QSAR of Cytokinin-Active *N*-Pyridinyl-*N'*-alkylureas and *N*-Oxides[1]

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Quantitative structure–activity relationships (QSAR) are derived for the chlorophyll retention activity in excised wheat leaves of *N*-(2-substituted 4-pyridinyl)-*N'*-alkylureas (**1**) and their corresponding pyridine *N*-oxides (**2**). The resulting equations provide strong evidence that the *N*-oxide functionality is directly involved in cytokinin receptor binding. The optimum length of the alkyl group for desoxypyridines **1** is 5.65 Å versus 4.59 Å for pyridine *N*-oxides **2**; the optimum π^R values are 1.09 and 1.64, respectively. Electronic (σ_{ortho}) and receptor binding (MR) properties of the pyridine ortho substituents of **2** are additional biological activity determinants. Optimum substitutions are embodied in compounds **1w**, **1x**, and **2r–2u**. Differences from the corresponding *N'*-arylureas are discussed. Reversed-phase TLC log *P* values for **1** and **2** are correlated with π and σ^* of the alkyl group.

Subsequent to our earlier examination of the chlorophyll retention activity of *N*-(2-substituted 4-pyridinyl)-*N'*-arylureas and their corresponding pyridine *N*-oxides (**1** and **2**, respectively, R = aryl) (Henrie et al., 1988), we elected to explore the analogous *N'*-alkylureas (**1** and **2**, R = alkyl). Our investigation was initiated despite statements in the literature that cytokinin-active *N*-4-pyridinylureas require an *N'*-aryl group (Okamoto et al., 1981, 1983). Contrary to these indications, we have discovered that the *N'*-alkylureas **1** and **2** possess good cytokinin activity in our wheat leaf chlorophyll retention bioassay. Therefore, quantitative structure–activity relationships (QSAR) were sought for comparison with those derived from the *N'*-arylurea series to provide additional information regarding the pyridinylurea–cytokinin receptor interaction.

QSAR of both cytokinin agonists and antagonists has been utilized recently to probe receptor site topology (Koshimizu and Iwamura, 1985). For example, the activity of N^6-alkyl-substituted purines **3** in tobacco callus culture is described by eq 1 (Iwamura et al., 1980). The cytokinin

$$\log (1/E_{50}) = 5.84\ (\pm 3.04) W_{max} - 0.56\ (\pm 0.29) W_{max}^2 + 1.66\ (\pm 1.02)\sigma^* - 14.67\ (\pm 7.81) \quad (1)$$

$$n = 12,\ r^2 = 0.83,\ s = 0.20,\ W_{max}(\text{opt}) = 5.21$$

agonist/antagonist activities of 4-substituted 2-methylpyrrolo[2,3-*d*]pyrimidines **4** in tobacco callus culture is represented by eq 2 (Iwamura et al, 1983). Finally, the

$$\log (1/I_{50}) = 2.98\ (\pm 1.86) W^R_{max} - 0.33\ (\pm 0.19) W^R_{max}{}^2 + 1.10\ (\pm 0.37) W^R_u - 0.35\ (\pm 0.20) L^R + 0.53\ (\pm 0.15)\pi + 1.21\ (\pm 0.65)\sigma^* - 1.09\ (\pm 0.27) W^{Ph}_{max} + 10.25\ (\pm 4.64) IN^{Ph} - 8.27\ (\pm 4.53) \quad (2)$$

$$n = 44,\ r^2 = 0.85,\ s = 0.39,\ W^R_{max}(\text{opt}) = 4.52$$

antagonist activity of 2-methylthio 4-substituted pyrido-[2,3-*d*]pyrimidines **5** in tobacco callus culture is described by eq 3a,b (Iwamura et al., 1979, 1985).

$$\log (1/I_{50}) = 8.75\ (\pm 2.67) W^R_{max} - 0.96\ (\pm 0.29) W^R_{max}{}^2 - 0.58\ (\pm 0.43)\pi - 18.32\ (\pm 5.86) \quad (3a)$$

