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# Synthesis and Screening of Anilides Having Olefinic and Alkyl Moiety in the Side Chain as Chemical Hybridizing Agents for Wheat (*Triticum aestivum* L.)

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Induction of male sterility by deployment of chemical hybridizing agents (CHAs) holds immense potential in heterosis breeding of wheat. A total of 21 anilides having different aromatic substitutions and side-chain variation were synthesized and screened as CHAs on three genotypes of wheat viz., PBW 343, HW 2046, and HD 2733, at winter season. Various anilides having vinyl moiety in the acyl side chain were synthesized by condensation between substituted anilines with different esters or acid chlorides. Another lead in the form of *N*-alkyl anilines also became evident. The percent male sterility data caused by CHAs revealed the significant contribution of anilides containing vinyl double bond incorporated in the form of closed ring structure viz., furyl moiety as the side chain. 4′-Fluoro-furyl anilide (**1**) and 4′-bromo-furyl anilide (**2**) are found to be promising lead CHAs for the design of highly active molecules. QSAR analysis revealed a direct relationship of field effect exemplified by the Swain−Lupton constant $F_p$ for the aromatic substitution but an inverse relationship of molar refractivity MR for the side chain. The negative influences of parachor for the acyl domain have been underlined. The real guiding principle for selectivity of CHA action was found to be the $\pi$ value. The CHAs act by mimicking UDP-glucose, the key substrate in the synthesis of callose, or lead to an imbalance in acid−base equilibrium in pollen mother cells resulting in dissolution of callose wall by premature callase secretion.

**KEYWORDS: Anilides; chemical hybridizing agents (CHAs); QSAR; wheat**

## INTRODUCTION

Vertical intensification through heterosis breeding is one of the most practical options for breaking the present yield plateau of wheat productivity. Through the infusion of hybrid technology, which has the potential of enhancing the productivity levels by 20−25%, one can achieve the projected production target of wheat. Even though the research efforts on the possibility of hybrids in wheat were initiated globally in the early fifties using a three-line system, it suffered major limitations of excessive height and biomass production because of the use of a tall parent, fewer seeds in hand pollination for hybrid seed production, and nonavailability of suitable restorer lines. Chemical hybridizing agents (CHAs) are important in heterosis breeding of self-pollinated crops (hermaphrodite) like wheat, wherein the male and female organs are in the same flower. Selective sterilization of the male organ (pollen) is of paramount importance in heterosis breeding. CHAs would be preferred over the existing cytoplasmic−genetic male sterility (CGMS) method because the former saves time and labor needed for transferring male sterility because male sterile and restorer lines are not required 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. Under situations where CHAs are unable to induce a very high degree of male sterility, they can still enable a breeder to produce a sufficient quantity of hybrid seed to test the value of hybrid population on a limited scale. The search for CHAs, known as gametocides in the fifties and sixties, was rather random and from the chemicals already used in agriculture such as plant growth regulators and herbicides (*1*). Prominent among the CHAs used in wheat includes maleic hydrazide, dalapon (*2*), DPX 3778 (*3*), Ethephon (*4*), LY 195259 (*5*), RH-531, RH-532, RH-2956, RH-4667 (*6*, *7*), SC-1058, and SC-1271 (*8*). Although the application of these chemicals at a specific plant growth stage was able to induce partial male sterility, several associated defects were observed. These include impaired female fertility in the majority of instances, phytotoxic effects, and growth inhibition. Ethyl oxanilates (*9*−*11*) and Clofencet (Genesis, MON 21200; 2-(4-chlorophenyl)-3-ethyl-2,5-dihydro-5-oxo-4-pyridazinecarboxylic acid) (*12*) were reported to be of practical value.

In earlier studies, we have seen that 6-membered heterocycles such as 1-aryl-4-pyridones (*N*-aryl-5-carbethoxy-4,6-dimethyl-1,2-dihydro-pyrid-2-one), 2-phenyl-4-oxonicotinates, and *N*-aralkyl-pyrid-2-ones containing an amido group and olefinic double bond in a cyclic system are promising as CHAs (*13*).

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This idea prompted us to explore the involvement of the olefinic double bond in the acyl side chain for the activity as CHAs. In one case, the vinylogous double bond was kept open to synthesize a series of (ar)-alkenyl anilides. In another case, we have introduced the N functional group at the third position of the double bond to synthesize a series of 3-amino alkenyl anilides. The side chain of anilides containing the vinylogous double bond has been further incorporated in the form of a furyl ring to produce a series of furyl anilides. Also, instead of an olefinic group, the side chain was varied in the form of an aryl ring and imides to synthesize phthalanilate/salicylanilide and phthalimides, respectively. Further, to compare the effect of an increasing number of bonds on the male sterility, a series of 3-methoxycarbonyl propiolanilides containing triple bonds in the alcoholic moiety have been prepared. In another study, we varied the *N*-acyl moiety by the *N*-alkyl group, and in this process, two *N*-alkylanilines [ethyl-(4-fluoroanilino) acetate and 4-fluoro-*N*-$\beta$-cyanoethyl aniline] have also been synthesized.

Quantitative structure−activity relationship (QSAR) analysis is a useful tool in elucidating essential structural features that govern the interaction of CHAs with the macromolecular receptor in wheat-controlling pollen formation and its viability. In this study, we report the syntheses, spectral data, and QSAR analyses of 21 anilides having olefinic and alkyl moiety in the side chain and their ability to induce male sterility on three genotypes of wheat (PBW 343, HW 2046, and HD 2733) were evaluated.

## MATERIALS AND METHODS

Substituted anilines, dimethyl acetylene dicarboxylate, *O*-acetyl methyl salicylate, methyl cinnamate, methylcrotonate, ethyl 3-amino crotonate, and acrylonitrile were procured from Aldrich Chemical Co. Inc. Furfural, acetic anhydride, phthalic anhydride, pyridine, dimethyl phthalate, and thionyl chloride were procured from E. Merck. The structures of synthesized compounds were confirmed by $^1$H NMR and mass spectroscopy. Melting points (mp's) were determined by using a sulfuric acid bath and were uncorrected. Anhydrous reactions were performed under an inert atmosphere. The setup was assembled and cooled under dry nitrogen. Unless otherwise noted, the 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 ($MgSO_4$), evaporated on a rotatory evaporator, and under reduced pressure. All reactions were monitored by UV fluorescence or staining with iodine. Thin-layer chromatography (TLC) was performed on 250 $\mu$m (60 Å) silica gel G plates, preactivated at 100 °C for 2 h and using hexane/ethyl acetate (4:1) as a developing medium. Preparative TLC was performed on 1.0 mm silica gel, 60 Å, 20 × 20 plates. GC data were recorded on a HP Series-II GC using a FID detector and 10 m × 0.53 mm i.d., 0.25 $\mu$m, OV-1 megabore column with the injector temperature maintained at 250 °C and the oven temperature programmed from 80 to 250 °C at 9.9 °C/min. The carrier gas used was $N_2$ with a flow rate of 30 mL/min. All solvents used in chromatography had been distilled. All test compounds gave the correct elemental analyses using Euro Vector elemental analyzer (model number EA3011). Mass spectral assays were obtained using a FISONS TRIO 1000 (HRGC Mega-2 coupled with 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 were 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 the internal standard (TMS, $\delta$ 0.0 ppm for $^1$H NMR). Chemical shifts ($\delta$) are expressed in parts per million, and the coupling constants (*J*) are expressed in hertz. 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 Furyl Anilides (1−3).** The following method illustrates the general scheme of synthesis of the title compounds using different substituted anilines and various esters.

To a solution of furoic acid (0.01 mol, 1.2 g) (obtained from furfural following the standard procedure using the Cannizaro reaction) in dichloromethane (10 mL) was added thionyl chloride (0.012 mol, 1.43 mL). The reaction mixture was stirred vigorously for 1 h in the presence of dry pyridine (0.011 mol, 1.11 mL) in cold conditions. The resultant solid intermediate (furoyl chloride) was solubilized in dichloromethane (10 mL), and then substituted aniline (0.01 mol) was added to that in cold conditions. The reaction mixture under an inert atmosphere of nitrogen was stirred at 0 °C for 2 h. Then, the mixture was diluted with diethyl ether (30 mL) and water (30 mL) and washed with ice-cold water (20 mL). The reaction was followed by TLC (hexane/ethyl acetate (4:1) as a developing medium) until completion. The organic phase was dried with magnesium sulfate, filtered, and evaporated to yield a solid residue, which was recrystallized with diethyl ether/hexane (1:1) to give a white crystalline solid of furyl anilides (**Figure 1**), which were homogeneous, by TLC.

