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Chemical Hybridizing Agents for Chickpea (*Cicer arietinum* L.): Leads from QSAR Analysis of Ethyl Oxanilates and Pyridones

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In the self-pollinated crops such as chickpea, induction of male sterility by deployment of chemical hybridizing agents (CHAs) facilitating "two-line" approach holds immense potential in heterosis breeding. A total of 40 test CHAs comprising 20 ethyl oxanilates and 20 pyridones were screened as potential CHAs on chickpea (variety BG 1088) at 500, 800, and 1000 ppm. Three test compounds mostly having either F (**4**)/Br (**5**)/CF_3 (**19**) at the *para* position of the aryl ring from a pool of 20 ethyl oxanilates were identified as the most potent CHAs causing ≥99% induction of pollen sterility and >90% total flower sterility at 1000-ppm test concentration. Among pyridone derivatives, *N*-(4-chlorophenyl)-5-carbethoxy-4,6-dimethyl, 1,2-dihydropyrid-2-one (**26**) was found to be the most active. Quantitative structure activity relationship (QSAR) analysis has revealed a direct involvement of Swain−Lupton field constant, F_p, with the target bioactivity which implied that field rather than resonance effect (*R*) had a positive effect on the activity. The real guiding principle for selectivity was found out to be the hydrophobic parameter π value. The QSAR models indicated that increased steric bulk at the 4-position on the phenyl ring is associated with enhanced activity. The CHAs appeared to act by mimicking UDP-glucose, the key substrate in the synthesis of callose, or lead to an imbalance in acid−base equilibrium in pollen mother cells resulting in dissolution of callose wall by premature callase secretion.

KEYWORDS: Chemical hybridizing agents (CHAs); chickpea; oxanilates; pyridones; quantitative structure activity relationship (QSAR)

INTRODUCTION

The production of hybrid chickpea (*Cicer arietinum* L.) offers an opportunity for increasing the yield of pulses. Among the pulses, chickpea is the third most important pulse crop in the world. At the present rate of consumption, the demand of pulses would increase annually by 3.3%. So, there is a need to step up the production on a sustainable basis by the development of hybrids. A viable and stable cytoplasmic-genetic male sterile (CGMS) system along with perfect restorer lines in chickpea is not in place though considerable research efforts are underway. In view of this, the other option by regulating pollen growth using chemical hybridizing agents (CHAs) needs to be pursued intensively (*1*). In the self-pollinated crops such as chickpea, wherein the male and female organs are in the same flower, selective sterilization of male organ (pollen) is of paramount importance in heterosis breeding, which can be achieved by the deployment of CHAs facilitating "two-line" approach. Male sterility supplements hand emasculation especially in a self-pollinated crop like chickpea where small flower size and flower dropping make crossing and emasculation a difficult task (*2*). Unlike the CGMS system, the CHAs have unique advantages of saving time and labor since no restorer−maintainer lines are required. Also, any profitable heterotic combination is apparently enormous (*3*). This is akin to the discovery of potent molecules from a large pool of synthetics. Lack of availability of safe and selective chemicals capable of induction of male sterility without causing any adverse effect on plant growth and development has been the constraint in the pursuit of this approach. Past attempts using herbicides and plant growth regulators, as gametocides, have not proved successful due to one reason or another (*4*). A number of CHAs have been reported for small grain cereals, viz. rice and wheat (*5*−*11*). Azetidines and aryl pyridones (RH-531) applied at the rate of 0.125−10 lb/acre induced >90% male sterility in wheat (*12*), but this was found to adversely affect the female fertility (*13*, *14*), where it retarded the stigmatal growth. Another CHA, viz. RH 532, was found to induce 100% male sterility in wheat at application rates of 1−3 kg/ha (*15*−*16*). However, it also adversely affected female fertility (*14*) and hybrid seed set on treated plants. The CHAs experimented on pulses are very few like NAA, IBA, Mendok (*17*), and dalapon, which have much less target activity. Thus, chemicals with targeted action are now being searched all over the world. Quantitative structure activity relationship (QSAR) analysis is a useful tool in elucidating essential structural features

