JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Quantitative Structure−Activity Relationship Analysis as a Tool To Evaluate the Mode of Action of Chemical Hybridizing Agents for Wheat (*Triticum aestivum* L.)

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Augmentation of wheat production calls for introduction of wheat hybrids in cultivation. In the absence of viable alternative technology of hybrid wheat development, chemical induction of male sterility mediated technology based on chemical hybridizing agents (CHAs) holds a great potential. The QSAR method was applied to two families of CHAs in the *N*-acylanilines and pyridone class of chemistry. The models for each CHA family gave good correlation between the variations in log percent of male sterility and the steric−electrostatic properties of the sets. QSAR analysis has revealed a direct relationship of the Swain−Lupton constant F_p and molecular mass but an inverse relationship of MR, ES, and Swain−Lupton resonance constant *R* in influencing the bioactivity in the *N*-acylanilines. QSAR analysis of four parent families consisting of two training sets each of pyrid-2-ones and prid-4-ones revealed the positive contributions of field effect exemplified by the Swain−Lupton field constant (*F*) and the negative contributions of the molar refractivity (MR) of aromatic substituents in all but one training set. The QSAR models also indicated that increased steric bulk at the 4-position on the phenyl ring is associated with enhanced activity. These leads will be useful in explaining the CHA binding fit in the macromolecular receptor site.

KEYWORDS: Chemical hybridizing agents; mode of action; *N*-acylanilines; pyridones; anilides; quantitative structure−activity relationship; wheat

INTRODUCTION

Chemical-induced male sterility is based on the use of certain chemicals called chemical hybridizing agents (CHAs) for induction of male sterility. The aim is to induce physiological male sterility by spraying the plant with chemicals to induce stamen sterility without harming the pistil. CHAs are an attractive alternative in heterosis breeding of small cereal grains such as wheat for which mechanical emasculation is impractical due to the small size and close proximity of male and female reproductive organs. There are, however, relatively few chemicals that are known to display activity as pollen suppressants (*1*). Of these a handful exhibit the selectivity, potency, and other production attributes needed to be commercially viable CHAs. 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. According to the scanty information available in the literature, disruption of meiosis leading to the degeneration of pollen mother cells is one such mechanism proposed. The rigor with which an agrophore can be developed depends on two factors: (a) the number and diversity of biologically active structures from which critical structural elements are deduced and (b) convincing biochemical evidence that the observed activity is due to a common mode of action. The field of CHAs is information poor in comparison with herbicide and fungicide chemistry (*2*). 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 useful tool in elucidating essential structural features that govern interaction of CHAs with the macromolecular receptor in the crop plants controlling pollen formation and its viability.

In a program of design and development of potential CHAs, we have undertaken the synthesis of several *N*-acylanilines and pyridones. Encouraging results have been obtained with the field trials of a few ethyl oxanilates on rice, wheat, and chickpea (*3*−*6*). The current QSAR modeling approach focuses to discern whether agrophores represented by our synthesized compounds (*N*-acylanilines and pyridones) are active via related mechanisms. Our focus is on (a) alkyl oxanilates, (b) malonanilates, (c) acetanilides, (d) acetoacetanilide, (e) *N*-aryl-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-ones, (f) *N*-aryl-4,6-dimethyl-

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10.1021/jf050187j CCC: $30.25
Published on Web 03/30/2005

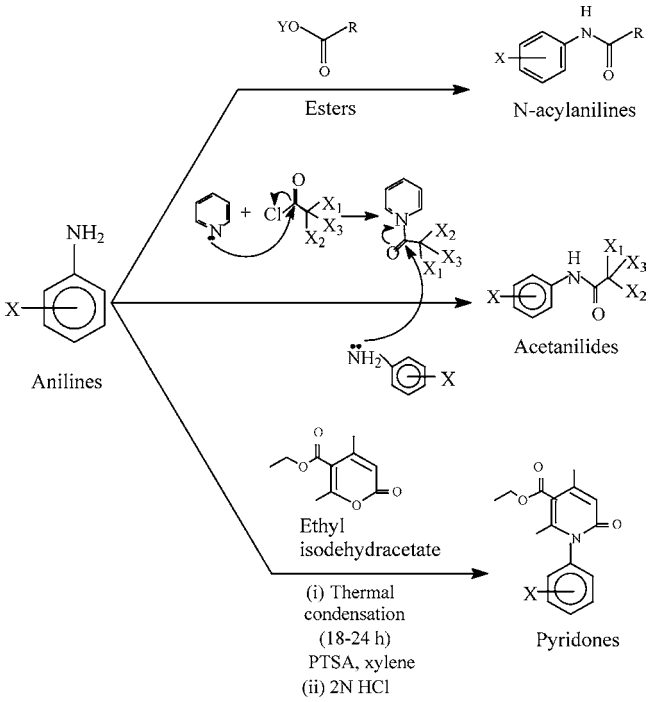


Figure 1. Synthetic scheme of *N*-acylanilines and pyridones.

1,2-dihydropyrid-2-one-5-carboxylic acid, (g) *N*-alkyl-6-methyl-2-phenyl-1,4-dihydro-4-oxonicotinic acid, and (h) *N*-aryl-5,6-dimethyl-1,4-dihydropyrid-4-one-3-carboxylic acid belonging to the pyridone group. We report in this paper the QSAR analysis of 117 compounds of these groups as potential CHAs for wheat.