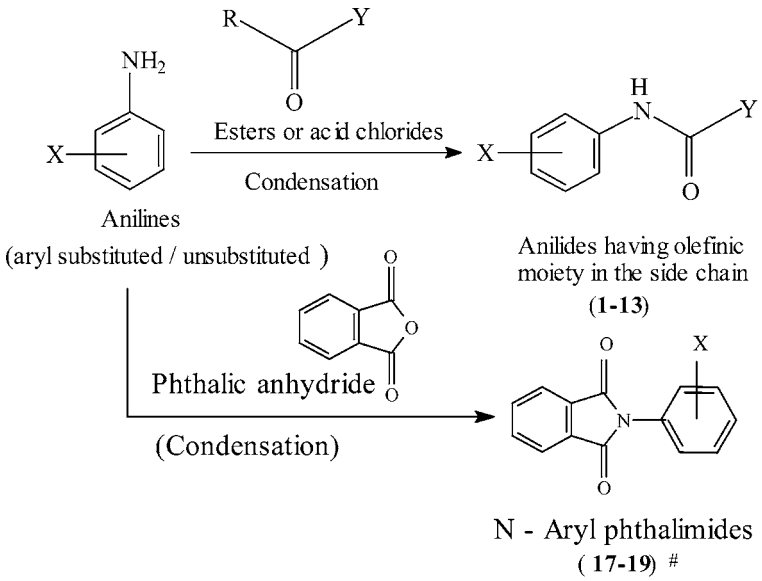
*4′-Fluoro-furyl Anilide (1).* To a stirred cold solution of furyl chloride (0.01 mol, 1.3 g) in dichloromethane (10 mL), 4-fluoroaniline (0.01 mol, 0.93 mL) was added under an inert atmosphere of nitrogen at 0 °C for 2 h following **Figure 1** to obtain white crystalline solid; yield, 1.46 g (71%); mp, 152 °C; TLC $R_f$, 0.62; GC $R_t$, 11.54 min; $^1$H NMR ($CDCl_3$-DMSO-$d_6$, $\delta$), 6.55 (m, 1H, $H_{bf}$), 7.20 (m, 2H, $H_{af}$, $H_{cf}$), 7.55 (m, 3H, $H_a'$,$H_b$, $H_b'$ aromatic), 7.55 (m, 1H, $H_a$ aromatic), 10.70 (s, 1H, *N*H); El-MS *m*/*z* (rel. int. %), 205 ($M^+$, 17), 111(17), 110(17), 109(4), 96(5), 95(*100*), 83(31), 75(5), 67(5), 57(12).

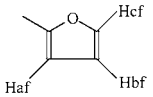
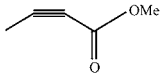
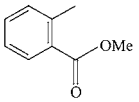
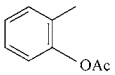
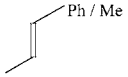
*4′-Bromo-furyl Anilide (2).* To a solution of furyl chloride (0.01 mol, 1.3 g) in dichloromethane (10 mL) at 0 °C was added 4-bromoaniline (0.01 mol, 1.7 g) following the general procedure to obtain white crystalline solid; yield, 1.91 g (72%); mp, 182−184 °C; TLC $R_f$, 0.60; GC $R_t$, 10.25 min; $^1$H NMR ($CDCl_3$-DMSO-$d_6$, $\delta$), 6.60 (m, 1H, $H_{bf}$), 7.10 (m, 2H, $H_{af}$, $H_{cf}$), 7.30 (m, 1H, $H_b'$ aromatic), 7.80 (m, 3H, $H_a'$, $H_a$, $H_b$ aromatic), 9.95 (s, 1H, *N*H); El-MS *m*/*z* (rel. int. %), 265 ($M^+$, 14), 263(11), 71(5), 156(7), 154(6), 143(22), 141(21), 135(5), 109(9), 95(*100*), 57(10).

*Furyl Acetanilide (3).* A stirred solution of furyl chloride (0.01 mol, 1.3 g) in dichloromethane (10 mL) at 0 °C was added to aniline (0.01 mol, 0.81 mL) under an inert atmosphere of nitrogen following **Figure 1** to obtain a white crystalline solid; yield, 1.05 g (56%); mp, 128−129 °C; TLC $R_f$, 0.69; GC $R_t$, 11.89 min; $^1$H NMR ($CDCl_3$-DMSO-$d_6$, $\delta$), 6.45 (dd, $J$ = 4 Hz, 1H, $H_{bf}$), 7.10 (d, $J$ = 4 Hz, 2H, $H_{af}$, $H_{cf}$), 7.70 (m, 1H, $H_c$ aromatic), 7.60 (m, 1H, $H_b$, $H_b'$ aromatic), 7.20 (m, 2H, $H_a$, $H_a'$ aromatic), 9.60 (s, 1H, *N*H); El-MS *m*/*z* (rel. int. %), 187 ($M^+$, 12), 110(73), 109(16), 95(*100*), 78(11), 77(15), 65(18), 57(2).

**General Procedure of Synthesis of 3-Methoxycarbonyl Propiolanilides (4−6).** A solution of aniline (0.012 mol) suspended in anhydrous benzene (10%, 14 mL) was added over a period of 30 min to a solution of dimethyl acetylene dicarboxylate (0.015 mol, 2.13 mL) in dry benzene (40 mL) kept at ≤10 °C with continuous stirring under an inert atmosphere of nitrogen. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as developing medium] until completion. The residue obtained was cooled, and hexane was added to get the precipitate. The precipitate was filtered off to get a solid product, which was diluted with cold ethyl acetate (100 mL) and treated with a saturated ammonium chloride solution (50 mL). The organic phase was washed with water (5 mL), dried, and evaporated to give a yellowish solid. The resulting crude solid product was filtered, 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 the title compound as a white crystalline solid of the title compound (3-methoxycarbonyl propiolanilide) (**Figure 1**), which was homogeneous by TLC.

*3-Methoxycarbonyl 4-Fluoro-propiolanilide (4).* To a stirred solution of 4-fluoro-aniline (0.012 mol) and dimethyl acetylene dicarboxylate (0.015 mol, 2.13 mL) in dry benzene (40 mL) kept at ≤10 °C were reacted following **Figure 1** to obtain a white crystalline solid of the title compound; yield, 1.10 g (~42%); mp, 180 °C (dec.); TLC $R_f$, 0.72; GC $R_t$, 12.62 min; $^1$H NMR ($CDCl_3$, $\delta$), 3.90 (s, 1H, $COOCH_3$), 6.85 (m, 1H, $H_b'$ aromatic), 7.15 (m, 1H, $H_b$ aromatic), 7.30 (m, 2H,




| Compound name (and number) | X | Y | R |
|---|---|---|---|
| *N*-Furyl anilides (**1-3**) | 4-F, 4-Br, H |   | -Cl |
| 3-Methoxycarbonyl propiolanilides (**4-6**) | 4-F, 4-Br, H |   | -OMe |
| Methyl phthalanilates (**7-9**) | 4-F, 4-Br, H |   | -OMe |
| *O*-acetyl 4'-fluoro salicylanilide (**10**) | 4-F |   | -OMe |
| (Ar)-alkenyl anilides (**11-13**) | 4-F, H |   | -OMe |

# X = 4-F, 4-Br, H

**Figure 1.** General procedure of the synthesis of anilides having olefinic moiety in the side chain.

$H_a$, $H_a'$ aromatic), 9.80 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 221 ($M^+$, 0.69), 138(64), 111(*100*), 110(83), 95(17), 83(4), 77(18), 76(9), 65(22), 64(8).

*3-Methoxycarbonyl 4-Bromo-propiolanilide (5).* The title compound (white crystalline solid) was made as described for the synthesis of 3-methoxycarbonyl propiolanilides (**Figure 1**) by the condensation of 4-bromo-aniline (0.012 mol) and dimethyl acetylene dicarboxylate (0.015 mol, 2.13 mL) under an inert atmosphere of nitrogen followed by crystallization; yield, 1.40 g (41%); mp, 186−188 °C (dec.); TLC $R_f$, 0.70; GC $R_t$, 12.39 min; $^1$H NMR ($CDCl_3$, $\delta$), 3.90 (s, 1H, *COOCH*$_3$), 7.20 (m, 1H, $H_b'$ aromatic), 7.45 (m, 3H, $H_a$, $H_a'$,$H_b$ aromatic), 9.80 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 282 ($M^+$, 2), 199(51), 197(46), 172(*100*), 170(89), 156(25), 154(28), 93(18), 77(25), 65(19), 59(38), 31(15).