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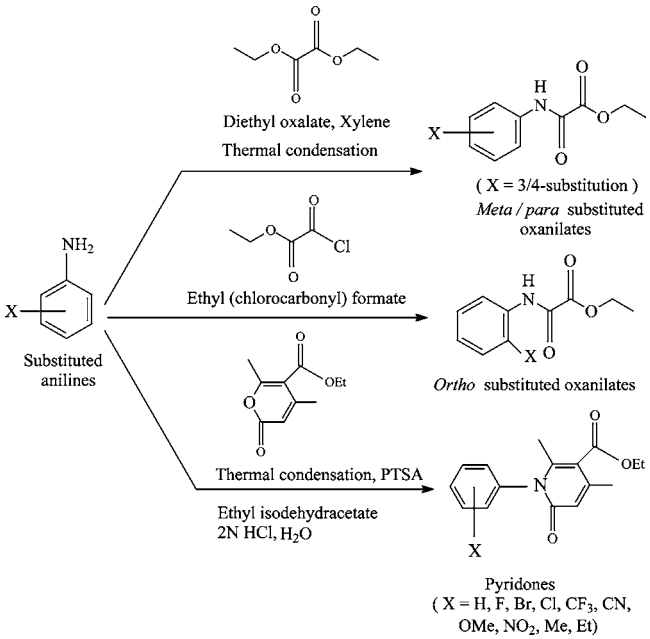


Figure 1. General procedure of synthesis of ethyl oxanilates (*ortho/para* and *meta* substituted) and pyridones.

that govern interaction of CHAs with the macromolecular receptor in the crop plants controlling pollen formation and its viability.

In a program of design and development of potential and targeted CHAs, we have already reported the deployment of *N*-acylanilines, anilides, and amino acid analogues in wheat (*18*−*21*). The present study consisted of synthesis, field evaluation, QSAR analysis, and possible mode of action of 40 test CHAs belong to two chemical classes, viz. ethyl oxanilates and pyridones, as potential CHAs for chickpea. On the basis of the preliminary screening, a short list of eight potent CHAs (showing ≥90% of induction of pollen sterility) were tested for their selectivity in action, and technology has been standardized toward the development of potent molecules as well as systematic ranking of CHAs on the basis of activity vis-à-vis selectivity.

MATERIALS AND METHODS

The compounds synthesized numbering 40 analogues belong to two broad chemical classes, viz. ethyl oxanilates and pyridones. The test compounds have been prepared by condensation between different substituted anilines with various esters. Ethyl oxanilates and pyridones containing variations at the acyl domain were synthesized by condensation of substituted anilines with appropriate diesters or acid chlorides or monoesters as described earlier (*18*−*21*). The compounds were purified using physical and chromatographic separation methods. The structures of synthesized compounds were confirmed by IR, ^{1}H NMR, and MS. Melting points (mp's) were determined by using a sulfuric acid bath and were uncorrected. All test compounds gave correct elemental analyses using a Euro Vector elemental analyzer (model EA3011).

General Procedure of Synthesis of Ethyl Oxanilates (1−20) and Pyridones (21−40). The following methods illustrate the general scheme of synthesis of the title compounds using different substituted anilines and various esters (**Figure 1**).

To a solution of aniline (0.025 mol) and toluene was added diethyl oxalate (0.03 mol). The reaction mixture was refluxed for 45 min, and ethanol was collected as an azeotrope. The reaction was followed by TLC (hexane−ethyl acetate (4:1) as developing medium) until completion. After cooling of the reaction mixture, a solid residue was obtained, which was triturated with boiling ethanol to give a white crystalline solid of *meta* and *para* aryl substituted ethyl oxanilates, which were homogeneous, by TLC (*18*). Ethyl 4′- fluoro oxanilate (**4**), a member of this family, was synthesized by thermal condensation of 4-fluoro aniline and diethyl oxalate. Yield 4.75 g (90%). Mp 118−119 °C (lit. 118.5 °C). TLC R_f: 0.50. GC R_t= 7.82 min. ^{1}HNMR ($CDCl_3$): δ 1.65 (t, J = 6 Hz, 3H, −CH_3), 4.70 (q, J = 6 Hz, 2H, −OCH_2), 7.40 (t, J = 6 Hz, 2H, H_b, $H_{b'}$ aromatic), 7.95 (d, J = 6 Hz, 1H, H_a aromatic), 8.10 (d, J = 6 Hz, 1H, $H_{a'}$ aromatic), 9.44 (s, 1H, *NH*). IR: 3333 (as. NH str), 1698 (C═O ester str), 1640 (amide-I band, C═O str), 1400 (CF str), 1295 (aromatic secondary δ_{CH}). EI-MS *m*/*z* (rel int %): 211 (M^+, 100), 139 (18), 138 (93), 137 (72), 110 (92), 75 (12), 83 (32), 63 (5).

