Synthesis and Pollen Suppressant Activity of Phenylcinnoline-3-carboxylic Acids

William J. Guilford,*,† Thomas G. Patterson, Rene O. Vega, Lawrence Fang, Yi Liang, Hal A. Lewis, and Jeffrey N. Labovitz

Sogetal, Inc., 3876 Bay Center Place, Hayward, California 94545

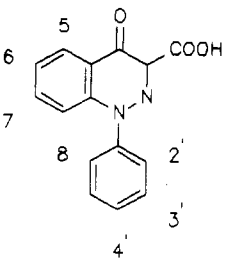
A series of 1,4-dihydro-4-oxo-1-phenylcinnoline-3-carboxylic acids were prepared, and their pollen suppression activity on wheat (*Triticum aestivum* L.) was evaluated. The substituents and substitution pattern on the cinnoline ring were varied systematically with particular interest shown to the substituent at C-5. Significant pollen suppressant activity is described for analogues bearing a heteroatom substituent, such as fluorine or alkoxy, at C-5 of the cinnoline ring. Compounds bearing an amino substituent showed little or no activity, and compounds with methyl substitution at C-5 were inactive. Variation in the substituents on the phenyl ring was carried out in parallel and was found to modulate the activity of the compounds. In addition to the pollen suppression activity measurement, the phytotoxic effect of the compounds on wheat was observed as chlorosis, plant height reduction, or necrosis. However, no effect on seed quality was seen. The leading compounds for use as chemical hybridization agents are members of the alkoxy series containing less than four carbon atoms, which have the best balance of high sterility and low phytotoxicity.

INTRODUCTION

The production of hybrid wheat (*Triticum aestivum* L.) may provide an opportunity for significant improvement in wheat quality, as well as a significant increase in wheat yield (Wilson, 1984). However, the commercial exploitation of the phenomenon of hybrid vigor in wheat is currently limited by the unavailability of suitable methods for the production of hybrid seed (Cubitt et al., 1988). Although cytoplasmic male sterile (CMS) lines have been used commercially to produce wheat hybrids, the lack of a consistent restorer system for genetic restoration of fertility and the time required to develop a CMS line remain major problems. An alternative that has received considerable attention is the use of chemical hybridization agents (CHA) which do not require either restoration or conversion of parental lines to a CMS background (Cross and Ladyman, 1991; Kaul, 1988; Wilson, 1984).

The chemical induction of male sterility through the suppression of pollen formation is a rapid and flexible method to prepare hybrids. Although several CHAs that induce complete pollen sterility in wheat have been reported in the literature, most agents induce strong phytotoxic responses in the plants, which cause considerable damage to the plant or result in poor seed quality (McRae, 1985). The key to the use of a CHA in the commercial production of hybrid wheat is achieving high male sterility while maintaining a high degree of female fertility.

Substituted analogues of 1,4-dihydro-4-oxo-1-phenylcinnoline-3-carboxylic acid (1) represent a new class of pollen suppressant agents for wheat (Labovitz et al., 1989; Labovitz and Fang, 1986, 1988). Although the synthesis of substituted cinnolines is well-known [see Castle (1973) for a review of the literature], no routes to the desired 1-aryl-1,4-dihydro-4-oxocinnoline-3-carboxylic acids (1) had been reported until relatively recently (Ames et al., 1983; Sandison et al., 1974). Even so, the synthesis of specifically substituted analogues of 1 has required the development of new synthetic schemes to overcome the lack of regiospecificity in the reported methods. The methods were expanded further with the rapid introduction of amino and alkoxy substitution at C-5 of the phenylcinnoline ring.



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In this paper we describe the results of our structure–activity optimization study to produce compounds having the best balance of pollen suppressant activity with minimal phytotoxicity.

MATERIALS AND METHODS

Chemical Section. Synthetic Methods. General Procedures. Proton NMR spectra were obtained on a JEOL FX90Q spectrometer with tetramethylsilane as an internal standard. Proton NMR spectra were obtained on all substituted phenylcinnoline analogues using DMSO-d_6 as solvent. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The purity of all substituted phenylcinnolines analogues was determined to be greater than 95% by HPLC analysis on a C-18 column using 35% tetrabutylammonium phosphate buffer (10 mM, pH 7.4) in methanol as solvent. All chemicals were of analytical grade (Aldrich, Milwaukee, WI). All solvents were of high purity grades and used without further purification.

The following procedure is an example of the route used to prepare the substituted phenylcinnoline analogues listed in Table I.