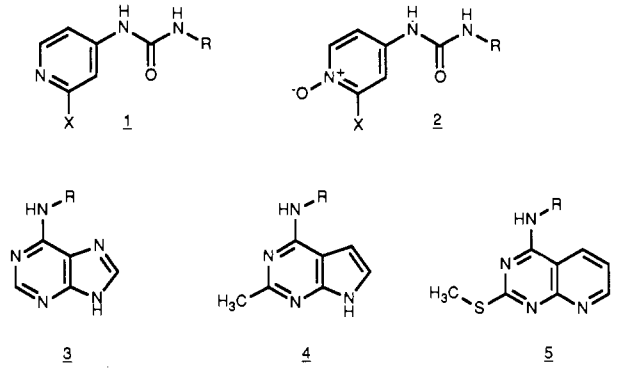
$$n = 10,\ r^2 = 0.92,\ s = 0.28,\ W^R_{max}(\text{opt}) = 4.56$$

$$\log (1/I_{50}) = 1.95 W^R_{max}\ (t = 3.68) - 0.28 W^R_{max}{}^2\ (4.44) - 0.67 W_u\ (3.79) + 0.45\pi\ (3.09) - 2.32\ (2.07) \quad (3b)$$

$$n = 18,\ r^2 = 0.72,\ s = 0.37,\ W^R_{max}(\text{opt}) = 3.48$$

All of the above equations for these fused bicyclic heteroaromatics show optima in the range 3.5–5.2 Å for the maximum width of the alkyl substituent, W^R_{max} [essentially Verloop's B_5 (Hansch and Leo, 1979)]. The rather large difference of 1.1 Å in W^R_{max}(opt) derived from eq 3a,b for the antagonist activity of the same chemical class has not been explained in the literature. The optimum W^R_{max} of 4.6 Å for **5** derived from eq 3a is more consistent with the other literature data presented above. L^R is stated to be a very poor substitute for W^R_{max} in all of the above equations.

From these equations based on tobacco callus culture data, a composite dimensional map for the cytokinin receptor has been sketched (Iwamura et al., 1985). A variety of structural types of both cytokinin agonists and antagonists can be accommodated by this receptor site model. Although pyridinylureas and pyridinyl *N*-oxide ureas **1** and **2** show somewhat different QSAR in our wheat leaf chlorophyll retention bioassay from the literature QSAR of **3–5**, potentially they may be accommodated by the same general receptor site model. Rigorous comparison is not possible, however, due to the different bioassays used.



MATERIALS AND METHODS

Chlorophyll retention assays, reversed-phase TLC log *P* determinations, and QSAR and statistical analyses were performed as described previously (Henrie et al., 1988). pSI_{50} values have a confidence interval of ±0.5; reversed-phase TLC values have a confidence interval of ±0.2. Statistical parameters are defined as referenced in Kakkis et al. (1984). All π values used in the QSAR analyses are *aromatic* substituent constants and were obtained from the tabulation of Hansch and Leo (1979) or calculated according to additivity principles (Hansch and Leo, 1983). Physicochemical parameters are defined in Hansch and Leo (1979) or in the original literature citations.

Synthesis of 1 and 2. The various methods used to prepare the target ureas are outlined in Scheme I; repre-

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[1] Dedicated to Professor Edward C. Taylor, Princeton University, Princeton, NJ, on the occasion of his 65th birthday.

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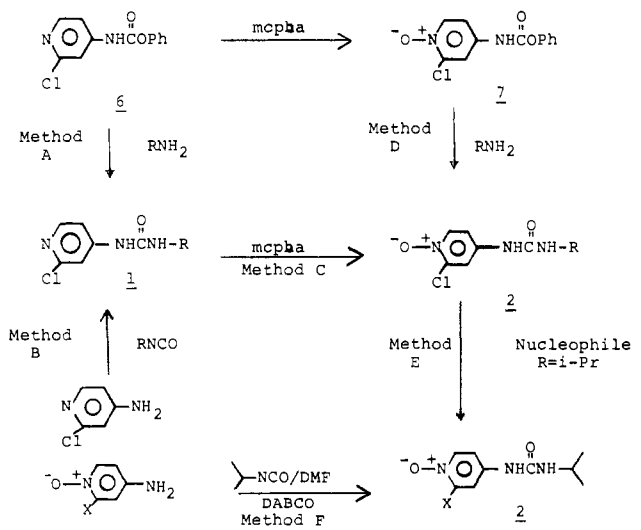
Table I. Data for Compounds 1 (X = Cl)