A solution of ethyl(chlorocarbonyl) formate (0.25 mol) in $CHCl_3$ (10 mL) was added over a period of 30 min to a solution of *ortho* substituted aniline (0.25 mol) in $CHCl_3$ kept at ≤10 °C with continuous stirring under an inert atmosphere of nitrogen. The reaction was followed by TLC (hexane−ethyl acetate (4:1) as developing medium) until completion. The resultant reaction mixture was poured into ice-cold water, and a solid residue was obtained after evaporating the $CHCl_3$ layer. Recrystallization of the residue with ethanol yielded *ortho* substituted ethyl oxanilates as white crystalline solids, which was homogeneous by TLC (*18*). Ethyl 2′-fluoro oxanilate (**2**), a member of this family, was synthesized by condensation between 2-fluoro aniline and ethyl(chlorocarbonyl) formate. Yield 4.91 g (93%). Mp103 °C. TLC R_f: 0.63. GC R_t: 4.79 min. ^{1}HNMR ($CDCl_3$): δ 1.35 (t, J = 6 Hz, 3H, CH_3), 4.35 (q, J = 6 Hz, 2H, OCH_2), 7.20 (m, 3H, H_b, $H_{b'}$ and H_c aromatic), 7.50(m, 1H, H_a aromatic), 9.15 (s, 1H, *NH*). EI-MS *m*/*z* (rel int %): 211 (M^+, 14), 139 (40), 111 (100), 91 (18), 84(45), 83 (63), 64(41), 63 (36), 57(59), 52(23), 50(16).

A solution of substituted anilines (0.03 mol), *p*-toluenesulfonic acid, and ethyl isodehydracetate (0.015 mol) suspended in dry xylene was heated to reflux for 18−24 h. The resulting suspension was washed with dilute HCl and water to remove excess aniline and dried over anhydrous Na_2SO_4. The crude product obtained after the removal of xylene was purified by silica column chromatography using acetone−hexane (4:6) to furnish *N*-aryl-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-ones/pyridones as dark brown oils (*19*). *N*-(4-Chlorophenyl)-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-one (**26**), a member of the pyridone family, was synthesized by thermal condensation of 4-chloro aniline and ethyl isodehydracetate using an acid catalyst. Yield 6.29 g (66%). TLC R_f 0.27. GC R_t 15.78 min. ^{1}HNMR ($CDCl_3$): δ 1.30 (t, J = 6 Hz, 3H, OCH$_2$ CH_3), 1.80 (s, 3H, ═C(CH_3)N−), 2.12 (s, 3H, −C (CH_3)═), 4.22 (q, J = 6 Hz, 2H, OCH_2), 6.13 (s, 1H, ═C(*H*)−), 7.00 (d, J = 6 Hz, 2H, H_b, $H_{b'}$ aromatic), 7.37 (d, J = 6 Hz, 2H, H_a, $H_{a'}$ aromatic). EI-MS: *m*/*z* (%) 305 (M^+, 31), 262 (8), 260 (24), 250 (15), 248 (45), 234 (7), 232 (26), 231 (20), 213 (17), 204-(22), 169 (14), 168 (55), 167 (16), 154 (36), 152 (69), 138 (11), 127 (12), 113 (35), 111 (100), 77 (35), 75 (58), 67 (16), 65 (17), 63 (17), 53 (30), 52 (47).

Screening of Synthesized Compounds as CHAs on Chickpea (Var BG 1088). The high-yielding *Kabuli* variety of chickpea (*Cicer arietinum* L.), viz. BG 1088, recommended for North Western Plain Zone of India, was chosen for evaluation of chemical induction of male sterility. The experiment was laid out in a randomized block design with three replicates along with the control. The material was planted in a row of 2.5 m length with a row-to-row distance of 50 cm and plant-to-plant distance of 35 cm for each treatment in three replications at the Indian Agricultural Research Institute experimental farm. The recommended package of practices was followed throughout the season for a good crop. Other optimum agronomic practices were also followed which included recommended fertilizer and irrigation schedule, timely weeding, and other cultural operations. A solvent suitable for formulation of CHAs was identified on the basis of the solubility characteristics of the test CHAs. A stock solution of 40 EC (40000 ppm) was prepared by dissolving 120 mg of CHA in 3 mL of cyclohexanone and Tween-80 (polyoxysorbitan monooleate) (5%) as emulsifier which was diluted to working solution of 30 mL by distilled water to prepare 4000 ppm concentration. Spray emulsion (2000, 1000, 800, and 500-ppm) was obtained by appropriate serial dilution of stock solution (4000 ppm) by distilled water. To ensure phytosafety of cyclohexanone, a blank solvent emulsion was sprayed on the crop well in advance. No serious