An Example of Synthesis of Substituted Benzoyl Chloride (B): 2,6-Difluorobenzoyl Chloride. A solution of 100 g of 2,6-difluorobenzoic acid (0.63 mol) in 210 mL of ether and 3 mL of dimethylformamide was stirred as 70 mL of neat oxalyl chloride (0.81 mol) was added at a rate to maintain the reaction

† Present address: ImmuLogic Pharmaceutical Corp., 855 California Ave., Palo Alto, CA 94304-1203.

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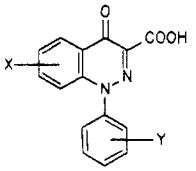
Table I. Physical Properties of Phenylcinnoline Analogues, A-Ring Variation



compd	X	Y	method	mp, °C	yield[a]
2	5-F	4′-CF_3	A	236–240	59
3	5-F	4′-OCF_3	A	205–209	46
4	5-F	2′,4′-diCl	A	225–230	48
5	5-F	2′-CF_3	A	225–230	49
6	5-F	3′,4′-diF	A	257–260	46
7	6-F	4′-Cl	A	274–277	56
8	6-F	2′-F	A	236–238	50
9	7-F	4′-Cl	A	293–295	43
10	7-F	4′-CF_3	A	292–294	53
11	8-F	4′-Cl	A	279–282	53
12	8-F	4′-CF_3	A	277–279	47
13	5-Cl	4′-Cl	B	247–251	46
14	5-Cl	4′-OCF_3	B	208–214	42
15	6-Cl	4′-OCF_3	A	265–269	45
16	6-Cl	2′-F	A	248–250	45
17	7-Cl	4′-Cl	A	295–298	40
18	8-Cl	4′-CF_3	A	235–248	29
19	8-Cl	4′-Cl	A	239–243	37
20	5-CH_3	4′-Cl	B	283–285	27
21	6-CH_3	4′-Cl	A	290–292	42
22	6-CH_3	2′-F	A	257–259	33
23	8-CH_3	4′-Cl	A	283–285	20
24	8-CH_3	4′-CF_3	A	288–291	37
25	5-OCH_3	4′-Cl,2′-F	B	265–268	49
26	5-OCH_3	4′-Cl	B	300–305	44
27	5-OCH_3	4′-CF_3	B	280–284	46
28	6-OCH_3	4′-CF_3	A	295–302	45
29	6-OCH_3	2′-F	A	277–280	45
30	7-OCH_3	4′-CF_3	A	303–307	43
31	7-OCH_3	4′-Cl	A	279–283	39
32	8-OCH_3	4′-Cl	A	301–303	41
33	8-OCH_3	2′-F	A	270–273	45
34	H	4′-Cl	A	277–279	51
35	H	4′-F	A	258–261	30

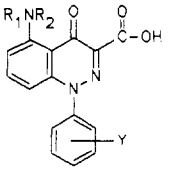
[a] Overall yield from starting benzoic acid.

temperature below 25 °C. The solvent was removed under reduced pressure. Excess oxalyl chloride was removed by codistillation with hexane, and 108 g of 2,6-difluorobenzoyl chloride was isolated in 95% yield: ^{1}H NMR ($CDCl_3$) δ 6.92 (d, 1 H), 7.02 (d, 1 H), 7.50 (m, 1 H).

An Example of the Synthesis of Substituted Methyl Benzoylacetate (C) via Route I: Methyl (2,6-Difluorobenzoyl)acetate. A solution of 89 g of Meldrum's acid (0.62 mol) and 150 g of 4-(dimethylamino)pyridine (DMAP, 1.23 mol) in 1 L of methylene chloride was stirred at 0 °C as 108 g of neat 2,6-difluorobenzoyl chloride (0.61 mol) was added under a nitrogen atmosphere. The reaction was stirred at 0 °C for an additional 3 h, before the solvent was removed under reduced pressure. The resulting thick slurry was stirred as 225 mL of methanol containing 38 mL (0.67 mol) of concentrated sulfuric acid was added. The reaction was heated at reflux for 3 h and solvent removed under reduced pressure. The residue was dissolved in dichloromethane (500 mL) and washed with water. The organic layer was diluted with 2 volumes of hexane and dried over sodium sulfate, and solvent was removed under reduced pressure to give 89 g (68%) of a clear liquid. The product was a 2:1 mixture of the keto and enol forms. Keto form: ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3 H), 3.95 (s, 2 H), 6.85 (d, 1 H), 7.03 (d, 1 H), 7.35 (m, 1 H). Enol form: ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3 H), 5.38 (s, 1 H), 6.82 (d, 1 H), 7.00 (d, 1 H), 7.34 (m, 1 H).

