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N-Acylanilines, Herbicide−CHA Chimera, and Amino Acid Analogues as Novel Chemical Hybridizing Agents for Wheat (*Triticum aestivum* L.)

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In the absence of viable alternative technology of hybrid wheat development, chemical induction of male sterility mediated technology based on chemical hybridizing agents (CHAs) holds a great potential. *N*-Acylaniline derivatives, namely, ethyl 4′-fluoro oxanilate (**1**) and ethyl 4′-trifluoromethyl oxanilate (**2**) containing halogen atoms in the para position of the aryl ring and substituted amide linkage (−CO−NH−) in the acyl side chain induced >98% spikelet sterility on three genotypes of wheat, namely, PBW 343, HD 2046 and HD 2733, at 1500 ppm. The active moieties were incorporated in the form of herbicide−CHA chimera and amino acid analogues using glycine and alanine as templates. The target activity was made more selective by synthesizing chimeric structure of herbicide (2,4-dichlorophenoxyacetic acid and dalapon) and the most active CHA templates, namely, 4-fluoroanilinyl and β-ethoxycarbonyl moieties. Among herbicide−CHA chimera ethyl 2′,4′-dichlorophenyl oxalate (**14**) induced 79.11% male sterility, whereas benzyl methyl 2-oxo-3-azaadipate (**20**) was the best, inducing 73.87% male sterility in HD 2733, among amino acid analogues. The CHAs were found to modify the reproductive biology to ensure cross-pollination in the cleistogamous wheat flowers, increasing the probability for the development of hybrids.

KEYWORDS: Chemical hybridizing agents (CHAs); wheat; *N*-acylanilines; herbicide−CHA chimera; amino acid analogues

INTRODUCTION

The production of hybrid wheat (*Triticum aestivum* L.) offers an opportunity for overcoming the stagnating yield plateau of wheat. There exists a yield gap of 2.0−2.3 t/ha over most of the 9.5 million hectares of the North East Plain Zone and 9.0 million hectares area under wheat in the North Western Plain Zone of India. There is a need to bridge this technology gap to achieve a quantum jump in production. Heterosis breeding offers a means to increase current yield levels in wheat. In self-pollinated crops such as wheat with the male and female organs in the same flower, selective sterilization of pollen is of paramount importance for heterosis breeding. Exploitation of heterosis at the commercial level depends on the availability of stable male sterile lines. A viable and stable cytoplasmic−genetic male sterile (CGMS) system along with perfect restorer lines in wheat is not in place, although considerable research efforts are underway. In view of this, the other option of regulating pollen growth using chemical hybridizing agents (CHAs) involving two-line hybrid breeding needs to be pursued intensively. The CHAs facilitate cross-breeding in plant species with perfect flowers by selectively sterilizing male sex cells or by interrupting microsporogenesis to prevent self-pollination and to promote fertilization (*1*, *2*). Induction of male sterility by CHAs may be a good alternative to hand emasculation in hybrid breeding as well as in population improvement programs. Unlike the CGMS system, the CHAs have the unique advantage of saving time and labor because no restorer−maintainer lines are required. Effective CHAs allow the sterilization of a large number of potential parents of wheat and permit the production of large numbers of hybrids. The lack of safe and selective chemicals capable of the induction of male sterility without adverse effects on plant development has been the main constraint of this approach. The second generations of CHAs were developed specifically for their pollen-suppressing activity. They provide much improved safety with significantly reduced phytotoxic effects. Earlier results with ethyl oxanilates on rice (*3*), wheat (*4*−*6*), and chickpea (*7*) revealed their paramount importance in heterosis breeding. Herbicides such as dalapon (*8*), 2, 4-dichlorophenoxyacetic acid (2, 4-D) (*9*), maleic hydrazide (*10*), and TIBA (*11*) were tested as possible CHAs in various studies, but were found to induce strong phytotoxic response on different agronomic parameters and female fertility. In a program of design and development of potential CHAs we have undertaken the synthesis of several *N*-acylanilines, amino acid analogues, and herbicide−CHA chimera. The target activity of the herbicides (2, 4-D and dalapon) was made more selective by synthesizing the chimeric structure of the herbicide and the

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most active agrophore CHA templates, namely, 4-fluoroanilinyl and β-ethoxycarbonyl moieties. In this group the dalapon analogue, namely, *N*-(2,2-dichloropropanoyl)-4-fluoroaniline (**13**), and the 2,4-D analogues, 2,4-dichlorophenyl oxalate, malonate and acetoacetanilide (**14**−**16**) were synthesized. The amino acid profiles of both sterile and fertile pollens have shown the deficiency of certain amino acids (*12*, *13*). A novel way to derive new analogues of glycine and alanine has been tried because they are absent in sterile pollens.

It is essential to develop selective and highly potent CHAs for the development of hybrid wheat. With this view in mind, we tried to get an insight into the synthesis, spectroscopic analysis, and structure−activity relationship governing *N*-acylanilines, herbicide−CHA chimera, and amino acid analogues as CHAs, and in this paper, we report our results.

MATERIALS AND METHODS

Substituted anilines, diethyl malonate, ethyl acetoacetate, ethyl pyruvate, and amino acids were procured from Aldrich Chemical Co. Inc. Other esters were prepared in the laboratory. Chloroacetyl chloride, dichloroacetyl chloride, and trichloroacetyl chloride were procured from E. Merck. The structures of synthesized compounds were confirmed by ^{1}H NMR and mass spectroscopy. Melting points (mp) were determined by using a sulfuric acid bath and are uncorrected. Anhydrous reactions were performed under an inert atmosphere, the setup assembled and cooled under dry nitrogen. Unless otherwise noted, starting material, reactant, and solvents were obtained commercially and were used as such or purified and dried by standard means. Organic solutions were dried over magnesium sulfate ($MgSO_4$) or sodium sulfate (Na_2SO_4), evaporated on a rotatory evaporator, and under reduced pressure. Thin-layer chromatography (TLC) was performed on 250 μm (60 Å) silica gel G plates, preactivated at 100 °C for 2 h and using hexane/ethyl acetate (4:1) as developing medium. All reactions were monitored by UV fluorescence or staining with iodine. Preparative TLC was performed on 1.0 mm silica gel, 60 Å, 20 × 20 plates. All solvents used in chromatography had been distilled. All test compounds gave correct elemental analyses using a Euro Vector elemental analyzer (model EA3011). Mass spectral assays were obtained using a Fisons Trio 1000 (HRGC Mega-2 coupled with an EI-mass detector) instrument under electron impact conditions using an ionization energy of 70 eV. A capillary column (30 m, HP-1, 0.32 mm i.d.) and helium (He) as the carrier gas at the flow rate of 2 mL/min was used in the mass spectrometer. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian EM-360, 60 MHz NMR apparatus. Samples were dissolved in deuteriochloroform ($CDCl_3$) or deuteriodimethyl sulfoxide (DMSO-d_6) for data acquisition using tetramethylsilane as internal standard (TMS, δ 0.0 for ^{1}H NMR). Chemical shifts (δ) are expressed in parts per million (ppm), and the coupling constants (J) are expressed in hertz (Hz). Multiplicities are described by the following abbreviations: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, and m for multiplet.

General Procedure of Synthesis of *N*-Acylanilines (1−12). The following method illustrates the general scheme of synthesis of the title compounds using different substituted anilines and esters.

