65.23), 126(66.99), 104(39.08), 79(70.97), 78(41.28), 63(100), 51(62.72).
4k	2.30(s, 3H, CH_3), 2.84—3.34(m, 2H, CH_2), 5.50(dd, $^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.86—8.56(m, 7H, C_6H_3 + C_5H_4N).	239(M^+, 7.38), 194(20.45), 134(79.42), 105(51.39), 104(50.34), 79(49.88), 78(100), 77(50.98), 51(72.66).

Continued

No.	^{1}H NMR (TMS, $CDCl_3$, δ)	MS (*m/z*, %)
4l	2.84—3.36(m, 2H, CH_2), 5.50(dd, $^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.90—8.56 (m, 8H, C_6H_4 + C_5H_4N).	225(M^+, 1.93), 120(55.67), 92(100), 79(37.94), 78(27.47), 63(46.18), 51(46.88), 39(29.69).
4m	2.96—3.38(m, 2H, CH_2), 5.56(dd, $^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.88—8.58 (m, 7H, C_6H_3 + C_5H_4N).	304(M^+, 4.75), 199(16.12), 197(16.50), 171(14.39), 169(14.12), 79(64.13), 78(31.60), 63(100), 51(50.85).
4n	2.76—3.26(m, 2H, CH_2), 5.50(dd, $^3J_{2,3a}$ = 10.8 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.92—8.76(m, 8H, C_6H_4 + C_5H_4N).	225(M^+, 24.11), 120(80.11), 92(100), 79(21.08), 78(15.13), 77(14.07), 63(38.60), 51(33.48), 39(19.51).
4o	2.74—3.26(m, 2H, CH_2), 5.50(dd, $^3J_{2,3a}$ = 10.8 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.86—8.72(m, 7H, C_6H_3 + C_5H_4N).	304(M^+, 15.83), 199(18.55), 197(17.50), 171(13.19), 169(12.67), 79(47.71), 78(26.28), 63(100), 51(46.94).
4p	2.30(s, 3H, CH_3), 2.74—3.24(m, 2H, CH_2), 5.46(dd, $^3J_{2,3a}$ = 10.8 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.86—8.72(m, 7H, C_6H_3 + C_5H_4N).	239(M^+, 23.54), 134(72.62), 105(67.00), 79(28.87), 78(100), 77(69.98), 63(20.14), 51(89.04), 39(32.74).
4q	2.74—3.26(m, 2H, CH_2), 5.50(dd, $^3J_{2,3a}$ = 10.8 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.94—8.76 (m, 7H, C_6H_3 + C_5H_4N).	260(M^+, 6.45), 258(15.70), 156(12.16), 154(35.71), 128(15.92), 126(49.85) 104(31.82), 79(43.33), 78(27.29), 77(22.52), 63(100), 51(59.13), 39(29.28).
4r	2.36(s, 3H, CH_3), 2.76—3.24(m, 2H, CH_2), 5.48(dd, $^3J_{2,3a}$ = 10.8 Hz, $^3J_{2,3e}$ = 5.4 Hz, 1H, CH), 6.82—8.72(m, 7H, C_6H_3 + C_5H_4N).	239(M^+, 25.00), 134(64.11), 105(59.09), 79(28.07), 78(100), 77(58.26), 63(20.67), 51(68.97), 39(34.63).
4s	2.72—3.20(m, 2H, CH_2), 3.80(s, 3H, OCH), 5.48(dd, $^3J_{2,3a}$ = 10.8 Hz, $^3J_{2,3e}$ = 5.34 Hz, 1H, CH), 6.44—8.70(m, 7H, C_6H_3 + C_5H_4N).	255(M^+, 56.50), 150(68.60), 122(79.02), 106(68.19), 79(92.89), 78(29.19), 77(24.44), 63(46.39), 51(100), 39(27.23).

General procedure for the preparation of 2-heteroaryl-4-chromanones **4**

A solution of chalone **3** (4 mmol) and NaOAc (3.2 g) in 40 mL of absolute ethanol with 3 drops of H_2O was refluxed for about 24 hours. The reaction mixture was diluted with cold water (20 mL) and extracted with CH_2Cl_2. The organic layer was washed with H_2O and saturated NaCl solution and dried ($MgSO_4$). The solution was evaporated to dryness, and the residue was chromatographed over Si gel to give the title compounds **4**.