An Example of the Synthesis of Substituted Methyl Benzoylacetate (C) via Route II: Methyl (2,6-Dimethylbenzoyl)acetate. A solution of lithium diisopropylamide in 200 mL of tetrahydrofuran, prepared from 17 mL of diisopropylamine (0.12 mol) and 42 mL of *n*-butyl lithium (2.6 M, 0.11 mol), was cooled

Table II. Physical Properties of 5-Aminophenylcinnoline



compd	R_1	R_2	Y	mp, °C	yield[a]
36	CH_3	CH_3	4′-Cl	271–276	93
37	CH_3	CH_3	4′-F	242–245	59
38	CH_3	CH_3	4′-CF_3	283–285	87
39	CH_3	H	4′-Cl	323–325	95
40	CH_3	CH_2CH_3	4′-Cl	233–237	53
41	CH_2CH_3	CH_2CH_3	4′-Cl	234–238	16
42	CH_2CH_3	CH_2CH_3	4′-F	191–201	67
43	$(CH_2)_2CH_3$	H	4′-Cl	245–247	92
44	$(CH_2)_2CH_3$	CH_3	4′-F	204–208	96
45	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	4′-Cl	180–187	81

[a] Yield from corresponding 5-fluorophenylcinnoline.

in a dry ice–2-propanol bath and stirred as a solution of methyl acetate (7.9 mL, 0.1 mol) in 8 mL of tetrahydrofuran was added dropwise. The solution was stirred for 15 min before the dropwise addition of a solution of 2,6-dimethoxybenzoyl chloride (11 g, 50 mmol) in 10 mL of tetrahydrofuran. The reaction was allowed to warm to room temperature over 3 h. After the addition of an equal portion of water, the solution was extracted with three equal portions of ether. The combined organic layers were twice washed with an equal portion of 0.1 N aqueous potassium hydroxide. The basic fractions were combined, neutralized with concentrated hydrochloric acid, and extracted three times with an equal portion of ether. The combined ether extracts were washed with water (two times) and a brine solution, dried over sodium sulfate, decolorized with activated carbon, filtered through silica gel, and concentrated under reduced pressure. The desired methyl (2,6-dimethoxybenzoyl)acetate was isolated in 68% yield (8.1 g), with a purity of over 95%: ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3 H), 3.81 (s, 6 H), 3.88 (s, 2 H), 6.55 (d, 2 H), 7.28 (dd, 1 H).

An Example of the Synthesis of Substituted Phenylcinnoline Analogues: 5-Fluoro-1-(4′-chlorophenyl)-1,4-dihydro-4-oxocinnoline-3-carboxylic Acid. A solution of 53 g of 4-chloroaniline (0.4 mol) in 420 mL of methanol was stirred in an ice–salt bath as 104 mL of concentrated hydrochloric acid and an aqueous solution of 32 g of sodium nitrite (0.5 mol) and 60 mL of water were added dropwise in that order. A solution of the methyl (2,6-difluorobenzoyl)acetate (88 g, 0.4 mol) and 82 g of potassium acetate (0.8 mol) was stirred in 420 mL of methanol as the 4-chlorophenyldiazonium chloride solution prepared above was added. The temperature was held below 10 °C throughout the addition. The resulting precipitate was isolated by filtration, washed with water, and dried in vacuo overnight to yield 150 g (99%) of the desired hydrazone: ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 3 H), 6.98 (t, 2 H), 7.46 (m, 5 H).

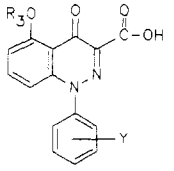
A solution of hydrazone (150 g, 0.4 mol) and a catalytic amount of 18-crown-6 was stirred in 300 mL of dry dimethylformamide as 59 g of solid potassium carbonate (0.43 mol) was added. The mixture was heated to and held at 100 °C for 1 h. The reaction mixture was cooled to room temperature and poured with 10 volumes of ice–water. The resulting precipitate was collected, washed with water, and dried in vacuo to yield 108 g (76%) of the desired phenylcinnoline: ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3 H), 6.58 (d, 1 H), 7.05 (dd, 1 H), 7.57 (m, 5 H).

A solution of phenylcinnoline methyl ester (108 g, 0.3 mol) in 450 mL of *p*-dioxane, 10 mL of water, and 8.3 mL of concentrated hydrochloric acid was heated at reflux for 4 h, cooled, and poured into 3 volumes of ice–water. The resulting solid was isolated by filtration and dried under reduced pressure to yield 72 g (72%) of a cream-colored solid: ^{1}H NMR (DMSO-d_6) δ 7.02 (d, 1 H), 7.35 (dd, 1 H), 7.70 (m, 5 H).

The following procedure is an example of the route used to prepare the 5-amino and 5-alkoxy compounds shown in Tables II and III, respectively.