MATERIALS AND METHODS

The test compounds have been prepared by condensation reaction between different substituted anilines with various esters. Several *N*-acylanilines containing variations at the acyl domain were synthesized by condensation of substituted anilines with appropriate diesters or acid chlorides or monoesters as the case might be. A large variation in the substitutions both in aromatic and side chain has been achieved (Figure 1). Various *N*-aryl-5-carbethoxy-4,6-dimethyl, 1,2-dihydropyrid-2-ones were prepared by thermal condensation of respective anilines with ethyl isodehydracetate. The compounds were purified using physical and chromatographic separation methods. The structures of synthesized compounds were confirmed by IR, ^{1}H NMR, and MS. Melting points (mp) were determined by using a sulfuric acid bath and were uncorrected. IR spectra (ν_{max} in cm^{-1}) were recorded on a Nicolet Impact 400 FT-IR spectrometer using a KBr disk, scanning from 625 to 4000 cm^{-1}. ^{1}H NMR spectra were recorded on a Varian EM-360, 60 MHz spectrometer using tetramethylsilane (TMS) as an internal reference; chemical shifts are reported in δ (parts per million) values relative to TMS, and *J*-values are expressed in hertz (Hz). Mass spectra were recorded under electron-impact (70 eV) conditions using a FISONS TRIO 1000 (HRGC Mega-2 coupled with EI-mass detector) with 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. Thin-layer chromatography (TLC) was performed on 250 μm silica gel G plates, preactivated at 100 °C for 2 h, with hexane−ethyl acetate (4:1) as developing medium. All test compounds gave correct elemental analyses using a Euro Vector elemental analyzer (Model No. EA3011).

Measurement of Male Sterility. The following example illustrates the general principles for measuring male sterility. For more detailed explanations, the reader is referred to our earlier references (*3*−*5*).

Three high-yielding varieties (HYVs) of bread wheat (*Triticum aestivum* L.), viz, PBW343, HW2046, and HD2733, recommended for timely sowing in North Western Plain Zone (NWPZ) of India, were chosen for evaluation of chemical induction of male sterility. The field trials were conducted in two winter seasons, viz., November 2000−April 2001 and November 2001−April 2002. The plants were fertilized and irrigated as appropriate. The test chemicals were sprayed at 1500 ppm as foliar sprays, using appropriate carrier solutions and surfactants at the premeiotic stage (60 days after sowing) when the length of the spike emerging out from the first node was 7−8 mm (*7*). After the spike emerged, but before anthesis, several spikes per plant were isolated by placing them in a bag to prevent out crossing at pre-emergence stage when awns were just emerging. Male sterility was calculated as percent inhibition of seed formation in isolated spikes of treated plants by the formula

$$\% \text{ sterility} = (S_c - S_f)/S_c \times 100$$

where S_c = the seeds per spikelet in bagged spikes of control plants and

S_f = the seeds per spikelet in bagged spikes of treated plants.

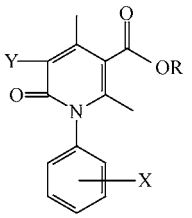
Pollen Sterility. The pollen sterility test was carried out by the acetocarmine (1%) or KI (2%) in iodine staining method as reported earlier (*5*).

Quantitative Structure−Activity Relationship Study (QSAR). *N-Acylanilines (**1**−**56**).* To observe the variability in the acyl side chain, the descriptor variables, namely, π, molecular weight (MW), molar refractivity (MR), molecular volume (MV), surface tension (ST), parachor (P), and polarizability (Pl) (ACD Chemsketch, Version 2), were taken (*8*−*10*). For the aromatic part, the following different descriptor variables that were proved to have significant contribution on the observed bioactivity based on our earlier studies (*5*) were selected: Swain−Lupton field constant for para substitution (F_p), Swain−Lupton resonance constant ($\sum R$), Taft steric parameter (E_s), molecular weight (MW), Verloop−Hoogenstraaten multidimensional steric parameters (L and B_4), descriptor variable (D), hydrophobic parameters (π, π^2), and other parameters, such as molar refractivity (MR) and δ^{13}C (*11*−*14*). Thirty-two independent variables were used in constructing the correlation matrix. The independent variables, which were found orthogonal to each other, were minimized. The "agrophore" data, viz., percent induction of spikelet sterility caused by test oxanilates tested at 1500 ppm on wheat variety PBW 343, were transferred into sin arc and used as the dependent variable (Ms % sin arc).

*Pyridones (**57**−**117**).* Two training sets each of pyrid-2-ones and pyrid-4-ones were selected for QSAR studies. Experimental data for one training set of pyrid-2-ones were generated in the present study. The male sterility data for other training sets, one from pyrid-2-one (*15*) and two from pyrid-4-one, were taken from the literature (*16*, *17*). The following descriptor variables for the substituents were used in constructing the correlation matrix, unless mentioned otherwise. Electronic parameters: Swain−Lupton field constant (F), Swain−Lupton resonance constant (R), Hammett constant (σ_m, σ_p), steric parameters like molar refractivity (MR), and hydrophobic parameters (π and π^2) (*18*). The Taft steric parameter (E_s), molecular weight (MW) (or substituent weight, SW), and ln(MW) were also included (*8*). Additional need-based index variables were used in each set. The descriptors F, R, and MR were further split into I_o, I_m, and I_p, where I represents any of these variables and the subscript denotes ortho, meta, and para positions, respectively. The additive nature of these variables was presumed for disubstitution.

