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Synthesis and Characterization of *N*-Acylaniline Derivatives as Potential Chemical Hybridizing Agents (CHAs) for Wheat (*Triticum aestivum* L.)

KAJAL CHAKRABORTY*,† AND C. DEVAKUMAR

Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110012, India

Induction of male sterility by deployment of chemical hybridizing agents (CHAs) are important in heterosis breeding of self-pollinated crops like wheat, wherein the male and female organs are in the same flower. Taking a lead from the earlier work on rice, a total of 25 *N*-acylanilines comprising of malonanilates, acetoacetanilides, and acetanilides (including halogenated acetanilides) were synthesized and screened as CHAs on three genotypes of wheat, viz., PBW 343, HD 2046, and HD 2733 at 1500 ppm in the winter of 2001−2002. The *N*-acylanilines containing variations at the acyl and aromatic domain were synthesized by condensation of substituted anilines with appropriate diesters, acid chlorides, or monoesters. The test compounds with highly electronegative groups such as F/Br at the *para* position of the aryl ring were identified as the most potent CHAs, causing higher induction of male sterility. A variation of N-substitution at the side chain generally furnished analogues like 4′-fluoroacetoacetanilide (**7**) and ethyl 4′-fluoromalonanilate (**1**), which induced 89.12 and 84.66% male sterility, respectively, in PBW 343. Among halogenated acetanilides, the increasing number of chlorine atoms in the side chain led to an increase in the activity of 4′-fluoro (**23**) and 4′-bromo (**24**) derivatives of trichoroacetanilides, which induced >87% male sterility. Quantitative structure−activity relationship (QSAR) models indicated the positive contributions of the field effect exemplified by the Swain−Lupton constant (F_p) and negative contributions of the Swain−Lupton resonance constant (R) for the aromatic substitution. The positive influences of parachor (P) for the acyl domain have been underlined. These leads will be significant in explaining the CHA fit in the macromolecular receptor site. The CHAs appeared to act by causing an imbalance in the acid−base equilibrium in pollen mother cells resulting in dissolution of the callose wall by premature callase secretion.

KEYWORDS: Chemical hybridizing agents; *N*-acylanilines; QSAR; malonanilates; anilides; wheat

INTRODUCTION

In the self-pollinated (hermaphrodite) crops like wheat, selective induction of male sterility by deployment of chemical hybridizing agents (CHAs) facilitating a "two-line" approach holds immense potential in heterosis breeding. 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 promote fertilization by an outside pollen source (*1*). When CHAs are used to induce male sterility, the maintainer line is not required, because the sterility is induced only when the chemical is sprayed on the plant to make its male sterile for hybrid seed production. When the chemical is not sprayed, the line is fully fertile and its seed can be multiplied by selfing. In this system, any pollinator, which is heterotic in combination with the female parent, irrespective of the presence of the fertility restorer gene (*Rf*), can be used for developing a hybrid. Thus, it broadens the choice of combinations that can be used for producing commercial hybrids. Because the system does not use sterile cytoplasm, the negative effect of the male sterile cytoplasm is also absent. In addition, the long and cumbersome method of developing a male sterile line can be overcome. The risks of a narrow genetic base for cytoplasmic−genetic male sterility (CGMS) being experienced in a three-line hybrid breeding program would cease to be a problem in this system. The hybrids based on a particular CGMS source are essentially half-sibs (have 50% of the genome in common) and therefore are generally more uniform, while in the case of CHA-based hybrids, one has the freedom to diversify hybrid combinations. CHAs would be preferred over CGMS because the former saves time and labor needed for transferring male sterility and sufficiently large quantity of seeds can be produced. An easy, economical, effective, and environment-friendly CHA technology would permit facile production of hybrid seeds (*2*, *3*). Under

* To whom correspondence should be addressed. Telephone: 91-484-2394867, 91-484-4069936, and 009447084867. Fax: 0091-0484-2394909. E-mail: kajal_iari@yahoo.com and kajal_cmfri@rediffmail.com.

† Current address: Division PNP, Central Marine Fisheries Research Institute, Ernakulam North, Post Office Box 1603, Cochin 682018, Kerala, India.

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situations where male sterility induction is not 100% or female fertility is slightly reduced, CHA could still enable a breeder to produce a sufficient quantity of hybrid seed to test the value of the hybrid population on a limited scale. The search for CHAs in the 1950s and 1960s was rather random and from the chemicals already used in agriculture such as plant-growth regulators and herbicides (*2*). Of the many chemicals investigated since the 1950s, ethephon was prominent in earlier work, with a series of products from Rohm and Hass and Shell dominating the field in the mid-1980s. A number of CHAs have been reported for cereals viz, rice and wheat. The azetidine group of CHAs rendered wheat male sterile (*4*). The fenridazon group of CHAs induced 95−100% male sterility in various wheat genotypes at application rates of 0.2−2.0 kg/ha (*5*). Ethrel is an effective CHA for wheat (*6*, *7*), but it induced high female sterility at application rates required for male sterility. Pyridine monocarboxylates were reported as potential CHAs for wheat (*8*). Prominent among other CHAs used in wheat includes MH, dalapon (*9*), DPX 3778 (*10*), LY 195259 (*11*), RH-531, RH-532, RH-2956, RH-4667, and SC-1058 (*12*, *13*).

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 the CHA mode of action. It is therefore necessary to explore molecular information on the possible site and mode of action of CHAs. Quantitative structure−activity relationship (QSAR) analysis is a powerful tool in elucidating essential structural features that govern the interaction of CHAs with the macromolecular receptor in the crop plants controlling pollen formation and its viability (*14*−*16*).

In a program of design and development of CHAs for crop plants, we have reported earlier the deployment of ethyl oxanilates in rice (*17*), wheat (*18*−*21*), and chickpea (*22*). It was therefore of interest to investigate a broader group of potential CHAs based on the leads and predictions from the earlier studies. In the present study, a total of 25 *N*-acylanilines having different aromatic substitutions and acyl side-chain variation were synthesized, characterized by different spectroscopic techniques [^{1}H nuclear magnetic resonance (NMR) and electronic impact gas chromatography/mass spectroscopy (EI−GC/MS)], and screened as CHAs on three genotypes of wheat, viz., PBW 343, HW 2046, and HD 2733 in the winter of 2001−2002. The current QSAR modeling focuses on discerning whether agrophores represented by *N*-acylanilines are active via related mechanisms. With this view in mind, we tried to get an insight into the structural features governing the activity and possible mode of action of the *N*-acylanilines as CHAs.