Synthesis of 1-(4′-Chlorophenyl)-1,4-dihydro-4-oxo-5-propoxycinnoline-3-carboxylic Acid. A slurry of hexane-washed

Table III. Physical Properties of 5-Alkoxyphenylcinnoline Analogues



compd	R_3	Y	mp, °C	yield[a]
46	CH_3	4'-F	300–305	95
47	CH_2CH_3	4'-Cl	271–274	96
48	CH_2CH_3	4'-F	236–238	87
49	$CH(CH_3)_2$	4'-Cl	276–280	95
50	$CH(CH_3)_2$	4'-F	235–240	88
51	$(CH_2)_2CH_3$	4'-Cl	251–258	76
52	$(CH_2)_2CH_3$	4'-F	238–243	99
53	$CH_2CH(CH_3)_2$	4'-Cl	271–273	81
54	$CH_2CH(CH_3)_2$	4'-F	236–242	86
55	$(CH_2)_3CH_3$	4'-Cl	238–242	99
56	$(CH_2)_3CH_3$	4'-F	234–237	99
57	$CH_2CH{=}CH_2$	4'-Cl	240–243	99
58	$CH_2CH{=}CH_2$	4'-F	228–231	99
59	CH_2CCH	4'-Cl	260–263	90
60	CH_2CCH	4'-F	262–265	90
61	$CH_2CH{=}CHCH_3$	4'-Cl	218–220	86
62	CH_2COOH	4'-F	281–284	85
63	CH_3	unsubst	289–250	95
64	$CH(CH_3)_2$	unsubst	248–250	95
65	CH_3	2'-F,4'-Cl	265–268	99
66	$CH(CH_3)_2$	2'-F,4'-Cl	269–272	82
67	CH_3	3',4'-diF	283–287	80
68	$CH(CH_3)_2$	3',4'-diF	260–264	89
69	CH_3	2',3'-diF	272–276	95
70	$CH(CH_3)_2$	2',3'-diF	260–265	93
71	CH_3	4'-F,3'-Cl	283–286	92
72	$CH(CH_3)_2$	4'-F,3'-Cl	236–243	96
73	CH_3	2'-OCH_3	261–264	95
74	$CH(CH_3)_2$	2'-OCH_3	231–233	96
75	CH_3	4'-OCH_3	297–302	92
76	$CH(CH_3)_2$	4'-OCH_3	275–278	95
77	CH_3	4'-CF_3	280–284	85
78	$CH(CH_3)_2$	4'-CF_3	283–288	90
79	CH_3	4'-CH_3	311–314	99
80	$CH(CH_3)_2$	4'-CH_3	278–280	99

[a] Yield from corresponding 5-fluorophenylcinnoline.

sodium hydride (1.8 g, 38 mmol) in 10 mL of *p*-dioxane was stirred as 20 mL of dry propanol was added dropwise. The resulting hot solution was stirred as 3 g (9.4 mmol) of solid 1-(4'-chlorophenyl)-1,4-dihydro-5-fluoro-4-oxocinnoline-3-carboxylic acid was added at once. The resulting slurry was stirred for 16 h at ambient temperature. The reaction was poured onto ice-cold 1 N hydrochloric acid and the resulting solid isolated by filtration. The solid was washed with water and dried at 50 °C under vacuum to yield 3.2 g (95%) of a cream-colored solid: ^{1}H NMR (DMSO-d_6) δ 1.09 (t, 3 H), 1.84 (q, 2 H), 4.12 (t, 2 H), 6.67 (d, 1 H), 7.11 (d, 1 H), 7.71 (m, 5 H).

An Example of 5-Aminophenylcinnolines: Synthesis of 1-(4'-chlorophenyl)-1,4-dihydro-5-(dimethylamino)-4-oxocinnoline-3-carboxylic Acid. A solution of 5 g of solid potassium 1-(4'-chlorophenyl)-1,4-dihydro-5-fluoro-4-oxocinnoline-3-carboxylate (14 mmol), 1.25 g of dimethylamine hydrochloride (15.4 mmol), and 2.9 g of potassium carbonate (21 mmol) in 100 mL of deionized water was heated at reflux for 36 h. The reaction was allowed to cool, acidified with 6 N hydrochloric acid, and extracted with methylene chloride. The organic layer was washed with brine, dried over sodium sulfate, and concentrated to approximately 20 mL of liquid. The desired 5-(dimethylamino)-cinnoline was precipitated upon addition of 5 volumes of petroleum ether, isolated by filtration, washed with petroleum ether, and dried under reduced pressure to yield 4.5 g (94%) of an orange solid: ^{1}H NMR (DMSO-d_6) δ 2.91 (s, 6 H), 6.42 (d, 1 H), 6.92 (d, 1 H), 7.42 (t, 1 H), 7.69 (m, 5 H).