To a solution of para-substituted anilines (0.025 mol) in toluene (50 mL) was added alkyl alkanoates (diethyl oxalate, dimethyl oxalate, diisopropyl oxalate, ethyl pyruvate, 2-methoxyethanol, diethyl malonate, and ethyl acetoacetate) (0.03 mol) in toluene (50 mL). The aliquot was added with boric acid (0.1 g) as catalyst to a round-bottom flask fitted with a Dean−Stark apparatus, over an oil bath, and refluxed for 0.5−4 h; the azeotrope was collected in the receiver tube. The product was cooled to 90 °C to let the product solidify, triturated with boiling ethanol, and refrigerated to allow for recrystallization of **1**−**8** (**Figure 1**), which were homogeneous, by TLC [hexane/ethyl acetate (4:1) as developing medium]. Acetanilides (including halogenated acetanilides) (**9**−**12**) were synthesized by condensation of acid chlorides with aniline. A solution of acid chloride (chloroacetyl chloride, dichloracetyl chloride, and trichloracetyl chloride) (0.03 mol) suspended in anhydrous toluene was added over a period of 45 min to a solution of 4-fluoroaniline (0.025 mol, 2.3 mL) and dry pyridine (0.05 mol, 3.96 mL) in toluene (50 mL) kept at ≤5 °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 residue obtained was cooled, and chilled hexane was added to get the precipitate. Toluene was removed under vacuum. The precipitate was filtered off to get a solid product, which was diluted with cold ethyl acetate (100 mL) and treated with saturated ammonium chloride solution (50 mL). The organic phase was washed with water, dried, and evaporated to give a yellowish solid. Recrystallization of the residue with a mixture of diethyl ether/hexane (1:1) yielded halogenated acetanilides (**9**−**12**) as crystalline solids, which were homogeneous, by TLC. The duration of reaction, yield, and physicochemical characteristics of the products thus prepared were as follows.

*Ethyl 4′-Fluoro Oxanilate (**1**).* To a solution of diethyl oxalate (0.03 mol, 4.06 mL) in toluene (50 mL) were added 4 -fluoroaniline (0.025 mol, 2.3 mL) and boric acid (0.1 g) under reflux for 0.5 h following **Figure 1** to obtain a white crystalline solid: yield, 4.96 g (94%); mp 118−119 °C; TLC R_f 0.50; GC t_R = 7.82 min; ^{1}H NMR ($CDCl_3$) 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*); EI-MS, *m*/*z* (rel intensity %) 211 (M^+, *100*), 139 (18), 138 (93), 137 (72), 110 (92), 75 (12), 83 (32), 63 (5).

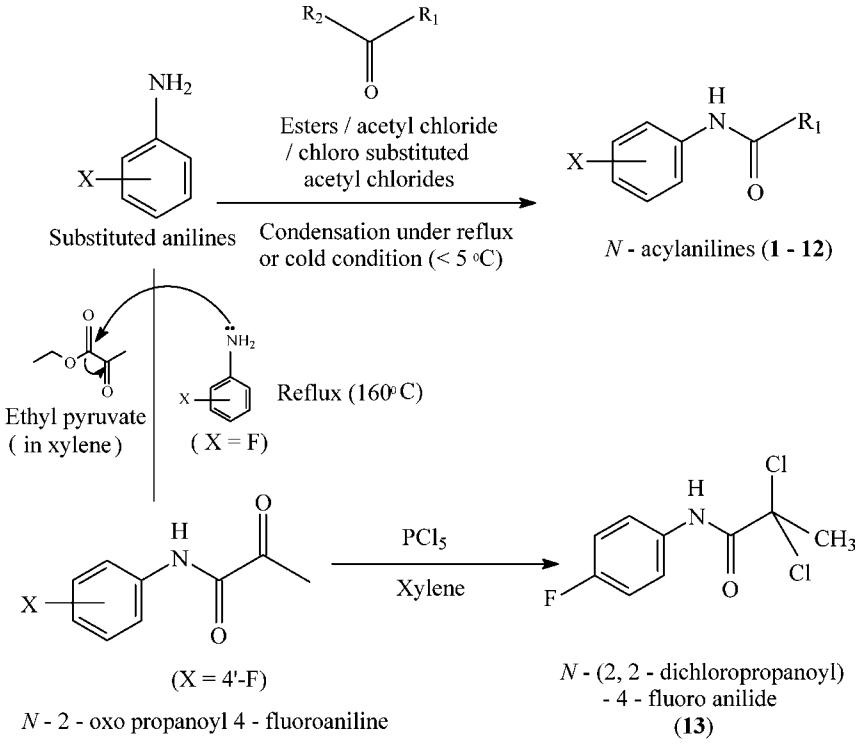
*Ethyl 4′-Trifluoromethyl Oxanilate (**2**).* A white crystalline solid of the title compound was obtained in a reaction between 4-trifluoromethylaniline (0.025 mol, 3.96 mL) and diethyl oxalate (0.03 mol, 4.06 mL) in toluene (50 mL) for 2 h: yield 5.81 g (89%); mp 139−141 °C; TLC R_f 0.66; GC t_R 10.78 min; ^{1}H NMR ($CDCl_3$) δ 1.35 (t, J = 6 Hz, 3H, CH_3), 4.35 (q, J = 6 Hz, 2H, OCH_2), 7.35 (m, 2H, H_b,H_b'-aromatic), 7.85 (m, 2H, H_a,H'_a-aromatic), 8.85 (s, 1H, *NH*); EI-MS, *m*/*z* (rel intensity %) 261 (M^+, 5), 207 (4), 188 (9), 145 (7), 59 (12), 58 (*100*).

*Methyl 4′-Fluoro oxanilate (**3**).* A stirred solution of 4-fluoroaniline (0.025 mol, 2.3 mL), dimethyl oxalate (0.03 mol, 3.5 g), and boric acid (0.1 g) suspended in toluene (50 mL) was heated to reflux for 1 h following the method of **Figure 1**. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as developing medium] until completion. The resulting suspension was filtered with diethyl ether (70 mL), washed with water (100 mL), and dried in a desiccator for 1 day. Recrystallization of the crude product with ethyl alcohol furnished the title compound (**3**) in the form of white crystals: yield 4.24 g (86%); mp 136−137 °C; TLC R_f = 0.37; GC t_R = 10.26 min; ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3H, OCH_3), 7.10 (dd, J = 6 Hz, 2H, H_b,H_b'-aromatic), 7.60 (dd, J = 6 Hz, 2H, H_a,H_a'-aromatic), 8.80 (s, 1H, *NH*); EI-MS, *m*/*z* (rel intensity %) 197 (M^+, 15), 138 (*100*), 139 (12), 137 (28), 112 (9), 110 (73), 77 (11), 75 (9), 65 (11).

*Isopropyl 4′-Fluoro Oxanilate (**4**).* 4-Fluoroaniline (0.025 mol, 2.3 mL) and diisopropyl oxalate (0.03 mol, 5.2 g) were refluxed in toluene for 3 h following the general procedure to furnish a white crystalline solid of the title compound: yield 4.27 g (76%); mp 107 °C; TLC R_f = 0.42; GC t_R = 12.26 min; ^{1}H NMR($CDCl_3$) δ 5.25 [m, 7H, $OCH(CH_3)_2$], 7.10 (dd, J = 6 Hz, 2H, H_b,H_b'-aromatic), 7.60 (dd, J = 6 Hz, 2H, H_a,H_a'-aromatic), 8.80 (s, 1H, *NH*); EI-MS, *m*/*z* (rel intensity %) 225 (M^+, 11), 166 (14), 138 (*100*), 120 (56), 110 (73), 107 (35), 94 (17), 93 (20), 77 (18), 75 (37), 65 (14), 59 (8).

*4′-Fluoro Pyruvanilide (**5**).* Reaction of 4-fluoroaniline (0.025 mol, 2.3 mL) in toluene (50 mL) with ethyl pyruvate (0.03 mol, 3.48 mL) following the method of **Figure 1** furnished a white crystalline solid of **5**: yield 3.39 g (75%); mp 180 °C (dec); TLC R_f = 0.39; GC t_R = 10.43 min; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J = 6 Hz, 3H, CH_3), 7.00 (m, 2H, H_b,H_b'-aromatic), 7.20 (m, 2H, H_a,H_a'-aromatic), 10.35 (s, 1H, *NH*); EI-MS, *m*/*z* (rel intensity %) 181 (M^+, 27), 139 (7), 138 (69), 137 (13), 110 (*100*), 77 (13), 65 (13).