MATERIALS AND METHODS

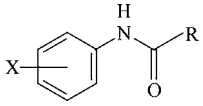
Chemicals and Reagents. Analar grade substituted anilines, diethyl malonate, and ethyl acetoacetate was procured from Aldrich Chemical Co., Inc. 2-Chloroacetyl chloride, 2,2-dichloroacetyl chloride, and 2,2,2-trichloroacetyl chloride were procured from E-Merck (Darmstadt, Germany). All solvents used for the sample preparation were of analytical grade, and the solvents used for MS analyses were of liquid chromatography (LC) grade from E-Merck. Double-distilled water was used throughout this work, while all reagents used were of analytical grade and purchased from E-Merck. Unless otherwise stated, starting material, reactants, and solvents were obtained commercially and were used as such or purified and dried by standard means. Organic solutions were dried over $Na_2SO_4/MgSO_4$, evaporated on a rotatory evaporator, and were under reduced pressure. Anhydrous reactions were performed under an inert atmosphere, with the setup assembled and cooled under dry N_2. Samples were evaporated under N_2 and redissolved in $CHCl_3$ at 50 μg/mL for analyses by GC/MS.

Instrumentation. The structures of synthesized compounds were confirmed by infrared (IR) spectroscopy, ^{1}H NMR, and MS. Melting points (mp) were determined by using a sulfuric acid bath and were uncorrected. ^{1}H NMR spectra were recorded on a Varian EM-360, 60 MHz NMR apparatus. Samples were dissolved in deuterochloroform ($CDCl_3$) or deuterodimethylsulfoxide (DMSO-d_6) for data acquisition using tetramethylsilane as an internal standard (Me_4Si, 0.0 ppm for ^{1}H NMR). 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 a developing medium. All reactions were monitored by UV fluorescence or staining with iodine. GC data were recorded on a Hewlett-Packard Series-II gas chromatograph equipped with an OV-1 (nonpolar) megabore column (10 m × 0.53 mm i.d., 0.25 μm film thickness) using a flame ionization detector (FID). The samples were injected (1 μL) into the GC using an initial column temperature of 80 °C, followed by 9.9 °C/min ramp to 250 °C. Ultrahigh purity N_2 (IOLAR grade-I, 99.999% purity) was used as the carrier gas at a flow rate of 30 mL/min. The injector temperature was maintained at 250 °C. The detector was isothermal at 290 °C. Peak retention times and areas were calculated by the integration of areas under the peaks. The qualitative GC−MS analyses were performed using EI ionization mode using a FISONS TRIO 1000 (HRGC Mega-2 coupled with an EI−mass detector). The GC apparatus was equipped with a capillary column of intermediate polarity (HP-1; 30 m × 0.32 mm i.d., 0.39 mm o.d., and 0.25 μm film thickness). The carrier gas was ultrahigh purity He (IOLAR grade-I, 99.999% purity) with a constant flow rate of 2 mL/min. The injector and detector temperature were maintained at 300 °C. The injection volume was 1 μL. Samples were injected in splitless mode at 290 °C into the capillary column similar to that used for the GC analyses, and the oven was identically programmed. The ion source and transfer line were kept at 300 °C. Electron ionization was produced by accelerating electrons from a hot filament through a potential difference at the standard value of 70 eV. All test compounds gave correct elemental analyses using Euro Vector elemental analyzer (model number EA3011).

General Methods of Syntheses and Purification of *N*-Acylanilines. A total of 25 *N*-acylanilines having variation at the acyl and aromatic domain were synthesized by condensation of substituted anilines (0.025 mol) with appropriate diesters or acid chlorides or monoesters (0.03 mol) as the case might be (**Table 1**). A large variation in the substitutions in both aryl and acyl side chains has been achieved to synthesize malonanilates, acetoacetanilides, and acetanilides (including halogenated acetanilides) (**Figure 1**). The duration of reaction, yield, and physicochemical characteristics of the products thus prepared are listed in **Table 1**. The ^{1}H NMR and mass spectral data of the test CHAs are listed in **Table 2**.

*Malonanilates (**1**−**6**).* In this series, five analogues having different aromatic substitutions (4-F, 4-Br, 2-OMe, 3-OMe, and 2-NO_2) and one unsubstituted malonanilate were synthesized. To a solution of aniline/substuted aniline (0.025 mol) in toluene/xylene was added diethyl ester of malonate (0.03 mol), and the reaction mixture was refluxed for 2−4 h over an oil bath. Ethanol was collected as an azeotrope. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as a developing medium] until completion. The resultant solid product was cooled to 90 °C to let the product solidify, triturated with boiling ethanol, and refrigerated to allow for recrystallization of the title compounds. For example, ethyl 4′-bromomalonanilate (**2**) was obtained as white crystalline solids by thermal condensation between 4-bromoaniline (0.025 mol) and diethyl malonate (0.03 mol). 2-Anisidine (0.025 mol) and 3-anisidine (0.025 mol) in toluene (10 mL) were used as the starting anilines to furnish ethyl 2′-methoxymalonanilate (**4**) and ethyl 3′-methoxymalonanilate (**5**), respectively. Other analogues in this series [ethyl 4′-fluoromalonanilate (**1**) and ethyl 2′-nitromalonanilate (**6**)] were synthesized in a similar way. TLC and GC monitored the course of

Table 1. Physicochemical Parameters of *N*-Acylanilines



compd no.	X	R	melting point (°C)	R_t (min)	R_f
1	4-F	$-CH_2COOEt$	72–73	6.12	0.58
2	4-Br	$-CH_2COOEt$	92–93	7.80	0.40
3	H	$-CH_2COOEt$	65–67	9.37	0.41
4	2-OMe	$-CH_2COOEt$	103	8.56	0.63
5	3-OMe	$-CH_2COOEt$	109	8.57	0.67
6	2-NO_2	$-CH_2COOEt$	89		0.78
7	4-F	$-CH_2COCH_3$	110	6.11	0.58
8	4-Br	$-CH_2COCH_3$	128	10.13	0.59
9	H	$-CH_2COCH_3$	86–88	9.85	0.63
10	2-Cl	$-CH_2COCH_3$	112	6.70	0.50
11	3-Cl	$-CH_2COCH_3$	106		
12	4-Cl	$-CH_2COCH_3$	107	8.25	0.47
13	3-NO_2	$-CH_2COCH_3$	135–137	7.19	0.62
14	3-CH_3	$-CH_2COCH_3$	56–58	8.28	
15	4-F	$-CH_3$	152	9.03	0.62
16	4-F	$-CH_2Cl$	109-112	9.02	0.49
17	4-Br	$-CH_2Cl$	136–137	9.17	0.51
18	H	$-CH_2Cl$	92–94	6.99	0.59
19	4-F	$-CHCl_2$	119–120	9.09	0.32
20	4-Br	$-CHCl_2$	142–144	10.30	0.43
21	H	$-CHCl_2$	102	11.27	0.53
22	2,4-(NO_2)$_2$	$-CHCl_2$	158–160		0.27
23	4-F	$-CCl_3$	128	13.42	0.29
24	4-Br	$-CCl_3$	172–175	13.59	0.31
25	H	$-CCl_3$	109–110	12.82	0.43