phytotoxic symptoms were visible two to 3 days after spray. The synthetic compounds were tested in winter 2001−02, and sprayed at premeiotic stage (60 days after sowing). The test formulations were sprayed on the foliage till drenching. While spraying, utmost precaution was taken not to spill the chemicals on the control lines. The spraying was done in late evening hours when wind speed was very low.

The sterility parameters such as pollen sterility and total flower sterility were used for assessing the effectiveness of CHAs in inducing male sterility in chickpea. Data on parameters such as plant height, 100-seed weight, number of pods per plant at maturity, and associated phytotoxicity were recorded on five plants of each treatment and untreated controls. Analysis of variance of factorial randomized block design was performed with all treatments. On the basis of the significance of the treatments, least-squares differences (LSD) at 5% level of significance ($p = 0.05$) were computed. Pollen sterility both from control and treated plants was tested by KI−I_2 (2%) staining method. Pollen sterility was studied in treated plants and control from fresh flower buds immediately after picking from the field. Five microscopic fields were selected for observation. Pollen sterility was calculated in percentage. Those which are of normal size and shape, well filled and fully stained, were taken to be fertile, and those that were not stained, partially stained, disfigured, and shriveled were considered as sterile. Total flower sterility was calculated as the ratio of the number of sterile flower buds/plants and total number of flower buds/plants multiplied by 100. It is not adequate that a chemical exhibits a very high degree of induction of male sterility, but it also should not cause any adverse side effects in the plant. The key to the use of a CHA in the commercial production of hybrid chickpea is to achieve a high degree of selectivity in terms of male sterility versus female fertility. To ascertain the associated effects of CHAs on agronomic traits, data were recorded in treated plants, for different parameters, viz. plant height, 100 seed weight, and seed yield per plant.

Quantitative Structure−Activity Relationship (QSAR) Study. The development of an agrophore model can serve as a powerful tool in discovering new leads based on existing active chemistry. The agrophore strategy involves identifying critical structural elements responsible for activity via a hypothetical mode of action. There has been very little progress in the mode of action of CHAs. This situation makes CHA-agrophore development difficult, but at the same time, it provides an opportunity to build a discovery program focused on developing new CHAs and on elucidating CHA mode of action. QSAR analysis is a useful tool in elucidating essential structural features that govern interaction of CHAs with the macromolecular receptor in the crop plants controlling pollen formation and its viability. The QSAR method applied to two families of CHAs in the ethyl oxanilate and pyridone classes of chemistry. The following descriptor variables were used for aromatic substituents for ethyl oxanilates and pyridones, viz. electronic parameters, Swain−Lupton field constant (*F*) (*22*), Hammett constant (σ_m, σ_p), σ^+, *R*, Taft steric parameter (E_s), molecular weight (MW), Verloop−Hoogenstraaten multidimensional steric parameters, *L*, and B4 (*23*, *24*); hydrophobic parameters, π, π^2; and other parameters such as molar refractivity (MR) (*25*), δ ^{13}C and index variable. The steric parameters, viz. E_s, MR, *L*, and B4 were designated according to their position, viz. *ortho*, *meta*, and *para* on the phenyl ring. The independent variables, which were found orthogonal to each other in the correlation matrix, were minimized. The "agrophore" data, viz. percent induction of pollen sterility (PS) caused by test CHAs tested at 1000 ppm, were transferred into *sin arc* and used as the dependent variable (PS % *sin arc*). The descriptor variables were used to generate multiple regression equations by autocorrelation. None of the independent variables appearing in the equations was ensured to be orthogonal.

RESULTS AND DISCUSSION

Synthesis and Spectral Analysis. Ethyl oxanilates and pyridones were synthesized by condensation of substituted anilines with diethyl oxalate/ethyl(chlorocarbonyl) formate and ethyl isodehydracetate, respectively (**Figure 1**). The characteristic features of ^{1}H NMR spectra of ethyl oxanilates were the presence of a triplet around δ 1.30−1.65 ppm and a quartet at δ 4.15−4.70 ppm. The anilide NH proton appeared as a broad singlet around δ 9.0−10.5 ppm. The olefinic proton in the pyridone moiety appeared as a singlet at δ 5.94 ± 0.17 ppm. The M^+ ion was conspicuous in the mass spectra of ethyl oxanilates and pyridones; the base peak was found to be either protonated aryl isocyanate moiety or an azatropylium (M − 101) ion.