Biology Section. The biological assay data shown in Table IV are for phenylcinnoline analogues which varied in the substitution pattern of the A ring and were obtained in the 1985–1986 season. The observed biological responses are consistent with greenhouse data and field trials carried out in subsequent years (data not shown). The field trials were carried out in Davis, CA, and Amity, OR, between 1985 and 1991 using the following procedure.

Seven-row plots were planted in a north–south direction at a seeding rate of 100 kg/ha (late October). Male and female plots were 1.2 m × 15.2 m and planted in a 1:1 ratio. Fertilizer amendments consisted of 39 kg/ha of nitrogen at planting (16–20–0) followed by a single application of 112 kg/ha nitrogen (urea) in the spring. In Oregon, weeds were controlled by 2.8 kg/ha Karmex (preemergence) and tank-mixed 0.02 kg/ha Glean applied in early spring. In Davis, a single treatment of Buctril at a rate of 0.28 kg/ha was applied before tillering.

Although the compounds were applied at various stages of development, the optimum effect was observed at a growth stage characterized by a spike length of 2.5 cm. Application of the compounds was carried out with continuous dilution of the active ingredient along the plot. The use of the log spray plot allowed for dose ranges either from 1 to 0.12 or from 2 to 0.24 kg/ha to be studied on a single plot. Treatments were applied with a hooded sprayer with drop shields fitted at the ends of sprayer shroud to protect the adjacent male plots. Spray solution was delivered with eight T-jet, twin fan nozzles at 30 psi and a delivery volume of 600 L/ha. Compounds were applied as aqueous solutions, pH 8–9, with 0.1% Triton X-100 as a wetting agent. No phytotoxicity was observed in control plots sprayed with the wetting agent. The analogues were rated both on the ability to suppress pollen formation and on the phytotoxicity.

Pollen suppression activity was measured by covering spikes with Glassine bags prior to pollen release. The number of self-pollinated seeds in the bagged spikes was compared to the number of seeds in the untreated control spikes. The results shown in Table IV are given in percent sterility calculated from

$$\% \text{ sterility} = 100 - \frac{\text{no. of seeds in treated spike} \times 100}{\text{no. of seeds in untreated spikes}} \quad (1)$$

The phytotoxic effect of the phenylcinnolines was visually rated using the A, B, and C phytotoxicity index (PI) in the field trial reported in Table IV. A PI rating of A indicated a range that included plots that appeared the same as the standard check plot to uniform plots with tertiary tillers emerged but reduced in length or delayed in maturity. The PI rating of B indicates that the plot is uniform in height, but tertiary tillers may be significantly reduced in length, not fully emerged, or necrotic or may show signs of female sterility. However, a B rating can also include a wheat stand which is not uniform with primary tillers reduced in length and maturity delayed; secondary and tertiary tillers may show indications of female sterility. At a PI rating of C, the range in the appearance of plots varies from having all tillers not fully emerged with foliage significantly burned and primary tillers showing signs of female sterility to plots that are necrotic. No effect on seed quality was seen at any of the doses or phytotoxicity levels observed.

The biological assay for the 5-amino- and 5-alkoxy-substituted phenylcinnoline analogues, listed in Tables II and III, was conducted in the greenhouse at Sogetal, Inc., in Hayward, CA, between 1986 and 1988. Although the effective dose in the greenhouse was significantly lower than the equivalent field dose, the sterility and phytotoxicity results were consistent. The results, shown in Table V, are for specific trials with care taken to obtain trials carried out at similar times of the year and plant growth stage. The compounds were applied to groups of potted plants to the foliage of wheat in a semiautomated spray chamber as aqueous solutions, pH 8–9, containing 0.2% Triton AG-98 as a wetting agent. A set of single-application rates were used to test each compound. Wheat plants (Yecora Rojo) were treated when the developing spike reached a length between 1.5 and 2.5 cm. After treatment, the plants were placed in a greenhouse maintained in a temperature range of 65–75 °F.