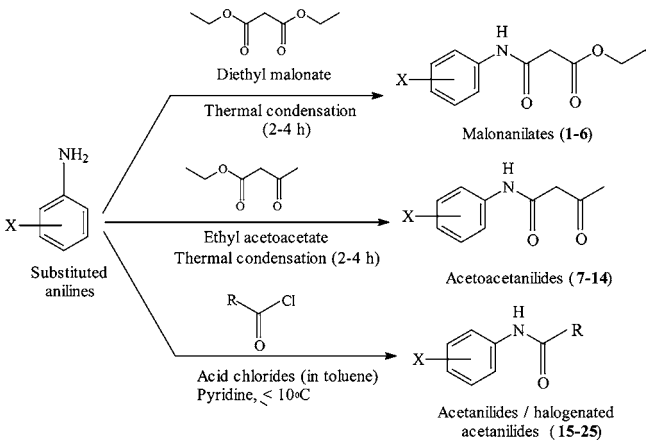


Figure 1. General synthetic scheme of *N*-acylanilines (**1**–**25**). The reaction conditions are as described in the Materials and Methods, and the details of the acyl (R) and aromatic substitution (X) patterns were described in **Table 1**.

the reaction, wherever required. The physicochemical characteristics and spectroscopic data of the compounds are listed in **Tables 1** and **2**, respectively.

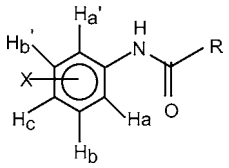
*Acetoacetanilides (**7**−**14**).* In this series, seven analogues having different aromatic substitutions (4-F, 4-Br, 2-Cl, 3-Cl, 4-Cl, 3-NO_2, and 3-CH_3) and one unsubstituted acetoacetanilide were synthesized. In a stirred solution of aniline/substituted anilines (0.025 mol) in dry toluene/xylene (50 mL), ethyl acetoacetate (0.03 mol) was added under reflux for 2−4 h in a round-bottomed flask fitted with a Dean−Stark apparatus, over an oil bath. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as a developing medium] until completion. The resulting product was cooled to 90 °C to let the products solidify and dried in a desiccator for a 1 day. Recrystallization of the crude product with boiling ethanol furnished the title compounds (**7**−**14**). For example, 4-bromoaniline and ethyl 3-oxobutanoate were refluxed in toluene for 2 h to furnish a white crystalline solid of 4′-bromoacetoacetanilide (**8**). Similarly, 3-nitroaniline and ethyl acetoacetate were thermally condensed using xylene as the solvent on an oil bath for 3 h to furnish deep yellow crystals of 3′-nitroacetoacetanilide (**13**). The reaction of 4-chloroaniline (0.025 mol) and ethyl acetoacetate (0.03 mol) in dry toluene following **Figure 1** furnished white crystals of 4′-chloroacetoacetanilide (**12**), which was further purified by column chromatography (using silica gel as an adsorbent), using 10% acetone in benzene as an eluant. Other chloro-substituted derivatives of acetoacetanilide [2′-chloroacetoacetanilide (**10**) and 3′-chloroacetoacetanilide (**11**)] were synthesized in a similar way. The physicochemical characteristics and spectroscopic data of the compounds are listed in **Tables 1** and **2**, respectively.

*Acetanilides (Including Halogenated Acetanilides) (**15**−**25**).* In this series, four series of analogues, viz., 4-fluoroacetanilide (**15**), chloroacetanilides (**16**−**18**), dichloroacetanilides (**19**−**22**), and trichloroacetanilides (**23**−**25**), comprising a total of 11 compounds were synthesized. A solution of substituted aniline (0.025 mol) in dry pyridine (0.05 mol, 3.96 mL) was added over a period of 30 min to a solution of acid chlorides (2-chloroacetyl chloride, 2,2-dichloroacetyl chloride, and 2,2,2-trichloroacetyl chloride) (0.03 mol) in dry toluene kept at ≤10 °C with continuous stirring under an inert atmosphere of nitrogen. The reaction mixture was kept under stirring until the product was formed as a solid. Toluene was removed under vacuum, and the residue obtained was recrystallized in diethyl ether/hexane to obtain acetanilides (including halogenated derivatives), which were homogeneous by TLC (**Figure 1**). For example, 4-bromochloroacetanilide (**17**) was synthesized by the reaction between 4-bromoaniline (0.025 mol) and 2-chloroacetyl chloride (0.03 mol) under stirring at ≤10 °C. Similarly, in dichloroacetanilide and trichloroacetanilide series, the analogues having different aromatic substitution [4-F, 4-Br, and 2,4-(NO_2)$_2$] were synthesized following the general procedure. The physicochemical characteristics and spectroscopic data of the compounds are listed in **Tables 1** and **2**, respectively.

Biological Activity. Three high-yielding common wheat (*Triticum aestivum* L.) cultivars, viz, PBW 343, HW 2046, and HD 2733, recommended in the northwestern plain zone of India were chosen for evaluation of spikelet sterility by the test CHAs in the winter of 2001−2002. The experiment was laid out in randomized block design (RBD) in three replicates. All of the details of agronomic practices mentioned in our earlier papers were followed (*18*−*21*). The test chemicals were sprayed at 1500 ppm as an oil-in-water emulsion containing cyclohexanone (1%) as the solvent and polyoxyethylene sorbitan monooleate (formula weight ∼ 1200) (Tween-80) (0.02%) as the emulsifier at a premeiotic stage. The spraying was carried out on three replicate plots of about 2 m lengths containing about 400 tillers, keeping the outermost two lines as pollinator lines (HW 2045). At the first signs of flower opening, 10 spikes were cross-pollinated, using the approach method, with the pollinator parent. A total of 10 spikes of each treated plants were covered immediately after the emergence. Remaining ear heads were left uncovered. Anthers from 3−4 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 a percentage. A total of 10 each of bagged and unbagged spikes including one control were harvested at maturity. As soon as the seeds became plainly visible, the number of seeds per spikelet was counted in both bagged and crossed spikes. To study the spikelet sterility, the number of fertile (filled) and sterile (unfilled) spikelets was counted and percent male sterility was computed as the percent inhibition of seed set in bagged spikes of treated plants as compared to that of the untreated control using the formula: percent male 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.