no.	R	synthesis method[c]	π	σ^*	L	TLC log P	pSI_{50} obsd	pSI_{50} calcd[a]	Δ
1a	1-methylbutyl	A	2.63	−0.22	6.17	2.67	6.60	5.00	1.60
1b	methyl	B	0.56	0.00	3.00	1.08	4.00	4.02	−0.02
1c	benzyl	A	2.01	0.23	3.63	2.41	4.30	4.39	−0.09
1d	cyclohexyl	A	2.79	−0.15	6.17	2.85	4.60	4.65	−0.05
1e	1-ethylpropyl	A	2.63	−0.23	4.73	2.51	4.80	4.83	−0.03
1f	isopentyl	A	2.63	−0.16	6.17	2.73	4.90	4.95	−0.05
1g	*tert*-pentyl	A	2.51	−0.33	4.92	2.74	5.10	5.22	−0.12
1h	CH_2CF_3	A	0.59	0.92	4.70	1.83	5.20	5.16	0.04
1i	neopentyl	A	2.51	−0.17	4.92	2.67	5.20	5.09	0.11
1j	1,2-dimethylpropyl	A	2.51	−0.23	5.09	2.46	5.20	5.21	−0.01
1k	ethyl	A	1.02	−0.10	4.11	1.45	5.50	5.66	−0.16
1l	cyclopropyl	A	1.14	−0.15	4.14	1.44	5.60	5.73	−0.13
1m	cyclopentyl	A	2.14	−0.20	4.97	2.41	5.60	5.67	−0.07
1n	cyclobutyl	A	1.65	−0.15	4.69	2.06	5.60	5.95	−0.35
1o	*tert*-butyl	A, B	1.98	−0.30	4.11	2.36	5.70	5.36	0.34
1p	*sec*-butyl	A	2.04	−0.21	5.05	2.41	5.70	5.83	−0.13
1q	allyl	A	1.10	0.23	5.11	1.56	5.80	6.03	−0.23
1r	3-chloropropyl	A	1.42	0.14	5.94	1.86	5.80	6.10	−0.30
1s	isobutyl	A	2.11	−0.13	5.05	2.22	5.80	5.69	0.11
1t	isopropyl	A, B	1.53	−0.19	4.11	1.86	5.90	5.62	0.28
1u	butyl	A, B	2.13	−0.13	6.17	2.35	5.90	5.69	0.21
1v	cyclopropylmethyl	A	1.62	0.01	4.90	2.19	6.00	5.96	0.04
1w	2-chloroethyl	A	0.82	0.39	5.57	1.71	6.30	5.95	0.35
1x	propyl	A, B	1.55	−0.12	5.05	1.76	6.40	6.16	0.24
1y	$CH(CH_2OH)CH(CH_3)_2$	A	0.52	0.08[b]	4.72		3.75	5.77	−2.02
1z	$C(CH_3)(CH_2CH_3)_2$	A	3.03	−0.33[b]	5.05		4.30	4.21	0.09
1aa	$CH(CH_2OH)CH_2CH_3$	A	0.12	0.10[b]	5.05	1.33	4.40	5.55	−1.15
1bb	$CH_2CH_2CH_2OH$	A	−0.29	0.06	6.02		4.50	5.10	−0.60
1cc	$CH_2CH_2CH_2OCH_3$	A	0.30	0.02	6.96		5.00	5.38	−0.38
1dd	$C(CH_3)_2CH_2OH$	A	−0.01	0.00[b]	4.11		5.10	4.88	0.22
1ee	$CH_2CH(CH_3)CH_2CH_3$	A	2.64	−0.13[b]	6.17	2.78	5.20	4.90	0.30
1ff	$C(CH_3)_2CH_2CH_2OH$	A	0.52	−0.30[b]	4.11		5.50	5.62	−0.12
1gg	$CH_2CH_2N(CH_3)_2$	A	0.17	0.13	5.58		5.70	5.69	0.01
1hh	$CH_2CF_2CF_3$	A	1.38	0.92[b]	5.35	2.28	5.90	5.49	0.41
1ii	phenyl	B	1.96	0.60	6.28	2.65	5.95	5.25	0.70

[a] Calculated using eq 7. [b] Estimated value. [c] Cf. Scheme I.