*2-Methoxyethyl 4′- Fluoro Oxanilate (**6**).* To a stirred solution of 2-methoxyethanol (0.03 mol) and dimethyl oxalate (0.03 mol) was added 4-fluoroaniline (0.025 mol, 2.3 mL) dissolved in dry toluene (50 mL), and the mixture was heated to reflux for 3 h following the general procedure to furnish grayish white crystals of the title compound: yield 3.78 g (63%); mp 113 °C; R_f = 0.31; t_R = 9.59 min; ^{1}H NMR ($CDCl_3$) δ 5.13 (s, 3H, OCH_3), 5.15 (s, 4H, C_2H_4), 6.90 (m,



Compound No.	X	R_1	R_2
1	4-F	-COOEt	-OEt
2	4-CF_3	-COOEt	-OEt
3	4-F	-$COOCH_3$	-OMe
4	4-F	-COOiPr	-OiPr
5	4-F	- $COCH_3$	-OEt
6	4-F	-COC_2H_4OMe	-OMe
7	4-F	-CH_2COOEt	-OEt
8	4-F	-CH_2COCH_3	-OEt
9	4-F	- CH_3	-Cl
10	4-F	- CH_2Cl	-Cl
11	4-F	- $CHCl_2$	-Cl
12	4-F	- CCl_3	-Cl

Figure 1. Synthesis of *N*-acylanilines and *N*-(2,2-dichloropropanoyl)-4-fluoroanilide.

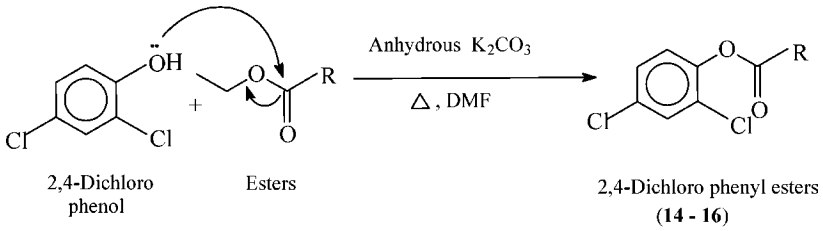
2H, H_b,H_b'-aromatic), 7.55 (m, 2H, H_a,H_a'-aromatic), 9.60 (s, 1H, *NH*); EI-MS, *m*/*z* (rel intensity %) 241 (M^+, 15), 210 (9), 196 (12), 166 (72), 110 (*100*), 137 (12), 77 (9), 65 (13).

Ethyl 4′-Fluoro Malonanilate (7). A solution of 4-fluoroaniline (0.025 mol, 2.3 mL) suspended in anhydrous xylene was added to a solution of diethyl malonate (0.03 mol, 4.8 mL) in xylene (50 mL), and the aliquot was refluxed for 1.5 h following the method of **Figure 1**. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as developing medium] until completion. The residue obtained was cooled, and hexane was added to get the precipitate. The precipitate was filtered off to get a solid product, which was filtered, washed with water (100 mL), and dried in a desiccator for 1 day. Recrystallization of the residue with ethyl alcohol yielded the title compound as a white crystalline solid, which was homogeneous by TLC: yield 4.65 g (83%); mp 72−73 °C; TLC R_f 0.58; GC t_R = 6.12 min; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, J = 6 Hz, 3H, CH_3), 3.46 (s, 2H, CH_2), 4.15 (q, J = 6 Hz, 2H, OCH_2), 7.11 (t, J = 6 Hz, 2H, H_b,H_b'-aromatic), 7.64 (dd, J = 6 Hz, 2H, H_a,H_a'-aromatic), 9.70 (s, 1H, *NH*); EI-MS, *m*/*z* (rel intensity %) 225 (M^+, 30), 180 (4), 111 (*100*), 110 (11), 109 (4), 95 (4), 83 (12), 75 (2), 69 (2).

4′-Fluoro Acetoacetanilide (8). 4-Fluoroaniline (0.025 mol, 2.3 mL) and ethyl acetoacetate (0.03 mol, 3.83 mL) were refluxed together using toluene as solvent for 2 h following the method of **Figure 1** to furnish a white crystalline solid: yield 4.00 g (89%); mp 110 °C; TLC R_f 0.58; GC t_R = 6.11 min; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, COCH_3), 3.70 (s, 2H, CH_2CO), 7.25 (dd, J = 6 Hz, 2H, H_b,H_b'-aromatic), 7.70 (dd, J = 6 Hz, 2H, H_a,H_a'-aromatic), 9.50 (s, 1H, *NH*); El-MS *m*/*z* (rel intensity %) 181 (M^+, 1), 137 (11), 124 (3), 112 (8), 111 (*100*), 97 (4), 83 (12).

4′-Fluoro Acetanilide (9). A stirred solution of acetyl chloride (0.03 mol, 2.3 mL) in toluene and 4-fluoroaniline (0.025 mol, 2.3 mL) in dry pyridine (0.05 mol, 3.96 mL) kept at ≤5 °C were reacted, followed by recrystallization of the residue in diethyl ether/hexane (1:1) following the method of **Figure 1** to obtain a white crystalline solid of the title compound: yield 3.40 g (89%); mp 152 °C; TLC R_f 0.62; GC t_R = 9.03 min; ^{1}H NMR ($CDCl_3$-d_6-DMSO) δ 2.12 (s, 3H, $COCH_3$), 7.10 (t, 2H, H_b,H_b'-aromatic), 7.65 (dd, 2H, H_a,H_a'-aromatic), 10.00 (s, 1H, *N*H); El-MS, *m*/*z* (rel intensity %) 153 (M^+, 12), 138 (29), 111 (*100*), 110 (9), 78 (26), 77 (67), 65 (58).

4-Fluoro Chloroacetanilide (10). The title compound (grayish crystalline solid) was made as described for the synthesis of halogenated acetanilides (**Figure 1**) by the condensation of 4-fluoroaniline (0.025 mol, 2.3 mL) and chloroacetyl chloride (0.03 mol, 3.35 mL) under an inert atmosphere of nitrogen followed by crystallization: yield 4.00 g (86%); mp 109−112 °C; TLC R_f 0.49; GC t_R = 9.02 min; ^{1}H NMR ($CDCl_3$-d_6−DMSO) δ 4.05 (s, 2H, CH_2Cl), 7.10 (m, 2H, H_b,H_b'-aromatic), 7.50 (m, 2H, H_a,H'_a-aromatic), 9.95 (s, 1H, *NH*); El-MS, *m*/*z* (rel intensity %) 187 (M^+, 2), 138 (30), 124 (12), 111 (*100*), 110 (69), 78 (11), 77 (75), 65 (81), 64 (15).



Compound No.	R
14	-COOEt
15	-CH_2COOEt
16	-$CH_2$$COCH_3$

Figure 2. Synthesis of 2,4-dichlorophenyl esters of oxalate, malonate, and acetoacetate.

*4-Fluoro Dichloroacetanilide (**11**).* 4-Fluoroaniline (0.025 mol, 2.3 mL) and dichloroacetyl chloride (0.03 mol, 4.4 mL) were reacted under stirring at ≤5 °C to provide the title compound as a grayish white crystalline solid: yield 4.70 g (85%); mp 119−120 °C; TLC R_f 0.32; GC t_R = 9.09 min; ^{1}H NMR ($CDCl_3$) δ 5.80 (s, 1H, −*CHCl*$_2$), 6.80 (m, 2H, H_b,H_b'-aromatic), 7.20 (m, 2H, H_a,H_a'-aromatic), 8.10 (s, 1H, *NH*); El-MS, *m*/*z* (rel intensity %) 221 (M^+, 5), 158 (5), 150 (7), 138 (28), 110 (799), 109 (8), 95 (19), 85 (33), 83 (*100*), 76 (28), 75 (15).

