Activity Optimization of Pyridinyl *N*-Oxide Urea Cytokinin Mimics[1]

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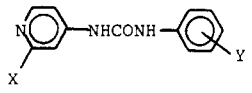
Quantitative structure–activity relationships have been derived for the chlorophyll retention activity in excised wheat leaves of *N*-(2-substituted 4-pyridinyl)-*N'*-arylureas **1** and *N*-(2-substituted 4-pyridinyl *N*-oxide)-*N'*-arylureas **2**. The resulting equations are predictive and are interpreted as indicating that N-oxidation of **1** to afford **2** affects cytokinin receptor binding. For optimum activity of *N*-oxides **2**, the pyridine 2-substituent should be a small, lipophilic, electronegative group and phenyl substitution should be limited to H or *m*-F; the most active compound identified is **2gg**, X = Cl, Y = 3,5-F_2. Reversed-phase TLC log *P* values are shown to be valid and useful parameters in regression analyses of the biological activity of **2**.

N-(4-Pyridinyl)-*N'*-arylureas **1**, discovered and developed by Okamoto, Isogai, Shudo, and co-workers, are now well-established cytokinin mimics with particularly good growth stimulatory activity in tobacco callus culture (Isogai, 1981; Koshimura and Iwamura, 1985; Okamoto et al., 1981, 1983). We have discovered that the *N*-oxides **2** possess strong senescence-delaying activity as evidenced by chlorophyll retention in excised wheat leaves. N-Oxidation of **1** to afford **2** generally increased chlorophyll retention activity from 10- to 1000-fold. Therefore, we chose to optimize the activity of **2** using Hansch analysis (Hansch and Leo, 1979). Concurrently, quantitative structure–activity relationship (QSAR) correlation equations for **1** using chlorophyll retention data were developed for comparison with those derived for **2**.



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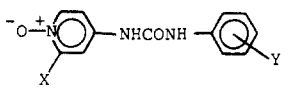

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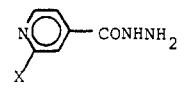

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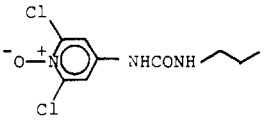

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No literature involving QSAR analyses of a pyridine *N*-oxide nucleus could be found; however, relevant QSAR studies of 2-substituted pyridines have been reported. Okamoto et al. (1981, 1983) have obtained the correlation equation (1) for the tobacco callus growth stimulation of

$$\log (1/C) = 3.81\ (\pm 1.01)\sigma^{x}_{meta} + 0.52\ (\pm 0.32)\pi^{x} + 6.50\ (\pm 0.28) \quad (1)$$

$$n = 11,\ r^2 = 0.95,\ s = 0.28$$

1 (Y = H; X = H, CH_3, CF_3, F, Cl, Br, NH_2, $NHCOCH_3$, OH, OCH_3, CN). This equation also adequately predicts the activity of several *N*-(2,6-disubstituted 4-pyridinyl)-*N'*-arylureas (Okamoto et al., 1983).

Seydel et al. (1976) have examined 2-substituted isoniazide derivatives **3** as tuberculostatic drugs (X = H, CH_3, C_2H_5, *n*-C_3H_7, *i*-C_4H_9, OCH_3, OC_2H_5, NH_2, $NHCOCH_3$, $CH_2NHCOCH_3$, $N(C_2H_5)_2$, F, Cl, Br, I, NO_2, CH_2Ph, $CH{=}CH_2$, Ph) and obtained the correlation equation (2),

$$\log (1/\mathrm{MIC}) = 0.232\mathrm{p}K_a(t = 5.79) - 1.073\pi(t = 6.57) - 1.454 \quad (2)$$

$$n = 19,\ r^2 = 0.78,\ s = 0.41,\ F = 28.3$$

where pK_a and π are strongly correlated with electronic and steric parameters, respectively (Moriguchi and Kanada, 1977; Tute, 1983). In-house we have generated eq 3 in the bona fide steric and electronic terms *L* and *F* using Seydel's data. [X = Ph and $CH_2NHCOCH_3$ were excluded from the equation development.]

$$\log (1/\mathrm{MIC}) = -1.97\ (\pm 0.34)F_{ortho}(t = 5.82) - 0.671\ (\pm 0.106)L(t = 6.32) + 1.03 \quad (3)$$

$$n = 17,\ r^2 = 0.82,\ s = 0.39,\ F = 32.6$$

Equation 3 should be more predictive than eq 2 since it is composed only of readily available physicochemical parameters rather than measured properties (i.e., pK_a).

Numerous QSAR studies of herbicidal ureas have appeared recently (Iwamura and Fujita, 1982; Cross et al., 1983; Takemoto et al., 1984, 1985; Mitsutake et al., 1986), and earlier data have been summarized by Hansch and co-workers in a detailed paper (Kakkis et al., 1984). QSAR for cytokinin activity of arylureas has also been published (Franke et al., 1981; Iwamura et al., 1980; Matsubara, 1980).

MATERIALS AND METHODS

Chlorophyll Retention Assay. The wheat leaf chlorophyll retention assay described by Yopp et al. (1986) was modified as follows. Compounds were assayed at four or five concentrations in the range 10^{-4}–10^{-9} M. For each concentration tested, three replicates consisting of five excised wheat leaves (*Triticum aestivum* cultivar Prodax or Northrup King 715) per replicate were placed in vials containing 1% aqueous acetone or DMSO solutions of **1** or **2** and dark-incubated at 30 °C for 4 days. Control treatments contained water only. Quantitation of chlorophyll followed either of two procedures.

Procedure A: Spectrophotometric Quantitation. Chlorophyll was extracted with hot methanol and quantitated at 652 nm and the A_{652} value converted to micrograms of chlorophyll/gram fresh weight. Ratios were taken with the zero time value (derived from frozen wheat leaves, extracted, and quantitated as above) to give percent chlorophyll retention. These data were plotted versus the negative log [concentration] to obtain a dose–response curve, and the pSI_{50} was graphically determined. The pSI_{50} value is defined as the negative log of the concentration

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[1] Dedicated to Professor Harold W. Heine, Bucknell University, Lewisburg, PA, on the occasion of his 65th birthday.

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required to inhibit senescence (reduce chlorophyll loss) by 50%. All pSI_{50} values reported in Tables II and III are normalized to a standard value of 7.5 for **2v**, which was included as the standard in each test. Actual pSI_{50} values for **2v** ranged from 7.0 to 8.0 for a valid test. Spectrophotometrically determined normalized pSI_{50} values have a confidence interval of ±0.4.

Procedure B: Visual Quantitation. Chlorophyll content was estimated visually with a numerical color rating of 1–5 (yellow to green) based on the following scale.

visual rating	% chlorophyll range
1	0–25
2	26–50
3	51–75
4	76–99
5	100

Using visual ratings, the percent chlorophyll retention was calculated by

$$\% \text{ chlorophyll retention} = \frac{\text{visual rating} - \text{control}}{\text{zero time} - \text{control}} \times 100$$

where visual rating = visual rating for the treatment, control = visual rating for the control, and zero time = visual rating for zero time. pSI_{50} values were determined and normalized as per procedure A. Visually determined normalized pSI_{50} values have a confidence interval of ±0.5.