*3-Methoxycarbonyl Propiolanilide (6).* Aniline (0.012 mol, 1.38 mL) and dimethyl acetylene dicarboxylate (0.015 mol, 2.13 mL) were reacted under stirring at ≤10 °C to provide the title compound as a white crystalline solid; yield, 1.05 g (43%); mp, 142 °C (dec.); TLC $R_f$, 0.74; GC $R_t$, 12.86 min; $^1$H NMR ($CDCl_3$, $\delta$), 3.90 (s, 1H, *COOCH*$_3$), 7.20 (m, 2H, $H_b$, $H_b'$ aromatic), 7.40 (m, 2H, $H_a$, $H_a'$ aromatic), 9.30 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 203 ($M^+$, 11), 119(44), 93(*100*), 77(45), 76(29), 65(13), 59(8), 31(16).

**Anilides Containing Aryl Moiety as a Side Chain (7−10).** In this series, two analogues viz., methyl phthalanilate (**7**−**9**) and *O*-acetyl salicylanilide (**10**), were synthesized.

*Methyl 4-Fluoro-phthalanilate (7).* A stirred solution of dimethyl phthalate (0.03 mol, 5.82 mg) and 4-fluoro-aniline (0.025 mol, 2.8 mL) suspended in dry THF (5 mL) was heated to reflux for 2 h under an inert atmosphere of nitrogen. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as a 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 diethyl ether/hexane (1:1) furnished the title compound in the form of white crystals. The solid was further crystallized with ethanol to furnish white crystals of methyl 4-fluoro-phthalanilate (**Figure 1**) (**7**); yield, 4.33 g (63%); mp, 182−183 °C; TLC $R_f$, 0.42; GC $R_t$, 14.42 min; $^1$H NMR ($CDCl_3$, $\delta$), 2.10 (s, 1H, *COOCH*$_3$), 7.00 (m, 1H, $H_3$ aromatic), 7.20 (m, 1H, $H_2$ aromatic), 7.35

(m, 1H, $H_4$ aromatic), 7.55 (m, 1H, $H_1$ aromatic), 7.60 (m, 1H, $H_b'$ aromatic), 7.80 (m, 3H, $H_a$, $H_a'$, $H_b$ aromatic), 9.95 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 273 ($M^+$, 8), 148(*100*), 136(51), 120(18), 111(67), 95(76), 78(5), 77(28), 66(12), 59(33).

*Methyl 4-Bromo-phthalanilate (8).* Reaction of dimethyl phthalate (0.03 mol, 5.82 mg) in dry THF (5 mL) with 4-bromo-aniline (0.025 mol, 2.8 mL) following **Figure 1** furnished white crystals of methyl 4-bromo-phthalanilate (**8**); yield, 5.59 g (67%); mp, 195 °C; TLC $R_f$, 0.41; GC $R_t$, 13.39 min; $^1$H NMR ($CDCl_3$, $\delta$), 3.80 (s, 1H, $COOCH_3$), 7.20 (m, 4H, phthalide aromatic), 7.60 (m, 1H, anilide aromatic), 9.60 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 334 ($M^+$, 12), 199(62), 197(58), 172(39), 170(35), 157(18), 155(14), 148(*100*), 136(61), 120(19), 115(27), 77(36), 65(9).

*Methyl Phthalanilate (9).* To a stirred solution of dimethyl phthalate (0.03 mol, 5.82 mg) was added aniline (0.025 mol, 2.3 mL) dissolved in dry THF (5 mL) and heated to reflux for 2 h under an inert atmosphere of nitrogen following the general procedure to furnish white crystals of the title compound (**9**); yield, 3.89 g (61%); mp, 143 °C; TLC $R_f$, 0.35; GC $R_t$, 15.58 min; $^1$H NMR ($CDCl_3$, $\delta$), 3.30 (s, 1H, $COOCH_3$), 7.15 (m, 4H, phthalide aromatic), 7.50 (m, 1H, anilide aromatic), 9.50 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 255 ($M^+$, 9), 148(*100*), 136(74), 115(63), 93(14), 77(41), 76(29), 65(9), 59(5).

*Synthesis of O-Acetyl-4′-fluoro-salicylanilide (10).* To a solution of *O*-acetyl-methyl salicylate (0.025 mol, 4.87 mL) in dichloromethane (10 mL) was added 4-fluoro-aniline (0.025 mol, 2.8 mL) suspended in dichloromethane. The reaction mixture was stirred vigorously at reflux for 2 h. Then, the mixture was diluted with diethyl ether (40 mL) and water (50 mL) and washed with water (10 mL). The organic phase was dried with magnesium sulfate, filtered, and evaporated to yield a solid compound (**Figure 1**). The residue was recrystallized with boiling ethanol to furnish white crystals of the title compound in 79% yield (5.39 g), which was homogeneous by TLC [hexane/ethyl acetate (4:1) as a developing medium]; mp, 156−158 °C; TLC $R_f$, 0.51; GC $R_t$, 13.25 min; $^1$H NMR ($CDCl_3$, $\delta$), 2.10 (s, 1H, $COCH_3$), 6.90 (m, 4H, aromatic), 9.27 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 273 ($M^+$, 13), 138(79), 137(22), 134(11), 148(*100*), 111(59), 78(27), 77(13), 65(9).

**(Ar)-Alkenyl Anilides (11−13).** In this series, three analogues viz., cinnamyl anilate (**11**), methyl crotonyl anilate (**12**), and 4-fluoro-methyl crotonyl anilate (**13**), were synthesized.

*Cinnamyl Anilate (11).* To a solution of aniline (0.025 mol) in DMF (1 mL) was added methyl cinnamate (0.03 mol, 4.86 mL) dissolved in a mixture of DMF/$H_2O$ (2:1.5 mL) under an inert atmosphere of nitrogen. The resulting mixture was stirred under reflux for 3 h, and methanol was collected as an azeotrope. The dark colored aliquot 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 compound (**Figure 1**) in the form of a white crystalline solid (3.24 g, 58% yield), which was homogeneous in TLC [hexane/ethyl acetate (4:1) as a developing medium]; mp, 112−115 °C; TLC $R_f$, 0.39; GC $R_t$, 8.64 min; $^1$H NMR ($CDCl_3$-DMSO-$d_6$, $\delta$), 6.10 (s, 1H, =*CHPh*), 6.40 (s, 1H, =*CHCO*), 7.25 (m, 10H, aromatic), 7.60 (m, 5H, aromatic), 10.60 (*s*, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 223 ($M^+$, 6), 146(8), 131(29), 130(11), 103(18), 102(25), 93(*100*), 78(62), 77(52), 64(25).

*Methyl Crotonyl Anilate (12).* To a stirred solution of aniline (0.025 mol) was added methylcrotonate in DMF and heated to reflux for 4 h following the general procedure (**Figure 1**) to furnish a solid compound. The resulting solid was recrystallized from ethanol to furnish a white crystalline solid of the title compound; yield, 1.93 g (48%); mp, 109 °C; TLC $R_f$, 0.48; GC $R_t$, 7.69 min; $^1$H NMR ($CDCl_3$, $\delta$), 6.35 (s, 1H, =*CH*), 6.40 (s, 1H, =*CHCO*), 6.60 (m, 3H, $H_b$, $H_b'$, $H_c$ aromatic), 6.80 (m, 2H, $H_a$ and $H_a'$ aromatic), 10.60 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 161 ($M^+$, 17), 145(8), 119(69), 93(*100*), 91(26), 78(65), 69(36), 41(48), 15(56).

*4-Fluoro-methyl Crotonyl Anilate (13).* A stirred solution of 4-fluoro-aniline (0.025 mol, 2.8 mL), in DMF (5 mL) and methylcrotonate (0.03 mol, 3.00 mL) was heated to reflux for 5 h under an inert atmosphere of nitrogen 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 solid; yield, 2.39 g (53%); mp, 112−115 °C; TLC $R_f$, 0.42; GC $R_t$, 9.25 min; $^1$H NMR ($CDCl_3$, $\delta$), 6.35 (s, 1H, =*CH*), 6.40 (s, 1H, =*CHCO*), 7.00 (m, 2H, $H_b$ and $H_b'$ aromatic), 7.25 (m, 2H, $H_a$ and $H_a'$ aromatic), 10.30 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 180 ($M^+$, 11), 139(58), 138(46), 110(*100*), 91(42), 78(61), 41(28), 15(20).