QSAR Study. The QSAR method applied to three families of *N*-acylanilines in the malonanilate, acetoacetanilide, and acetanilide (including halogenated acetanilide) classes of chemistry. The following descriptor variables were used for aromatic substituents, viz., electronic parameters, Swain−Lupton field constant for *para* substitution (F_p) (*23*), Swain−Lupton resonance constant (ΣR), Taft steric parameter (E_s), Verloop−Hoogenstraaten multidimensional steric parameters, *L* and *B*4 (*24*, *25*), descriptor variable (*D*); hydrophobic parameter π, and other parameters, such as molar refractivity (MR) (*26*). To observe the

Table 2. Spectral Data (^{1}H NMR and EI–MS) of *N*-Acylanilines



compd no.[a]	^{1}H NMR (δ, ppm)	EI–MS *m/z* (relative intensity, %)
1	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 and H_b', aromatic), 7.64 (dd, J = 6 Hz, 2H, H_a and H_a' aromatic), 9.70 (s, 1H, *NH*)	225 (M^+, 30), 180 (4), 111 (*100*), 110 (11), 109 (4), 95 (4), 83 (12), 75 (2), 69 (2)
2	1.40 (t, J = 6 Hz, 3H, CH_3), 3.45 (s, 2H, CH_2), 4.40 (q, J = 6 Hz, 2H, OCH_2), 7.35 (m, 1H, H_b' aromatic), 7.50 (m, 2H, H_a', H_b aromatic), 7.60 (m, 1H, H_a aromatic), 9.80 (s, 1H, *NH*)	286 (M^+, 9), 241 (82), 240 (4), 201 (10), 200 (3), 199 (11), 198 (3), 174 (96), 172 (*100*), 171 (3), 156 (2), 146 (4), 145 (5), 75 (3), 69 (2), 65 (6), 64 (3)
3	1.20 (t, J = 6 Hz, 3H, CH_3), 3.45 (s, 2H, CH_2), 4.13 (q, J = 6 Hz, 2H, OCH_2), 6.90 (t, J = 6 Hz, 2H, H_b and H_b', aromatic), 7.55 (dd, J = 6 Hz, 2H, H_a and H_a' aromatic), 9.90 (s, 1H, *NH*)	208 (M^+, 7), 162 (7), 121 (13), 94 (*100*), 93 (28), 78 (7), 65 (10)
4	1.21 (t, J = 6 Hz, 3H, OCH_2CH_3), 3.89 (s, 3H, OCH_3), 3.56 (s, 2H, CH_2), 4.0 (q, J = 6 Hz, 2H, OCH_2CH_3), 6.95 (m, 1H, H_b aromatic), 7.01 (m, 1H, H_c aromatic), 7.07 (m, 1H, H_b' aromatic), 8.34 (dd, 1H, H_a' aromatic), 9.52 (s, 1H, *NH*)	237 (M^+, 38), 192 (7), 150 (6), 135 (4), 124 (9), 123 (*100*), 120 (7), 108 (67), 92 (7), 80 (11), 6.5 (14)
5	0.75 (t, J = 6 Hz, 3H, OCH_2CH_3), 3.23 (s, 3H, OCH_3), 3.15 (s, 2H, CH_2), 3.75 (q, J = 6 Hz, 2H, OCH_2), 6.40 (m, 1H, H_c aromatic), 6.55 (m, 1H, H_b' aromatic), 6.90 (m, 1H, H_a' aromatic), 7.50 (m, 1H, H_a' aromatic), 8.55 (s, 1H, *NH*)	237 (M^+, 37.4), 192 (8.5), 149 (10.8), 135 (4.6), 123 (100), 120 (9.2), 108 (70.0), 80 (11.5), 77 (4.6), 65 (13.8)
6	1.50 (t, J = 6 Hz, 3H, CH_3), 4.20 (s, 2H, CH_2), 4.55 (q, J = 6 Hz, 2H, OCH_2), 7.40 (m, 1H, H_c aromatic), 7.80 (m, 1H, H_b' aromatic), 8.50 (t, 1H, H_b aromatic), 9.10 (t, 1H, H_a' aromatic), 10.30 (s, 1H, *NH*)	unstable
7	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*)	195 (M^+, 1), 123 (11), 110 (3), 98 (8), 97 (*100*), 83 (4), 69 (12)
8	3.00 (s, 3H, COCH_3), 3.00 (s, 2H, CH_2CO), 7.40 (m, 3H, H_b, H_b', H_a' aromatic), 7.60 (m, 1H, H_a aromatic), 10.05 (s, 1H, *NH*)	256 (M^+, 2), 222 (21), 221 (7), 199 (3), 172 (*100*), 156 (13), 138 (22), 93 (8), 66 (8), 64 (3)
9	2.20 (s, 3H, $COCH_3$), 3.40 (s, 2H, CH_2CO), 6.95 (m, 3H, H_b, H_b', H_c aromatic), 7.55 (m, 2H, H_a, H_a' aromatic), 9.90 (s, 1H, NH)	177 (M^+, 1), 143 (9), 142 (2), 120 (6), 93 (100), 92 (6), 77 (23), 65 (1)
10	2.45 (s, 2H, CH_2CO), 3.75 (s, 2H, CH_2CO), 7.35 (m, 1H, H_c aromatic), 7.60 (m, 2H, H_b, H_b' aromatic), 8.60 (m, 1H, H_a aromatic), 9.93 (s, 1H, NH)	211 (M^+, 8), 177 (5), 176 (38), 129 (31), 127 (100), 99 (8), 92 (6), 91 (5), 85 (7), 75 (4), 71 (4), 69 (6), 65 (7), 63 (8)
11	2.35 (s, 3H, $COCH_3$), 3.50 (s, 2H, CH_2CO), 7.40 (m, 1H, H_c aromatic), 7.50 (m, 1H, H_b', H_a aromatic), 7.65 (m, 1H, H_a aromatic), 9.40 (s, 1H, NH)	unstable
12	2.50 (s, 3H, $COCH_3$), 3.72 (s, 2H, CH_2CO), 7.60 (m, 2H, H_b, H_b' aromatic), 7.80 (m, 2H, H_a, H_a' aromatic), 9.60 (s, 1H, NH)	211 (M^+, 0.29), 196 (5), 154 (5), 153 (6), 127 (100), 111 (4), 93 (6), 92 (6), 65 (10)
13	2.40 (s, 3H, COCH_3), 3.70 (s, 2H, CH_2CO), 7.40 (m, 1H, H_c aromatic), 7.55 (m, 2H, H_b', H_a' aromatic), 7.90 (m, 1H, H_a aromatic), 9.60 (s, 1H, *NH*)	222 (M^+, 2), 138 (*100*), 122 (2), 121 (13), 108 (14), 104 (13), 92 (2), 65 (12)
14	1.35 (s, 3H, ArCH_3), 2.45 (s, 3H, COCH_3), 3.70 (s, 2H, CH_2CO), 7.20 (m, 1H, H_c aromatic), 7.40 (m, 1H, H_b' aromatic), 7.50 (m, 2H, H_a, H_a' aromatic), 9.60 (s, 1H, *NH*)	191 (M^+, 1), 157 (13), 156 (7), 147 (9), 134 (11), 107 (*100*), 92 (16), 91 (22), 73 (5), 65 (9)
15	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)	153 (M^+, 12), 138 (29), 111 (*100*), 110 (9), 78 (26), 77 (67), 65 (58)
16	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*)	187 (M^+, 2), 138 (30), 124 (12), 111 (*100*), 110 (69), 78 (11), 77 (75), 65 (81), 64 (15)
17	4.05 (s, 2H, CH_2Cl), 7.15 (m, 2H, H_b, H_b' aromatic), 7.50 (m, 2H, H_a, H'_a aromatic), 10.05(s, 1H, *NH*)	248 (M^+, 6), 199 (21), 185 (8), 172 (*100*), 171 (71), 79 (5), 78 (59), 66 (63), 65 (6)
18	4.10 (s, 2H, CH_2Cl), 7.20 (m, 2H, H_b, H_b' aromatic), 7.40 (m, 2H, H_a, H'_a aromatic), 8.30 (s, 1H, *NH*)	169 (M^+, 1), 120 (25), 106 (14), 93 (*100*), 92 (61), 78 (18), 77 (85), 65 (95), 63 (28)
19	5.80 (s, 1H, $CHCl_2$), 6.80 (m, 2H, H_b and H_b' aromatic), 7.20 (m, 2H, H_a and H_a' aromatic), 8.10 (s ,1H, *NH*)	222 (M^+, 5), 159 (5), 151(7), 139 (28), 111 (80), 110 (8), 84 (*100*), 77 (28)
20	5.85 (s, 1H, $CHCl_2$), 6.85 (m, 2H, H_b and H_b' aromatic), 7.20 (m, 2H, H_a and H_a' aromatic), 8.15 (s ,1H, *NH*)	282 (M^+, 3), 171 (80), 170 (12), 156 (118), 146 (21), 144 (*100*), 137 (30), 136 (11)
21	5.90 (s, 1H, $CHCl_2$), 6.70 (m, 2H, H_c and H_b' aromatic), 7.30 (m, 2H, H_b and H_a' aromatic), 7.80 (m, 1H, H_a aromatic), 8.65 (s, 1H, *NH*)	204 (M^+, 3), 120 (12), 93 (62), 92 (8), 78 (9), 68 (21), 66 (*100*), 59 (29), 58 (7)
22	4.40 (s, 1H, $CHCl_2$), 6.60 (m, 2H, H_b and H_b' aromatic), 7.25 (m, 1H, H_a' aromatic), 8.10 (s, 1H, *NH*)	unstable
23	7.00 (m, 4H, aromatic), 9.80 (s, 1H, *NH*)	257 (M^+, 7), 169 (22), 119 (37), 111 (*100*)
24	7.00 (m, 2H, H_b and H_b' aromatic), 7.05 (m, 2H, H_a and H_a' aromatic), 9.85 (s, 1H, *NH*)	317 (M^+, 6), 283 (18), 248 (15), 201 (41), 199 (8), 190 (*100*), 189 (10), 110 (17), 77 (12), 65 (3)
25	6.95 (m, 4H, aromatic), 8.90 (s, 1H, *NH*	unstable