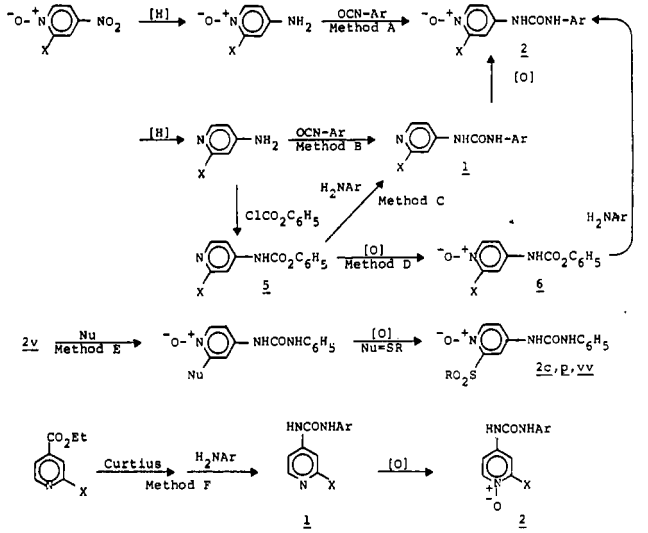
QSAR Analyses. Physicochemical parameters were obtained from literature sources or calculated using additivity principles (Hansch and Leo, 1979). When a variety of steric parameters could function adequately in a regression analysis (e.g., MR, B_4, L in the TLC log P correlations), generally only the equations in MR are reported since biological activity correlations invariably were found to be better with MR as the regression parameter, even though the cross correlation L/MR is high (e.g., 0.91 for the subset used to generate eq 13 and 14a–c). Apparently for the chlorophyll retention activity of **2**, MR is not a pure steric factor but represents London dispersion interactions involved in the formation of a substrate–cytokinin receptor complex (Charton, 1983, 1984). For TLC log P correlations, MR is considered to represent a steric factor since it is exchangable with Verloop L. Preliminary correlation analysis involved regression of lipophilicity (π, TLC log P), electronic (σ, F, R) and steric (MR, L, B_1, B_4) physicochemical parameters versus TLC log P or pSI_{50} using an adaptation of Purcell's program (Purcell et al., 1973). Statistical analyses were performed with BMDP (Dixon, 1983) software on a VAX 785 computer. Statistical parameters are defined as referenced in Kakkis et al. (1984).

Synthesis of 2. The various methods used to synthesize the target areas are outlined in Scheme I; representative examples follow.

Method A. Hydrogenation of 6.0 g (39 mmol) of 2-methyl-4-nitropyridine N-oxide (Fairfield Chemical Co., Inc.) over 0.26 g of 10% Pd/C in 150 mL of 4% acetic acid in ethanol using a Parr shaker afforded crude product in quantitative yield. The crude solid was chromatographed over silica gel using 25% methanol/chloroform and the product recrystallized from acetone to give 2.0 g (41%) of 4-amino-2-methylpyridine N-oxide. Reaction of the aminopyridine N-oxide with phenyl isocyanate in DMF provided **2q** in 50% yield; mp 236–239 °C dec. Anal. Calcd for $C_{13}H_{13}N_3O_2$: C, 64.19; H, 5.39; N, 17.27. Found: C, 63.94; H, 5.10; N, 17.26.

Compounds **2j**, **l**, **q**, **uu**, and **xx** were prepared in this manner.

Scheme I



Method B. N-(2-Chloro-4-pyridinyl)-N'-(3-fluorophenyl)urea (3.4 g, 13 mmol) in 200 mL of warm EtOAc was treated with 3.6 g (ca. 18 mmol, 85% pure) of m-chloroperoxybenzoic acid (mcpba). The precipitated product (3.1 g, 87% yield) was recrystallized from aqueous acetone to afford 2.0 g (55%) of **2ff**, mp 203.5 °C dec. Anal. Calcd for $C_{12}H_9N_3O_2ClF$: C, 51.17; H, 3.22; N, 14.92; F, 6.75. Found: C, 51.19; H, 3.18; N, 14.96; F, 6.49.

Compounds **2b**, **k**, **o**, **u**, **v**, **z**, **bb–oo**, and **ss** were prepared in this manner.

Method C. Under dry nitrogen a solution of 12.0 mL (96 mmol) of phenyl chloroformate in 300 mL of dichloromethane was added dropwise to an ice-cold stirred solution of 10.2 g (79 mmol) of 4-amino-2-chloropyridine and 16.5 mL (120 mmol) of triethylamine in 300 mL of dichloromethane. Workup involving washing of the reaction mixture with water and chromatography over silica gel eluting with 4% acetone/methylene chloride provided 7.4 g (38%) of phenyl N-(2-chloro-4-pyridinyl)carbamate (**5**), mp 171–172 °C.

A mixture of 4.0 g (16 mmol) of the above carbamate, 2.4 mL (19 mmol) of 3-methylmercaptoaniline, and 2.2 mL (16 mmol) of triethylamine in 50 mL of THF was refluxed for 18 h. The product was chromatographed over silica gel with 5% methanol/dichloromethane as eluent to provide 3.5 g (74%) of **1z**, mp 68–75 and 134–136 °C. Anal. Calcd for $C_{13}H_{12}N_3OClS$: C, 53.15; H, 4.12; N, 14.30; S, 10.91; Cl, 12.07. Found: C, 52.85; H, 3.90; N, 14.04; S, 10.78; Cl, 12.06. Oxidation of **1z** with excess mcpba in ethyl acetate at 45–50 °C provided **2qq** as a precipitate in 89% yield; mp 212–214 °C dec. Anal. Calcd for $C_{13}H_{12}N_3O_4SCl$: C, 45.69; H, 3.54; N, 12.29. Found: C, 45.80; H, 3.42; N, 11.99.

Compounds **2x** and **qq** were prepared by this method.

Method D. The carbamate from method C was oxidized with mcpba in ethyl acetate to produce 72% of the carbamate N-oxide **6** as a precipitate, mp 184–184.5 °C dec.

A suspension of 1.0 g (3.8 mmol) of the above carbamate N-oxide, 0.50 g (4.6 mmol) of 3-aminophenol, and 1.0 mL (17 mmol) of triethylamine in 50 mL of acetonitrile was treated in an ultrasonic bath for 16 h. After being diluted with 50 mL of diethyl ether, the precipitate was filtered to yield 0.75 g (71%) of **2w**, mp 240–250 °C dec.

Compounds **2w**, **y**, **aa**, and **pp** were prepared in this manner.

Method E. A solution of 2.0 g (7.7 mmol) of **2v** in 20 mL of DMSO plus 20 mL of n-butylamine was stirred at room temperature for 6 days. The excess amine was

Table I. TLC log *P* Standards

compd	lit. log *P* (shake flask av)	compd	lit. log *P* (shake flask av)
p-aminophenol	0.04	monuron	1.93
m-aminophenol	0.20	fluometuron	2.28
o-aminophenol	0.58	atrazine	2.56
phenylurea	0.83	diuron	2.77
fenuron	0.97	linuron	2.98
(*p*-chlorophenyl)urea	1.70	terbutryne	3.73

evaporated and water (100 mL) added to precipitate the product. Purification included silica gel chromatography using 20% methanol/dichloromethane as eluent and recrystallization from methanol/diethyl ether to afford 1.1 g (49%) of **2e**, mp 186.5–189 °C dec. Anal. Calcd for $C_{16}H_{20}N_4O_2$: C, 63.98; H, 6.71; N, 18.65. Found: C, 64.10; H, 6.77; N, 18.47. For X = SR (**2h, m, ww**) subsequent oxidation with mcpba in methanol/ethyl acetate mixtures afforded the corresponding sulfones (**2c, p, vv**).