**3-Amino Alkenyl Anilides and Analogues (14−16).** In this series, three analogues viz., 3-(*N*-acetamino)-4-fluoro-crotonanilide (**14**), 3-(*N*-trityl)amino-4-fluoro-crotonanilide (**15**), and 3-amino-4-bromo-crotonanilide (**16**), were synthesized.

*3-(N-Acetamino)-4-fluoro-crotonanilide (14).* A mixture of ethyl 3-amino crotonate (0.05 mol, 6.45 g), acetic anhydride (0.05 mol), and pyridine was kept stoppered for overnight at room temperature to be followed by the addition of 5 mL of HPLC-grade methanol. The resulting intermediate (ethyl 2-acetamino crotonate) was recrystallized from ether/hexane (1:1). A stirred solution of the resulting ethyl 2-acetamino crotonate (0.025 mol, 4.28 g) and 4-fluoro-aniline (0.025 mol, 2.8 mL) suspended in dry xylene (5 mL) was heated to reflux for 3.5 h under an inert atmosphere of nitrogen. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as a developing medium] until completion. Chilled hexane (20 mL × 2) was added to the mixture to remove xylene to obtain a solid, which was recrystallized in diethyl ether/hexane to furnish a buff solid of the title compound **14** (**Figure 2**); yield, 3.51 g (59%); mp, 178−179 °C; TLC $R_f$, 0.32; GC $R_t$, 11.38 min; $^1$H NMR ($CDCl_3$, $\delta$), 2.30 (s, 3H, $CH_3$), 2.55 (s, 3H, $COCH_3$), 5.00 (s, 1H, =*CHCO*), 7.00 (m, 2H, $H_b$, $H_b'$ aromatic), 7.30 (m, 2H, $H_a$, Ha′ aromatic), 10.56 (s, 1H, *NH*), 10.60 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 236 ($M^+$, 2), 174(28), 173(69), 159(*100*), 138(72), 110(56), 86(19), 78(27), 77(13), 58(12), 43(31).

*3-(N-Trityl)amino-4-fluoro-crotonanilide (15).* To a mixture of ethyl 3-amino crotonate (0.05 mol, 6.45 g) in dry $CHCl_3$ (15 mL), triethylamine (0.05 mol, 5.5 mL), and trityl chloride (0.01 mol, 2.78 g) were added, and the mixture was allowed to react for 6 h at room temperature with continuous stirring. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as a developing medium] until completion. The product was then washed with water and dried with $Na_2SO_4$. The complete removal of $CHCl_3$ was ensured by adding a few milliliters of methanol, and reconcentration was done in a vacuum. The residue was recrystallized from methanol to obtain grayish-white crystals of the intermediate (ethyl 2-tritylamino crotonate). To a solution of the resultant solid intermediate (ethyl 2-tritylamino crotonate; 0.025 mol, 9.28 g) was added 4-fluoro-aniline (0.025 mol, 2.8 mL) in xylene, and the reaction mixture was stirred vigorously at reflux for 4.5 h. Chilled hexane (20 mL × 2) was added to the mixture to remove xylene. The excess hexane was evaporated off and the mixture was poured into ice-cold water followed by recrystallization in ether/hexane (1:1) to obtain a grayish-white solid of **15** (**Figure 2**), which was homogeneous, by TLC; yield, 3.00 g (28%); mp, 216−218 °C; TLC $R_f$, 0.48; GC $R_t$, 9.57 min; $^1$H NMR ($CDCl_3$, $\delta$), 2.50 (s, 3H, $CH_3$), 5.20 (s, 1H, =*CHCO*), 7.00 (m, 2H, $H_b$, $H_b'$ aromatic), 7.50 (m, 2H, $H_a$, $H_a'$ aromatic), 10.85 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 437 ($M^+$, 6), 243(19), 174(57), 167(21), 159(*100*), 158(77), 138(69), 110(85), 78(67), 77(52), 64(21).

*3-Amino-4-bromo-crotonanilide (16).* 4-Bromo-aniline (0.045 mol) and ethyl 3-amino crotonate were reacted together for 4 h following the general procedure (**Figure 2**) to furnish a white crystalline solid of the title compound; yield, 4.00 g (35%); mp, 226 °C; TLC $R_f$, 0.51; GC $R_t$, 12.06 min; $^1$H NMR ($CDCl_3$, $\delta$), 1.50 (s, 3H, $CH_3$), 2.30 (s, 2H, $NH_2$), 4.50 (s, 1H, =*CHCO*), 7.40 (m, 4H, aromatic), 8.90 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 255 ($M^+$, 13), 253(11), 235(88), 233(85), 220(*100*), 218(92), 199(42), 197(39), 171(27), 169(25), 155(72), 119(48), 91(55), 78(61), 77(13), 56(17).

**General Method of Synthesis of *N*-Aryl Phthalimides (17−19).** To a suspension of phthalic anhydride (0.025 mol, 3.7 g) in dry toluene (4 mL) under inert nitrogen atmosphere was slowly added a solution of substituted aniline (0.025 mol) under stirring. The resulting mixture was stirred for 1 h to furnish *N*-aryl phthalimide as a solid product. The reaction was followed by TLC [hexane/ethyl acetate (4:1) as a developing medium] until completion. The resultant solid material was filtered to furnish the crude product, which was recrystallized with acetone to give solid products of the title compounds (**Figure 1**).

*N-(4′-Fluoro-phenyl)phthalimide (17).* The title compound was made as described for the synthesis of the *N*-aryl phthalimides by the

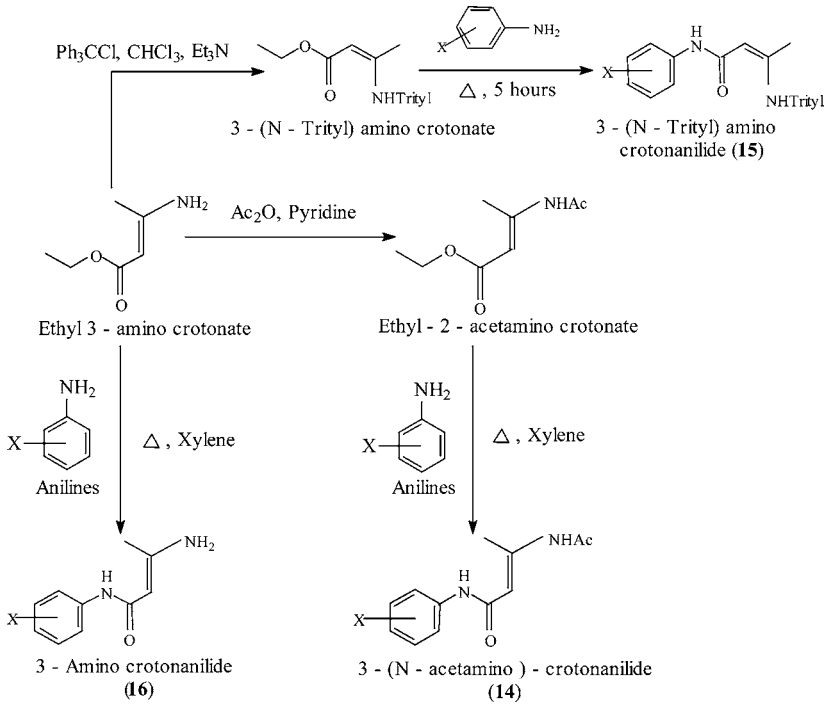



**Figure 2.** Synthesis of 3-amino alkenyl anilides and analogues.

condensation of 4-fluoro-aniline (0.025 mol, 2.8 mL) and phthalic anhydride (0.025 mol, 3.7 g) followed by recrystallization from acetone to yield a grayish amorphous solid of the title compound **17**; yield, 5.59 g (92%); mp, 139−140 °C; TLC $R_f$, 0.63; GC $R_t$, 11.22 min; $^1$H NMR ($CDCl_3$, $\delta$), 7.10 (m, 2H, $H_b$, $H_b'$ aromatic), 7.35 (m, 2H, $H_a$, $H'_a$ aromatic), 7.80 (m, 4H aromatic protons in phthalimide ring); El-MS *m*/*z* (rel. int. %), 241($M^+$, 21), 147(*100*), 138(73), 120(35), 111(39), 97(64), 91(76), 78(22), 66(35).