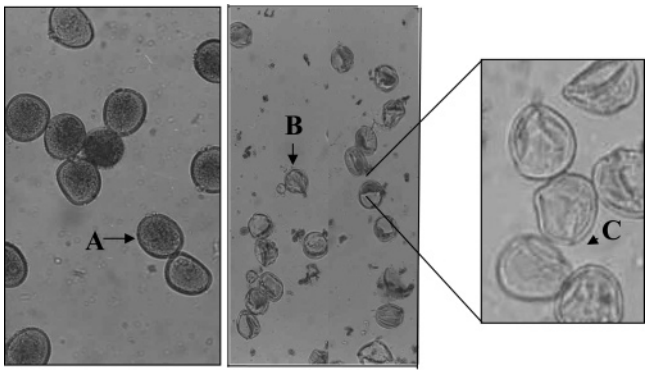


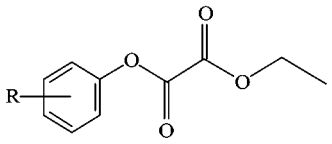
Figure 2. Sterile pollens of chickpea (BG 1088) vis-à-vis fertile pollens from control plants as revealed from KI–I_2 stain test: (**A**) fertile pollens with fully developed cytoplasm of *C. arietinum* in untreated plants, (**B**) sterile pollen grains of *C. arietinum* in CHA-treated plants, (**C**) magnified view of sterile pollen grains showing sterile transparent cytoplasm.

Effect of CHAs on Pollen Sterility. The results of induction of pollen sterility on chickpea caused by test CHAs at three test concentrations (500, 800, and 1000 ppm) on BG 1088 are given in **Table 1**. *Para* substitution with highly electronegative groups, i.e., fluoro, cyano, or trifluoromethyl, can give rise to analogues having a high level of activity in ethyl oxanilates. Ethyl 4′-fluoro oxanilate (**4**), ethyl 4′-bromo oxanilate (**5**), ethyl 4′-trifluoromethyl oxanilate (**19**), and ethyl 4′-cyano oxanilate (**16**) containing F, Br, CF_3, and CN, respectively, at the *para* position of the aromatic ring were found to be the best in that order when considered across three test concentrations. The other substituents at the *para* position influenced the activity in the following order: OMe (**9**) > NO_2 (**12**) > Cl (**6**). Except CF_3 containing analogues, in all cases the influence of aromatic substituents on the induction of pollen sterility was in the following order: *para* > *ortho* > *meta* (**Table 1**). The steric effect seems to be operating for the higher activity of the *para* and not the *ortho* substituent. The substituents at the *para* position in pyridones had a positive effect on induction of pollen sterility in the following order: Cl (**26**) > CF_3 (**39**) > CN (**36**) > F (**24**) > Br (**25**). Thus, the notable change is the dominant effect of chloro as aromatic substituent in pyridones (**Table 1**).

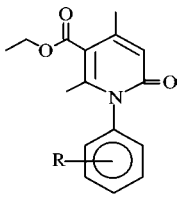
From the stain test, it was seen that the sterile pollen grains were transparent, thereby confirming the disintegration of cytoplasm and nucleus. In contrast, fertile pollens from control plots stained uniform deep blue color in the KI−I_2 stain test, thus confirming the induction of male sterility in various treatments (**Figure 2**). The negative color in the KI−I_2 stain test shown by sterile pollens was indicative of the absence of starch. The absence of starch could occur either due to inhibition of its synthesis or its premature dissolution. An important observation with chickpea was that pollen sterility was concurrent with the loss of anthocyanin. In other words, the flowers containing sterile pollens were devoid of pigments. It was therefore easy to count the male sterile flowers. These would become barren once left as such.