Compounds **2a, c, e–h, m, p, rr, vv,** and **ww** were prepared in this manner.

Method F. 2-Phenylisonicotinic acid hydrazide was prepared as described (Libermann et al., 1958) and subjected to Curtius rearrangement conditions (Smith, 1946) in 1,2-dichloroethane to form the isocyanate in situ. Addition of aniline provided the urea **1b** in 76% yield after silica gel chromatography using 5% methanol/dichloromethane. N-Oxidation as in procedure B provided **2d** in 87% yield; mp 232–235 °C dec.

Compounds **2d, i, n, r–t, tt, yy,** and **zz** were prepared in this manner.

Reversed-Phase TLC log *P*. The procedure of Ellgehausen et al. (1981) was modified to allow determination of log *P* values in the range 0–4. Standards (Table I) and experimentals were spotted on a 20 × 20 cm Whatman C-18 reversed-phase TLC plate with fluorescent indicator. The plate was developed in 70/30 methanol/water (v/v) for at least 15 cm. Approximately 35 compounds per plate, including standards, could be determined in this manner. Standards were selected from the on-line log *P* database of Hansch and Leo (1983), averages of multiply determined shake flask (octanol/water) log *P* values (Hansch and Leo, 1979) being used (Table I). Herbicidal ureas and heterocycles were selected as a relevant standard set on the basis of structural analogies with **1** and **2**. TLC log *P* values have a confidence interval of ±0.1. Various data for pyridinylureas **1** and *N*-oxides **2** are summarized in Tables II and III, respectively.

For validation of the TLC procedure, *N*-oxides **2e, 2v, 2gg,** and **4** (Table IV) were chosen as a standard set for chemical class **2** (Eadsforth, 1986), and their shake flask log *P* values were determined. Reversed-phase HPLC-derived log *P* values for a subset of *N*-oxides (**2a–v, ff, gg, kk, ll**) were then measured by this class **2** standard set. Plotting TLC log *P* versus HPLC log *P* showed excellent correlation of the two data sets. Linear regression analysis provided eq 4. TLC-derived log *P* values (using a herbicide standard set) are 0.50 unit higher than their corresponding HPLC-derived log *P* values (utilizing a class **2** *N*-oxide urea standard set). Since TLC log *P* values were already available from ongoing work, HPLC-derived log *P* values were not measured for all compounds.

$$\text{TLC log } P = 0.997\ (\pm 0.054)\ \text{HPLC log } P + 0.501 \quad (4)$$

$$n = 26,\ r^2 = 0.93,\ s = 0.15,\ F = 341.3$$

Table II. Data for Compounds 1

no.	X	Y	π^x	F^x_{ortho}	MR^x	B^x_4	$\sum\pi^y$	$\sum F^y$	$\sum R^y$	$\sum MR^y$	pSI_{50}	TLC log *P*	calcd pSI_{50}[a]	Δ
1a	H	H	0.00	0.00	1.03	1.00	0.00	0.00	0.00	5.15		2.38		
1b	C_6H_5	H	1.96	0.10	25.36	3.11	0.00	0.00	0.00	5.15		3.48		
1c	CH_3	H	0.56	−0.05	5.65	2.04	0.00	0.00	0.00	5.15	4.10	2.62	4.34	−0.24
1d	*t*-C_4H_9	H	1.98	−0.09	19.62	2.97	0.00	0.00	0.00	5.15	4.20	3.34	4.46	−0.26
1e	C_2H_5	H	1.02	−0.06	10.30	2.97	0.00	0.00	0.00	5.15	4.80	2.90	4.56	0.24
1f	CO_2CH_3	H	−0.01	0.41	12.87	3.36	0.00	0.00	0.00	5.15	5.90	2.25	6.32	−0.42
1g	Cl	H	0.71	0.51	6.03	1.80	0.00	0.00	0.00	5.15	5.95	2.64	6.25	−0.30
1h	OC_2H_5	H	0.38	0.27	12.47	3.36	0.00	0.00	0.00	5.15	6.10	2.89	5.83	0.27
1i	Br	H	0.86	0.55	8.88	1.95	0.00	0.00	0.00	5.15	6.20	2.67	6.43	−0.23
1j	O-*n*-C_5H_{11}	H	2.04	0.31	26.26	5.73	0.00	0.00	0.00	5.15	6.20	4.52	6.62	−0.42
1k	$CONH_2$	H	−1.49	0.30	9.81	3.07	0.00	0.00	0.00	5.15	6.30	1.78	5.86	0.44
1l	O-*n*-C_3H_7	H	1.05	0.27	17.06	4.30	0.00	0.00	0.00	5.15	6.50	3.21	6.09	0.41
1m	I	H	1.12	0.50	13.94	2.15	0.00	0.00	0.00	5.15	6.70	2.94	6.31	0.39
1n	$CO_2C_2H_5$	H	0.51	0.41	17.47	4.29	0.00	0.00	0.00	5.15	6.70	2.62	6.58	0.12
1o	Cl	4-OCH_3	0.71	0.51	6.03	1.80	−0.02	0.26	−0.51	11.99		2.49		
1p	Cl	3-OCH_3	0.71	0.51	6.03	1.80	−0.02	0.25	−0.18	11.99		2.72		
1q	Cl	4-F	0.71	0.51	6.03	1.80	0.14	0.43	−0.34	5.04		2.79		
1r	Cl	2-CH_3	0.71	0.51	6.03	1.80	0.56	−0.05	−0.11	9.77		2.82		
1s	Cl	2-F	0.71	0.51	6.03	1.80	0.14	0.54	−0.29	5.04		2.98		
1t	Cl	3-F	0.71	0.51	6.03	1.80	0.14	0.42	−0.12	5.04		3.04		
1u	Cl	3-NO_2	0.71	0.51	6.03	1.80	−0.28	0.66	0.06	11.48		3.07		
1v	Cl	4-CH_3	0.71	0.51	6.03	1.80	0.56	−0.04	−0.13	9.77		3.09		
1w	Cl	3-CF_3	0.71	0.51	6.03	1.80	0.88	0.37	0.07	9.14		3.20		
1x	Cl	2-OCH_3	0.71	0.51	6.03	1.80	−0.02	0.32	−0.44	11.99		3.21		
1y	Cl	4-NO_2	0.71	0.51	6.03	1.80	−0.28	0.67	0.16	11.48		3.22		
1z	Cl	3-SCH_3	0.71	0.51	6.03	1.80	0.61	0.20	−0.06	17.94		3.37		
1aa	Cl	2-SCH_3	0.71	0.51	6.03	1.80	0.61	0.25	−0.16	17.94		3.37		
1bb	Cl	2-NO_2	0.71	0.51	6.03	1.80	−0.28	0.84	0.14	11.48		3.43		
1cc	Cl	4-Cl	0.71	0.51	6.03	1.80	0.71	0.41	−0.15	10.15		3.45		
1dd	Cl	3,5-F_2	0.71	0.51	6.03	1.80	0.28	0.84	−0.24	4.93		3.51		
1ee	Cl	2-Cl	0.71	0.51	6.03	1.80	0.71	0.51	−0.13	10.15		3.58		
1ff	Cl	3-Cl	0.71	0.51	6.03	1.80	0.71	0.40	−0.05	10.15		3.59		
1gg	Cl	2-CF_3	0.71	0.51	6.03	1.80	0.88	0.47	0.16	9.14		3.74		
1hh	Cl	3,4-Cl_2	0.71	0.51	6.03	1.80	1.42	0.81	−0.20	15.15		4.28		
1ii	Cl	3-CH_3	0.71	0.51	6.03	1.80	0.56	−0.04	−0.05	9.77		3.10		