Fungicidal test

The four fungi used, *Rhizoctonia solani* (*R. s.*), *Fusarium oxysporum* (*F. o.*), *Physalospora piricola* (*P. p.*) and *Cercospora beticola* (*C. b.*) belong to the group of field fungi and were isolated from various grains. The effect of synthetic flavanone analogues and 4′,5,7-trihydroxyflavanone on growth of the test fungi was investigated in solid medium. The medium contained malt extract, 30; agar, 15; peptone 3 g/L in distilled water. Samples were dissolved in acetone, so that the final concentration of solvent in the medium was

11 mL/L. Medium (15 mL) including the particular sample in required concentrations was transferred to Petri dishes (90 mm) and inoculated with one small piece of mycelium (1 mm in diameter). The dishes were incubated at 25℃ in the dark. Mycelial diameters of *Rhizoctonia solani* (*R. s.*), *Fusarium oxysporum* (*F. o.*), *Physalospora piricola* (*P. p.*) and *Cercospora beticola* (*C. b.*) were measured after 3 days. Evaluation was carried out by measuring the diameter of the colonies three times at different sites. The mean value of six repetitions for each fungus was used for calculation. The data were evaluated by analysis of variance. Probability of single differences was calculated at the 5% level. All data are statistically significant at this level.

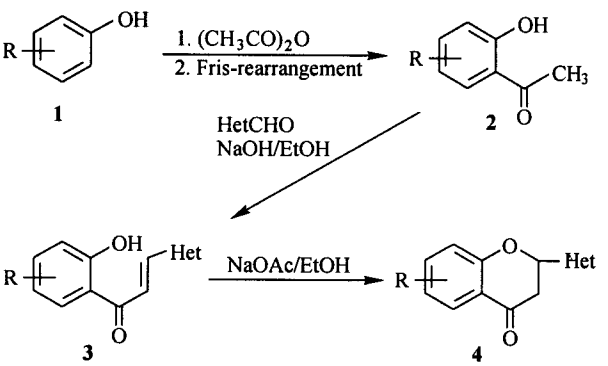
Results and discussion

Synthesis and characterization of the title compounds

The synthetic pathway for the title compounds is outlined in Scheme 1. The synthesis started with substituted phenols **1**, which were acetylized with acetic anhydride followed by Fris-rearrangement at 110—120℃ to give substituted acetophenones **2** according to the reported method.[12] Condensation of **2** with heterocyclic aldehydes proceeded in aqueous alcoholic alkali yielding chalcones **3**. Compounds **3** were cyclized by refluxing in a solution of NaOAc in EtOH to give the 2-heteroaryl-4-chromanones **4**.

All the products **4** were purified by chromatography over Si gel. The structures of the products **4** were confirmed by ^{1}H NMR and MS spectroscopy as well as elemental analyses. The experimental data for **4** were listed in Tables **1** and **2**, respectively. Taking **4a** as a representative example, its ^{1}H NMR spectrum showed methine and methylene protons of the oxygen heterocyclic ring at δ 5.68 as a quartet ($^3J_{2,3a}$ = 9.0 Hz, $^3J_{2,3e}$ = 5.4 Hz) and at δ 2.84—3.35 as a multiplet ($^3J_{3a,2}$ = 9.0 Hz, $^3J_{3e,2}$ = 5.4 Hz), respectively. The aromatic protons displayed a multiplet at δ 6.82—7.98. In addition, the EI-MS spectra of **4** demonstrate the existence of the molecular ion peaks. All the fragmentation ions are consistent with their structures and can be clearly assigned. For example, compound **4q**, under electron impact, gives the molecular ion peak *m/z* (%): 259 (15.70), and the other conspicuous peaks: 181(4.33), 154(35.71), 126(49.85), 105(31.82), 91(3.93), 79(43.33), 63(100).

Scheme 1



Fungicidal activities

Fungicidal activities of some compounds against *Rhizoctonia solani* (*R. s.*), *Fusarium oxysporum* (*F. o.*), *Physalospora piricola* (*P. p.*) and *Cercospora beticola* (*C. b.*) were evaluated *in vitro* at a concentration of 50 ppm and 4′, 5, 7-trihyoxylflavanone was used as a reference. The test result was shown in Table 3. From Table 3 we can conclude that compounds **4h** and **4k** display much better inhibitory activities against *P. p.* and *F. o.* than 4′, 5, 7-trihydroxyflavanone,

Table 3 Fungicidal activities of some compounds (50 ppm, % difference in mycelial diameter as compared with the control)

	Inhibition rate (%)																	
Compd.	**4a**	**4b**	**4c**	**4e**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**	**4m**	**4n**	**4o**	**4p**	**4q**	**4r**	**4s**	Ref*
R. s.	22	16	18	37	10	40	—	26	0	8	27	—	22	70	20	8	6	67
F. o.	51	28	60	11	56	60	44	3	100	70	2	80	—	—	15	17	4	51
P. p.	54	54	—	14	3	90	50	51	52P	41	—	60	54	52	51	41	3	75
C. b.	31	16	62	63	15	70	—	7	56	15	32	40	3	54	56	70	64	69

* 4′,5,7-trihydroxyflavanone.

which indicates the feasibility of structural modification of flavanone phytoalexins via bioisosterism substitution. Further studies on fungicidal activities *in vivo* and quantitative structure-activity relationships are on the way.

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(E200010229 JIANG, X.H.; DONG, L.J.)