[a] The identity of the compound number was mentioned under **Table 1**.

variability in the acyl side chain, the descriptor variables, viz., π, molecular weight (MW), MR, and parachor (*P*) (ACD Chemsketch, version 2.0) were taken (*16*). The "agrophore" data, viz., percent induction of spikelet sterility (SS) caused by test CHAs tested at 1500 ppm were transferred into sin arc and used as the dependent variable (SS % sin arc). The descriptor variables were used to generate multiple regression equations (MLR) by autocorrelation using the computer software package SPSS (version 10.0) program. None of the independent variables appearing in the equations was ensured to be orthogonal.

Statistical Analyses. Analysis of variance (ANOVA) of factorial

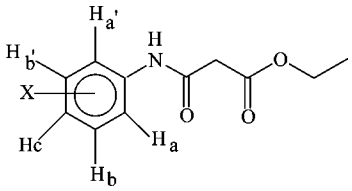
RBD was performed with all treatments. On the basis of the significance of the treatments, critical differences (CD) at a 5% level of significance ($p = 0.05$) were computed.

RESULTS AND DISCUSSION

Chemical Syntheses and Spectroscopic Analyses. The compounds synthesized numbering 25 belong to three broad chemical classes, viz., malonanilates, acetoacetanilides, and acetanilides (including halogenated acetanilides). The CHAs were synthesized by condensation with appropriate diesters/monoesters or acid chlorides with substituted anilines. Several *N*-acylanilines containing variations at the acyl domain were prepared having various substituents in the aromatic ring (**1**−**25**). The compounds were purified using physical and chromatographic separation methods. Their structures were confirmed using ^{1}H NMR and MS.