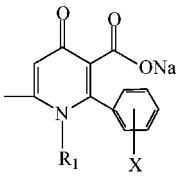
*N-Aryl-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-ones (**57**−**82**).* The mean percent spikelet sterility caused by 26 test compounds tested at 1500 ppm in wheat var. PBW 343 in winter 2001−02 were transformed into sin arc and used as the dependent variable (Ms %). In addition to F, σ_m, σ_p, R, MR, π, π^2, E_s, and MW, the index variable D_0 was included with arbitrary values of 1, 2, and 3 for ortho, para, and meta positions, respectively. The "Leave-out" method yielded the best equations for 20 and 23 analogues.

N-Aryl-4,6-dimethyl-1,2-dihydro-pyrid-2-one-5-carboxylic Acids and Their Na$^+$ Salts. The reported data on the mean percent spikelet sterility caused by 17 test compounds tested at 1500 ppm in wheat (*15*) were transformed into sin arc and used as the dependent variable (Ms %). In addition to F, σ_m, σ_p, R, MR, π, π^2, E_s, and SW, two index variables, D_1 and D_2, were introduced to account for substituents in the pyridone ring, viz., $D_1 = 1$ for Br, $D_1 = 2$ for H, and $D_2 = 1$ for Na salt, $D_2 =$



Co. No.	X	R	Y
83	H	H	H
84	4-CH_3	H	H
85	4-F	H	H
86	4-OMe	H	H
87	4-CH_3, 3-Cl	H	H
88	H	H	Na
89	4-CH_3	H	Na
90	4-CH_3, 3-Cl	H	Na
91	4-Br	Br	Na

Figure 2. List of *N*-aryl-4,6-dimethyl-1,2-dihydropyrid-2-one-5-carboxylic acids and their Na^+ salts.



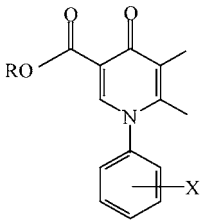
Co. No.	X	R_1
92	H	CH_3
93	2-Cl	CH_3
94	4-Cl	CH_3
95	3,4-Cl_2	CH_3
96	2,4-Cl_2	CH_3
97	3-F	CH_3
98	3-F	C_2H_5
99	4-F	C_2H_5
100	3-CH_3	C_2H_5
101	H	nC_3H_7
102	4-Cl	nC_3H_7
103	3,4-Cl_2	nC_3H_7
104	3-Cl	nC_4H_9

Figure 3. List of *N*-alkyl-6-methyl-2-phenyl-1,4-dihydro-4-oxonicotinic acids and their Na^+ salts.

2 for acid. After performing the leave-out method, only nine compounds (**83**−**91**) were found to be statistically significant (**Figure 2**).

N-Alkyl-6-methyl-2-phenyl-1,4-dihydro-4-oxonicotinic Acids and Their Na^+ Salts. The reported data on mean percent spikelet sterility caused by 20 test compounds at 1500 ppm on wheat (*17*) were transformed into sin arc and used as the dependent variable (Ms %). In addition to *F*, σ_m, σ_p, *R*, MR, π, π^2, E_s, and SW mentioned above, parachor (*P*), which is a measure of the bulk of the substituent, was included to account for substituents in the pyridone ring as well as the *N*-alkyl group. For the *N*-alkyl group, in addition, Fr and Taft's steric parameter (E_s) were used additionally. After performing the leave-out method, only 13 compounds (**92**−**104**) were found to be significant (**Figure 3**).

N-Aryl-5,6-dimethyl-1,4-dihydropyrid-4-one-3-carboxylic Acids and Their Na^+ Salts. The reported data on mean percent spikelet sterility



Co. No.	X	R
105	4-Cl	H
106	4-CH_3	H
107	4-CF_3	H
108	3-Cl	H
109	3-F	H
110	2-Cl	H
111	3,4-diMe	H
112	2,4-Cl_2	H
113	3-CF_3	H
114	4-Cl	Na
115	H	Na
116	4-OMe	Na
117	2,4-Cl_2	Na

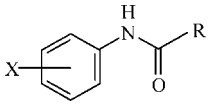
Figure 4. List of *N*-aryl-5,6-dimethyl-1,4-dihydropyrid-4-one-3-carboxylic acids and their Na^+ salts.

caused by 30 test compounds tested at 1500 ppm in wheat (*16*) were transformed into sin arc and used as the dependent variable (Ms %). In addition to *F*, σ_m, σ_p, *R*, MR, π, π^2, E_s, and SW, an additional index variable D_1 was introduced to account for substituents in the pyridone ring, *viz.*, $D_1 = 1$ for Na salt, $D_1 = 2$ for acid. After performing the leave-out method, only 13 compounds (**105**−**117**) were found to be significant (**Figure 4**).