In the greenhouse, the pollen suppression activity was measured by visual scoring of spikes for male sterility. Florets open wide in the male sterile spikes but remain tightly closed in fertile spikes. A sterility rating score of "–" indicated that the florets in the treated spikes remained closed and no sterility was induced. A rating score of "+" indicated that approximately 50% of the

Table IV. Sterility Score[a] (Numerical Value) and Phytotoxicity Value[b] (Letter) for Phenylcinnoline Analogues Varying on the A Ring at Different Doses

	dose, kg/ha									
compd	0.12	0.24	0.35	0.45	0.57	0.7	0.8	0.96	1.35	1.92
2		86 A	94 A	96 A	96 A	99 A	100 B			
3	50 A	84 A	89 A	95 B	82 B	100 B	100 B	98 B		
4	80 B	100 B	100 B	100 B						
5	4 A	19 B	24 A	16 A	19 A	38 A	31 A	29 A		
6	88 A	90 A	100 A	100 A						
7	13 A	4 A	9 A	32 A	10 A	28 A	14 A	9 A		
8		14 A		27 A		48 A		45 A	55 A	25 A
9										NA
10										NA
11										NA
12										NA
13	60 B	98 B	96 C	95 C						
15										NA
16										NA
17										NA
18										NA
19										NA
20										NA
21										NA
22										NA
23										NA
24										NA
25				100 C		100 C		100 C	100 C	
26		98 B		87 A		100 B		100 B	100 B	
27		74 A		91 A		93 A		99 A	99 A	
28		66 A								
29										NA
30										NA
31										NA
32										NA
33										NA
34										NA
35										NA

[a] Sterility values are based on seed count and are calculated according to eq 1. NA means no sterility or phytotoxicity was observed up to 2 kg/ha. [b] Visual phytotoxicity scores range between A and C.

treated spikes exhibited male sterility, and a rating of "++" indicated that all spikes were sterile. The phytotoxicity values were based on visual evaluation of the plants. The scoring system was based on a three-point scale: a value of A indicated no effect, a value of B indicated slight chlorosis or height reduction, and a value of C was indicative of severe chlorosis, severe height reduction, or necrosis.

The results for the 5-amino- and 5-alkoxyphenylcinnolines are summarized in Table V.

RESULTS AND DISCUSSION

Chemical Studies. The synthesis of the phenylcinnoline ring structure is divided into two parts: preparation of the substituted benzoylacetate (Scheme I) and hydrazone formation with cyclization to the cinnoline structure (Scheme II). The substitution pattern on the benzo portion of the cinnoline ring is determined by the substitution pattern on the starting substituted benzoic acid (A). The only limitation on the substitution pattern was the presence of a fluoro, methoxy, or nitro group at C-2 of the benzoic acid, since this group will be eliminated in the cyclization step later in the synthesis. The use of methoxy as a leaving group in a nucleophilic aromatic substitution reaction had been reported earlier using Grignard or alkyllithium reagents on activated aryl ethers (Meyers et al., 1975). The substitution on the phenyl ring is determined by the substitution pattern on the starting aniline and is limited by the availability of the aniline and the ability to convert the aniline to the corresponding diazonium salt.

Substituted methyl benzoylacetates (C) were prepared by one of two routes (Scheme I). In cases where the benzoyl ring was substituted with fluorine or the substitution pattern was 2,3-, 2,4-, or 2,5-substituted, route I was used. Conversion of the desired substituted benzoic acid (A) to the corresponding acid chloride (B) was carried out using oxalyl chloride in ether with a catalytic amount of dimethylformamide in excellent yield. Condensation of B with Meldrum's acid using (dimethylamino)pyridine (DMAP) as catalyst gave the desired substituted benzoylacetates (C, Scheme I, route I) (Oikawa et al., 1978). An alternative synthesis was required in cases where one of the two substituents on the 2,6-disubstituted benzoic acid was sterically bigger than fluorine (Scheme I, route II). In the alternative method, the enolate of methyl acetate was used instead of the enolate derived from Meldrum's acid. The desired substituted methyl benzoylacetate (C) was prepared in similar purity and yield.

The substituent and substitution pattern of the cinnoline ring was formed according to the route shown in Scheme II. The diazonium salt, prepared from the corresponding aniline, underwent condensation with substituted methyl benzoylacetate C to yield the desired hydrazone D. Cyclization to the desired cinnoline ring via an intramolecular nucleophilic aromatic substitution reaction was carried out in dimethylformamide using potassium carbonate as a base in excellent yield. In the final three steps of the synthesis, the products were isolated by filtration at a purity level of greater than 95%. This method was independently discovered and reported by Ames (Ames et al., 1983).