Effect of CHAs on Flower Sterility. Flower sterility was used as an index of induction of male sterility. Percent pollen sterility was found to have a high correlation ($r = 0.97$) with

Table 1. Percent Pollen Sterility Induced by Ethyl Oxanilates (**1**–**20**) and Pyridones (**21**–**40**) on Chickpea (var BG 1088) at Three Different Concentrations



(1-20)



(21-40)

ethyl oxanilates					pyridones				
compd no.	*R* (aromatic substituent)	500 ppm	800 ppm	1000 ppm	compd no.	*R* (aromatic substituent)	500 ppm	800 ppm	1000 ppm
1	H	68.14	71.65	74.89	21	H	57.28	63.87	68.08
2	2-F	70.82	75.64	78.43	22	2-F	71.41	75.68	78.24
3	3-F	56.99	60.33	75.10	23	3-F	50.37	52.34	58.51
4	4-F	98.62	100.0	100.0	24	4-F	82.59	85.28	86.52
5	4-Br	95.89	98.70	99.96	25	4-Br	80.04	82.39	85.87
6	4-Cl	20.95	25.16	31.57	26	4-Cl	96.34	96.82	98.25
7	2-OMe	7.36	12.98	28.96	27	2-OMe	8.58	11.39	20.41
8	3-OMe	6.07	12.57	23.92	28	3-OMe	8.06	9.70	19.26
9	4-OMe	24.39	33.12	65.76	29	4-OMe	20.14	28.57	50.39
10	2-NO_2	12.67	33.52	40.39	30	2-NO_2	8.37	15.22	20.43
11	3-NO_2	6.95	30.87	32.55	31	3-NO_2	8.15	13.92	20.65
12	4-NO_2	20.51	30.58	57.69	32	4-NO_2	21.43	25.67	32.11
13	3-Me	2.92	2.95	6.41	33	3-Me	3.95	4.74	7.28
14	2-CN	82.64	85.22	85.98	34	2-CN	79.15	85.62	88.25
15	3-CN	71.74	86.31	88.82	35	3-CN	69.18	82.84	83.05
16	4-CN	83.25	96.13	96.79	36	4-CN	82.36	86.33	88.51
17	2-CF_3	83.37	86.49	88.33	37	2-CF_3	75.38	80.85	85.63
18	3-CF_3	85.98	93.46	95.29	38	3-CF_3	82.58	90.67	90.81
19	4-CF_3	96.54	97.07	99.18	39	4-CF_3	96.15	96.50	96.84
20	4-Et	4.59	8.42	15.63	40	4-Et	2.44	5.81	10.52
emulsion control:		1.87	2.15	2.30			1.87	2.15	2.30
LSD ($p = 0.05$):		1.38	2.62	0.96			2.10	1.39	2.55

Table 2. Effect of CHAs on Percent Induction of Flower Sterility on Chickpea (var BG 1088) Tested in Winter 2001–02 at Three Different Concentrations

ethyl oxanilates				pyridones			
compd no.	500 ppm	800 ppm	1000 ppm	compd no.	500 ppm	800 ppm	1000 ppm
1	70.16	72.59	72.90	21	62.59	68.77	72.68
2	72.05	73.11	79.24	22	68.28	69.66	72.95
3	56.61	59.52	73.88	23	41.81	45.29	56.14
4	89.28	94.36	95.64	24	79.33	81.18	82.65
5	80.14	93.22	97.69	25	75.25	75.96	78.29
6	13.40	18.86	28.38	26	82.71	85.23	88.66
7	5.97	8.21	20.03	27	6.84	6.52	15.22
8	3.35	8.24	15.39	28	5.89	6.11	10.05
9	28.57	30.11	59.72	29	15.27	19.15	23.28
10	14.86	26.03	38.90	30	7.66	10.25	16.62
11	6.31	21.55	25.46	31	4.56	6.91	12.74
12	18.53	29.52	45.15	32	18.83	21.16	29.46
13	3.11	3.91	5.28	33	2.95	3.18	3.65
14	75.27	79.39	81.65	34	65.29	70.81	73.22
15	65.84	71.63	75.06	35	62.75	68.24	68.55
16	76.21	82.22	82.50	36	69.72	71.10	73.38
17	73.91	78.44	81.56	37	68.15	68.90	72.51
18	78.52	82.69	86.26	38	70.97	75.39	76.03
19	80.62	92.83	94.52	39	74.38	76.27	83.51
20	3.26	5.66	12.81	40	2.04	4.11	6.28
emulsion control:	1.85	2.39	2.50		1.85	2.39	2.50
LSD ($p = 0.05$):	2.51	2.28	1.84		0.94	2.11	1.58

the total flower sterility. The results showed that, among ethyl oxanilates, ethyl 4′-fluoro (**4**), 4′-bromo (**5**), and 4′-trifluoromethyl (**19**) derivatives at 1000 ppm test concentration were found to be very active, and they induced >94% flower sterility on BG 1088 (**Table 2**). Among pyridones, *N*-(4-chlorophenyl)-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-one (**26**) caused 98.25% induction of total flower sterility at 1000 ppm. It was generally observed that nodal position of pod setting from the base increased as a result of CHA treatment.