RESULTS AND DISCUSSION

Synthesis and Spectral Analysis. The compounds synthesized, numbering over 100, belong to chemical classes such as anilates, anilides, and pyridone analogues. An array of anilates and anilides were synthesized by condensation with appropriate diesters or acid chlorides or monoesters with substituted anilines. 5-Ethoxycarbonyl-*N*-aryl-4,6-dimethyl-[1*H*]-2-pyridones were synthesized in which the side chain constituted a heterocyclic moiety. IR spectra showed characteristic peaks for symmetrical NH stretching vibrations and the C═O stretching vibration of an ester at 3339 ± 16 and 1712 ± 12 cm^{-1}, respectively for *N*-acylanilines. The characteristic feature of the ^1H NMR spectra in *N*-acylanilines was a broad singlet that appeared at a range between δ 8.85 and 11.8 ppm, corresponding to the anilido (NH) proton. The descending order of shielding of different side chains in *N*-acylanilines was $COOCH_3$ (−1.2 ppm) > COOiPr (−1.0 ppm) > $C_6H_4OCOCH_3$ (−0.73 ppm) compared to the base compound (4′-fluoroacetanilide; $\delta H_a = 7.65$ ppm, $\delta H_b = 7.10$ ppm, $\delta NH = 10.00$ ppm). The methylene and methine protons had chemical shifts of δ 4.0−4.05 and δ 4.4−6.2 ppm, respectively, in chloroacetanilides and dichloroacetanilides, corresponding to the electronegativity effect of the chlorine atom. In *N*-aryl-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-ones, the methyl protons ortho to the N-atom of the pyridone ring were more shielded than the 4-methyl protons surrounded as they are both by an electron-deficient *N*-aryl group as well as an ethoxycarbonyl moiety, and they appeared at δ 2.05 ± 0.14 and δ 2.27 ± 0.11 ppm, respectively. The presence of the ethyl ester group was confirmed by the spin−spin coupling

Table 1. Percent Spikelet Sterility (% Ss) of *N*-acylanilines Tested in Winter 2001–02 at 1500 ppm Spray Concentrations on PBW 343



ethyl oxanilates (R = COOEt)			elkyl oxanilates			
no.	X	Ss (%)	no.	X	R	Ss (%)
1	H	84.18	28	4-F	COOMe	84.32
2	2-F	68.13	29	4-F	COOiPr	67.15
3	3-F	50.04	30	4-F	$COCH_3$	51.71
4	3-Cl,4-F	77.91	31	4-F	$COOC_2H_4OMe$	87.07
5	4-F	99.97	32	4-F	CH_2COOEt	84.66
6	4-Br	99.96	33	4-Br	CH_2COOEt	83.40
7	2-Cl	50.25	34	H	CH_2COOEt	62.97
8	3-Cl	44.25	35	2-OMe	CH_2COOEt	25.51
9	2,4-Cl_2	41.56	36	3-OMe	CH_2COOEt	20.54
10	4-Cl	72.09	37	2-NO2	CH_2COOEt	12.53
11	2-OMe	78.02	38	4-F	CH_2COCH_3	89.12
12	3-OMe	39.07	39	4-Br	CH_2COCH_3	83.65
13	4-OMe	84.39	40	H	CH_2COCH_3	64.52
14	2,4-$(OMe)_2$	63.43	41	2-Cl	CH_2COCH_3	34.98
15	2-NO_2	61.00	42	3-Cl	CH_2COCH_3	15.53
16	3-NO_2	32.13	43	4-Cl	CH_2COCH_3	58.65
17	4-NO_2	79.06	44	3-NO_2	CH_2COCH_3	22.13
18	2,4-$(NO_2)_2$	82.37	45	3-CH_3	CH_2COCH_3	2.94
19	3-Me	23.29	46	4-F	CH_3	61.35
20	2-CN	81.43	47	4-F	CH_2Cl	39.92
21	3-CN	71.74	48	4-Br	CH_2Cl	39.45
22	4-CN	96.63	49	H	CH_2Cl	32.16
23	2-CF_3	84.59	50	4-F	$CHCl_2$	63.75
24	3-CF_3	94.86	51	4-Br	$CHCl_2$	62.38
25	4-CF_3	99.57	52	H	$CHCl_2$[a]	34.85
26	4-Et	18.48	54	4-F	CCl_3	89.61
27	4-iPr	9.41	55	4-Br	CCl_3	88.09
			56	H	CCl_3	46.87
emulsion control		0.46				0.46
CD ($p = 0.05$)		0.59				0.60

[a] Compound no. **53** denotes 2,4-dinitrodichloroacetyl chloride.

system, viz., a triplet centered at δ 1.33 ± 0.06 ppm for CH_3 and a quartet centered at δ 4.24 ± 0.04 ppm for OCH_2-. The olefinic proton in the pyridone moiety appeared as a singlet at δ 5.94 ± 0.17 ppm. The substituents 4′-ethyl,2,4′-dichloro, and 4′-methoxy caused marked shielding due to the +R effect by 0.38, 0.43, and 0.35 ppm, respectively. The M^+ ion was conspicuous in the mass spectra of alkyl oxanilates; the base peak was found to be either the protonated aryl isocyanate moiety or an azatropylium (M − 101) ion, whereas in malonanilates the breakdown to parent anilines (M − 114) appeared to be the base peak. The M^+ ion was conspicuous in the El-MS spectra of pyridones. The aryl side chain in one form or another dominated as the base peak.

Spikelet Sterility and QSAR Analyses. *N-Acylanilines.* The results of induction of spikelet sterility on bread wheat (PBW 343) caused by ethyl oxanilates in *N*-acylanilines series at 1500 ppm are mentioned in **Table 1**. There was no marked variation in the response of different genotypes. Because of that, only the variety PBW 343 sprayed at 1500 ppm concentration was taken for QSAR analysis. The para-substituted oxanilates, containing F (**5**), Br (**6**), CF_3 (**25**), and CN (**22**), respectively, were found to be the best in that order when considered across three test concentrations and 2-year trial data. The other substituents at the para position influenced the activity in the following order OMe (**13**) > NO_2 (**17**) > Cl (**10**). Alkyl substitution in oxanilates gave the least effect. It can be inferred that para substitution with highly electronegative groups such as F, CN, or CF_3 can give rise to analogues having a high level of activity. QSAR analysis in toxicology and medicine is usually carried out using the log median dose as the dependent variable. It would have meant in our case number of samples in field trials too large to be handled within the constraints of our resources. We have circumvented this problem by resorting to the direct use of sin arc transformed male sterility percent as the dependent variable, because log dose is thus related in probit analysis. Results of the multiple regression analysis carried out using the leave-out method 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 the equations were found to be statistically significant at $p < 0.01\%$.