*N-(4′-Bromo-phenyl)phthalimide (**18**).* To a stirred suspension of 4-bromo-aniline (0.025 mol, 4.28 mL) was made to react with phthalic anhydride following the general procedure to yield a grayish amorphous solid; yield, 6.48 g (86%); mp, 168 °C; TLC $R_f$, 0.60; GC $R_t$, 10.38 min; $^1$H NMR ($CDCl_3$, $\delta$), 7.35 (m, 4H, aromatic), 7.75 (m, 4H aromatic protons in phthalimide ring); El-MS *m*/*z* (rel. int. %), 302 ($M^+$, 13), 301(11), 147(*100*), 199(58), 197(55), 171(29), 169(27), 119(15), 97(31), 91(16), 78(48), 66(25).

*N-Phenylphthalimide (**19**).* A stirred suspension of phthalic anhydride (0.025 mol, 3.7 g) and aniline (0.025 mol, 2.3 mL) in dry toluene (4 mL) was stirred under an inert atmosphere of nitrogen to furnish *N*-phenylphthalimide as a solid product of the title compound; yield, 4.58 g (82%); mp, 105 °C; TLC $R_f$, 0.53; GC $R_t$, 12.18 min; $^1$H NMR ($CDCl_3$, $\delta$), 7.20 (m, 2H, $H_c$, $H_b'$ aromatic), 7.40 (m, 3H, $H_a$, $H'_a$, $H_b$ aromatic), 7.80 (m, 4H aromatic protons in phthalimide ring); El-MS *m*/*z* (rel. int. %), 224 ($M^+$, 16), 147(*100*), 119(32), 97(46), 91(68), 77(32).

***N*-Alkylanilines (20−21).** In this series, two analogues viz., ethyl-(4-fluoroanilino)acetate (**20**) and 4-fluoro-*N*-$\beta$-cyanoethyl-aniline (**21**), were synthesized.

*Ethyl-(4-fluoroanilino)acetate (**20**).* To a stirred solution of ethyl bromo-acetate (0.03 mol, 5.01 mL), 4-fluoro-aniline (0.025 mol, 2.8 mL), and triethylamine (0.03 mol, 3.03 mL) in dichloromethane (12 mL) was heated to reflux for 45 min at 100 °C (**Figure 3**). The product was cooled to 70 °C to solidify, recrystallized with boiling ethanol, and refrigerated to allow recrystallization of the title compound as a white crystalline solid, which was homogeneous by TLC [hexane/ethyl acetate (4:1) as a developing medium]; yield, 3.87 g (78%); mp, 92−93 °C; TLC $R_f$, 0.54; GC $R_t$, 7.18 min; $^1$H NMR ($CDCl_3$, $\delta$), 1.20 (t, $J$ = 6 Hz, 3H, $CH_3$), 1.75 (s, 2H, $CH_2CO$), 4.20 (q, $J$ = 6 Hz, 2H, $OCH_2$), 6.70 (m, 3H, $H_a'$, $H_b$, $H_b'$ aromatic), 7.00 (m, 1H, $H_a$ aromatic), 9.65 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 197 ($M^+$, 4), 124(18), 111(*100*), 91(25), 87(31), 78(64), 73(15), 66(49), 45(38).

*4-Fluoro-N-$\beta$-cyanoethyl-aniline (**21**).* To a solution of 4-fluoroaniline (0.025 mol, 2.8 mL) in DMSO (10 mL) was added excess

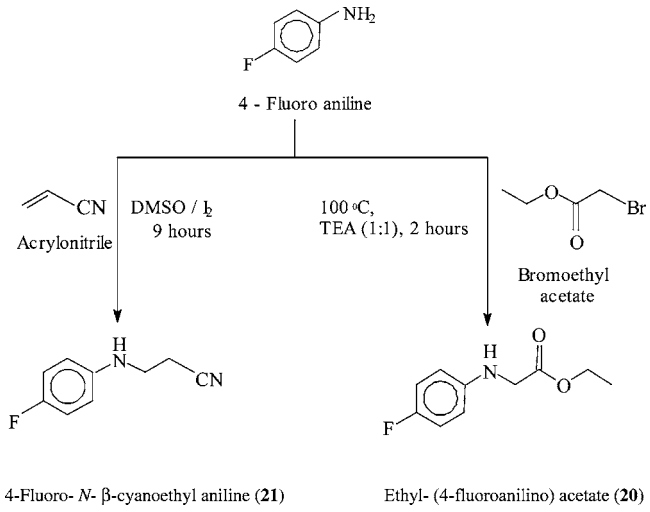



**Figure 3.** Synthesis of *N*-alkylaniline analogues.

acrylonitrile (0.03 mol, 1.3 mL) with iodine as a catalyst. The reaction mixture was stirred vigorously at reflux for 9 h. The reaction mixture was cooled, and the residue was recrystallized with boiling ethanol to give grayish white flakes of the title compound, which was homogeneous by TLC [hexane/ethyl acetate (4:1) as a developing medium]; yield, 1.16 g (28%); mp, 132−140 °C; TLC $R_f$, 0.29; GC $R_t$, 12.38 min; $^1$H NMR ($CDCl_3$-DMSO-$d_6$, $\delta$), 2.90 (m, 4H, $NHCH_2$ and $CH_2CN$), 7.20 (m, 2H, $H_b$, $H_b'$ aromatic), 7.55 (m, 2H, $H_a$, $H_a'$ aromatic), 8.20 (s, 1H, *NH*); El-MS *m*/*z* (rel. int. %), 164 ($M^+$, 15), 124(39), 111(*100*), 96(55), 91(67), 77(84), 76(62), 54(20), 52(13), 40(11).

**Field Evaluation of CHAs.** Three high-yielding varieties of bread wheat (*Triticum aestivum* L.) viz., PBW 343, HW 2046, and HD 2733, recommended for timely sowing were chosen for evaluation of chemical induction of male sterility. The experiment was laid out following randomized block design 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 at Indian Agricultural Research Institute (IARI), New Delhi. Other optimum agronomic practices were also followed, which included recommended fertilizer schedule, timely weeding, and other cultural operations. Five irrigations were given at different stages of crop growth viz., crown root initiation (CRI), late tillering, late jointing, flowering, and dough

stage. Row−row distance was kept at 30 cm. Five rows of 2 m in length were taken as a plot in which the outermost two rows were treated as the pollen parents and the inner three rows as the female parents. The incoming foreign pollens were prevented to enter 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) using polyoxyethylene sorbitan monooleate (FW ∼ 1200) (Tween-80) (1 g), as an emulsifier, was prepared after trial and error with different solvents. To ensure phytosafety of cyclohexanone, a blank solvent emulsion without CHAs was sprayed on the crop well in advance. No serious phytotoxic symptoms were visible 2−3 days after the spray. The synthetic compounds were sprayed at premeiotic stage in the winter of 2001−2002 at a concentration of 1500 ppm when the length of the spike emerging out from the first node was about 6−9 mm in length (60 days after sowing (*14*). While spraying, utmost precaution was taken not to spill the chemicals on the tillers of the pollinator lines. Crossing of the female parents was done by generous dusting of the pollens from HW 2045. To ensure cross pollination, pollens from the male parent were also manually dusted on the sterilized female spike and bagging was done immediately to prevent any further cross pollination by any undesirable foreign pollens. Because the degree of synchrony of flowering varied with the variety, care was taken to tag the treated tillers of an appropriate stage. A total of 10 spikes of each treated plants were covered immediately after emergence. 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 were smeared together over a drop of acetocarmine (1%) or KI (2%) in iodine and examined under a light microscope. Pollen sterility was calculated in percentages. A total of 10 of each bagged and unbagged spikes from each treatment including one control were harvested at maturity. Male sterility was calculated as the percent inhibition of seed set in bagged spikes of treated plants. Data were recorded on pollen sterility and spikelet sterility under bagged/unbagged conditions as detailed elsewhere (*10*, *11*).

Analysis of variance (ANOVA) of factorial randomized block design (RBD) was performed with all treatments. On the basis of the significance of the treatments, critical differences (CDs) at a 5% level of significance ($p = 0.05$) were computed.