Scheme I



sentative examples follow. All reactions were conducted under dry nitrogen. Melting points are uncorrected. Chromatography refers to the flash column chromatography technique (Hostettmann et al., 1986) using Merck silica gel 60 as adsorbant. DABCO refers to 1,4-diazabicyclo[2.2.2]octane; mcpba refers to *m*-chloroperoxybenzoic acid.

Method A. A solution of 16.0 g (64 mmol) of phenyl *N*-(2-chloro-4-pyridinyl)carbamate (**6**; Scheme I; Henrie et al., 1988) and 8.0 mL (76 mmol) of *tert*-butylamine in 300 mL of acetone was refluxed for 3 h. Evaporation of solvent and chromatography of the residual oil (10% acetone/CH_2Cl_2) provided 14.6 g (100%) of **1o** (Table I), mp 154–156 °C. Anal. Calcd for $C_{10}H_{14}ClN_3O$, MW 227.70: C, 52.75; H, 6.20; N, 18.45; Cl, 15.57. Found: C, 53.05; H, 6.22; N, 18.47; Cl, 15.42.

Method B. A solution of 2.6 g (20 mmol) of 4-amino-2-chloropyridine (Henrie et al., 1988), 0.53 g (5 mmol) of DABCO, and 3.9 mL (30 mmol) of isopropyl isocyanate in 20 mL of dry DMF was stirred at ambient temperature for 5 days. The DMF was removed in vacuo and chased with absolute EtOH. The residual solid was triturated with hot H_2O and cooled to provide 4.1 g (96%) of **1t** (Table I), mp 142–146.5 °C. Anal. Calcd for $C_9H_{12}ClN_3O$, MW 213.67: C, 50.59; H, 5.66; N, 19.67; Cl, 16.60. Found: C, 50.80; H, 5.39; N, 19.72; Cl, 16.37.

Method C. A solution of 1.2 g (5.6 mmol) of **1t** and 2.0g (10 mmol, ca. 85% pure) of mcpba in 45 mL of CH_2Cl_2 plus 100 mL of Et_2O was refluxed for 24 h. The precipitated solid was collected and chromatographed, eluting first with Et_2O to remove *m*-chlorobenzoic acid, followed by 15% MeOH/CH_2Cl_2, affording 0.82g (64%) of **2s** (Table II), mp 170 °C dec. Anal. Calcd for $C_{19}H_{12}ClN_3O_2$, MW 229.66: C, 47.07; H, 5.27; N, 18.30; Cl, 15.44. Found: C, 46.98; H, 5.31; N, 18.01; Cl, 15.29.

Method D. A mixture of 16.5 g (62 mmol) of the carbamate *N*-oxide **7** (Henrie et al., 1988) and 7.9 mL (75 mmol) of *tert*-butylamine in 750 mL of dry THF was refluxed for 16 h. The precipitated product was collected by filtration, affording 13.6g (89.7%) of **2p** (Table II), mp 170–173 °C dec. Anal. Calcd for $C_{10}H_{14}ClN_3O_2$, MW 243.69: C, 49.29; H, 5.79; N, 17.24; Cl, 14.55. Found: C, 49.50; H, 5.91; N, 16.96; Cl, 14.59.

Table II. Data for Compounds 2 (X = Cl)