Effect of CHAs on Performance Evaluation. Besides high and selective induction of pollen sterility, the most potent CHAs had shown neither any adverse effects on various growth nor yield parameters for chickpea. The most potent CHAs, viz. ethyl 4′-fluoro oxanilate (**4**), ethyl 4′-bromo oxanilate (**5**), ethyl 4′-

Table 3. Effect of CHAs on Various Agronomic Traits of Chickpea (Var BG 1088) at 1000-ppm Test Concentration

compd no.	plant height (cm)	100-seed weight (g)	seed yield /plant (g)
4	55.24	25.38	90.75
5	56.39	26.14	88.36
16	42.10	19.21	52.44
18	56.45	23.40	85.28
19	56.93	25.25	91.05
26	49.27	21.37	82.27
38	54.88	24.86	88.39
39	55.62	24.79	89.42
emulsion control:	59.60	23.85	94.18
LSD ($p = 0.05$):	1.85	0.89	1.35

trifluoromethyl oxanilate (**19**), ethyl 3′-trifluoromethyl oxanilate (**18**), ethyl 4′-cyano oxanilate (**16**), *N*-(4-chlorophenyl)-5-carbethoxy-4,6-dimethyl,1,2-dihydropyrid-2-one (**26**), *N*-(4-trifluoromethyl phenyl)-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-one (**39**), and *N*-(3-trifluoromethyl phenyl)-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-one (**38**), were tested for the adverse effects on chickpea. It was found out that Br, F, and CF_3 analogues of oxanilates and pyridones are most effective and selective CHAs for wheat. The CN analogues, though effective, were found to be wanting in selectivity. A very moderate to low reduction in plant height was recorded in most of the treatments (**Table 3**). Interestingly, a marked increase in 100-seed weight was observed in the treated plants as compared to untreated controls in case of ethyl 4′-fluoro oxanilate (**4**). There was marked reduction in seed yield at 1000 ppm by cyano analogues. The negative effect on seed yield per plant is obvious due to the reduction in sink of plant as a result of flower sterility or suppression of flowering process.

Quantitative Structure−Activity Relationship Study (QSAR). The results of induction of pollen sterility on chickpea (BG 1088) caused by ethyl oxanilates and pyridones at 1000-ppm were mentioned in **Table 1**. There was no marked variation in the trend of activity at different concentrations. Because of that BG 1088 sprayed at 1000 ppm test concentration was taken for QSAR analysis. Results of the multiple regression analysis carried out are given along with the statistical values (N = number of compounds; r = multiple regression coefficient; s = standard deviation; F = Fisher's ratio of significance index with respect to the equation). All the equations were found to be statistically significant at $p < 0.01\%$. QSAR analysis of the 40 analogues thus generated was carried out by using a combination of chemical descriptors for aromatic substitutions, and the correlation matrix was constructed. The models for each CHA family gave good correlation between the variations in log percent of spikelet sterility and the steric−electrostatic properties of the sets. In ethyl oxanilates, the observed bioactivity (pollen sterility, PS) could be collectively explained in the form of the multiple linear regression equations using a total of 14 independent variables.

$$\text{PS } (\textit{sin arc } \%) = 24.15F_p - 8.20\Sigma\text{MR} - 3.65\Sigma R - 832.59 \quad (1)$$

where $N = 20$; $r = 0.83$; $s = 8.58$; $F = 36.72$

$$\text{PS } (\textit{sin arc } \%) = 15.79F_p - 5.91\Sigma\text{MR} - 2.87D - 4.92\Sigma R - 22.15\Sigma\pi + 18.71\text{MW} - 253.32 \quad (2)$$

where $N = 20$; $r = 0.95$; $s = 5.65$; $F = 31.48$

The best equation (eq 2) was the one which combined the independent variables F_p for aromatic substitution and ΣMR with r values of 0.95. In pyridones, the observed induction of pollen sterility could also be explained by the negative influence of MR and positive contributions of F_P (eq 3).