**QSAR Study.** The development of an agrophore model can serve as a powerful tool in discovering new leads based on existing active chemistry. The agrophore strategy involves identifying critical structural elements responsible for activity via a hypothetical mode of action. There has been very little progress in the mode of action of CHAs. This situation makes CHA-agrophore development difficult, but at the same time, it provides an opportunity to build a discovery program focused on developing new CHAs and on elucidating a CHA mode of action. The QSAR method applied to two families of CHAs in the anilides containing olefinic moiety in the side chain and *N*-alkyl aniline class of chemistry. To observe the variability in the olefinic and alkyl side chain, the physicochemical descriptors, namely, $\pi_a$, molecular weight ($MW_a$), molar refractivity ($MR_a$), parachor (P), and polarizability (Pl), were generated using ACD Chemsketch (version 2) software. The following descriptive variables were used for aromatic substituents viz., electronic parameters: Swain−Lupton field constant or inductive parameter (*F*) (*15*), Hammett constants ($\sigma_m$ and $\sigma_p$) to define electron-donating and -withdrawing tendencies, partial atomic charges and electrostatic field densities, and Swain−Lupton resonance constant *R* (*16*); steric parameters: Taft steric parameter ($E_s$), molecular weight ($MW_b$), and Verloop−Hoogenstraaten multidimensional steric parameters, *L* and *B*4 (*17*, *18*); hydrophobic parameter: $\pi_b$; and other parameters such as molar refractivity ($MR_b$), and index variable (*D*).

A total of 15 independent variables were used in constructing the correlation matrix. The independent variables, which were found orthogonal to each other in the correlation matrix, were minimized. The "agrophore" data viz., percent induction of spikelet sterility caused by test CHAs tested at 1500 ppm, were transferred into *sin arc* and used as the dependent variable (PS % *sin arc*). The descriptor variables were used to generate multiple linear regression equations (MLR) by autocorrelation using the SPSS program (version 10.0). None of the independent variables appearing in the equations was ensured to be orthogonal.

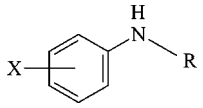
## RESULTS AND DISCUSSION

**Syntheses and Spectral Analyses.** The synthesis of different series of CHAs belonging to anilides having olefinic and alkyl moiety in the side chain involves condensation of anilines with esters, acid chlorides, anhydrides, and nitrile as detailed under the earlier section. The substituent fluoro at the *para* position in furyl anilide caused deshielding of the $H_a$ and $H_a'$ aromatic protons by about 0.90 and 0.35 ppm, respectively. The effect of bromine on the deshielding of the same is comparatively less being 0.60 ppm for both $H_a$ and $H_a'$ as compared to that of the unsubstituted furyl anilide ($\delta$ 7.20 ppm) (**Figure 1**). It was observed that there was an overall deshielding effect of halogen atoms on the chemical shifts of furyl protons ($H_{af}$, $H_{bf}$, and $H_{cf}$). The α-olefinic proton in cinnamyl anilate appeared as a singlet at $\delta$ 5.56 ± 0.84 ppm, with the highest deshielding effect being experienced both by the olefinic proton geminal to the methyl or phenyl group in methyl crotonyl and cinnamyl anilides ($\delta$ = 6.40 ppm). In *N*-aryl phthalimide, the anilido protons experienced a marked variation in the chemical shift because of their proximity with the halogen substituents. In the 4′-fluoro analogue, the $H_a$ and $H_b$ protons were shielded by 0.05 and 0.30 ppm, respectively, perhaps because of the predominant +M effect of the fluorine atom. The *NH* proton in *N*-*β*-cyanoethyl aniline appeared upfield at $\delta$ 4.40 ppm in contrast to the same in ethyl-4-(fluoroanilino)acetate, which appeared significantly downfield at $\delta$ 9.65 ppm. The methylene protons ($CH_2$CO) in ethyl-(4-fluoroanilino)acetate appeared at $\delta$ 1.75 ppm as a sharp singlet. In 4-fluoro-*N*-*β*-cyanoethylaniline, the $CH_2$CN protons appeared upfield at about $\delta$ 2.90 ppm.

In the mass fragmentation pattern of furyl anilides, the base peak was found to be the furyl acylium moiety (*m*/*z* 95). The parent aniline was assigned to be the base peak in the mass fragmentation of 3-methoxycarbonyl propiolanilide. Methyl phthalanilates gave the mass fragmentation pattern similar to *O*-acetyl-(4-fluoro)salicylanilde, and the base peak was assigned to be the indane dione moiety. The spectra further contained lower *m*/*z* fragment viz., *m*/*z* 111, 78, 77, 65, etc., characteristic of the aromatic skeleton. In the mass fragmentation pattern of 3-amino alkenyl anilides and analogues, fission of ethoxycarbonyl moiety gave rise to the protonated aryl isocyanate moiety. The mass fragment at *m*/*z* 159 (4′-fluoro-phenyl-pyridone moiety) was assigned to be the base peak in this series. In *N*-aryl phthalimides, phthalimide (*m*/*z* 147) was assigned to be the base peak.

**Effect of CHAs on Spikelet Sterility.** As may be seen from **Table 1**, the maximum male sterility was induced by 4-fluoro-furyl anilide (**1**) followed by 4-bromo-furyl anilide (**2**) on three genotypes of wheat. In HD 2733, the trend of activity was the same as in the other two genotypes, but the magnitude of the effect was found to be more pronounced. Incorporation of the acetylinic bridge in the amide side chain did not improve the activity as compared to the olefinic double bond. Among aryl-substituted propiolanilides, 4-fluoro propiolanilide (**4**) induced greater male sterility than the other analogues (i.e., 4-bromo and unsubstituted), with the trend of activity being 4F (**4**) > 4Br (**5**) ≫ H (**6**). It was observed that 4-fluoro-phthalanilate (**7**) induced greater activity as compared to *O*-acetyl-4-fluoro-salicylanilide (**10**). A series of compounds of *N*-acylanilines with an (ar)-alkenyl side chain were prepared among which cinnamyl analogues (**12** and **13**) were found to be more active than the

**Table 1.** Effect of Side-Chain Variation in Anilides on the Percent Induction of Spikelet Sterility at 1500-ppm Test Concentrations on Three Genotypes of Wheat on Winter 2001–2002




| | | | % spikelet sterility | | |
|---|---|---|---|---|---|
| compound number | X | R | PBW 343 | HW 2046 | HD 2733 |
| **1** | 4-F | $COC_4H_3O$ | 82.81 | 79.68 | 83.48 |
| **2** | 4-Br | $COC_4H_3O$ | 82.45 | 79.46 | 83.22 |
| **3** | H | $COC_4H_3O$ | 54.53 | 50.82 | 55.66 |
| **4** | 4-F | COC≡CCOOMe | 67.47 | 66.79 | 68.54 |
| **5** | 4-Br | COC≡CCOOMe | 65.42 | 64.58 | 66.76 |
| **6** | H | COC≡CCOOMe | 49.72 | 48.65 | 52.61 |
| **7** | 4-F | $COC_6H_4COOMe$ (ortho) | 70.87 | 69.99 | 73.64 |
| **8** | 4-Br | $COC_6H_4COOMe$ (ortho) | 65.01 | 63.32 | 67.50 |
| **9** | H | $COC_6H_4COOMe$ (ortho) | 26.17 | 22.67 | 27.78 |
| **10** | 4-F | $COC_6H_4OCOCH_3$ (ortho) | 40.48 | 35.16 | 42.28 |
| **11** | H | COCH═CHPh | 55.97 | 51.37 | 57.69 |
| **12** | H | COCH═CHMe | 48.51 | 45.95 | 51.17 |
| **13** | 4-F | COCH═CHMe | 52.99 | 50.16 | 55.07 |
| **14** | 4-F | COCH═C(Me)NHAc | 39.41 | 34.70 | 39.59 |
| **15** | 4-F | COCH═C(Me)NHTri | 23.66 | 20.48 | 24.42 |
| **16** | 4-Br | $COCH═C(Me)NH_2$ | 21.29 | 18.07 | 22.31 |
| **17** | 4-F | $CONCOC_6H_4CO$ | 74.21 | 71.89 | 74.83 |
| **18** | 4-Br | $CONCOC_6H_4CO$ | 69.38 | 65.82 | 71.87 |
| **19** | H | $CONCOC_6H_4CO$ | 51.50 | 48.09 | 52.73 |
| **20** | 4-F | $NHCH_2COOEt$ | 41.84 | 41.43 | 47.33 |
| **21** | 4-F | $NHCH_2CH_2CN$ | 59.29 | 56.69 | 61.23 |
| | emulsion control | | 0.46 | 0.33 | 0.49 |
| | CD ($p = 0.05$) | | 1.02 | 2.21 | 0.95 |