[a] Calculated from eq 9a.

Table III. Data for Compounds 2

no.	X	Y	π^x	F^x_{ortho}	MR^x	$\sum\pi^y$	$\sum F^y$	$\sum R^y$	$\sum MR^y$	pSI_{50}	TLC log P	HPLC log P	calcd pSI_{50}[a]	Δ	calcd pSI_{50}[b]	Δ
2a	$NHCH_2$-C_6H_5	H	1.00	−0.14[c]	34.73	0.00	0.00	0.00	5.15	4.80	3.23	2.90	4.62	0.18	4.66	0.14
2b	$CONH_2$	H	−1.49	0.30	9.81	0.00	0.00	0.00	5.15	5.40	1.36	1.01	5.46	−0.06	5.54	−0.14
2c	$SO_2C_6H_5$	H	0.27	0.70	33.20	0.00	0.00	0.00	5.15	5.40	2.15	1.66	5.76	−0.35	5.72	−0.32
2d	C_6H_5	H	1.96	0.10	25.36	0.00	0.00	0.00	5.15	5.60	2.65	1.98	5.99	−0.39	5.96	−0.36
2e	NH-*n*-C_4H_9	H	1.45	−0.35	24.26	0.00	0.00	0.00	5.15	5.65	3.37	3.07	5.05	0.60	5.09	0.56
2f	$N(CH_3)_2$	H	0.18	0.12	15.55	0.00	0.00	0.00	5.15	5.70	2.11	1.67	5.68	0.02	5.71	−0.01
2g	$NHCH_3$	H	−0.47	−0.14	10.33	0.00	0.00	0.00	5.15	5.75	2.09	1.63	5.20	0.55	5.29	0.46
2h	SCH_3	H	0.61	0.25	13.82	0.00	0.00	0.00	5.15	5.75	1.81	1.25	6.20	−0.45	6.19	−0.44
2i	*i*-C_3H_7	H	1.53	−0.06	14.98	0.00	0.00	0.00	5.15	5.80	2.53	1.86	6.07	−0.27	6.06	−0.26
2j	*n*-C_3H_7	H	1.55	−0.07	14.96	0.00	0.00	0.00	5.15	5.80	2.47	1.88	6.06	−0.26	6.06	−0.26
2k	OCH_3	H	−0.02	0.32	7.87	0.00	0.00	0.00	5.15	5.85	1.51	1.05	6.32	−0.47	6.33	−0.48
2l	C_2H_5	H	1.02	−0.06	10.30	0.00	0.00	0.00	5.15	5.90	2.15	1.53	6.07	−0.16	6.08	−0.18
2m	SC_2H_5	H	1.07	0.29	18.42	0.00	0.00	0.00	5.15	6.00	2.30	2.05	6.25	−0.24	6.22	−0.22
2n	*t*-C_4H_9	H	1.98	−0.09	19.62	0.00	0.00	0.00	5.15	6.05	2.88	2.30	5.99	0.06	5.97	0.01
2o	H	H	0.00	0.00	1.03	0.00	0.00	0.00	5.15	6.10	1.44	1.01	6.16	−0.06	6.21	−0.11
2p	SO_2CH_3	H	−1.63	0.67	13.49	0.00	0.00	0.00	5.15	6.10	1.20	1.07	5.82	0.28	5.86	0.24
2q	CH_3	H	0.56	−0.05	5.65	0.00	0.00	0.00	5.15	6.20	1.84	1.26	6.10	0.10	6.14	0.07
2r	OC_2H_5	H	0.38	0.27	12.47	0.00	0.00	0.00	5.15	6.30	1.79	1.07	6.19	0.11	6.19	0.11
2s	O-*n*-C_5H_{11}	H	2.04	0.31	26.26	0.00	0.00	0.00	5.15	6.60	3.29	2.55	6.34	0.26	6.27	0.33
2t	I	H	1.12	0.50	13.94	0.00	0.00	0.00	5.15	6.65	1.81	1.25	6.86	0.21	6.80	−0.15
2u	Br	H	0.86	0.55	8.88	0.00	0.00	0.00	5.15	7.40	1.80	1.35	7.09	0.31	7.03	0.37
2v	Cl	H	0.71	0.51	6.03	0.00	0.00	0.00	5.15	7.50	1.73	1.37	7.10	0.40	7.05	0.45
2w	Cl	3-OH	0.71	0.51	6.03	−0.67	0.28	−0.22	6.97	4.70	0.84		5.98	−1.28		
2x	Cl	2-SO_2CH_3	0.71	0.51	6.03	−1.63	0.67	0.19	17.61	4.70	2.27		4.79	−0.09		
2y	Cl	3-OCH_3	0.71	0.51	6.03	−0.02	0.25	−0.18	11.99	5.50	1.88		5.34	0.16		
2z	Cl	3-CH_3	0.71	0.51	6.03	0.56	−0.04	−0.05	9.77	5.60	1.90[d]		6.23	−0.63		
2aa	Cl	2-OCH_3	0.71	0.51	6.03	−0.02	0.32	−0.44	11.99	5.80	1.61[d]		4.65	1.15		
2bb	Cl	3-Cl	0.71	0.51	6.03	0.71	0.40	−0.05	10.15	6.00	2.68		6.58	−0.58		
2cc	Cl	2-CH_3	0.71	0.51	6.03	0.56	−0.05	−0.11	9.77	6.00	1.93		6.05	−0.04		
2dd	Cl	2-F	0.71	0.51	6.03	0.14	0.54	−0.29	5.04	6.70	1.95		6.80	−0.10		
2ee	Cl	2-Cl	0.71	0.51	6.03	0.71	0.51	−0.13	10.15	6.90	2.53		6.44	0.46		
2ff	Cl	3-F	0.71	0.51	6.03	0.14	0.42	−0.12	5.04	7.80	2.12	1.62	7.19	0.61	7.57	0.23
2gg	Cl	3,5-F_2	0.71	0.51	6.03	0.28	0.84	−0.24	4.93	8.30	2.62	2.13	7.28	1.02	8.08	0.22
2hh	H	2,3,5,6-F_4	0.00	0.00	1.03	0.56	1.92	−0.82	4.71	5.10	1.37		5.75	−0.65		
2ii	H	2-F	0.00	0.00	1.03	0.14	0.54	−0.29	5.04	5.20	1.72		5.86	−0.66		
2jj	H	3-Cl	0.00	0.00	1.03	0.71	0.40	−0.05	10.15	5.30	2.42		5.65	−0.34		
2kk	H	3-F	0.00	0.00	1.03	0.14	0.42	−0.12	5.04	6.72	1.78	1.27	6.25	0.47	6.72	0.00
2ll	H	3,5-F_2	0.00	0.00	1.03	0.28	0.84	−0.24	4.93	6.90	2.27	1.59	6.34	0.56	7.24	−0.34
2mm	Cl	4-F	0.71	0.51	6.03	0.14	0.43	−0.34	5.04	6.30	1.85		6.56	−0.26		
2nn	Cl	3-CF_3	0.71	0.51	6.03	0.88	0.37	0.07	9.14		2.22					
2oo	Cl	2-CF_3	0.71	0.51	6.03	0.88	0.47	0.16	9.14		2.79					
2pp	Cl	4-OH	0.71	0.51	6.03	−0.67	0.29	−0.64	6.97		0.56					
2qq	Cl	3-SO_2CH_3	0.71	0.51	6.03	−1.63	0.53	0.08	17.61		1.67					
2rr	$NHCH_3$	3-F	−0.47	−0.14	10.33	0.14	0.42	−0.12	5.04	5.50	2.41				5.80	−0.30
2ss	CO_2CH_3	H	−0.01	0.41	12.87	0.00	0.00	0.00	5.15	5.60	2.31				6.21	−0.61
2tt	*n*-C_3F_7	H	1.49	0.55[c]	13.44	0.00	0.00	0.00	5.15	5.90	3.17				7.07	−1.17
2uu	2,6-$(CH_3)_2$	H	1.12	−0.10	11.30	0.00	0.00	0.00	5.15	5.90	2.01				6.01	−0.11
2vv	$SO_2C_2H_5$	H	−1.11	0.67[c]	18.14	0.00	0.00	0.00	5.15	6.00	1.48				5.84	0.16
2ww	SC_6H_5	H	2.32	0.46[c]	34.29	0.00	0.00	0.00	5.15	6.30	2.77				6.20	0.10
2xx	CH_3	3-F	0.56	−0.05	5.65	0.14	0.42	−0.12	5.04	6.60	2.11				6.65	−0.05
2yy	C_2F_5	H	1.23	0.55	9.23	0.00	0.00	0.00	5.15	6.30					7.18	−0.88
2zz	CF_3	H	0.88	0.47	5.02	0.00	0.00	0.00	5.15	6.50					7.12	−0.62