*4-Fluoro Trichloroacetanilide (**12**).* The title compound (grayish white crystalline solid) was made as described for the synthesis of halogenated acetanilides (**Figure 1**) by the condensation of 4-fluoroaniline (0.025 mol, 2.3 mL) and trichloroacetyl chloride (0.03 mol, 5.32 mL) under an inert atmosphere of nitrogen followed by crystallization in diethyl ether/hexane (1:1): yield 4.10 g (69%); mp 128 °C; TLC R_f 0.29; GC t_R = 13.42 min; ^{1}H NMR ($CDCl_3$) δ 7.00 (m, 4H, aromatic), 9.80 (s,1H, *NH*); El-MS, *m*/*z* (rel intensity %) 238 (M^+, 7), 204 (15), 169 (22), 121 (10), 119 (37), 111 (*100*), 110 (17), 78 (13), 77 (8), 65 (10).

Synthesis of *N*-(2,2-Dichloropropanoyl)-4-Fluoro Anilide (13). To a solution of 4-fluoroaniline (0.025 mol, 2.3 mL) in xylene (50 mL) was added ethyl pyruvate (0.03 mol, 3.48 mL). The reaction mixture was refluxed for 3 h at 160 °C to obtain a dark colored aliquot, which after cooling gave a solid product. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as developing medium] until completion. The resultant solid intermediate (*N*-2-oxopropanoyl-4-fluoroaniline; 0.025 mol, 4.5 g) was chlorinated using PCl_5 in xylene (10 mL) to furnish a solid residue. Chilled hexane was added to get a precipitate, which was filtered off to get a solid product that was diluted with cold ethyl acetate (50 mL) and treated with a saturated ammonium chloride solution (50 mL). The organic phase was washed with water (5 mL), dried with magnesium sulfate, filtered, and evaporated to give a solid. The resulting crude solid product was washed with water (100 mL) and dried in a desiccator for 1 day. Recrystallization of the residue with a mixture of diethyl ether/hexane (1:1) yielded grayish white crystals of the title compound [*N*-(2,2-dichloropropanoyl)-4-fluoro anilide] (**Figure 1**), which was homogeneous by TLC: yield 3.4 g (58%); mp 165 °C; TLC R_f 0.37; ^{1}H NMR ($CDCl_3$-d_6−DMSO) δ 5.65 (s, 3H, CCl_2CH_3), 7.20 (m, 2H, H_b,H_b'-aromatic), 8.30 (m, 2H, H_a,H_a'-aromatic), 9.40 (s, 1H, *NH*); El-MS, *m*/*z* (rel intensity %) 236 (M^+, 12), 224 (8), 222 (19), 138 (*100*), 128 (10), 126 (26), 125 (10), 111 (52), 107 (39), 100 (51), 98 (63), 97 (36), 95 (28), 91 (86), 77 (24), 65 (20).

General Procedure of Synthesis of 2,4-Dichlorophenyl Esters (14−16). In this series three analogues, namely, ethyl 2′,4′-dichlorophenyl oxalate (**14**), ethyl 2′,4′-dichlorophenyl malonate (**15**), and 2′,4′-dichlorophenyl acetoacetate (**16**) were synthesized.

To a stirred solution of 2,4-dichlorophenol (0.025 mol, 4.08 g) in DMF (5 mL) was added diethyl esters of oxalate, malonate, or ethylacetoacetate (0.03 mol). The resulting mixture was refluxed in the presence of anhydrous K_2CO_3 (0.025 mol, 3.5 g) for 3−5 h, and ethanol was collected as an azeotrope. The dark colored aliquot so produced was cooled below 90 °C to solidify. The resulting solid was filtered, washed with water (100 mL), and dried in a desiccator for a day. Recrystallization of the residue with boiling ethanol furnished the title compounds (**Figure 2**) in the form of crystalline solids, which were homogeneous in TLC [hexane/ethyl acetate (4:1) as developing medium].

*Ethyl 2′,4′-Dichlorophenyl Oxalate (**14**).* To a stirred solution of 2,4-dichlorophenol (0.025 mol, 4.08 g) was added diethyl oxalate (0.03 mol, 4.06 mL) in DMF (5 mL), and the mixture was heated to reflux for 4 h following the general procedure (**Figure 2**) to furnish a solid residue, which was recrystallized from ethanol to furnish a creamy white crystalline solid of the title compound: yield 3.49 g (53%); mp 189−192 °C; TLC R_f 0.29; ^{1}H NMR ($CDCl_3$-d_6−DMSO) δ 1.40 (t, J = 6 Hz, 3H, OCH_2CH_3), 4.30 (q, J = 6 Hz, 2H, OCH_2), 7.45 (m, 1H, H_b'-aromatic), 7.65 (m, 1H, H_b-aromatic), 7.90 (m, 1H, H_a'-aromatic); El-MS, *m*/*z* (rel intensity %) 263 (M^+, 18), 165 (52), 163 (*100*), 162 (25), 148 (12), 146 (39), 145 (7), 121 (41), 114 (8), 112 (21), 101 (8), 94 (55), 75 (12), 73 (40), 72 (8), 65 (51), 63 (86), 62 (49), 61 (40), 60 (25).

*Ethyl 2′,4′-Dichlorophenyl Malonate (**15**).* A stirred solution of 2,4-dichlorophenol (0.025 mol, 4.08 g) in DMF (5 mL) and diethyl malonate (0.03 mol, 4.8 mL) was heated to reflux for 5 h to furnish a suspension, which was filtered, washed with water (100 mL), and dried in a desiccator. Recrystallization of the residue with boiling ethanol furnished the title compound in the form of a white crystalline solids: yield 3.12 g (45%); mp 159−160 °C (dec); TLC R_f 0.32; ^{1}H NMR ($CDCl_3$-d_6−DMSO) δ 1.40 (t, J = 6 Hz, 3H, OCH_2CH_3), 2.20 (s, 2H, CH_2CO), 4.40 (q, J = 6 Hz, 2H, OCH_2), 7.45 (m, 1H, H_a'-aromatic), 7.25 (m, 2H, H_b,H_b'-aromatic); El-MS, *m*/*z* (rel intensity %) 277 (M^+, 10), 165 (34), 163 (*100*), 148 (16), 146 (47), 135 (42), 121 (28), 114 (14), 112 (38), 77 (11), 75 (31), 73 (52), 72 (20), 65 (39), 63 (55), 62 (35).

*2′,4′-Dichlorophenyl Acetoacetate (**16**).* 2,4-Dichlorophenol (0.025 mol, 4.08 g) and ethylacetoacetate in DMF (5 mL) were reacted together for 4 h following the general procedure (**Figure 2**) followed by recrystallization with boiling ethanol to furnish a grayish white crystalline solid of the title compound: yield 2.97 g (48%); mp 173 °C (dec); TLC R_f 0.35; ^{1}H NMR ($CDCl_3$-d_6−DMSO) δ 3.70 (s, 2H, CH_2CO), 3.40 (t, J = 6 Hz, 3H, $COCH_3$), 6.90 (m, 2H, H_b,H_b'-aromatic), 7.20 (m, 1H, H_a'-aromatic); El-MS, *m*/*z* (rel intensity %) 247 (M^+, 18), 165 (35), 163 (*100*), 148 (11), 146 (37), 138 (38), 77 (9), 75 (22), 73 (14).

General Procedure of Synthesis of Amino Acid Analogues (17−21). In this series five analogues, namely, benzyl glycine *p*-toluenesulfonic acid salt (**17**), benzyl glycine (**18**), benzyl β-alanine *p*-toluenesulfonic acid salt (**19**), benzyl methyl 2-oxo-3-aza-adipate (**20**), and trityl glycine (**21**), were synthesized.