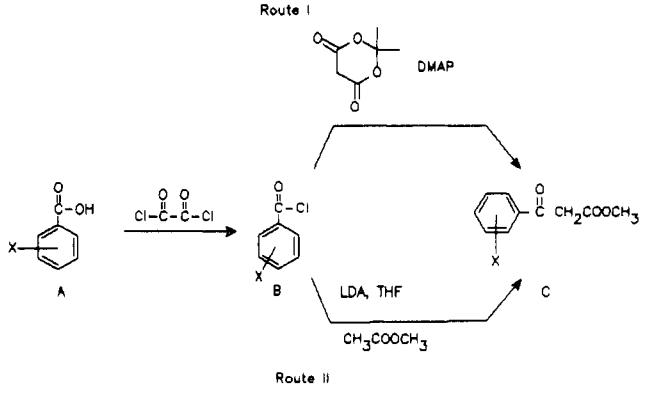
The synthesis of the 5-aminophenylcinnoline analogues was carried out through a nucleophilic aromatic substitution reaction between a mono- or disubstituted amine group and a 5-fluoro-1-aryl-4-oxo-1,4-dihydrocinnoline-

Table V. Sterility Score[a] (Numerical Value) and Phytotoxicity Value[b] (Letter) for 5-Amino- and 5-Alkoxyphenylcinnoline Analogues at Different Doses

	dose, g/ha									
compd	6	25	50	75	100	150	200	300	400	600
36			-A		-A		++B		++C	
37			-A		+B		++B		++C	
38				+A		++A		++B		++C
39				-A		-A		-A		-A
40			-A		-A		-A		+A	
41			-A		-A		-A		+A	
42			-A		+A		+A		+A	
43			-A				+A		+A	
44			-A		-A		-A		-A	
45			-A		+A		+A		+A	
46				+A		+A		++A		++A
47	-A	++B	++C		++C					
48	-A	++A	++B	++C						
49			+A		++A		++A		++A	
50	-A	+A	++A	++C						
51		+A	++A		++B		++B			
52		+A	++A		++A		++B			
53			-A		-A		++A		++A	
54			-A		+A		++A		++A	
55			-A		-A		-A		-A	
56			-A		-A		-A		-A	
57		++A	++B		++C		++C			
58		++B	++C		++C		++C			
59		++A	++B		++B					
60					-A		+A		++A	++B
61			+A		++A		++A		++A	
62			-A		-A		-A		-A	
63									+A	++A
64		++A	++B		++C		++C			
65			-A		-A		++A		++A	
66		++B	++C		++C		++C			
67		-A		-A		+A		++A		
68		+A	++B		++C		++C			
69									++A	++A
70		++B	++C		++C					
71		-A			+A		++A		++A	
72			+A		++A		++B			
73				-A		-A		-A		-A
74		-A	+A		++A		++A			
75			-A		+A		++A		++A	
76		+A	++A		++B		++C			
77				++A		++A		++A		
78			++C		++C		++C		++C	
79				-A		-A		-A		
80			++A		++A		++B		++C	

[a] Sterility values are based on visual observations made in a greenhouse. Scale defined as - for inactive, + for partial activity, and ++ for high sterility. [b] Phytotoxicity values are based on visual observations made in a greenhouse. Scale defined as A, no effect, B, minimal effect, and C, highly toxic.

Scheme I

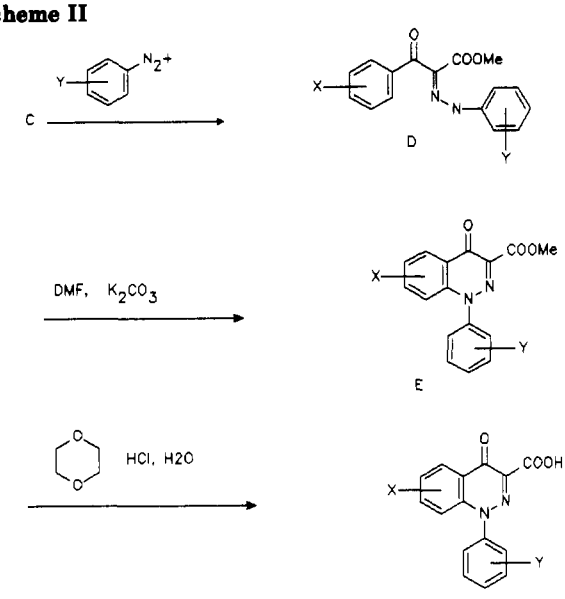


3-carboxylic acid (1) (Scheme III). In a similar fashion, the 5-alkoxyphenylcinnoline analogues were prepared by the substitution of an alcohol group for a fluorine at C-5 of the phenylcinnoline ring (Scheme IV). Variation in the substitution pattern on the phenyl ring was made through an appropriately substituted compound 1. This method of synthesis allowed for the rapid preparation of a series of analogues varying at C-5 from a single phenylcinnoline precursor.