no.	R	synthesis method[c]	π	σ^*	L	TLC log P	pSI_{50} obsd	pSI_{50} calcd[a]	Δ
2a	cycloheptyl	D	3.33	−0.13	7.19	2.45	3.80	3.84	−0.04
2b	CH_2CH_2OH	D	−0.77	0.21	4.79		4.00	0.06	−0.06
2c	$CH_2CH_2OCH_3$	D	−0.23	0.24	6.03	0.55	4.40	4.32	0.08
2d	$CH_2CH(CH_3)CH_2CH_3$	D	2.63	−0.13[b]	6.17	1.90	4.70	4.84	−0.14
2e	$C(CH_3)(CH_2CH_3)_2$	D	3.03	−0.33[b]	5.05	2.40	4.80	4.93	−0.13
2f	$C(CH_3)_2CH_2OH$	D	−0.01	0.00[b]	4.11		4.80	4.75	0.05
2g	butyl	C	2.13	−0.13	6.17	1.57	5.00	5.02	−0.02
2h	cyclohexyl	C	2.79	−0.15	6.17	2.00	5.00	4.76	0.24
2i	2-chloroethyl	D	0.82	0.39	5.57	0.89	5.00	5.12	−0.12
2j	propyl	C	1.55	−0.12	5.05	1.17	5.10	5.38	−0.28
2k	ethyl	C	1.02	−0.10	4.11	0.54	5.10	5.29	−0.19
2l	*sec*-butyl	D	2.04	−0.21	5.05	1.43	5.20	5.34	−0.14
2m	cyclobutyl	D	1.65	−0.15	4.69	1.28	5.20	5.41	−0.21
2n	neopentyl	D	2.51	−0.17	4.92	1.80	5.20	5.22	−0.02
2o	*tert*-pentyl	D	2.51	−0.33	4.92	2.01	5.20	5.22	−0.02
2p	*tert*-butyl	D	1.98	−0.30	4.11	1.57	5.30	5.35	−0.05
2q	CH_2CF_3	D	0.59	0.92	4.70	1.32	5.30	5.15	0.15
2r	cyclopropylmethyl	D	1.62	0.01	4.90	1.58	5.50	5.39	0.11
2s	isopropyl	C	1.53	−0.19	4.11	0.96	5.60	5.38	0.22
2t	isobutyl	C	2.11	−0.13	5.05	1.46	5.60	5.33	0.27
2u	cyclopentyl	C, D	2.14	−0.20	4.97	1.63	5.60	5.33	0.27
2v	3-chloropropyl	D	1.42	0.14	5.94	1.13	4.70	5.15	−0.45
2w	isopentyl	D	2.63	−0.16	6.17	2.01	5.40	4.84	0.56
2x	$CH(CH_2OH)CH_2CH_3$	D	0.12	0.10[b]	5.05	0.57	4.00	4.84	−0.84
2y	allyl	D	1.10	0.23	5.11	0.90	4.80	5.30	−0.50
2z	1-ethylpropyl	D	2.63	−0.23	4.73	1.80	3.90	5.17	−1.27
2aa	phenyl	C	1.96	0.60	6.28	1.73	7.50	5.01	2.49

[a] Calculated using eq 8. [b] Estimated value. [c] Cf. Scheme I.

Table III. Data for Compounds 2 (R = Isopropyl)

no.	X	synthesis method[c]	$\sum\sigma$	$\sum$MR	TLC log P	pSI_{50} obsd	pSI_{50} calcd[a]	Δ
2bb	SCH_3	E	0.21	16.91	1.05	4.00	4.13	−0.13
2cc	OCH_3	F	−0.37	10.96	0.74	4.10	3.85	0.25
2dd	CH_3	F	−0.13	8.74	0.91	4.40	4.52	−0.12
2ee	2-pyridinyl	F	1.50	26.12	1.19	5.20	5.24	−0.04
2s	Cl	C	0.67	9.12	0.96	5.60	5.83	−0.23
2ff	Br	C	0.70	11.97	1.03	5.60	5.55	0.05
2gg	2,6-Cl_2	F	1.34	14.12	1.24	6.60	6.38	0.22
2hh	C_2F_5	F	0.81[b]	12.32		4.10	5.69	−1.59
2ii	CF_3	F	0.81	8.11		4.90	6.19	−1.29

[a] Calculated using eq 9. [b] Estimated value. [c] Cf. Scheme I.

Method E. A solution of 3.3 g (14 mmol) of **2s** and 2.5 g (36 mmol) of $NaSCH_3$ in 20 mL of dry DMF was heated to 50–55 °C for 24 h. After evaporation of the DMF in vacuo, the residue was chromatographed (15% MeOH/CH_2Cl_2), providing 2.3 g (65%) of **2bb** (Table II), mp 222–224 °C dec. Anal. Calcd for $C_{10}H_{15}N_3O_2S \cdot {}^1/_2H_2O$, MW 250.31; C, 47.98; H, 6.44; N, 16.79. Found: C, 48.29; H, 5.99; N, 16.90.