To a mixture of glycine (0.25 mol, 18.8 g) in dry benzene (50 mL) were added fresh benzyl alcohol (100 mL), *p*-toluenesulfonic acid (PTSA) (0.26 mol, 48.5 g) and dry benzene (50 mL), and the mixture was distilled to reflux for 3 h with continuous stirring. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as developing medium] until completion. The distillate was diluted with ether and

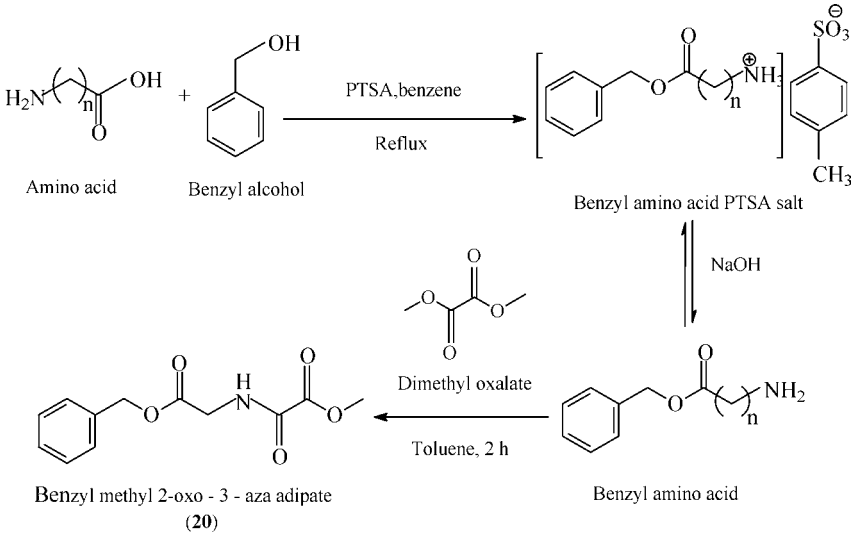


Figure 3. General procedure of synthesis of benzyl amino acids and benzyl methyl 2-oxo-3-aza-adipate.

was cooled to obtain a solid residue, which was washed with water and dried with Na_2SO_4. The product was recrystallized from methanol to obtain grayish white crystals of the intermediate salt (**17**) (benzyl glycine *p*-toluenesulfonic acid salt). To the suspension of the resultant solid intermediate in benzene was added an aqueous solution of 5 N NaOH, and the reaction mixture was stirred vigorously at reflux for 4.5 h until benzyl glycine (free base) (**18**) was oiled out. Chilled hexane (20 mL × 2) was added to the mixture to remove benzene. The complete removal of benzene was ensured by adding a few milliliters of methanol, and reconcentration was done in a vacuum. The excess hexane was evaporated off, and benzyl glycine was condensed with dimethyl oxalate for 3 h in toluene to furnish a solid residue, which was poured into ice-cold water followed by recrystallization in solvent ether/MeOH (5:2) to obtain white crystalline solids of benzyl methyl 2-oxo-3-aza-adipate (**20**) (**Figure 3**), which was homogeneous, by TLC. In another series, to a stirred solution of a β-amino acid (β-alanine) (0.25 mol, 22.3 g) were added PTSA (0.26 mol, 48.5 g) and benzyl alcohol (100 mL) suspended in dry benzene (50 mL) and heated to reflux for 2 h. The resulting suspension was filtered with diethyl ether (50 mL), washed with water, and dried in a desiccator for a day. Recrystallization of the crude product with diethyl ether/hexane (1:1) furnished benzyl β-alanine *p*-toluenesulfonic acid salt (**19**) in the form of white crystals. The details regarding yield and physicochemical characteristics of the products thus prepared are as follows.

*Benzyl Glycine p-Toluenesulfonic Acid Salt (**17**).* To a solution of glycine (0.25 mol, 18.8 g) were added fresh benzyl alcohol (100 mL), *p*-toluenesulfonic acid (PTSA) (0.26 mol, 48.5 g), and dry benzene (50 mL), reacted following **Figure 3** to obtain grayish white crystals of the title compound: yield 26.98 g (32%); mp 132−134 °C; TLC R_f = 0.21; ^{1}H NMR ($CDCl_3$) δ 4.95 (s, 2H, $-OCH_2$), 3.70 (s, 2H, $-COCH_2$), 2.20 (s, 3H, $-CH_3$), 7.60 (m, 2H, H_a,H_a'-aromatic), 7.20 (m, 1H, H_b-aromatic), 7.10 (m, 2H, H_b',H_c-aromatic), 9.01 (s, 1H, *NH*).

*Benzyl Glycine (**18**).* The title compound (yellowish oil) was made as described for the synthesis of benzyl glycine (**Figure 3**) by the addition of an aqueous solution of 5 N NaOH to a stirred suspension of PTSA−benzyl glycine salt (0.025 mol) in benzene: yield 1.6 mL (39%); TLC R_f = 0.49; ^{1}H NMR ($CDCl_3$) δ 1.05 (s, 2H, $-COCH_2$), 1.70 (s, 2H, $-OCH_2$), 5.00 (s, 1H, NH_2), 7.20 (m, 5H, aromatic); El-MS, *m*/*z* (rel intensity %) 165 (M^+, 19), 164 (70), 148 (25), 107 (59), 58 (12), 107 (*100*), 91 (62), 77 (72), 65 (22).

*Benzyl β-Alanine p-Toluenesulfonic Acid Salt (**19**).* To a stirred solution of β-alanine (0.25 mol, 22.28 g) were added PTSA (0.26 mol, 48.5 g) and benzyl alcohol (100 mL) in dry benzene (50 mL), and the mixture was heated to reflux following the general procedure to furnish a solid compound. The resulting solid was recrystallized from diethyl ether/hexane (1:1) to furnish a white crystalline solid of the title compound: yield 28%; TLC R_f = 0.16; ^{1}H NMR ($CDCl_3$) δ 4.97 (s, 2H, $-OCH_2$), 2.20(s, 3H, $-CH_3$), 7.80 (m, 2H, H'_a,H_a-aromatic), 7.20 (m, 3H, H_b,H_b',H_c-aromatic).

*Benzyl Methyl 2-Oxo-3-aza-adipate (**20**).* Benzyl glycine (0.015 mol) was condensed with dimethyl oxalate for 3 h in toluene followed by recrystallization in solvent ether/MeOH (5:2) following the method of **Figure 3** to furnish white crystalline solids of the title compound: yield 1.58 g (42%); mp 68−71 °C; TLC R_f = 0.49; ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 2H, $-OCH_2$), 2.30 (s, 3H, $-OCH_3$), 4.50 (s, 2H, $-COCH_2$), 5.20 (s, 1H, *NH*), 7.25 (m, 5H, aromatic); El-MS, *m*/*z* (rel intensity %) 251 (M^+, 10), 179 (13), 163 (26), 148 (57), 136 (46), 107 (22), 103 (11), 91 (*100*), 77 (15), 65 (12).

*Trityl Glycine (**21**).* A solution of triethylamine (10 mL, 10% v/v) suspended in dry $CHCl_3$ was added dropwise over a period of 30 min to a chilled solution of trityl chloride (3 g) in dry $CHCl_3$ (30 mL) kept at ≤5 °C with continuous stirring to make reagent 1. A solution of glycine (0.75 g) in triethylamine (1 mL) suspended in dry $CHCl_3$ (10 mL) was added to reagent 1 kept at ≤10 °C with continuous stirring followed by the addition of diethylamine (10% v/v) in $CHCl_3$ (7 mL). The resulting solution was diluted with ammonium chloride solution (25 mL, 10% w/v), and the organic phase was separated and dried over anhydrous Na_2SO_4. Chilled hexane (50 mL) was added to the organic phase to get the precipitate of trityl glycine. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as developing medium] until completion. The precipitate was filtered off to get a solid product, which was washed with water (50 mL) and dried with anhydrous Na_2SO_4. The complete removal of $CHCl_3$ was ensured by adding a few milliliters of methanol, and reconcentration was done in a vacuum. The excess hexane was evaporated off, and the mixture was poured into ice-cold water followed by recrystallization of the residue in ethanol to obtain a white crystalline solid of **9**, which was homogeneous, by TLC; yield 1.09 (23%); mp 178−179 °C; TLC R_f = 0.33; ^{1}H NMR ($CDCl_3$) δ 1.60 (d , J = 6 Hz, 2H, $-CH_2$), 7.30 (m, 15H, aromatic), 10.20 (s, 1H, *NH*); El-MS, *m*/*z* (rel intensity %) 315 (M^+, 24), 259 (32), 243 (*100*), 167 (15), 107 (29), 91 (74), 77 (31), 65(14).