crotonate analogue (**11**). Even the unsubstituted cinnamyl anilate induced a higher (55.97) percent of male sterility in PBW 343 than crotonyl anilate, which induced 52.99%. Also, functionalization of the third carbon position in a series of 3-amino alkenyl anilides and analogues did not improve their performance as CHAs (male sterility < 40%). Among 3-(*N*-trityl-amino)-4-fluoro-crotonanilide (**15**) and 3-(*N*-acetamino)crotonanilide (**14**), the later had shown comparatively higher activity than the former. Among the *N*-aryl phthalimides, *N*-(4-fluorophenyl)phthalimides (**17**) had shown greater induction of male sterility on three genotypes of wheat (**Table 1**). Among *N*-alkylanilines, 4-fluoro-*N*-$\beta$-cyanoethyl aniline (**21**) exhibited comparatively higher activity than ethyl-(4-fluoro-anilino)acetate (**20**) (**Table 1**). It is significant that the CHAs belonging to furyl anilides not only induced 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 increasing the probability for the development of hybrids. Wheat is a self-pollinated crop having a closed floret. Florets of male sterile wheat opened twice to facilitate the cross fertilization. First floret opening with the action of lodicules lasted only for a shorter period. Second floret opening started after the lodicules have collapsed, and the carpels in the sterile floret continued to grow palea and lemma apart. This second opening lasted for more 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. To ensure cross pollination, there is no need to cut the palea and lemma, because second floret opening is sufficient for cross pollination to occur. Further, peak period for stigma receptivity lasted for about 3−4 days, which helped in planning for hybrid seed production. The CHA technology was optimized in terms of variation in genotype, choice of CHA, stage of spray, number of spray, type of formulation, and dose. The premeiotic stage (6−8 mm of spike length) was found to be very ideal. The single spray was adequate and safe. Oil in water emulsion was used. Cyclohexanone was found to be the best among the solvents tested. As far as the aspect of toxicity of the CHAs is concerned, no residue of CHA has been noticed in the $F_2$ seeds of wheat, and thus, it has no outreach to the consumer in the form of crop produce.

From the stain test, it was seen that the sterile grains obtained from the treatment were transparent, thereby confirming the disintegration of cytoplasm and nucleus in the sterile pollen. In contrast, fertile pollen from control plots stained uniform deep red or blue color depending on the stain, confirming the induction of male sterility in various treatments. In some cases, the sterile pollen grains became shriveled and the mass of cytoplasm and nucleus contracted in the pollen keeping the external pollen wall (exine) intact.

**QSAR Study.** Results of the multiple regression analysis carried out are given along with the statistical values ($N$ = number of compounds; $r$ = multiple regression coefficient; $s$ = standard deviation; and $F$ = Fisher's ratio of significance index with respect to the equation). All of the equations were found to be statistically significant at $p < 0.01\%$.

In this study, the acyl side chain has been varied with various chemical groups such as $C_4H_3O$, $-CCOOMe$, $-C(Cl)_2CH_3$, $-C_6H_4COOCH_3$, $-C_6H_4OCOCH_3$, $-CH═CHPh$, $-CH═CHCH_3$, $-CH═C(CH_3)NHAc$, $-CH═C(CH_3)NHTri$, and $-CH═C(NH_2)CH_3$ with concurrent variation in the aromatic substitution mostly by F, Br, or unsubstituted as well. Replacing the alcohol moiety from furoyl ($-C_4H_3O$) (**1**−**3**) to methoxycarbonyl propiolanilide ($C≡CCOOCH_3$) (**4**−**6**) led to a decrease in the target activity. Inspired by the enhancing effect of the electronegative groups (F and Br) in our earlier studies (*10*, *11*), *O*-acetyl-(4′-fluoro)salicylanilide (**10**) and methyl phthalanilides (**7**−**9**) containing different substituents in the aromatic ring (4′-F, 4′-Br, and unsubstituted) were synthesized. While

methyl 4′-fluoro-phthalanilide (**7**) induced 70.87% male sterility, *O*-acetyl-(4′-fluoro)salicylanilide (**10**) induced remarkably low male sterility, i.e., 40.48%. It therefore appears that methoxycarbonyl rather than the acetoxy group in the *ortho* position of the side-chain aromatic ring had an activity-enhancing effect. Functionalization of the ethoxycarbonyl group in the side chain by way of cinnamyl and crotonyl groups has caused a severe reduction in the male sterility percentage. Functionalization of the amino group in **16** with acetyl (−*NHAc*) and trityl (−*NHTri*) groups as in **14** and **15** led to the enhancement of activity as compared to that of the parent compound (**16**).

QSAR analysis of the 21 analogues thus generated was carried out by using a combination of chemical descriptors both for aromatic substitution and side-chain variation, and the correlation matrix was constructed. The models for each CHA family gave a good correlation between the variations in log percentages of spikelet sterility and the steric−electrostatic properties of the sets. In the anilides synthesized, the observed bioactivity could be collectively explained in the form of two multiple linear regression (MLR) equations using a total of 15 independent variables:

$$\text{spikelet sterility } (\textit{sin arc}\ \%) = 0.32F_p - 2.19P + 99.52 \quad (1)$$

where $N = 21$, $r = 0.73$, $s = 15.63$, and $F = 6.480$

$$\text{spikelet sterility } (\textit{sin arc}\ \%) = 34.71F_p - 8.44\sum MR_b - 5.27P + 99.517 \quad (2)$$

where $N = 21$, $r = 0.92$, $s = 6.14$, and $F = 28.63$.

The best equation (eq 2) was the one that combined the independent variables $F_p$ for aromatic substitution and parachor ($P$) and $\sum MR_b$ for the side chain with $r$ values of 0.92. The direct involvement of Swain−Lupton field constant for *para* substitution ($F_p$) with the target bioactivity in the best equation implied that inductive (field or polar) rather than resonance effect ($R$) of the substituent is the key factor influencing the induction of male sterility. It can be inferred that *para* substitution with highly electronegative groups such as F or 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. Swain and Lupton studied the inverse correlation of $F_p$ and $R$ (*19*) in the form of the equation viz., $\sigma_p = \alpha F + R$, where $\sigma_p$ is the Hammett constant for *para* substitution and $\alpha$ is a constant. Parachor in the olefinic side chain appeared to influence the bioactivity in a significant manner and was found to be the most dominant descriptor, which could guide us to devise better CHAs. It is apparent from the study that the closed ring structure in the side chain as in furyl analogues has a comparatively lesser surface area resulting in a lower value of parachor than 3-amino alkenyl anilides and (ar)-alkenyl anilides, having an open olefinic side chain of comparatively more surface area. It is hypothesized that the extended olefinic structure at the acyl side chain has a less efficient macromolecular receptor-fit at the enzyme active site than the closed ring structure viz., furyl anilides. It has generally been assumed that a positive coefficient with a molar refractivity (MR) term in a correlation equation suggests a binding action via dispersion forces. Such binding could produce a concomitant conformation change in a macromolecular binding site. If the conformational change favored the process under study, one would certainly expect a positive coefficient with the detrimental; a negative coefficient could result from the MR term. In competitive