[a] Calculated by eq 12b. [b] Calculated by eq 14b. [c] Estimated value. [d] Calculated by eq 7.

Table IV. log *P* Data for *N*-Oxide Standards

no.	shake flask log *P*	TLC log P	Δ
4	0.92	1.32	0.40
2v	1.37	1.73	0.36
2gg	2.13	2.62	0.49
2e	3.07	3.37	0.30

RESULTS AND DISCUSSION

TLC log *P*. TLC log *P* values were found to correlate with physicochemical parameters. Multiple linear regression analysis of several subsets of **1** and/or **2** provided the following correlation equations (subsets were based on those analyzed for pSI_{50} correlations, vide infra).

Unoxidized ureas **1a–n** (Y = H):

$$\text{TLC log } P = 0.447\ (\pm 0.063)\pi^x - 0.944\ (\pm 0.316)R^x_{ortho} + 0.152\ (\pm 0.044)B^x_4 + 1.96 \quad (5a)$$

$$n = 14,\ r^2 = 0.94,\ s = 0.18,\ F = 55.9$$

Note that L^x and MR^x also substitute adequately for B_4, e.g.

$$\text{TLC log } P = 0.323\ (\pm 0.082)\pi^x - 1.27\ (\pm 0.32)R^x_{ortho} + 0.0288\ (\pm 0.0094)MR^x + 2.09 \quad (5b)$$

$$n = 14,\ r^2 = 0.94,\ s = 0.19,\ F = 48.8$$

N-Oxides **2a–v** (Y = H):

$$\text{TLC log } P = 0.250\ (\pm 0.048)\pi^x - 0.807\ (\pm 0.151)F^x_{ortho} + 0.0400\ (\pm 0.0051)MR^x + 1.52 \quad (6)$$

$$n = 22,\ r^2 = 0.92,\ s = 0.19,\ F = 73.8$$

Note that L^x also serves as a useful steric term, but B_4 is poor.

1g,o–hh plus **2v–y,bb–gg,mm–qq** (X = Cl; *N* is an indicator variable, 0 for unoxidized ureas 1 and 1 for *N*-oxides **2**):

$$\text{TLC log } P = 0.513\ (\pm 0.073)\sum\pi^y + 1.03\ (\pm 0.18)\sum F^y + 0.944\ (\pm 0.224)\sum R^y - 1.03\ (\pm 0.09)N + 2.73 \quad (7)$$

$$n = 36,\ r^2 = 0.90,\ s = 0.26,\ F = 73.4$$

Note from the coefficient of N that N-oxidation lowers the log P by ca. 1.0 unit. $\sum\sigma^y$ is a poor substitute electronic term for $\sum F^y$ and $\sum R^y$.

N-Oxides **2a**–**11**:

$$\text{TLC log } P = 0.344\ (\pm 0.062)\pi^x - 0.395\ (\pm 0.159)F^x_{ortho} + 0.0291\ (\pm 0.0063)MR^x + 0.582\ (\pm 0.126)\sum\pi^y + 1.06\ (\pm 0.20)\sum F^y + 2.54\ (\pm 0.46)\sum R^y + 1.61 \quad (8)$$

$$n = 38,\ r^2 = 0.82,\ s = 0.27,\ F = 22.9$$

Unfortunately, due to the subset analyzed, the cross correlation of $\sum F^y$ and $\sum R^y$ is 0.79. This may account for the difference in the coefficients of $\sum R^y$ in eq 7 and 8. As expected L^x could also serve as a useful steric parameter.

Equations 5–8 highlight the importance to TLC log P of lipophilic, electronic, and steric terms for the pyridine X substituent and lipophilic and electronic terms for the aryl Y group. From the work of Fujita and Hansch, equations involving lipophilicity and electronic parameters had been anticipated (Fujita, 1983a, b; Kakkis et al., 1984), but the incorporation of MR^x (or B^x_4 or L^x) with a positive coefficient was rather surprising. For the data sets analyzed, the following cross correlations between π and steric parameters were found: $\pi^x/B^x_4 = 0.30$ (eq 5a), $\pi^x/MR^x = 0.65$ (eq 5b), $\pi^x/MR^x = 0.41$ (eq 6). Since the pyridine 2-substituent is buttressed against the strongly hydrophilic *N*-oxide, a positive steric factor may be important for effective interaction of the X group with a hydrophobic phase during partitioning. The negative coefficient of F^x_{ortho} indicates that dipolar effects involving interactions with the *N*-oxide are important to lipophilicity. This also accounts for the difference in preference for R^x_{ortho} for the unoxidized ureas 1 (eq 5a,b) versus F^x_{ortho} for the *N*-oxides **2** (eq 6). The positive coefficients of $\sum\pi^y$, $\sum F^y$, and $\sum R^y$ (eq 7 and 8) are in line with the bidirectional effects observed by Fujita (1983b) for anilines, formanilides, and acetanilides.