Field Evaluation of CHAs. Three high-yielding varieties of bread wheat (*Triticum aestivum* L.), namely, PBW 343, HW 2046, and HD 2733, recommended for the North Western Plain Zone of India, were chosen for chemical induction of male sterility. The experiment was a randomized block design (RBD) with three replicates. Seeds of the genotypes (pollen and female parents) of the wheat varieties were sown at a 100 kg/ha seed rate in November 2001 under drilling. Row to row distance was kept at 30 cm. Five rows of 2 m length were taken as a plot in which the outermost two rows were treated as the pollen parents and the inner three rows as female parents. The incoming foreign pollens were prevented from entering the plot by planting two border rows of oat (*Avena sativa* L.) on the surroundings of the experimental plot. An emulsifiable concentrate (5 EC) of test CHAs (1.5 g) in cyclohexanone (7 mL) containing Tween-80 (1 g) was prepared. From EC 5 stocks appropriate dilutions with water furnished a spray emulsion of 1500 ppm for trials. The synthetic compounds were tested in the winter of 2001−2002, sprayed at premeiotic stage when the length of the spike

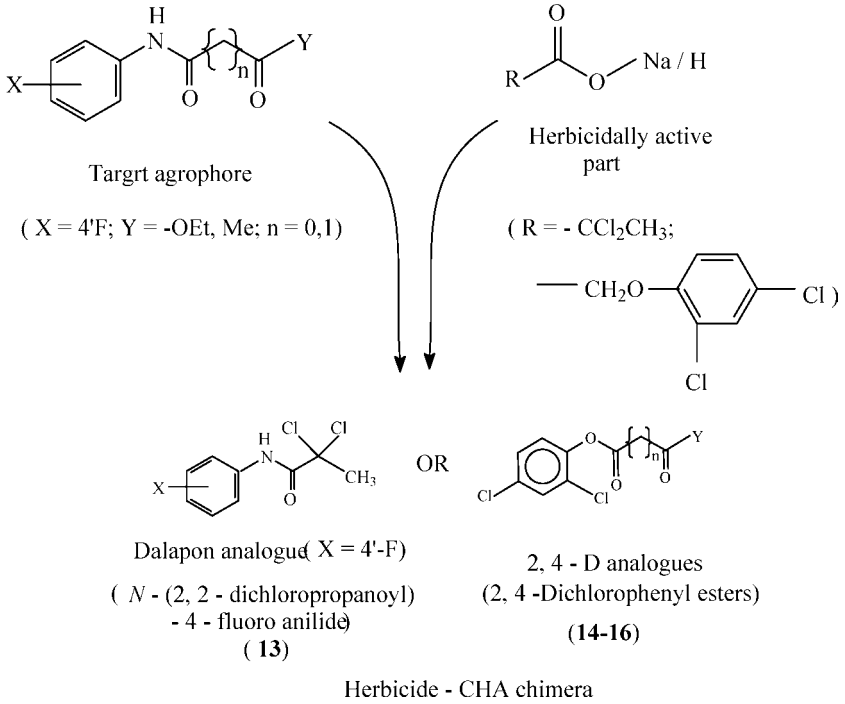


Figure 4. Sketch model of herbicide–CHA chimera in dalapon and 2,4-D analogue.

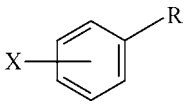
emerging from the first node was 6−9 mm (60 days after sowing) (*14*). Pollen from the pollinator was dusted on 10 spikes of female parents, and bagging was done immediately to prevent any further cross-pollination. Remaining ear heads were left uncovered. The efficacy of the test chemicals was studied using pollen sterility and seed set under bagged (male sterility) conditions. Anthers from three to four florets of the sprayed genotypes were smeared together on a glass slide over a drop of acetocarmine (1%) and/or KI−I_2 (2%) and examined under a light microscope. Pollen sterility was calculated as percentage. To study the floret fertility, the numbers of fertile (filled) and sterile (unfilled) grains per spike were counted, and percent male sterility was computed using the following formula: % floret sterility = $(S_c - S_f)/S_c \times 100$, where S_c = seeds per spike in control plants and S_f = seeds per spike in bagged and treated plants. Data were subjected to statistical analysis, and on the basis of the significance at 5% levels ($p = 0.05$) the results were compared.

RESULTS AND DISCUSSION

Synthesis and Spectral Analyses. Several *N*-acylanilines containing variations at the acyl domain were prepared by essentially keeping fluoro at the para-position of the aromatic ring and screened as CHAs. The *N*-acylanilines (**1**−**12**) were synthesized by condensation of substituted anilines with appropriate diesters or acid chlorides or monoesters as the case might be as detailed under the earlier section. Dalapon ($H_3CCH_2COO^-Na^+$) was reported to have CHA activity (*9*), albeit with adverse phytotoxic effects. It was of interest to understand whether the target gametocidal activity could be made more selective by using a chimeric structure drawn from dalapon and 4-fluoro anilinyl moieties (**Figure 4**). This chimeric compound was synthesized from the synthon *N*-2-oxopropanoyl-4-fluoroaniline, which was chlorinated by PCl_5 to obtain *N*-(2,2-dichloropropanoyl)-4-fluoroanilide (**Figure 1**) (**13**). 2,4-Dichorophenol and diethyl esters of oxalate, malonate, or ethylacetoacetate containing anhydrous K_2CO_3 were refluxed to obtain ethyl 2′,4′-dichlorophenyl oxalate, malonate, and acetoacetate (**Figure 2**) (**14**−**16**). The amino acid analogues (**17**−**20**) synthesized appeared to act as mimics of two essential precursors for sporopollenin synthesis in pollen grains, namely, glycine or alanine, thus competitively inhibiting the active site of the enzyme required to synthesize the amino acids. Moreover, *N*-acylaniline derivatives containing a substituted amide linkage [−CO−NH− or −CO−$(CH_2)_n$−NH− moiety] in the acyl side chain were found to be essential to impart CHA activity of the compounds as obvious from the very high activity (>98% spikelet sterility) of ethyl 4′-fluoro oxanilate (**1**). This idea prompted us to utilize these templates in synthesizing various amino acid analogues, namely, the benzyl ester of glycine (**18**) and *N*-tritylglycine (**21**), by means of C- and N-protection strategies. Benzyl methyl 2-oxo-3-aza-adipate (**20**) was synthesized by condensation between dimethyl ester of oxalate and benzyl glycine (**Figure 3**).