Equation 1 contains the least number of independent variables (F_p and $\sum$MR) with the multiple correlation coefficient of 0.70.

$$\text{Ms (sin arc \%)} = 49.99F_p - 2.39\sum\text{MR} + 64.73 \quad (1)$$

$$r = 0.7,\ s = 14.10,\ F = 11.75\ (0.00)$$

Stepwise multiple regression accounts for the other equations.

$$\text{Ms (sin arc \%)} = 39.73F_p - 3.24\sum\text{MR} + 0.32\text{MW} - 0.91 \quad (2)$$

$$r = 0.76,\ s = 13.20,\ F = 10.43\ (0.02)$$

$$\text{Ms (sin arc \%)} = 43.74F_p - 3.04\sum\text{MR} + 0.36\text{MW} - 5.63D - 0.7 \quad (3)$$

$$r = 0.81,\ s = 12.10,\ F = 10.41\ (0.00)$$

$$\text{Ms (sin arc \%)} = 44.61F_p - 2.93\sum\text{MR} + 0.65\text{MW} - 5.78D + 8.02\sum E_s - 56.94 \quad (4)$$

$$r = 0.86,\ s = 10.80,\ F = 12.05\ (0.00)$$

$$\text{Ms (sin arc \%)} = 35.56F_p - 2.96\sum\text{MR} + 0.85\text{MW} - 4.94D + 10.36\sum E_s - 10.00\sum\pi - 96.48 \quad (5)$$

$$r = 0.90,\ s = 9.37,\ F = 14.49\ (0.00)$$

The observed biological activity could be best explained by incorporating F_p, $\sum$MR, MW, D, $\sum R$, and $\sum\pi$ (eq 6) with $r = 0.91$.

$$\text{Ms (sin arc \%)} = 39.59F_p - 2.86\sum\text{MR} + 0.67\text{MW} - 5.11D - 2.57\sum R - 16.91\sum\pi - 64.28 \quad (6)$$

$$r = 0.91,\ s = 8.78,\ F = 16.99\ (0.00)$$

The descriptive variables F_p (the Swain−Lupton constant) and $\sum$MR (molar refractivity index) are involved in all the equations. The individual contributions of the independent variables to R^2 were 27.29, 22.19, 8.17, 8.23, 8.29, and 7.13%, respectively, in that order.

Both F_p and MR have shown a great influence on the bioactivity. A negative sign of the coefficients in MR, D, and π indicates the inverse relationship with the bioactivity. In other words, the lesser the hydrophobic character, the higher the percent sterility will be. Chemicals with high water solubility would be preferred. In fact, many of the CHAs reported in the literature are alkali salts of carboxylic acids. The negative sign of D implied that substitution is preferred at ortho rather than meta, ignoring other factors contributing to activity. The following parameters occurring in the equations were found to

be correlated: $\sum$MR vs $\sum B_4$, $r = 0.8$ and $\sum E_s$ vs $\sum R$, $r = 0.79$. It is now possible to carry out lead optimization via aromatic substitution of ethyl oxanilates as CHAs using eqs 5 and 6. We have carried out such an exercise with the side chain part of the molecule as well.

Three classes of compounds having variation in the acyl side chain have been synthesized, viz., alkyl oxanilates (esters), malonanilates, and anilides. Among the alkyl oxanilates, methyl 4′-fluorooxanilate (**28**) and 2-methoxyethyl 4′-fluorooxanilate (**31**) were found to be the most effective in the three genotypes of wheat, inducing on an average 83.57 and 86.17% male sterility at 1500 ppm (Table 1). Among malonanilates, ethyl 4′-fluoromalonanilate (**32**) was the best. Among acetoacetanilides, 4′-fluoroacetoacetanilide (**38**), and 4′-bromoacetoacetanilide (**39**) induced a significantly high level of male sterility. Among chloroacetanildes, the increasing number of chlorine atoms in the side chain led to an increase in the activity. Incidentally, all of them have fluoro or bromo as the substituent at the *para* position of the aromatic ring. The presence of a carboxy group in the side chain did not appear merely to serve as an electronegative group, as evidenced by the moderate activity of **47** and **50** containing the electronegative chloro group. Twenty-nine *N*-acylanilines were evaluated on PBW 343 at 1500 ppm. QSAR analysis of the 29 analogues thus generated was carried out by using a combination of chemical descriptors both for aromatic substitution and side chain variation. For the side chain variants, the physicochemical descriptors such as MR, molar volume (MV), *P*, surface tension (ST), and polarizability were generated using the ACD-Freeware-Chemsketch, and the correlation matrix was constructed using 29 analogues. It is seen that the above chemical descriptors for side chain variants are orthogonal to each other. Three multiple linear regression (MLR) equations were generated using 19 independent variables (molecular weight (MW), ln(MW), MR, MV, *P*, ST, polarizability, *L*, B_1, B_4, E_s, π side chain, π aromatic, index variable (*D*), MR aromatic, MR total, Swain−Lupton constant *R*, F_p, and *F*).