Biological Activity. Unoxidized ureas **1c**–**n** (Y = H):

$$pSI_{50} = 3.52\ (\pm 0.50)F^x_{ortho} + 0.275\ (\pm 0.098)B^x_4 + 3.96 \quad (9a)$$

$$n = 12,\ r^2 = 0.86,\ s = 0.38,\ F = 27.9$$

$$pSI_{50} = 3.51\ (\pm 0.59)F^x_{ortho} + 0.0401\ (\pm 0.0225)MR^x + 4.29 \quad (9b)$$

$$n = 12,\ r^2 = 0.81,\ s = 0.45,\ F = 18.8$$

Inclusion of MR^x in eq 9b is not statistically warranted ($t = 1.78$) but is reported for comparison with QSAR for the *N*-oxides **2** below. In eq 9a and 9b the F^x_{ortho} term is dominant.

N-Oxides **2a**–**v** (Y = H):

$$pSI_{50} = 1.56\ (\pm 0.17)\ \text{TLC log } P + 2.62\ (\pm 0.24)F^x_{ortho} - 0.113\ (\pm 0.010)MR^x + 3.93 \quad (10a)$$

$$n = 22,\ r^2 = 0.90,\ s = 0.21,\ F = 55.4$$

$$pSI_{50} = 0.415\ (\pm 0.086)\pi^x + 1.40\ (\pm 0.27)F^x_{ortho} - 0.0519\ (\pm 0.0091)MR^x + 6.29 \quad (10b)$$

$$n = 22,\ r^2 = 0.75,\ s = 0.33,\ F = 18.2$$

N-Oxides **2v**–**gg** (X = Cl):

$$pSI_{50} = 1.29\ (\pm 0.33)\ \text{TLC log } P - 0.237\ (\pm 0.043)\sum MR^y + 5.85 \quad (11a)$$

$$n = 12,\ r^2 = 0.82,\ s = 0.55,\ F = 20.2$$

Inclusion of several pyridine 2-unsubstituted (X = H) compounds provides eq 11b.

N-Oxides **2o**,**v**–**11** (X = H, Cl; IN is an indicator variable, 0 for X = H and 1 for X = Cl):

$$pSI_{50} = 1.29\ (\pm 0.30)\ \text{TLC log } P - 0.245\ (\pm 0.042)\sum MR^y + 0.970\ (\pm 0.312)IN + 4.96 \quad (11b)$$

$$n = 18,\ r^2 = 0.76,\ s = 0.56,\ F = 15.1$$

N-Oxides **2a**–**11**:

$$pSI_{50} = 1.28\ (\pm 0.17)\ \text{TLC log } P + 1.37\ (\pm 0.30)F^x_{ortho} - 0.0812\ (\pm 0.0107)MR^x - 0.250\ (\pm 0.029)\sum MR^y + 5.28 \quad (12a)$$

$$n = 38,\ r^2 = 0.77,\ s = 0.42,\ F = 27.1$$

$$pSI_{50} = 0.491\ (\pm 0.115)\sum\pi^{xy} + 1.68\ (\pm 0.35)F^x_{ortho} - 0.0532\ (\pm 0.0128)MR^x + 0.826\ (\pm 0.412)\sum F^y + 2.88\ (\pm 0.93)\sum R^y - 0.210\ (\pm 0.037)\sum MR^y + 7.29 \quad (12b)$$

$$n = 38,\ r^2 = 0.64,\ s = 0.54,\ F = 9.1$$

In eq 12b if π^x and π^y are separated, their coefficients are identical within the confidence limits (0.468 ± 0.131 and 0.581 ± 0.263, respectively); therefore, π^x and π^y represent contributions to overall molecular lipophilicity.

Chlorophyll retention activity, as measured by pSI_{50} values, was observed to be a function of the same lipophilic, electronic, and "steric" factors as for TLC log P. Reanalysis of pSI_{50} equations containing a TLC log P term using only the relevant physicochemical parameters provided biological activity equations of predictive value (vide infra).

For the unoxidized ureas 1, the wheat leaf pSI_{50} (chlorophyll retention) equation (9a) compared to the literature equation derived from tobacco callus growth stimulation (eq 1) shows that electron-withdrawing substituents improve their respective biological activities; however, a positive steric term (B_4) for chlorophyll retention contrasts with a positive lipophilicity (π) term for callus growth stimulation. Differences in the two equations may be due to the particular set analyzed, different biological species, and/or assay methods. Apparently in wheat leaves a large cytokinin binding site exists in the region of the X substituent, making high B_4 a desirable component for activity. Note that for our set (eq 9a) the cross correlation between π and B_4 is only 0.24; B_4 is not a substitute for π. The same is true for Okamoto's set (eq 1): cross correlation $\pi/B_4 = 0.15$.

Upon N-oxidation to afford **2** the effect of MR^x on chlorophyll retention activity is reversed (eq 10a,b). This may reflect differences in binding to the cytokinin receptor (Iwamura et al., 1985) introduced by the *N*-oxide functionality. A positive lipophilicity term (π^x or TLC log P) appears in the QSAR for **2** because the log P of **2** is ca. 1 unit lower than for the corresponding **1**. The electron-withdrawing character of the pyridine X substituent remains the only common activity determinant for both series. The effect of phenyl (Y) substitution on the chlorophyll retention activity of **1** was not rigorously examined but appears to be the same as for **2**.

The phenyl ring occupies a sterically sensitive receptor site. Para substitution greatly reduced or totally abolished chlorophyll retention activity: even a para fluoro (compound **2mm**) reduced activity more than 10-fold with respect to the parent. Therefore, only Y substituents in the

Table V. Correlation Matrix for eq 13

	log P	π^x	$\sum\pi^Y$	F^x_{ortho}	MR^x
log P	1.0000				
π^x	0.7481	1.0000			
$\sum\pi^Y$	0.0800	−0.1164	1.0000		
F^x_{ortho}	−0.4565	−0.2918	0.0923	1.0000	
MR^x	0.6341	0.4271	−0.4505	−0.0531	1.0000

ortho and/or meta positions could be considered in the regression analyses. The dominant term in eq 11a and 11b is $\sum MR^y$ with a relatively large negative coefficient; a term of the same magnitude appears in summary eq 12a and 12b. Bulky phenyl substitution either inhibits receptor binding or disables the cytokinin receptor–substrate complex form undergoing requisite conformational change (Hansch and Leo, 1979; Iwamura et al., 1985). This is a directional effect, much greater in the para direction than in the ortho or meta directions. Iwamura et al. (1980) have observed analogous negative steric effects (in Verloop L) for diphenylurea cytokinins.