The compounds were purified using physical and chromatographic separation methods. Their structures were confirmed using ^{1}H NMR and mass spectroscopy. The characteristic feature of the ^{1}H NMR spectra of *N*-acylanilines was the presence of a broad singlet due to the anilide *NH* proton around δ 8.68 ± 2.58. The 4′-fluoro and trifluoromethyl substituents in the aryl ring of *N*-acylanilines caused marked deshielding of the highly exchangeable *NH* protons due to its electron-withdrawing −I effect. The group $-COC_2H_4OMe$ as in **6** caused deshielding of H_a by about 0.15 ppm and of H_b and H'_b aromatic protons by about 0.70 and 0.50 ppm when compared to that with 4′-fluoroacetanilide ($\delta H_a = 7.65$, $\delta H_b = 7.10$, $\delta NH = 10.00$). The deshielding effect was due to the strong electron-withdrawing phenyl and carboxymethyl groups, which withdraw the electron cloud from the aromatic ring, resulting in the deshielding of the aromatic protons. The descending order of shielding of different side chains was $COOCH_3$ (−1.2 ppm) > COOiPr (−1.0 ppm) > $COC_2H_4OCH_3$ (−0.73 ppm) compared to the base compound. The methylene and methine protons had chemical shifts of δ 4.0−4.05 and δ 4.4−6.2, respectively, in chloroacetanilides and dichloroacetanilides, corresponding to the electronegativity effect of the chlorine atom. In *N*-(2,2-dichloropropanoyl)-4-fluoro anilide, the side chain $-C(Cl)_2CH_3$ caused substantial deshielding of the aromatic protons, due to the electronegative chlorine atom. In benzyl glycine the aromatic

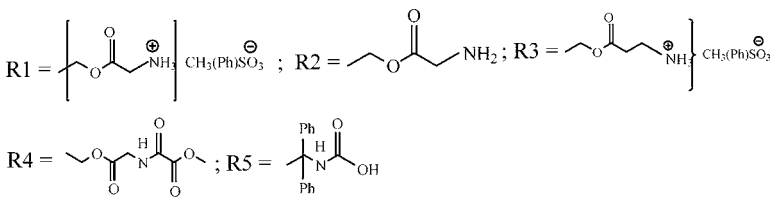
Table 1. Effect of *N*-Acylanilines, Herbicide–CHA Chimera, and Amino Acid Analogues on the Percent Induction of Spikelet Sterility at 1500 ppm Test Concentrations on Three Genotypes of Wheat in Winter 2001–2002



			spikelet sterility (%)		
Compound Number	X	R	PBW 343	HW 2046	HD 2733
1	4-F	–NHCOCOOEt	99.97	99.48	99.99
2	4-CF_3	–NHCOCOOEt	99.57	99.83	99.98
3	4-F	–NHCOCOOCH$_3$	84.32	81.69	84.71
4	4-F	–NHCOCOOiPr	67.15	64.20	69.29
5	4-F	–NHCOCOCH$_3$	51.71	50.38	52.35
6	4-F	–NHCOCOC$_2$H$_4$OMe	87.07	84.06	87.38
7	4-F	–NHCOCH$_2$COOEt	84.66	80.30	84.46
8	4-F	–NHCO CH$_2$COCH$_3$	89.12	87.00	89.36
9	4-F	–NHCOCH$_3$	61.35	59.33	64.13
10	4-F	–NHCOCH$_2$Cl	39.92	38.17	41.28
11	4-F	–NHCOCHCl$_2$	63.75	56.05	65.10
12	4-F	–NHCOCCl$_3$	89.61	85.00	91.20
13	4-F	–NHC(Cl)$_2$CH$_3$[a]	75.28 (76.29)	71.33 (68.45)	76.83 (75.22)
14	2,4-di-Cl	–OCOCOOEt[b]	77.77 (69.15)	75.10 (65.71)	79.11 (71.58)
15	2,4-di-Cl	–OCOCH$_2$COOEt	61.79	58.00	65.02
16	2,4-di-Cl	–OCOCH$_2$COCH$_3$	63.01	60.99	67.87
17	H	R1[c]	41.15	38.10	42.75
18	H	R2[c]	51.13	44.16	53.86
19	H	R3[c]	37.22	30.83	38.93
20	H	R4[c]	71.53	68.07	73.87
21	H	R5[c]	28.16	26.49	30.24
control			0.33	0.33	0.49
CD ($p = 0.05$)			6.96	3.82	5.22

[a] Corresponding values of dalapon are in parentheses. [b] Corresponding values of 2,4-D are in parentheses.

[c]



protons appeared at about δ 7.20 as a singlet and the aromatic protons did not split up significantly. The order of deshielding of aromatic protons in benzyl β-alanine PTSA salt appeared to be the same as that of benzyl glycine PTSA salt, the order of deshielding being $H_a = H_b > H_c > H_a' = H_b'$. In benzyl methyl-2-oxo-3-aza-adipate (**20**) the aromatic protons did not split up much and appeared as a multiplet at δ 7.25.

The M^+ ion was conspicuous in the mass spectra of alkyl oxanilates; the base peak was found to be either a protonated aryl isocyanate moiety (*m*/*z* 138) or an azatropylium ion, whereas in malonanilates and chloroacetanilides the breakdown to parent anilines (*m*/*z* 111) appeared to be the base peak. In *N*-(2,2-dichloropropanoyl)-4-fluoroanilide (**13**), the aryl isocyanate moiety was found to be the base peak, whereas in 2,4-dichlorophenyl esters, 2,4-dichlorophenol (*m*/*z* 163) appeared to be the base peak. Azatropylium (*m*/*z* 91) ion was determined to be the base peak in benzyl methyl 2-oxo-3-aza-adipate (**20**).

Effect of CHAs on Spikelet Sterility. The results of induction of spikelet sterility on bread wheat caused by test chemicals at 1500 ppm on three genotypes of wheat are given in **Table 1**. The para-substituted ethyl oxanilates (**1** and **2**) containing F and CF_3, respectively, were found to be the best in that order. Among *N*-acylanilines, 2-methoxyethyl 4′-fluoro oxanilate (**6**) was found to induce 86.2 ± 1.5% male sterility in the wheat genotypes. An increasing number of chlorine atoms in the side chain of chloroacetanilides (**10**−**12**) led to an increase in the activity. 4′-Fluoro trichloroacetanilide (**12**) showed the highest induction of male sterility (88.6 ± 2.6%) in the three genotypes of wheat. As may be seen from **Table 2**, dalapon was phytotoxic to wheat at 1500 ppm, but the herbicide analogue, namely, *N*-(2,2-dichloropropanoyl)-4-fluoro anilide (**13**), was not phytotoxic. Moreover, it had shown profound effect on male sterility on the three genotypes of wheat (74.5 ± 2.3%). Among 2,4-dichlorophenyl ester analogues, maximum male sterility was induced by ethyl 2′,4′-dichlorophenyl oxalate (**14**) followed by 2′,4′-dichlorophenyl acetoacetate (**16**).

Benzyl glycine (**18**) as well as its *p*-toluenesulfonic acid salt (**17**) exhibited higher induction of male sterility as compared to benzyl β-alanine PTSA salt (**19**) and trityl glycine (**21**). Maximum male sterility (73.87% in HD 2733) was induced by benzyl methyl 2-oxo-3-azaadipate (**20**). This offers the opportunity of exogenous application of amino acid or their derivatives containing substituted amide linkage in the side chain to induce male sterility. It was of interest to note that amidate showed induction of >70% male sterility, making this series of compounds deserving of further investigations.

Effect of CHAs on Pollen Sterility. An assessment of pollen sterility using KI−I_2 (2%) or acetocarmine (1%) staining showed that pollen sterility was highly correlated ($r = 0.9872$) with spikelet sterility. Sterile pollen grains were transparent and

Table 2. Effect of CHAs on Different Agronomic Traits and Female Fertility on PBW 343 at 1500 ppm Test Concentration

Compound Number	plant ht (cm)	spike length (cm)	1000-grain wt (g)	female fertility (%)	germination (%)
1	66.22	10.30	38.70	96.52	97.93
2	69.25	10.92	38.65	96.81	98.89
8	74.44	10.73	36.83	93.99	97.13
12	63.07	8.53	29.73	79.96	81.66
dalapon	45.97	7.26	24.14	47.21	57.73
2,4-D	48.62	7.53	25.83	44.08	52.59
13	62.55	9.34	38.83	85.61	92.58
14	63.81	9.59	38.24	82.93	88.21
15	66.70	10.42	36.81	84.25	95.60
16	60.25	10.90	31.88	89.57	84.72
17	56.32	8.55	28.73	66.92	70.91
18	64.01	9.64	33.11	89.43	82.55
19	58.25	8.02	28.57	67.88	68.39
20	66.11	10.83	34.12	85.00	88.60
21	64.29	9.73	37.91	87.71	91.15
emulsion control	69.89	10.99	39.82	99.88	99.80
CD ($p = 0.05$)	6.48	1.26	0.94	2.51	0.72

[a] Compound names of the corresponding compound number are mentioned in **Table 1**.

clearly distinguishable from fertile pollens, which stained uniform deep red or blue in the acetocarmine or KI−I_2 stain test, respectively (**Figure 5**). The sterile pollen grains showed with irregular shape and increasing disintegration. The absence of any starch material in the sterile pollen grains as shown by the KI−I_2 stain test could be indicative of either of the processes leading to starch depletion or blocking its synthesis. This could provide an important lead in unraveling the mode of action of the CHAs.