$$\text{PS } (\textit{sin arc } \%) = 32.25F_p - 3.81\Sigma\text{MR} - 4.46D - 1117.35 \quad (3)$$

where $N = 20$; $r = 0.91$; $s = 3.65$; $F = 28.96$

The guiding principles both for activity and selectivity of CHAs were designed by utilizing different descriptor variables (Swain−Lupton field constant (F_p), hydrophobic parameter (π), molar refractivity, bulk descriptor parachor, etc.) using ACD Chemsketch software (version 1.50). The direct involvement of F_p with the target bioactivity in the best equation implied that inductive (field or polar) rather than resonance effect (R) of the substituent is the key factor influencing the induction of male sterility. In a competitive binding at the bioreceptor site, a negative F_p term could mean unfavorable conformational changes in an enzyme−inhibitor complex as compared to favorable conformational changes caused by the enzyme−substrate complex. Ethyl 4′-bromo oxanilate (**5**) was very active because bromine has a very high F_p value ($F_p = 0.44$). A positive coefficient with a molar refractivity (MR) term in a correlation equation suggests a binding action via dispersion forces. Such binding could produce a concomitant conformational change in the macromolecular binding site. If the conformational change favored the process under study, one would certainly expect a positive coefficient with the detrimental; a negative coefficient could result for the MR term. The negative sign of the coefficient in MR indicated that less hydrophobicity of the aromatic substituents would enhance the target activity. The real guiding principle for selectivity was found out to be the π value. For the less selective cyano analogues, the π value is negative (ethyl 4′-cyano oxanilate; $\pi(\text{CN}) = -0.57$) as compared to the positive value in the more selective bromo analogues (ethyl 4′-bromo oxanilate; $\pi(\text{Br}) = 0.86$). It is apparent from the study that decreased hydropobicity (π) as in ethyl 4′-cyano oxanilate has a less efficient macromolecular receptor fit at the enzyme active site.

The solubility and log(octanol/water) value of ethyl 4′-fluoro oxanilate (**4**) were experimentally recorded to be 0.5332 g/L and 1.506 at 25 °C which indicate the high hydrophilicity of the CHA. Unlike chemicals such as DDT and HCH, which are highly persistent in tissues due to lipid solubility, the CHAs developed in this study are excreted from the body due to their high polarity (water solubility). It can be concluded with certainty that the CHAs appeared to have no lasting residual effects on avians and mammals, including humans. So, the CHA appeared to be ecologically very safe, and it has no outreach to the consumer in the form of crop produce.

The compounds in the ethyl oxanilate or pyridone series having an ethyl ester moiety in the side chain are immediately transformed in the leaves to give the free acids. The high hydrophilicity of the free acids (viz. oxanilic acid obtained from the hydrolysis of ethyl oxanilates) results in an easy water solubility and phloem transport, which add to the efficiency of the active CHAs. The acidic pH generated by the hydrolysis of esters to their respective acids leads to an imbalance of acid−base equilibria in the pollen mother cells resulting in dissolution of microsporocyte callose wall during the meiotic-I prophase by premature callase (1,3-β glucanase) secretion. In light of the importance of callose deposition vis-à-vis callase activity, it is likely that the CHA may either block the synthesis of callose or accelerate the premature release of callase. The absence of

any starch material in the sterile pollen grains as shown by the $KI-I_2$ pollen stain test could be indicative of either of the processes leading to starch depletion or blocking its synthesis. The first step in the synthesis of cell-wall polysaccharides is catalyzed by UDP−glucose pyrophosphorylase, and this synthesis is inhibited by the substrates such as UDP-glucose. The CHAs containing the *N*-aryl moiety can possibly mimic UDP−glucose that also contains similar groups, viz. a uridinyl group, and an isosteric glucose moiety. Incidentally, the molar volumes of bromophenyl (105.6 cm^3) and glucosyl moieties (106.2 cm^3) are almost similar. Only the field effect (F_p) rather than the resonance effect of aromatic substitution can give an appropriate isosteric effect with the glucosyl group as predicted by QSAR analysis. The efforts in this direction may be of significance in heterosis breeding as has happened in the cases of rice and wheat. The reason for the optimism is the existence of up to 200% heterosis and reports of open flower mutants in chickpea.

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Supporting Information Available: General synthetic procedures and experimental data for ethyl oxanilates (**1**−**20**), *ortho*-substituted anilates, and pyridones **21**−**40**. This material is available free of charge via the Internet at http://pubs.acs.org.

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