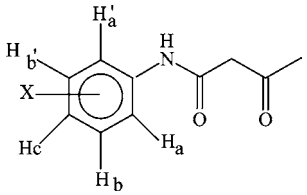
In *N*-acylanilines, the effect of different side chains on the chemical shifts of different protons was investigated by keeping the aromatic moiety constant. In malonanilate series, the signal corresponding to the methylene group (−CH_2−) flanked by two carbonyl groups appeared as a sharp singlet centered at δ 3.54 ± 0.32 ppm. The presence of the ethyl ester group was confirmed by the signal, viz., a triplet centered at δ 1.22 ± 0.24 ppm and a quartet centered at δ 4.16 ± 0.26 ppm for −OCH_2−. A broad singlet corresponding to the −NH proton appeared at δ 9.56 ± 0.52 ppm. The 4′-fluoro and 4′-bromo analogues of malonanilate caused substantial deshielding of the −NH proton (**Table 3**). Ethyl 4′-bromomalonanilate also had a strong deshielding effect on H_b and H'_b aromatic protons by about 0.60 and 0.45 ppm, respectively, as understood from its −I effect. Ethyl 4′-fluoromalonanilate followed the same trend as its bromo counterpart. In acetoacetanilides, the methyl group appeared as a sharp singlet at δ 2.48 ± 0.22 ppm. The chemical-shift values of the 4′-bromo analogue appeared downfield by 0.52 ppm, whereas those in unsubstituted acetoacetanilide (**9**) appeared upfield by about 0.28 ppm for $COCH_3$ protons in the side chain. The −NH proton appeared as a broad singlet at δ 9.59 ± 0.29 ppm in acetoacetanilides. The 4′-bromo substituent caused marked deshielding of the highly exchangeable −NH protons by 0.46 ppm because of its electron-withdrawing −I effect. The unsubstituted acetoacetanilide recorded substantial shielding by 0.54 ppm. The substituent nitro at the *meta* position caused substantial deshielding by 0.45 ppm ($\Delta\delta$) of the H_a aryl proton as compared to the same in acetoacetanilide (**9**). All of the substituents, namely, F, Cl, and Br groups having a −I effect, exhibited a deshielding effect on the H_a aryl proton. In comparison, the methyl group having a +I effect exerted a comparative shielding effect and its chemical shift (δ 7.50 ppm) value matches that of the unsubstituted analogue. The 2′- and 4′-chloro analogues exerted a strong deshielding effect on the H_b and H'_b aromatic protons by about 0.65 ppm as compared to that of the unsubstituted one. The 3′-nitro and 3′-chloro analogues, by virtue of their −M and −I effects, exerted a strong deshielding effect on H_c aryl protons by 0.45 ppm, as compared to that of acetoacetanilide (**Table 3**). In unsubstituted acetanilide, the methylene protons (CH_2Cl) appeared at δ 4.00 ppm. The methylene protons in 4-fluorochloroacetanilide (**16**) and 4-bromochloroacetanilide (**17**) were deshielded by 0.05 ppm as compared to the unsubstituted one. Because of the halogen substitution (F/Br) in the aryl moeity, it withdraws the electron cloud by virtue of their −I effect operating in them, from the aryl as well as the methylene protons resulting in the deshielding of the same. In unsubstituted dichloroacetanile (**18**), the methine ($CHCl_2$) proton appeared at δ 5.90 ppm. In 4-bromodichloroacetanilide (**20**), substantial deshielding by about 0.30 ppm of the $CHCl_2$ proton as compared to the same in the unsubstituted one was observed. In 4-fluorodichloroacetanilide (**19**), the methine proton was shielded by 0.10 ppm perhaps because of the very small size of the electronegative fluorine atom. The amido proton in trichloroacetanilide was more deshielded (δ 10.45 ppm) as compared to that of dichloroacetanilide (δ 8.65 ppm) and chloroacetanile (δ 9.90 ppm) because of the presence of more electronegative chlorine atoms in the side chain. The amido (NH) proton in 4-fluorotrichloroacetanilide (**23**) and 4-bromotrichloroacetanilide (**24**) was shielded by 0.65 and 0.60 ppm, respectively, perhaps because of the halogen substitution in the aromatic moiety. In regard to the influence of substituents on the chemical shift of aromatic protons, the δ aryl of chloroacetanile was taken as the base value. It was seen that there was no difference in the chemical shift of H_a and H'_a aromatic protons (δ 7.50 ppm) in 4-fluorochloroacetanilide (**16**) and 4-bromochloroacetanilide (**17**) as compared to their unsubstituted counterpart. The substituent bromo and fluoro at the *para* position caused deshielding respectively by about 0.10 and 0.05 ppm ($\Delta\delta$) of H_b and H'_b aryl protons as compared to the same in chloroacetanilide (δ 7.50 ppm). It was observed that the halogen substitution at the aromatic moiety caused shielding of the H_a aryl proton by 0.30 and 0.60 ppm in 4-fluorodichloroacetanilide (**19**) and 4-bromodichloroacetanilide (**20**) as

Table 3. Change in Chemical-Shift Values ($\Delta\delta$) of Aromatic Protons of *N*-Acylanilines as Compared to Unsubstituted Analogues



Unsubstituted Malonanilate (Compd No. **3**)
H_a = 7.50 ppm, H_b = 6.90 ppm, and H_c = 6.20 ppm

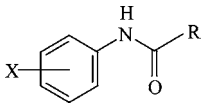
		Δδ (ppm)				
compd no.	X	H_a	H'_a	H_b	H'_b	H_c
1	4-F	0.14	0.14	0.21	0.21	
2	4-Br	0.00	0.00	0.60	0.45	
4	2-OCH_3		0.84	0.05	0.17	0.81
5	3-OCH_3	0.00	–0.60		0.35	0.20
6	2-NO_2		1.60	1.60	0.90	1.20



Unsubstituted Acetoacetanilide (Compd No. **9**)
H_a = 7.45 ppm, H_b = 6.95 ppm, and H_c = 6.95 ppm

		Δδ (ppm)				
compd no.	R	H_a	H'_a	H_b	H'_b	H_c
7	4-F	0.25	0.25	0.30	0.30	
8	4-Br	0.15	–0.05	0.45	0.45	
10	2-Cl		1.15	0.65	0.65	0.40
11	3-Cl	0.20	0.05		0.55	0.45
12	4-Cl	0.35	0.35	0.65	0.65	
13	3-NO_2	0.45	0.10		0.60	0.45
14	3-CH_3	0.05	0.05		0.45	0.25

Table 4. Effect of *N*-Acylanilines on the Percent Induction of Spikelet Sterility at 1500 ppm Spray Concentrations on Three Genotypes of Wheat in the Winter of 2001–2002



compd no.	X	R	spikelet sterility (%) PBW 343	HW 2046	HD 2733
		Malonanilates			
1	4-F	$-CH_2$ COOEt	84.66	80.30	84.46
2	4-Br	$-CH_2$ COOEt	83.40	79.15	83.48
3	H	$-CH_2$ COOEt	62.97	57.73	64.62
4	2-OMe	$-CH_2$ COOEt	25.51	24.18	29.16
5	3-OMe	$-CH_2$ COOEt	20.54	18.07	27.51
6	2-NO_2	$-CH_2$ COOEt	12.53	10.42	14.66
		Acetoacetanilides			
7	4-F	$-CH_2$ $COCH_3$	89.12	87.00	89.36
8	4-Br	$-CH_2$ $COCH_3$	83.65	80.01	83.82
9	H	$-CH_2$ $COCH_3$	64.52	62.47	66.42
10	2-Cl	$-CH_2$ $COCH_3$	34.98	32.39	36.69
11	3-Cl	$-CH_2$ $COCH_3$	15.53	10.57	16.76
12	4-Cl	$-CH_2$ $COCH_3$	58.65	50.57	61.04
13	3-NO_2	$-CH_2$ $COCH_3$	22.13	14.04	23.09
14	3-CH_3	$-CH_2$ $COCH_3$	2.94	2.29	3.40
		Acetanilides/Halogenated Acetanilides			
15	4-F	$-CH_3$	61.35	59.33	64.13
16	4-F	$-CH_2Cl$	39.92	38.17	41.28
17	4-Br	$-CH_2Cl$	39.45	38.26	40.73
18	H	$-CH_2Cl$	32.16	26.11	33.83
19	4-F	$-CHCl_2$	63.75	56.05	65.10
20	4-Br	$-CHCl_2$	62.38	57.13	62.75
21	H	$-CHCl_2$	34.85	29.71	36.97
22	2,4-$(NO_2)_2$	$-CHCl_2$	24.86	22.49	26.81
23	4-F	$-CCl_3$	89.61	85.00	91.20
24	4-Br	$-CCl_3$	88.09	84.35	89.12
25	H	$-CCl_3$	46.87	42.47	49.22
	emulsion control		0.46	0.33	0.49
	CD ($p = 0.05$)		0.84	1.28	1.07

compared to dichloroacetanile (δ 7.80 ppm). In 4-fluorotrichloroacetanilide (**23**) and 4-bromotrichloroacetanilide (**24**), the aromatic protons (H_a, H'_a, and H_b) were comparatively more shielded than those in trichloroacetanilide (**25**).