QSAR analysis of *N*-acylanilines has given the three best equations. Two equations (eqs 7 and 8) were obtained using 23 analogues containing two and three descriptor variables, respectively.

$$\text{Ms (sin arc \%)} = 51.40F_p - 1.99\sum R + 35.74 \quad (7)$$

$N = 29$, $r = 0.75$, $r^2 = 0.56$; $s = 11.94$, F (probability) = 16.39 (0.0000)

$$\text{Ms (sin arc \%)} = 62.76F_p - 1.66\sum R - 6.39D + 43.38 \quad (8)$$

$N = 29$, $r = 0.81$, $r^2 = 0.65$, $s = 10.79$, F (probability) = 15.65 (0.0000)

The best equation (eq 9) was the one that combined the independent variables F_p, $\sum R$, and *D* for aromatic substitution and parachor (*P*) for the side chain, with *r* values of 0.86.

$$\text{Ms (sin arc \%)} = 67.54F_p - 1.67\sum R - 6.59D + 0.13P + 15.37 \quad (9)$$

$N = 29$, $r = 0.86$, $r^2 = 0.74$, $s = 9.55$, F (probability) = 16.97 (0.0000)

In the absence of *P*, eq 8 was found to be the next best, with an r-value of 0.81. It therefore appears that in *N*-acylanilines, the substitution in the aromatic part has an overriding effect. For this reason, most of the patented CHAs contain this part of the molecule as a common moiety. Apportioning of the contributions of the descriptor variables to the R^2-term revealed that F_p (38%), $\sum R$ (18%), and parachor (9%) were the major factors influencing the target activity.

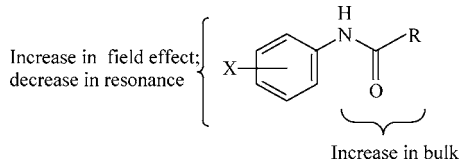


Figure 5. Sketch model of *N*-acylanilines.

The improvement of the above best equation was attempted by resorting to the leave-out method. A plot of residuals (the difference between observed and predicted percent male sterility) revealed considerable (>10%) deviations of the observed activity from the predicted values in nine cases. The analogues that were underestimated were methyl 4′-fluorooxanilate (**28**), *p*-fluoroacetaoacetanilide (**38**), ethyl malonanilate (**34**), 3′-nitroacetaoacetanilide (**44**), and 4′-fluorotrichloroacetanilide (**54**), and those that were overestimated were ethyl 2′-methoxymalonanilate (**35**), *m*-methylacetoacetanilide (**45**), and *p*-fluoro- (**47**) and *p*-bromochloroacetanilide (**48**). MLR equations were obtained using the remaining 20 analogues, out of which two were improvements of the earlier equations with high *r* values of 0.86 and 0.92.

$$\text{Ms (sin arc \%)} = 74.63F_p - 42.49\sum R + 43.88 \quad (10)$$

$N = 20$, $r = 0.86$, $r^2 = 0.74$, $s = 8.43$; F (probability) = 24.28 (0.0000)

$$\text{Ms (sin arc \%)} = 78.21F_p - 43.83\sum R + 0.12P + 17.51 \quad (11)$$

$N = 20$, $r = 0.92$, $r^2 = 0.85$, $s = 6.66$, F (probability) = 29.64 (0.0000)

Equation 11 teaches us that the aromatic substituent must have a dominant field effect with the less resonance effect (**Figure 5**), and this clue must provide useful leads in the synthesis of new analogues.

Equation 11 was obtained using 20 analogues refined by the leave-out method and contains only four descriptor variables. In this equation the contribution of the R^2 term was 37% (F_p), 37% ($\sum R$), and 11% (*P*). Thus, the dominating factors of the substituents are lower values of *R* and higher values of inductive effect (F_p) and parachor. These equations teach us that the aromatic substituent must have high value of F_p but lower values of *R*. Both electronic and steric effects have a significant influence in the bioactivity. Inductive rather than resonance effect seemed to contribute to the activity. It is significant that parachor rather than E_s best represents the steric effect of the acyl domain. It is the inductive (field) effect of the substituent that is the key factor influencing the induction of male sterility. The equations essentially highlight the primary importance of the field effect of the aromatic substituents, rather than the resonance effect as in eqs 7−9. In summary, the QSAR analysis of *N*-acylaniline class of CHAs thus has given rise to the following conclusions:

(i) Parachor in the acyl side chain appeared to influence the bioactivity in a significant manner.

(ii) The aromatic substituent must have higher F_p value but lower *R* values.

(iii) In all the cases, the field rather than the resonance effect of the substituent appears to be important.

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 fit of the CHA in the macromolecular receptor site.

In a competitive binding at the bioreceptor site, a negative MR term could mean unfavorable conformational changes in the enzyme−inhibitor complex as compared to favorable conformational changes caused by the enzyme−substrate complex (*18*).

Pyridones. Maximum male sterility was induced by 5-carbethoxy-*N*-(4-chlorophenyl)-4,6-dimethyl-1,2-dihyropyrid-2-one (simply *N*-(4-chlorophenyl)-2-pyridone) (**66**) followed closely by *N*-(4-fluorophenyl)-2-pyridone (**61**) and *N*-(4-bromophenyl)-2-pyridone (**62**) in PBW 343 (Table 2). *N*-(4-trifluoromethylphenyl)-2-pyridone (**81**), *N*-(4-cyanophenyl)-2-pyridone (**78**), and *N*-(3-trifluoromethylphenyl)-2-pyridone (**80**) closely followed them. Except CF_3-containing analogues, in all cases the influence of aromatic substituents on the induction of male sterility was in the order para > ortho > meta. The substituents at the para position had a positive effect on induction of male sterility in the order Cl (**66**) = Br (**62**) > F (**61**) > CF_3 (**81**) > CN (**78**) > OMe (**69**) > NO_2 (**73**) > ethyl (**82**).