Method F. A solution of 1.5 g (12 mmol) of 4-amino-2-methylpyridine *N*-oxide (Henrie et al., 1988), 1.6 mL (16 mmol) of isopropyl isocyanate, and 0.25 g (2.2 mmol) of DABCO in 20 mL of anhydrous DMF was stirred at ambient temperature for 24 h. The precipitated product was collected and washed with acetone to afford 1.9 g (76%) of white **2dd** (Table II), mp 229–231 °C dec. Anal. Calcd for $C_{10}H_{15}N_3O_2$, MW 209.28: C, 57.39; H, 7.24; N, 20.08. Found: C, 57.24; H, 7.29; N, 19.95.

RESULTS AND DISCUSSION

TLC log *P* Correlations. As observed in our previous study with the related *N'*-arylureas (Henrie et al., 1988), TLC log P values for **1** and **2** are dependent on the lipophilic and electronic properties of the N'-substituent (Kakkis et al., 1984; Fujita, 1983a,b). Equations 4–6 summarize the dependence of the TLC log P of **1** and **2** (R = alkyl) on π^R and σ^{*R} (data in Tables I–III).

Desoxyureas **1a–1x** (X = Cl):

$$\text{TLC log } P = 0.807\ (\pm 0.051)\pi^R + 0.588\ (\pm 0.128)\sigma^{*R} + 0.713 \quad (4)$$

$$n = 24,\ r^2 = 0.94,\ s = 0.13,\ F = 154.2$$

N-Oxide ureas **2a**, **2g–2w**, **2y**, **2z** (X = Cl):

$$\text{TLC log } P = 0.820\ (\pm 0.069)\pi^R + 0.795\ (\pm 0.168)\sigma^{*R} - 0.035 \quad (5)$$

$$n = 20,\ r^2 = 0.91,\ s = 0.15,\ F = 84.2$$

Combined sets (X = Cl; N = indicator variable, 0 for desoxyureas **1** and 1 for *N*-oxide ureas **2**):

$$\text{TLC log } P = 0.824\ (\pm 0.043)\pi^R + 0.698\ (\pm 0.105)\sigma^{*R} - 0.746\ (\pm 0.043)N + 0.698 \quad (6)$$

$$n = 44,\ r^2 = 0.95,\ s = 0.14,\ F = 231.4$$

Note that N-oxidation of the pyridine reduces the log P by 0.75 when X = Cl, which compares favorably with a difference of 1.0 log P unit for R = aryl (Henrie et al., 1988).

Table IV. Development of Equation 7: $pSI_{50} = aL^R + bL^{R2} + c\pi^{R2} + d\sigma^{*R} + e\pi^R + f$

a	b	c	d	e	f	n	s	r^2	F
0.253					4.20	23	0.58	0.11	2.68
(0.154)									
3.829	−0.373				−4.14	23	0.49	0.40	6.74
(1.156)	(0.120)								
4.073	−0.378	−0.156			−4.64	23	0.36	0.69	14.37
(0.851)	(0.088)	(0.037)							
4.109	−0.370	−0.252	−1.153		−4.72	23	0.25	0.86	27.43
(0.593)	(0.061)	(0.033)	(0.251)						
3.399	−0.301	−0.588	−0.781	1.284	−4.01	23	0.22	0.90	30.52
(0.582)	(0.060)	(0.131)	(0.260)	(0.489)					

Table V. Development of Equation 8: $pSI_{50} = aL^{R2} + b\pi^R + c\pi^{R2} + dL^R + e$

a	b	c	d	e	n	s	r^2	F
−0.031				5.87	21	0.40	0.34	9.73
(0.010)								
−0.042	0.213			5.79	21	0.35	0.54	10.39
(0.009)	(0.077)							
−0.020	0.795	−0.248		5.22	21	0.20	0.85	32.88
(0.007)	(0.106)	(0.041)						
−0.133	0.759	−0.232	1.216	2.00	21	0.18	0.89	31.04
(0.053)	(0.098)	(0.038)	(0.567)					

Table VI. Development of Equation 9: $pSI_{50} = a\sum\sigma_{ortho} + b\sum MR + c$

a	b	c	n	s	r^2	F
1.067		4.47	7	0.64	0.62	8.28
(0.371)						
1.702	−0.118	5.89	7	0.22	0.96	52.33
(0.167)	(0.019)					

Table VII. Correlation Matrix for Equation 7

	pSI_{50}	π	π^2	L	L^2	σ^*
pSI_{50}	1.000					
π	−0.222	1.000				
π^2	−0.335	0.983	1.000			
L	0.336	0.429	0.432	1.000		
L^2	0.274	0.417	0.428	0.994	1.000	
σ^*	0.007	−0.641	−0.580	−0.017	−0.013	1.000