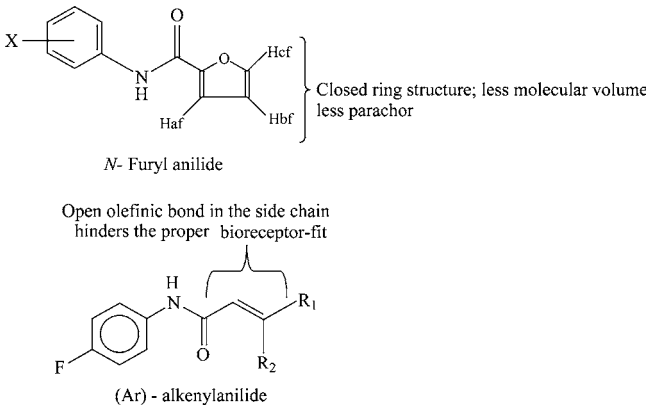



**Figure 4.** Sketch model of closed vis-à-vis open vinylogous double bond of anilides.

binding at the bioreceptor site, a negative MR term in the best equation could mean unfavorable conformational changes in the enzyme−inhibitor complex as compared to favorable conformational changes caused by the enzyme−substrate complex (*19*). It is known that, for representing bulk rather than directional effects, molar refractivity would explain better than Taft's steric constant and sterimol parameters. The negative sign of the coefficient in MR indicated that less hydrophobicity of the olefinic or alkyl moiety would enhance the target activity. 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 or energetic metabolism disruption (uncoupling effect on stamen mitochondria). The observed effects were the result of leaf transcuticular uptake and possible phloem transport. The compounds in the anilide series having olefinic moiety in the side chain are esters or substituted amides, which very likely are immediately transformed in the leaves to give the free acids. All of the free acids studied here 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. These products can therefore be suspected to concentrate inside the stamen through that way. Moreover, the acidic pH generated by the hydrolysis of anilides lead to an imbalance of acid−base equilibria in the pollen mother cells resulting in dissolution of microsporocyte callose wall during the meiotic I prophase by premature callase (1,3-$\beta$ glucanase) secretion. The high polarity of the acids (viz., furoic acid obtained from the hydrolysis of 4-fluoro-furyl anilide) results in easy water solubility and phloem transport, which add to the efficiency of the active CHAs. Therefore, chemicals with high water solubility would be preferred as active CHAs. The equation essentially highlights the negative effect of the steric parameter viz., parachor of the side chain containing the vinylogous double bonds. Interestingly, in eq 1, the sign of the coefficient of the steric variable is reversed unlike in *N*-acylaniline derivatives (*11*). It implies that acyl linkage in the side chain is directly proportional to their bulk, unlike in open vinylogous side chains where the unsaturation hinders the proper fitting in the macromolecular receptor site (**Figure 4**). There is ample scope for the development of potent CHA analogues based on the leads postulated and predicted in the present studies.

Two distinct types of histological changes (type 1 and type 2) have been established in the induction of male sterility (*20*). In type 1, callose synthesis is inhibited, and because of nonsecretion of callose, the sporogenous cells did not undergo meiosis to form tetrads and microspores, leading to the absence

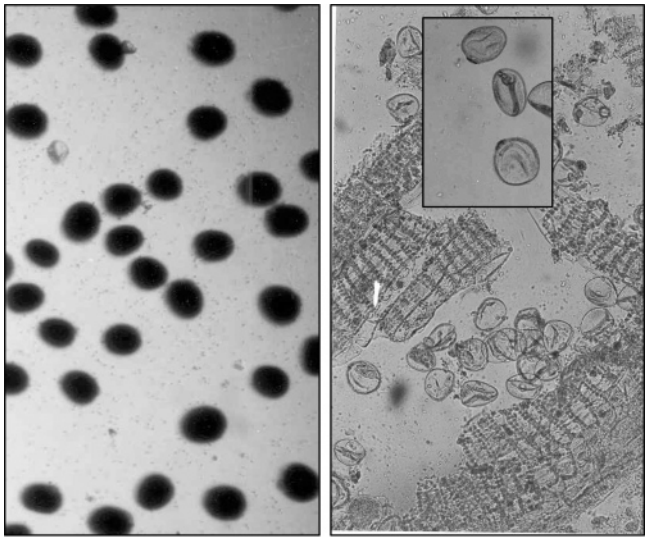
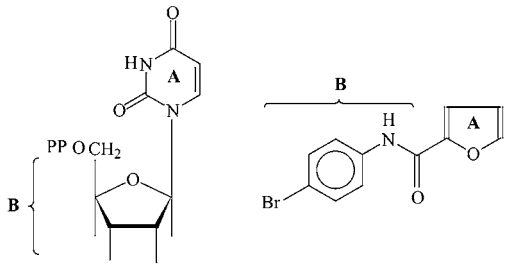



**Figure 5.** Sterile pollens of wheat because of the treatment of CHAs vis-à-vis fertile pollens as observed in KI–$I_2$ stain test.

of a pollen grain. In type 2, alterations in the timing of callase (endo-$\beta$-1,3-glucanase) expression leads to abnormal dissolution of the tetrad callose walls, which has been shown to be a primary cause of male sterility in cytoplasmic male sterile lines of petunia (*21*) and sorghum (*22*). In light of the importance of callose deposition vis-à-vis callase activity it is likely that the CHA may either block the synthesis of callose inducing type 1 action or accelerate the premature release of callase causing type 2 disruption. The absence of any starch material in the sterile pollen grains as shown by the KI$-I_2$ pollen stain test could be indicative of either of the processes leading to starch depletion or the blocking of its synthesis (**Figure 5**). The first step in the synthesis of cell-wall polysaccharides is catalyzed by UDP-glucose pyrophosphorylase (EC 2.7.7.9; monomer of an approximately 55-kDa polypeptide), and this synthesis is inhibited by the substrates such as UDP-glucose (*22*) and the most active CHA in the study. 4-Fluoro-furyl anilide (**2**) of the present invention has a dissociation constant for the reversible association with the enzyme, $K_I$, of $1.3 \pm 0.1$ $\mu$M. Because 4-fluoro-furyl anilide has a very low $K_I$ value, it is an efficient inhibitor of UDP-glucose pyrophosphorylase. A comparison of CHAs vis-à-vis UDP-glucose is depicted with the labeling of different regions in **Figure 6**. The CHAs containing the heterocyclic furyl ring and *N*-aryl moiety can possibly mimic UDP-glucose that also contains similar groups viz., uridinyl group, and isosteric glucose moiety. Incidentally, the molar volumes of bromophenyl (105.6 $cm^3$) and glucosyl moieties (106.2 $cm^3$) are almost similar. Only the field effect ($F_p$) rather than the resonance effect of aromatic substitution can give an appropriate isosteric effect with the glucosyl group as predicted by QSAR analysis.

Besides high and selective induction of male sterility, the most potent CHAs had neither shown any adverse effects on growth parameters such as plant height and germination percentage nor on yield parameters viz., spike length, female fertility, and thousand-grain weight, and thus have a great potential as CHAs for wheat. It was found that anilides with closed vinyl double bond viz., furyl anilides and *N*-alkyl aniline analogues, are most selective CHAs for wheat (**Table 2**). The real guiding principle for selectivity was found to be the $\pi$ value. For the less selective 3-(*N*-trityl)amino-4-fluoro-crotonanilide (**15**), and 3-(*N*-acetamino)-4-fluoro-crotonanilide (**14**) analogues, the $\pi$ values are negative




(**A** = Heterocyclic furyl ring or uridinyl group as in furyl anilides or UDP respectively; **B** = *N*-aryl moiety or isosteric glucose moiety as in furyl anilides or UDP respectively)

**Figure 6.** Comparison between UDP-glucose and furyl anilides with the binding of different regions bound to UDP-glucose pyrophosphorylase thus inhibiting the enzyme.

**Table 2.** Performance of Fluoroaryl Substituted Anilides for Important Traits of Wheat (PBW 343) Tested in Winter 2001–2002 at 1500-ppm Test Concentration

| compound number | female fertility (%) | 1000-grain weight (g) | spike length (cm) |
|---|---|---|---|
| **1** | 92.60 | 37.20 | 10.42 |
| **4** | 89.15 | 34.72 | 8.46 |
| **7** | 47.70 | 25.15 | 7.53 |
| **10** | 58.54 | 33.29 | 8.10 |
| **13** | 79.07 | 33.41 | 9.04 |
| **14** | 52.80 | 28.32 | 7.81 |
| **15** | 45.26 | 26.01 | 7.26 |
| **17** | 92.11 | 36.97 | 10.38 |
| **20** | 93.01 | 37.25 | 10.59 |
| **21** | 94.29 | 38.78 | 10.85 |
| emulsion control | 99.88 | 39.82 | 10.99 |
| CD ($p = 0.05$) | 1.39 | 0.86 | 1.42 |

($\pi = -0.69$ and $-0.48$, respectively) for the olefinic side chain as compared to the positive value in the more selective furyl analogues [$\pi$ (furyl) = 0.31]. Therefore, it can be concluded that more hydrophobicity in the olefinic side chain of the molecule lead to higher selectivity. The study concludes how an array of compounds can be brought down to a narrow range of active compounds using QSAR analyses. The leads derived from the present study can be valuable in exploring the primary site and mode of action of these CHAs.

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