Effect of CHAs on Performance Evaluation. The chemical induction of male sterility through the suppression of pollen formation is a rapid and flexible method to prepare hybrids. The first generation of chemical compounds to be tested as CHAs generally caused a high degree of phytotoxicity at rates required for effective sterility. This often resulted in poor female receptivity or fertility or failed to produce adequate male sterility. The risk of commercial production or their utility in developing breeding populations is unacceptable with any of this class of compounds. The second generations of CHAs developed by this study provide for improved seed quality and are able to be used on a wide array of genotypes. The most potent CHAs had less impact on various agronomic features and female fertility. Ethyl 4′-trifluoromethyl oxanilate (**2**) appeared to be a highly selective CHA in this study (**Table 2**). The guiding principles both for activity and for selectivity of CHAs were designed by utilizing different descriptor variables, namely, Swain−Lupton field constant (F_p), hydrophobic parameter π, molar refractivity, bulk descriptor parachor, etc. As far as activity was concerned, F_p appeared to be most important. Ethyl 4′-fluoro (**1**) and trifluoromethyl oxanilate (**2**) were very active because F and CF_3 have very high F_p values ($F_p = 0.43$ and 0.38, respectively). In a competitive binding at the bioreceptor site, a negative F_p term could mean unfavorable conformational changes in the enzyme−inhibitor complex as compared to favorable conformational changes caused by the enzyme−substrate complex. The lead derived from the present study can be valuable in exploring the primary site and mode of action of these CHAs. The guiding principle for selectivity was the π value. For the more selective trifluoromethyl analogue (**2**), the π value is positive [ethyl 4′-trifluoromethyl oxanilate; $\pi(CF_3) = 0.88$]. It is apparent from the study that increased hydropobicity (π) as in ethyl 4′-trifluoromethyl oxanilate has an efficient macromolecular receptor fit at the enzyme active site. The compounds in the *N*-acylaniline series are lipophilic esters allowing a better penetration inside wheat. The esters appeared to be transformed in the leaves to give the free acids, which are very probably able to move and concentrate inside the phloem sieve tubes and be transported to the organs presenting a high metabolic activity, as is the case of stamen at this stage. However, the amino acid analogues, namely, **17**−**21**, seem to be highly hydrophilic, and their uptake is likely to be difficult. They are also probably submitted to hydrolysis in the apoplastic space. That might explain why these compounds are poorly effective as CHA.

The acidic pH generated by the hydrolysis of the ester moiety in *N*-acylaniline series of CHAs led to an imbalance of acid−base equilibria in the pollen mother cells, resulting in dissolution of the microsporocyte callose wall during the meiotic −I prophase by premature callase secretion. The high hydrophilicity of the acids (viz., oxanilic acid obtained from the hydrolysis of *N*-acylanilines, **1**−**5**) results in an easy water solubility and phloem transport, which add to the efficiency of the active

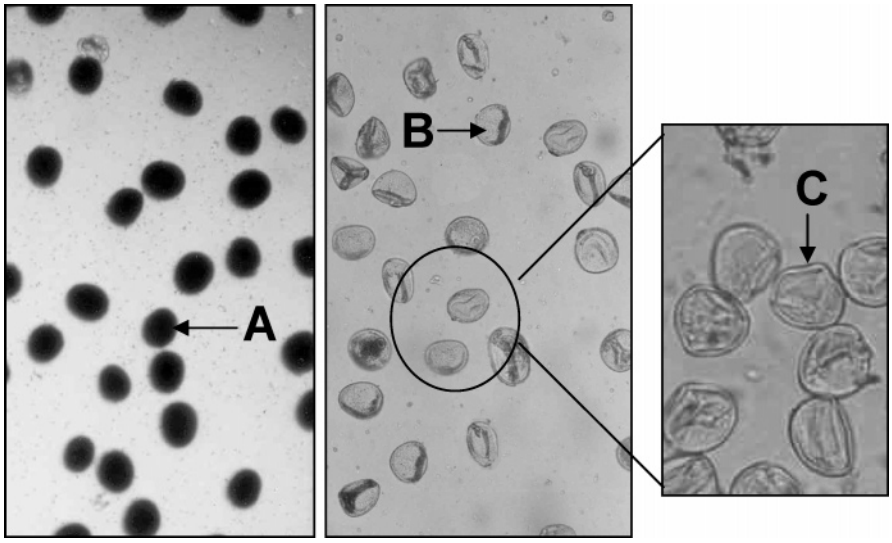


Figure 5. Sterile pollens of wheat due to treatment of *N*-(2,2-dichloropropanoyl)-4-fluoroanilide (13) vis-à-vis fertile pollens as revealed from KI–I_2 stain test: (**A**) fertile pollens with fully developed cytoplasm of *T. aestivum* in untreated plants; (**B**) sterile pollen grains of *T. aestivum* in CHA-treated plants; (**C**) magnified view of sterile pollen grains showing sterile transparent cytoplasm.

CHAs. Therefore, chemicals with high water solubility would be preferred as active CHAs. The solubility and log(octanol/water) value of ethyl 4′-fluoro oxanilate (**1**) were experimentally recorded to be 0.5332 g/L and 1.506 at 25 °C, which indicate the high hydrophilicity of the CHA. The chimeric synthon derived from herbicides (dalapon and 2,4-D) and CHA agrophores (4-fluoro anilinyl and β-ethoxycarbonyl moieties) appeared to be more selective than their parent herbicides. It is apparent that unlike dalapon, the herbicide−CHA chimera, *N*-(2,2-dichloropropanoyl)-4-fluoro anilide (**13**), did not show any significant reduction in plant height (7.34 cm with respect to the emulsion control). Among amino acid analogues, the hydrophilic salts (e.g., benzyl glycine *p*-toluenesulfonic acid salt) were found to be less selective in action as compared to their neutral analogues (**Table 2**). The key to the use of a CHA in the commercial production of hybrid wheat is to achieve a high degree of selectivity in terms of male sterility versus female fertility. Past attempts using herbicides and plant growth regulators, as gametocides have not proved to be successful due to less selectivity, namely, induction of very high female sterility. The data pertaining to female fertility revealed that *N*-(2,2-dichloropropanoyl)-4-fluoro anilide (**13**) and 2,4-dichlorophenyl ester analogues (**14**−**16**) showed a marginal reduction in female fertility. Among amino acid derivatives, benzyl glycine (**18**) and benzyl methyl 2-oxo-3-aza-adipate (**20**) exhibited a moderate (10−15%) reduction in female fertility at 1500 ppm. Dalapon and 2,4-D, in comparison, had marked detrimental effect on female fertility, showing a reduction of 52−55% in PBW 343. There is ample scope for the development of potent CHA analogues based on the leads postulated and predicted in the present study. The CHAs not only induce a very high degree of male sterility but also modify the reproductive biology in such a fashion to ensure cross-pollination in the cleistogamous wheat flowers and increase the probability for the development of hybrids. Second floret opening started after the lodicules had collapsed; the carpels in the sterile floret continued to grow palea and lemma apart. This second opening lasted for longer periods (5−6 days) and was normally sufficient for cross-pollination to take place by the pollen source. The extent of cross-pollination without cutting of palea and lemma is indicative of natural floret opening, stigma receptivity, and out-crossing percentage.

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