Table VIII. Correlation Matrix for Equation 8

	pSI_{50}	π	π^2	L	L^2
pSI_{50}	1.000				
π	−0.180	1.000			
π^2	−0.191	0.910	1.000		
L	−0.555	0.388	0.552	1.000	
L^2	0.582	0.392	0.563	0.996	1.000

Table IX. Correlation Matrix for Equation 9

	pSI_{50}	$\sum\sigma_{ortho}$	$\sum MR$
pSI_{50}	1.000		
$\sum\sigma_{ortho}$	0.790	1.000	
$\sum MR$	0.042	0.628	1.000

Biological Activity Correlations.

Desoxyureas **1b–1x** (X = Cl):

$$pSI_{50} = 1.28\ (\pm 0.49)\pi^R - 0.588\ (\pm 0.131)\pi^{R2} + 3.40\ (\pm 0.58)L^R - 0.301\ (\pm 0.059)L^{R2} - 0.781\ (\pm 0.260)\sigma^{*R} - 4.01 \quad (7)$$

$$n = 23,\ r^2 = 0.90,\ s = 0.22,\ F = 30.5$$

$$\pi^R(\text{opt}) = 1.09,\ L^R(\text{opt}) = 5.65,\ pSI_{50}(\text{opt}) \approx 6.5$$

N-Oxide ureas **2a–2u** (X = Cl):

$$pSI_{50} = 0.759\ (\pm 0.098)\pi^R - 0.232\ (\pm 0.038)\pi^{R2} + 1.22(\pm 0.57)L^R - 0.133\ (\pm 0.053)L^{R2} + 2.00 \quad (8)$$

$$n = 21,\ r^2 = 0.89,\ s = 0.18,\ F = 31.0$$

$$\pi^R(\text{opt}) = 1.64,\ L^R(\text{opt}) = 4.59,\ pSI_{50}(\text{opt}) = 5.4$$

N-Oxide ureas **2s**, **2bb–2gg** (R = isopropyl):

$$pSI_{50} = 1.70\ (\pm 0.17)\Sigma\sigma^x_{ortho} - 0.118\ (\pm 0.019)\Sigma MR^x + 5.77 \quad (9)$$

$$n = 7,\ r^2 = 0.96,\ s = 0.22,\ F = 52.3$$

The development of eq 7–9 is presented in Tables IV–VI, and correlation matrices for the equations are shown in Tables VII–IX, respectively. Parabolic relationships in both π^R and L^R were observed for the chlorophyll retention activity of 1 and 2 (Figures 1–4). Verloop *L* proved to be the steric parameter of choice; neither B_4, B_5, nor MR provided useful equations.

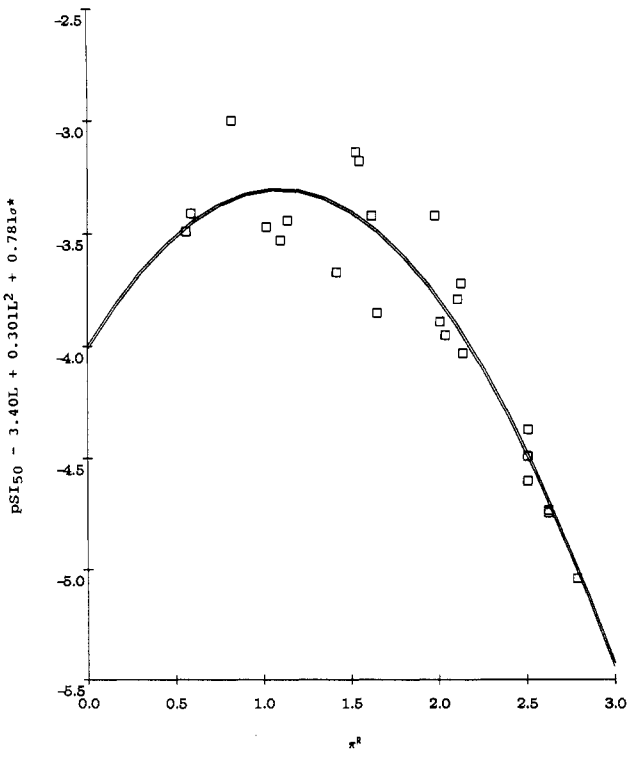


Figure 1. Parabolic behavior in π^R of eq 7.