In the mass spectroscopic analysis of malonanilates and acetoacetanilides, the base peak was dominated by the aryl side chain in one form or another. Apparently, the molecular ion (M^+) loses the ethoxy radical ($EtO^{\cdot}$) to give rise to the acylium ion radical (M-45), which upon cyclization became 4-hydroxy carbostyril. The loss of CH_2COOEt or CH_2COCH_3 as in malonanilates and acetoacetanilides, respectively, led to the formation of the protonated aryl isocyanate moiety ($ArNCO^{+\cdot}$), which, very likely in turn, eliminated CO from the side chain to give rise to azatropylium ion species. The formation of aryl isocyanate (M-88) can be visualized as a result of the McLafferty rearrangement in malonanilates. The formation of aniline (M-114) stems from the loss of CO from aryl isocyanate. In the mass fragmentation pattern of acetanilides, fission of the $-CH_2$-Cl, $-CH_3$, $-CHCl_2$, or $-CCl_3$ moieties led to the azatropylium ion species via protonated aryl isocyanate. Further fragmentation could explain the formation of substituted aniline species, which in most cases was found to be the base peak.

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 (PBW 343, HW 2046, and HD 2733) are given in **Table 4**. There was no marked variation in the response of three genotypes. *Para* substitution with highly electronegative groups, i.e., F/Br, can give rise to analogues having a high level of activity in malonanilates. Ethyl 4′-fluoromalonanilate (**1**) and ethyl 4′-bromomalonanilate (**2**) containing F and Br, respectively, at the *para* position of the aromatic ring were found to be the best in that order when considered across three genotypes of wheat. The other aromatic substituents influenced the activity in the following order: H (**3**) > 2-OMe (**4**) > 3-OMe (**5**) > 2-NO_2 (**6**). In all cases, the influence of aromatic substituents on the induction of spikelet sterility was in the order: *para* > *ortho* > *meta* (**Table 4**). Among anilides, two types of compounds were synthesized, viz., acetoacetanilides and chloroacetanilides. Among acetoacetanilides, 4-fluoro (**7**) and 4-bromo derivatives (**8**) induced 88.5 ± 1.3 and 82.5 ± 2.2% spikelet sterility, respectively, in the genotypes, which was significantly higher as compared to other members in this series. The other substituents influenced the activity in the order Cl > NO_2 > CH_3. Among chloro-substituted acetoacetanilides, *para* substitution was found to be more effective followed by *ortho* and *meta* substitution. It can be inferred that *para* substitution with highly electronegative groups such as F or Br can give rise to analogues having a high level of activity. The steric effect seems to be operating for the higher activity of *para* and not *ortho* substituents. Increasing the number of chlorine atoms in the side chain of chloroacetanilides (**16**−**18**) led to an increase in the activity. 4-Fluorotrichloroacetanilide (**23**) exhibited the highest induction of male sterility (88.6 ± 2.6%).

In a program of design and development of CHAs for crop plants, we earlier reported the deployment of ethyl oxanilates in rice, wheat, and chickpea. Incidentally, the highly active compounds have F/Br as substituents at the *para* position of the aromatic ring inducing >98% spikelet sterility when considered across two test concentrations, three genotypes of wheat, and 2 year trial data. Earlier results with rice (variety Pusa 150) indicated similar trends governing structure−activity relationship inducing >80% male sterility (*17*). The ethyl oxanilate class of CHAs mostly having F/Br/CF_3 at the *para* position of the aryl ring were identified as the most potent compounds, causing ≥99% induction of pollen sterility at the 1000 ppm test concentration on chickpea (variety BG 1088) (*22*). On the basis of the leads obtained in the earlier studies, we have synthesized the CHAs with electronegative groups (F/Br) in the *para* position of aryl rings and varying the acyl side chain. It is interesting to note that elongation of the acyl side chain in ethyl 4′-fluoro-oxanilate by introducing a methylene bridge ($-CH_2-$) as in ethyl 4′-fluoromalonanilate (**1**) has caused a reduction in the spikelet sterility apparently because of the induction of steric hindrance in the macromolecular receptor site. Earlier, ethyl 4′-fluoro-oxanilate was screened on 29 genotypes of wheat at a 0.15% test concentration and was observed to induce 99.76 ± 0.37% male sterility (*20*), whereas ethyl 4′-fluoromalonanilate (**1**) exhibited 83.14 ± 2.46% spikelet sterility in the wheat genotypes in the present study.

The percent pollen sterility was found to have a high correlation ($r = 0.98$) with the spikelet sterility. Thus, pollen sterility appears to be the direct cause of spikelet sterility. From the stain test, it was seen that the sterile grains were transparent, thereby confirming the disintegration of the cytoplasm and nucleus in the sterile pollen. In contrast, fertile pollens from control plots were stained a uniform deep blue color in the KI−I_2 stain test, thus confirming the induction of male sterility in various treatments. The negative color in the KI−I_2 stain test shown by sterile pollens was indicative of the absence of starch. The absence of any starch material in the sterile pollen grains

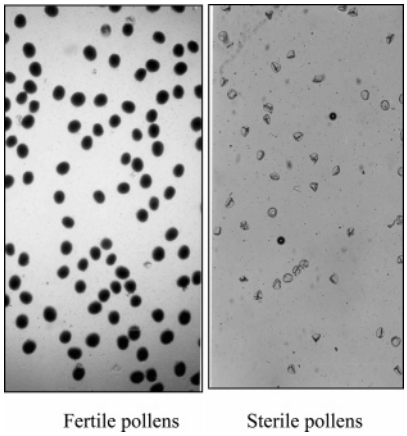


Figure 2. Sterile pollens of wheat because of the treatment of CHA vis-à-vis fertile pollens as revealed from KI–I_2 stain test.

could be indicative of either of the processes leading to starch depletion or blocking of its synthesis (**Figure 2**). This could provide an important lead in unraveling the mode of action of the CHAs.

QSAR and Mode of Action of CHAs. QSAR is a useful tool in elucidating essential structural features governing the induction of male sterility. Apparently, CHAs exert their biological effects (inducing male sterility) by participating in a series of events, which include transport binding with the macromolecular receptor and metabolism. Because the interaction mechanisms between the molecule and the putative receptor are unknown in most cases (i.e., no bound crystal structure), one is reduced to making inferences from properties, which can easily be obtained (molecular properties and descriptors), to explain these interactions for known molecules. Once the relationship is defined, it can be used to aid in the prediction of new or unknown molecules.