The coefficients of $\sum\pi^y$, $\sum F^y$, and $\sum R^y$ in summary eq 12b are identical with those in the corresponding TLC log P eq 8 within the confidence limits. Thus, in addition to the negative effect of MR^y on pSI_{50}, a secondary influence of phenyl substitution can be attributed to a positive log P effect. The summation of these two effects leads to the conclusion that only meta fluoro substitution will result in an increase in chlorophyll retention activity. This has indeed been observed in our own work (see compounds **2ff,gg,kk,ll**). The same observation was made for **1** by the Japanese group using the tobacco callus system (Okamoto et al., 1981; Isogai, 1981); however, they provided no rationale for their observation. Meta fluoro has also been noted to be an outlier in the QSAR of urea herbicides, being 7 times more active than predicted (Kakkis et al., 1984; Camilleri et al., 1987). It is clear from the present work that meta fluoro combines optimum steric and electronic qualities, the latter of which increases lipophilicity.

In light of the above observations, those compounds having Y = meta fluoro (**2ff,gg,kk,ll**) were added to the N-oxide subset used for generating eq 6 and 10a,b (varying pyridine X substitution), and TLC log P and pSI_{50} correlation equations were recalculated. Note that steric terms in Y are insignificant due to the comparable size of hydrogen and fluorine.

N-Oxides **2a–v,ff,gg,kk,ll** (Y = H, meta F):

$$\text{TLC log } P = 0.272\ (\pm0.047)\pi^x + 3.18\ (\pm0.52)\sum\pi^y - 0.693\ (\pm0.138)F^x_{ortho} + 0.0404\ (\pm0.0051)MR^x + 1.47 \quad (13)$$

$$n = 26,\ r^2 = 0.91,\ s = 0.19,\ F = 56.0$$

$$pSI_{50} = 1.38\ (\pm0.11)\ \text{TLC log } P + 2.43\ (\pm0.18)F^x_{ortho} - 0.103\ (\pm0.006)MR^x + 4.18 \quad (14a)$$

$$n = 26,\ r^2 = 0.94,\ s = 0.21,\ F = 115.8$$

$$pSI_{50} = 0.447\ (\pm0.082)\pi^x + 3.68\ (\pm0.90)\sum\pi^y + 1.55\ (\pm0.24)F^x_{ortho} - 0.0527\ (\pm0.0088)MR^x + 6.26 \quad (14b)$$

$$n = 26,\ r^2 = 0.86,\ s = 0.32,\ F = 33.5$$

$$pSI_{50} = 1.34\ (\pm0.16)\ \text{HPLC log } P + 2.25\ (\pm0.24)F^x_{ortho} - 0.102\ (\pm0.009)MR^x + 4.97 \quad (14c)$$

$$n = 26,\ r^2 = 0.88,\ s = 0.30,\ F = 55.15$$

Correlation matrices and equation development data for eq 13, 14a, and 14b are presented in Tables V–X. TLC log P appears to function somewhat better than HPLC log P as a predictor of chlorophyll retention activity. The reason for this is unclear at the present.

Table VI. Correlation Matrix for eq 14a

	pSI_{50}	log P	F^x_{ortho}	MR^x
pSI_{50}	1.0000			
log P	−0.1318	1.0000		
F^x_{ortho}	0.4656	−0.4565	1.0000	
MR^x	−0.5767	0.6341	−0.0531	1.0000

Table VII. Correlation Matrix for eq 14b

	pSI_{50}	π^x	$\sum\pi^y$	F^x_{ortho}	MR^x
pSI_{50}	1.0000				
π^x	0.0534	1.0000			
$\sum\pi^y$	0.6339	−0.1164	1.0000		
F^x_{ortho}	0.4656	−0.2918	0.0923	1.0000	
MR^x	−0.5767	0.4271	−0.4505	−0.0531	1.0000

Table VIII. Development of eq 13

$$\text{TLC log } P = a\pi^x + bMR^x + c\sum\pi^y + dF^x_{ortho} + e$$

a	b	c	d	e	n	s	r^2	F
0.472				1.87	26	0.40	0.56	30.51
(0.086)								
0.368	0.025			1.60	26	0.35	0.68	24.53
(0.082)	(0.008)							
0.345	0.037	2.90		1.35	26	0.27	0.81	31.57
(0.065)	(0.007)	(0.74)						
0.272	0.040	3.18	−0.69	1.47	26	0.19	0.91	55.99
(0.047)	(0.005)	(0.52)	(0.14)					

Table IX. Development of eq 14a

$$pSI_{50} = aMR^x + bF^x_{ortho} + c\ \text{TLC log } P + d$$

a	b	c	d	n	s	r^2	F
−0.051			6.93	26	0.67	0.33	11.96
(0.015)							
−0.049	1.23		6.66	26	0.58	0.52	12.57
(0.013)	(0.41)						
−0.103	2.43	1.38	4.18	26	0.21	0.94	115.84
(0.006)	(0.18)	(0.11)					

Table X. Development of eq 14b

$$pSI_{50} = a\sum\pi^y + bF^x_{ortho} + cMR^x + d\pi^x + e$$

a	b	c	d	e	n	s	r^2	F
6.24				6.03	26	0.64	0.40	16.13
(1.55)								
5.87	1.16			5.82	26	0.55	0.57	15.18
(1.35)	(0.39)							
4.28	1.15	−0.032		6.31	26	0.49	0.67	15.02
(1.35)	(0.35)	(0.012)						
3.68	1.55	−0.053	0.45	6.26	26	0.32	0.86	33.54
(0.90)	(0.24)	(0.009)	(0.08)					

In eq 14a and 14b the $\sum\pi^y$ term almost serves as an indicator variable since only three values are possible for the presence of 0–2 meta fluorines: 0, 0.14, 0.28. The effective π value of 0.445 (calculated from eq 13) for meta fluoro corresponds well with the value of 0.46 tabulated by Fujita (1983b) for the acetanilide series and represents a composite of intrinsic lipophilicity and bidirectional electronic effects.

The predictability of eq 14a and 14b was tested on a number of compounds not included in the original equation development (**2rr–zz**); in general the agreement between calculated and observed values was excellent. Compound **2ss**, bearing a methyl ester substituent, was somewhat less active than predicted. This same trend has been observed for ester substituents in other arylurea cytokinin series, including **1** (Isogai, 1981; Iwamura et al., 1980). Reduced activity has been ascribed to in vivo hy-

drolysis. Note that the 2,6-dimethyl *N*-oxide **2uu** is well predicted using the summation of physicochemical parameters for the pyridine X substituents. The corresponding 2,6-dimethyl analogue of **1** was poorly predicted by eq 1 (Okamoto et al., 1983).

Perfluoroalkyl *N*-oxides **2tt**, **2yy**, and **2zz** are much less active than predicted. The difference in predicted versus observed values can be directly related to the fluorine content of the perfluoroalkyl group ($\Delta = -0.2$ pSI_{50} unit per fluorine). The reason for this unique effect of fluorine substitution in the pyridine 2-position of *N*-oxides **2** is not obvious.

CONCLUSION

N-Oxides **2** are potent cytokinin mimics as evidenced by chlorophyll retention in excised wheat leaves. Biological activity is sensitive to both pyridine and phenyl substitution. QSAR for **1** in different biological systems is observed to be different, which may be attributed to varying structural requirements for each specific cytokinin receptor involved. QSAR is also different for the *N*-oxides **2** versus the unoxidized ureas **1** in the same system (wheat leaf chlorophyll retention), particularly with regard to the pyridine X group. This is not surprising since the strongly hydrophilic dipolar *N*-oxide functionality buttresses the pyridine 2-X substituent in **2** and may cause variations in receptor binding modes between the two classes. QSAR indicates the following activity constraints on substituents X and Y for *N*-oxides **2**: For X, small, lipophilic, electronegative groups enhance activity, and for Y, meta fluorination uniquely increases activity.