N-Aryl-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-one. QSAR analysis gave three best equations. Equations 12 and 13 were obtained using 23 analogues containing five and six descriptor variables, respectively. Apportioning of the contributions of the descriptor variables to the R^2-term revealed that ∑MR (38%), F_p (28%), and R_p (12%) were the major factors influencing the target activity.

$$\text{Ms (sin arc \%)} = 38.46F_p - 3.25\sum\text{MR} + 0.68\text{MW} - 4.56D + 10.20\sum E_s - 118 \quad (12)$$

$$N = 23,\ s = 5.73,\ r = 0.95,\ F = 31.80$$

$$\text{Ms (sin arc \%)} = 42.46F_p - 3.85\sum\text{MR} + 0.48\text{MW} - 42.46R_p - 3.49D + 658\sum E_s - 57.98 \quad (13)$$

$$N = 23,\ s = 5.97,\ r = 0.95,\ F = 26.23$$

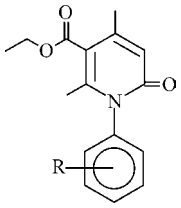
Equation 14 was obtained using 20 analogues refined by the leave-out method and contains only four descriptor variables. In this equation, the contribution of the R^2 term was 38% (∑MR), 33% (F_p), 9% (MW), and 8% (∑R). Thus, the dominating factors of the substituents are lower values of MR and resonance effect (R) and higher values of inductive effect (F) and molecular weight.

$$\text{Ms (sin arc \%)} = 38.14F_p - 1.39\sum R - 3.67\sum\text{MR} + 0.26\text{MW} - 2.65 \quad (14)$$

$$N = 20,\ s = 6.25,\ r = 0.94,\ F = 26.71$$

These equations teach us that the aromatic substituent must have a high value of F_p but lower values of MR and R. Both electronic and steric effects exert a significant influence on the bioactivity (**Figure 6**). An inductive rather than resonance effect seemed to contribute to the activity. It is significant that the steric effect is best represented by molar refractivity rather than E_s or Verloop sterimol parameters. The induction of male sterility is compared on the basis of constant dose measured in gram weight rather than molecular weight. The appearance of molecular weight term in the equations accounts for this conversion/correction factor. The direct influence of MW can be inferred as the preference of molecules with less volatility within this class.

Table 2. Percent Spikelet Sterility (% Ss) Induced by 2-Pyridones (1500 ppm) on PBW 343 Tested in Winter 2001–02



no.	R	% Ss	no.	R	% Ss
57	H	77.04	70	2,4-$(OMe)_2$	39.59
58	2-F	72.68	71	2-NO_2	50.07
59	3-F	52.91	72	3-NO_2	39.99
60	3Cl,4-F	75.60	73	4-NO_2	67.58
61	4-F	98.61	74	2,4-$(NO_2)_2$	35.75
62	4-Br	98.44	75	3-Me	16.66
63	2-Cl	73.73	76	2-CN	69.96
64	3-Cl	55.69	77	3-CN	53.26
65	2,4-Cl_2	48.09	78	4-CN	89.06
66	4-Cl	98.88	79	2-CF_3	74.33
67	2-OMe	59.68	80	3-CF_3	87.15
68	3-OMe	44.54	81	4-CF_3	96.22
69	4-OMe	72.63	82	4-Et	19.74
emulsion control		0.46			
CD ($p = 0.05$)		0.46			

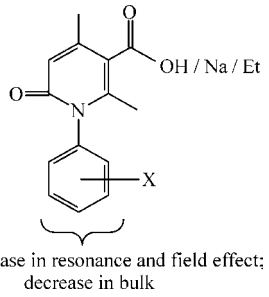


Figure 6. Sketch model of *N*-aryl-5-carbethoxy-4,6-dimethyl-1,2-dihydropyrid-2-ones and *N*-aryl-5-carboxy-4,6-dimethyl-1,2-dihydropyrid-2-ones.

N-Aryl-4,6-dimethyl-1,2-dihydropyrid-2-one-5-carboxylic acids and Na^+ Salts. Percent spikelet sterility data of 17 analogues were available for QSAR analysis. Equation 4 was obtained as a result of the leave-out method.

$$\text{Ms (\%sin arc)} = 0.49\text{SW} - 26.05\sum F + 10.11\sum R + 15.10 \quad (15)$$

$$N = 9,\ r = 0.99,\ s = 0.87,\ F = 230.59$$

where SW = molar mass of substituent.

It contains three independent variables, viz., SW, F, and R terms. Interestingly, in this equation, the signs of the coefficients of F and R are reversed, unlike in eqs 12−14. It implies that in the case of 5-carboxy analogues, the resonance rather than the field effect of the substituent in the aromatic position appears to dominate the target activity (**Figure 6**). However, with the number of analogues amenable to QSAR analysis being limited, the conclusion thus drawn cannot be overly relied on.

N-Alkyl-6-methyl-2-phenyl-1,4-dihydro-4-oxonicotinic Acids and Their Na^+ Salts. Percent spikelet sterility data for 20

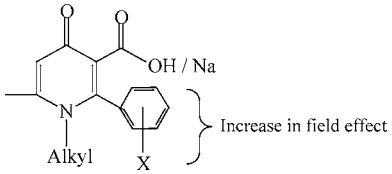


Figure 7. Sketch model of *N*-alkyl-6-methyl-2-phenyl-1,4-dihydro-4-oxonicotinates.