No significant variation in the response of different genotypes for CHAs was apparent in this study (**Table 4**). Therefore, the variety PBW 343, sprayed at a 1500 ppm concentration was taken for QSAR analysis. Results of the multiple regression analyses carried out are given along with the statistical values (N = number of compounds; r = multiple regression coefficient; s = standard deviation, and F = Fisher's ratio of significance index with respect to the equation). All of the equations were found to be statistically significant at $p < 0.01\%$. Using a combination of chemical descriptors both for aromatic substitution and side-chain variation as detailed in the earlier section carried out QSAR analysis of the 25 analogues of *N*-acylanilines thus generated, and the correlation matrix was constructed. The models for each CHA family gave a good correlation between the variations in sin arc percent of the spikelet sterility and the steric−electrostatic properties of the sets. In the CHAs synthesized, the observed bioactivity could be collectively explained in the form of two MLR equations containing two and three descriptor variables, respectively, using a total of 12 independent variables

$$\text{spikelet sterility (sin arc \%)} = 108.71F_p - 10.53D + 44.52 \quad (1)$$

where $N = 25$, $r = 0.82$, $r^2 = 0.68$, $s = 16.21$, and F (probability) = 21.77 (0.0000), and

$$\text{spikelet sterility (sin arc \%)} = 108.54F_p - 0.83\Sigma R - 9.66D + 1.52P + 14.68 \quad (2)$$

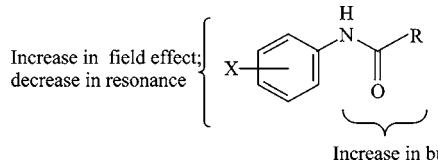


Figure 3. Sketch model of *N*-acylanilines. Induction of male sterility is directly proportional to the inductive (field or polar, F_p) effect and inversely related to the resonance effect (R) of the aromatic substituent. The positive influences of the steric parameter (P) for the acyl domain have been underlined.

where $N = 25$, $r = 0.84$, $r^2 = 0.71$, $s = 16.12$, and F (probability) = 11.56 (0.0000).

The best equation (eq 2) was the one that combined the independent variables F_p, ΣR, and D for aromatic substitution and parachor (P) for the acyl side chain with $r = 0.84$. Both electronic and steric effects have a significant influence on the bioactivity. The direct involvement of the Swain−Lupton field constant for *para* substitution (F_p) with the target bioactivity in the best equation implied that the inductive (field or polar) (F_p) rather than the resonance (R) effect of the aromatic substituent appears to be the key factor influencing the induction of male sterility. 4-Bromotrichloroacetanilide (**24**) found to be highly active because of bromine has a very high F_p value ($F_p = 0.44$). It can be inferred that *para* substitution with highly electronegative groups (F/Br) withdraw the electron cloud by the inductive effect (−I effect) from the aromatic ring as well as substituted amide moiety (−CO−NH) of the most active CHAs, thus acting as the nucleophilic center of the molecules resulting in a high level of activity.

In competitive binding at the bioreceptor site, a negative R term could mean unfavorable conformational changes in the enzyme−inhibitor complex as compared to favorable conformational changes caused by the enzyme−substrate complex. The equation essentially highlights the positive effect of the steric parameter, viz., parachor of the side chain. It implies that bulk in acyl side chain is directly proportional to the induction of male sterility (**Figure 3**). There is ample scope for the development of potent CHA analogues based on the leads postulated and predicted in the present studies. These leads will be significant in explaining the CHA fit in the macromolecular receptor site and exploring the primary site and mode of action of these CHAs.

The mode of action of these substances is supposed to be due to specific inhibition in the meiosis mechanism, leading to degeneration of pollen mother cells. The observed effects were the result of leaf transcuticular uptake and possible phloem transport. The compounds in malonanilate series have ethyl ester moiety in the side chain, which are very likely immediately transformed in the leaves to give the free acids. Similarly, hydrolysis of 4-fluorotrichloroacetanilide (**23**) in pholoem sap may likely result in *N*-(4-fluorophenyl)oxanilic acid through the intermediates, viz., 2,2-dichloro-2-hydroxy-4-fluoroacetanilide and 2-(4-fluoroanilino)-2-oxoethanoyl chloride (**Figure 4**). The high hydrophilicity of the free acids results in an easy water solubility and phloem transport, which add to the efficiency of the active CHAs. The free acids are very likely 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 the stamen at this stage. Moreover, *N*-phenyl oxanilic acid results to an imbalance in the pH equilibrium in the pollen mother cells, leading to the dissolution of the microsporocyte callose wall during early stages of meiosis (meiotic I prophase) by premature callase (1,3-β glucanase) secretion. Alterations

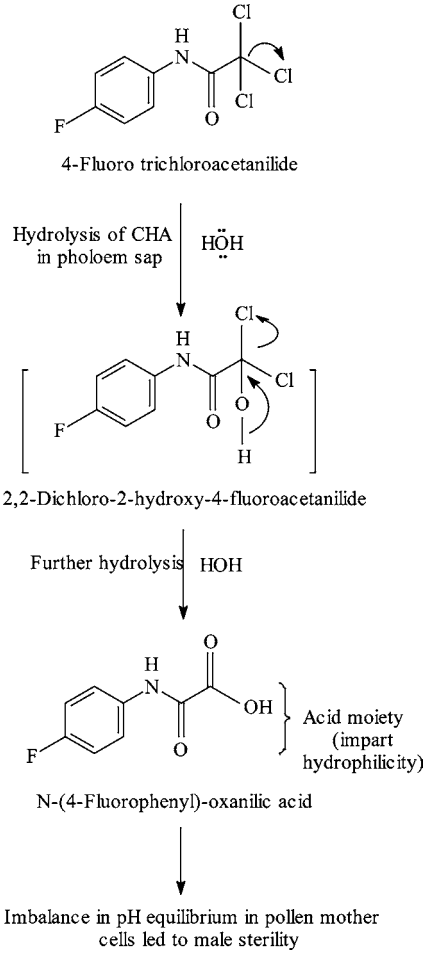


Figure 4. Mode of action of CHAs. Hydrolysis of 4-fluorotrichloroacetanilide in pholoem sap results in *N*-(4-fluorophenyl)oxanilic acid through the possible intermediates, viz., 2,2-dichloro-2-hydroxy-4-fluoroacetanilide and 2-(4-fluoroanilino)-2-oxoethanoyl chloride (structure not shown). Apparently, *N*-(4-fluorophenyl)oxanilic acid results in the imbalance in pH equilibrium in pollen mother cells resulting in premature callase secretion from tapetum and dissolution of the microsporocyte callose wall during early stages of meiosis (meiotic I prophase) with the breakdown of microsporocyte callose wall.

in the timing of callase expression leads to abnormal dissolution of the tetrad callose walls, which has been shown to be a primary cause of male sterility.

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