The latter effect is adequately explained by a combination of negative steric and positive lipophilicity effects. The optimum substitutions are embodied in compound **2gg**: X = Cl and Y = 3,5-F_2. Finally, reversed-phase TLC log *P* values are shown to be valid and useful parameters in regression analyses of molecules represented by generic structure **2**. Apparently these correlations are the first such studies to be reported for pyridine *N*-oxides.

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Registry No. **1a**, 1932-35-0; **1aa**, 113548-51-9; **1b**, 113548-40-6; **1bb**, 113548-52-0; **1c**, 68197-48-8; **1cc**, 68197-46-6; **1d**, 113548-41-7; **1dd**, 110944-18-8; **1e**, 113548-42-8; **1ee**, 68157-48-2; **1f**, 75279-46-8; **1ff**, 68157-47-1; **1g**, 68157-60-8; **1gg**, 113548-53-1; **1h**, 113548-43-9; **1hh**, 113548-54-2; **1i**, 75279-37-7; **1ii**, 68157-50-6; **1j**, 113567-66-1; **1k**, 113548-44-0; **1l**, 113548-45-1; **1m**, 113548-46-2; **1n**, 113548-47-3; **1o**, 113567-67-2; **1p**, 76947-72-3; **1q**, 76963-45-6; **1r**, 68157-51-7; **1s**, 70696-31-0; **1t**, 76963-44-5; **1u**, 113548-48-4; **1v**, 68157-49-3; **1w**, 97966-84-2; **1x**, 113548-49-5; **1y**, 113567-68-3; **1z**, 113548-50-8; **2a**, 113548-55-3; **2aa**, 113548-68-8; **2b**, 97985-38-1; **2bb**, 97967-04-9; **2c**, 97985-21-2; **2cc**, 97967-15-2; **2d**, 113548-56-4; **2dd**, 97967-13-0; **2e**, 97967-06-1; **2ee**, 97967-11-8; **2f**, 97985-26-7; **2ff**, 97967-03-8; **2g**, 97967-05-0; **2gg**, 113548-69-9; **2h**, 97985-27-8; **2hh**, 113548-70-2; **2i**, 113548-57-5; **2ii**, 113548-71-3; **2j**, 113548-58-6; **2jj**, 97967-09-4; **2k**, 97985-18-7; **2kk**, 97967-08-3; **2l**, 113548-59-7; **2ll**, 113548-72-4; **2m**, 113548-60-0; **2mm**, 97967-14-1; **2n**, 113548-61-1; **2nn**, 97967-17-4; **2o**, 97985-34-7; **2oo**, 113548-73-5; **2p**, 97985-40-5; **2pp**, 113548-66-6; **2q**, 97967-18-5; **2qq**, 113548-74-6; **2r**, 113548-62-2; **2rr**, 113548-75-7; **2s**, 113548-63-3; **2ss**, 97985-19-8; **2t**, 113548-64-4; **2tt**, 113548-58-6; **2u**, 97967-10-7; **2uu**, 113548-76-8; **2v**, 97985-35-8; **2vv**, 97985-40-5; **2w**, 113548-65-5; **2ww**, 97985-20-1; **2x**, 113548-66-6; **2xx**, 97967-18-5; **2y**, 113548-67-7; **2yy**, 113548-77-9; **2z**, 97967-16-3; **2zz**, 113548-78-0; **3** (X = $-CH(CH_3)_2$), 113548-81-5; **3** (X = $-C(CH_3)_3$), 100133-20-8; **3** (X = $-OCH_2CH_3$), 58481-00-8; **3** (X = $-O(CH_2)_4CH_3$), 113548-82-6; **3** (X = -I), 29247-87-8; **3** (X = $-CF_2CF_3$), 113548-84-8; 2-methyl-4-nitropyridine *N*-oxide, 5470-66-6; 4-amino-2-methylpyridine *N*-oxide, 14045-17-1; phenyl isothiocyanate, 103-72-0; 2-isopropyl-4-nitropyridine *N*-oxide, 113548-79-1; 2-ethyl-4-nitropyridine *N*-oxide, 38594-62-6; 2,6-dimethyl-4-nitropyridine *N*-oxide, 4808-64-4; 3-fluorophenyl isocyanate, 404-71-7; phenyl chloroformate, 1885-14-9; 4-amino-2-fluoropyridine, 18614-51-2; phenyl *N*-(2-chloro-4-pyridinyl)-carbamate, 76947-86-9; 3-(methylthio)aniline, 1783-81-9; 2-(methylthio)aniline, 2987-53-3; phenyl *N*-(2-chloro-4-pyridinyl)carbamate *N*-oxide, 113567-69-4; *N*-phenyl-*N'*-(2-isopropylpyridin-4-yl)urea, 113548-80-4; *N*-phenyl-*N'*-(2-*tert*-butylpyridin-4-yl)urea, 113548-41-7; *N*-phenyl-*N'*-(2-(pentafluoroethyl)pyridin-4-yl)urea, 113548-83-7.

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Ring Transformation of Michael Adducts of 4-Benzylidene-5-oxazolones and 3-Mercapto-*s*-triazoles to 2,3-Dihydro-4*H*-*s*-triazolo[3,4-*b*][1,3]thiazin-4-ones with Some Antifungal Activity

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Michael addition of 3-mercapto-*s*-triazoles Ia–e to 4-benzylidene-5-oxazolones IIa,b followed by ring transformation yielded a novel class of compounds, the 2,3-dihydro-2,6-diaryl-3-benzamido-4*H*-*s*-triazolo[3,4-*b*][1,3]thiazin-4-ones IVa–j, in one pot. The compounds IVa–j were compared with Dithane M-45, a commercial fungicide, for their antifungal activity against *Aspergillus niger* and *Fusarium oxysporium*, and the results have been correlated with the structural features of the tested compounds.

Among the most widely used antibiotics, cephalosporins essentially contain the 1,3-thiazine nucleus. Likewise, the *s*-triazole ring is also associated with various useful pesticidal activities (Greenfield et al., 1970; Okano and Yasujaga, 1970; Reisser, 1969; Bucchel and Draber, 1971). In view of these facts and with the hope of achieving antifungal compounds of high potency, we have fused the biolabile 1,3-thiazine and *s*-triazole nuclei to probe how this combination could enhance the antifungal action. Further, all of these compounds possess a fluoroaryl moiety, which might be expected to enhance their antifungal activity (Filler and Kobayashi, 1983). The investigation appeared quite interesting as the 2,3-dihydro-4*H*-*s*-triazolo[3,4-*b*][1,3]thiazin-4-ones IVa–j reported here constitute a hitherto unknown class of nitrogen-bridged heterocyclic compounds.

3-Mercapto-*s*-triazoles Ia–e were refluxed with 4-benzylidene-5-oxazolones IIa,b in dioxane for 2 h to furnish

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