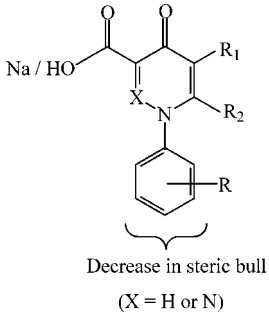


Figure 8. Sketch model of *N*-aryl-5,6-dimethylpyrid-4-one-3-carboxylic acids (R = H) or RH 0007 family (R = N).

compounds were used for QSAR analysis, and the following MLR equations were obtained by the leave-out method.

$$\text{Ms (\%sin arc)} = 45.77\sum F - 22.76R_p + 28.15 \quad (16)$$

$$N = 13,\ r = 0.96,\ s = 5.13,\ F = 53.45$$

In eq 16, only electronic parameters, viz., F and R_p, accounted for the observed activity. The nature of the *N*-alkyl group did not apparently influence the bioactivity. The equation essentially highlights the primary importance of the field effect of the aromatic substituents (**Figure 7**), rather than the resonance effect, as in eqs 12−14.

N-Aryl-5,6-dimethyl-1,4-dihydropyrid-4-one-3-carboxylic Acids and Na^+ Salts. The QSAR analysis has given the following eq 17, wherein the MR and SW terms could account for 80% of the observed data. The negative influence of MR of the aromatic substituent in pyridones, both 2- and 4-ones, have been underlined (**Figure 8**).

$$\text{Ms (\% sin arc)} = -7.24\sum \text{MR} + 0.48\text{SW} + 72.75 \quad (17)$$

$$N = 13,\ R = 0.90,\ s = 10.86,\ F = 22.29$$

where SW = molar mass of substituent.

In summary, the QSAR analysis of the pyridone class of CHAs thus has revealed the following molecular clues:

(i) The *N*-substitution could be either aryl/alkyl, and when it is alkyl, an aryl substitution in the pyridone ring has been included.

(ii) The aromatic substituent must have higher F value but lower MR values, and the field effect of the substituent appears to be important.

In a related study (*19*), 2,6-bis(trifluoromethyl)-4-hydroxypyridine-3-carboxylates were identified as the potent CHAs for wheat. Therefore, it appears that ring substitution rather than *N*-substitution is more essential. The 3-D QSAR method of comparative molecular field analysis (CoMFA) of the pyridazine group of CHAs was carried out (*2*) and there are many common findings with the present study. They found that the observed sterility rates were generally higher for the carboxylate anions than their esters. In pyridones, too, this trend was discernible. The acidic pH generated by the hydrolysis of 5-carbethoxy-*N*-aryl-4,6-dimethyl-1,2-dihydropyrid-2-ones to their respective acids results in a spurt of callase (1,3β-glucanase) secretion by the tapetal cells of pollen. The callase in turn results in premature dissolution of microsporocyte callose wall during the early stages of meiosis (meiotic I prophase) with the breakdown of microspore mother cells. Similar conclusions can be drawn on the influence of electronic and steric effects in three different pyridazine systems. A decrease in the steric bulk in the aromatic region has been unanimously predicted to favor enhancement of induction of male sterility in the RH0007 family as well as the analogous 5,6-dimethyl-*N*-aryl-3-carboxypyrid-4-ones (**Figure 8**).

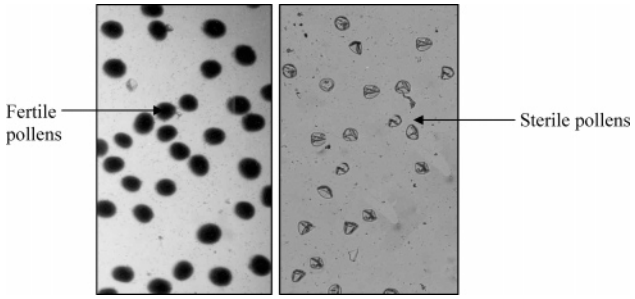


Figure 9. Sterile pollens of wheat due to treatment of CHA vis-à-vis fertile pollens as revealed from KI–I_2 stain test.

Percent pollen sterility was found to have high correlation (r = 0.9988) 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 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 starch could occur either due to inhibition of its synthesis or its premature dissolution. This could provide an important lead in unraveling the mode of action of the CHAs. 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 (**Figure 9**). In a competitive binding at the bioreceptor site, a negative MR term could mean unfavorable conformational changes in the enzyme−inhibitor complex as compared to favorable conformational changes caused by the enzyme−substrate complex. It is known that molar refractivity would explain better than Taft's steric constant and sterimol parameters the bulk rather than directional effects. The lead derived from the present study can be valuable in exploring the primary site and mode of action of these CHAs.

ACKNOWLEDGMENT

The authors are thankful to the Director, IARI, New Delhi, India, for providing necessary facilities to carry out the work. Thanks are due to Dr. Eugene Sebastian J. Nidiry of IIHR, Bangalore, India for some information on descriptor variables.

Supporting Information Available: Physicochemical and spectral data and chemical descriptors for alykyl oxanilates, ethyl oxanilates, and 5-carbethoxy-*N*-aryl-4,6-dimethyl-1,2-dihydropyrid-2-ones. This information is available free of charge via the Internet at http://pubs.acs.org.

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Received for review January 26, 2005. Revised manuscript received February 22, 2005. Accepted February 25, 2005.

JF050187J