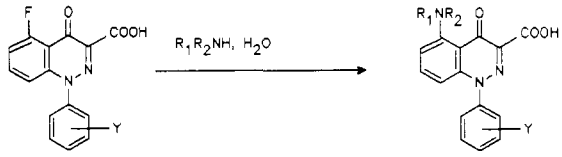
Biological Evaluation. Our initial studies have concentrated on wheat, a self-pollinating crop, although chemical hybridization agents are applicable to other crops (Patterson, patent applied for). In the method, two parent strains to be crossed are planted in alternating strips in a field. The female parent is treated with CHA to render this parent male sterile. Pollen from the male (untreated) parent then fertilizes the female parent. The seed produced by the female parent is an F1 hybrid, which is then harvested according to conventional techniques.

The first question addressed was the requirements for the substituent and substitution pattern on the cinnoline ring to obtain the best balance of sterility without phytotoxicity. The effect is clearly shown in data summarized in Table IV. Pollen suppression activity of greater than 98% for analogues was obtained for compounds bearing a fluorine group at C-5, **2–4** and **6**, a chlorine group at C-5, **13**, or a methoxy group, **25–28**. Related compounds

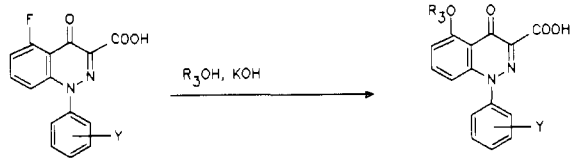
Scheme II



Scheme III



Scheme IV



5, 7, and 8 showed partial sterility. Compounds substituted at C-5 with a methyl, **20**, or unsubstituted compounds, such as **34** and **35**, or compounds with a hydrogen at C-5 and another substituent at C-6, C-7, or C-8 were inactive.

In addition to the sterility data, a visual phytotoxicity score is given for each compound at each dose. A phytotoxicity score of A indicates the compound could induce sterility with minimal toxic side effects. Six compounds, **2–4**, **6**, **26**, and **27**, showed the desired combination of high male sterility and no visible phytotoxicity over several doses. These compounds are candidates for use as chemical hybridization agents.

With the importance of the heteroatom substituent at C-5 of the cinnoline ring to achieve the desired biological response established, the effect of amino substituent, the size and shape requirements of the alkoxy substituent, and the influence of the phenyl ring substituent were probed.

In the 5-amino series, only compounds bearing a dimethylamino substituent, **36–38**, showed the desired male sterility (Table V). Biological activity fell off sharply as the length of the alkyl chain was increased to ethyl and propyl, regardless of the substituent on the phenyl ring. With the possible exception of compound **38**, no compound showed the desired combination of high male sterility and low phytotoxicity.

The size and shape requirements for biological activity were probed through the synthesis of a wide range of alkoxy substituents, compounds **46–64**. All of the 5-alkoxy analogues having less than four carbons in their side chain showed excellent male sterility induction. The most active compounds, i.e., compounds that required the lowest doses to completely suppress pollen formation, carried either an ethoxy, **47** and **48**, a 2-propoxy, **49** and **50**, an allyloxy, **57** and **58**, or a propargyloxy substituent, **59**, at C-5.

The influence of the phenyl ring substituent on the biological activity is demonstrated by a comparison of two series of phenyl-substituted analogues, one bearing a 2-propoxy group at C-5 and the other bearing a methoxy group at C-5 (**65–80**). Compounds bearing a 2-propoxy group at C-5 were more than 4 times as active as the corresponding compound bearing a methoxy group at C-5, except in the case of compounds **77** and **78** where the lowest dose to induce male sterility was similar. The phytotoxicity scores for the methoxy compounds were lower, in general, than those of the 2-propoxy substituent over a wide range of substituents including the halogens and fluoroalkyl. However, when the phenyl ring was substituted with methoxy, **76**, or methyl, **80**, little or no phytotoxicity was observed at doses having excellent male sterility. These results illustrate the dominant role played by the C-5 substituent in determining biological activity, with the phenyl ring substituent playing a modifying role. In addition, the importance of optimizing the substituent on both the cinnoline and phenyl ring to obtain the optimum biological activity is demonstrated.

Conclusion. As a result of our structure–activity optimization study, a new series of phenylcinnoline analogues bearing a fluorine or alkoxy group containing less than four carbon atoms at C-5 have been identified as candidates for use as chemical hybridization agents. However, the desired activity is seen only when the compound is applied to wheat at a particular plant stage and at specific doses. The best phenyl ring substituent in the 2-propoxy series was alkoxy or alkyl. Alternatively, compounds bearing a methoxy substituent at C-5 gave best results with a trifluoromethyl group on the phenyl ring. Several compounds showed the desirable combination of high sterility and low phytotoxicity needed for commercially useful chemical hybridization agents.

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