The predictability of eq 7 and 8 was examined with compounds not included in the original training sets.

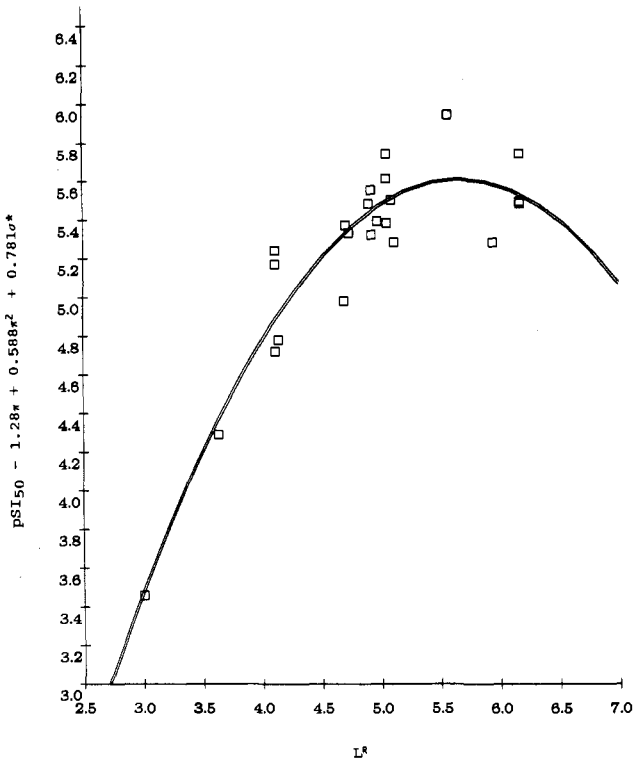


Figure 2. Parabolic behavior in L^R of eq 7.

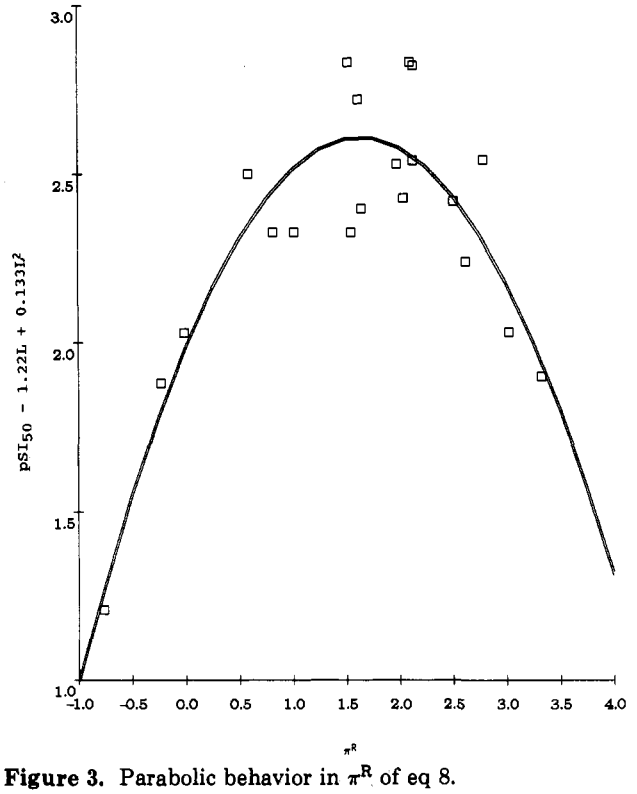


Figure 3. Parabolic behavior in π^R of eq 8.

Specifically, for desoxyureas 1, R groups with low (negative) π^R values were sought to validate eq 7 in this region of physicochemical parameter space. Therefore, compounds **1y**–**1hh** were synthesized and evaluated. The agreement between calculated and observed values was excellent with the exception of sterically unhindered alcohols **1y**, **1aa**, and **1bb**, which are potentially subject to metabolic deactivation by conjugation. Neopentyl alcohol **1dd** and its homologue **1ff** are well predicted by eq 7, apparently being protected from rapid metabolism by the geminal dimethyl substitution. A surprising exception to

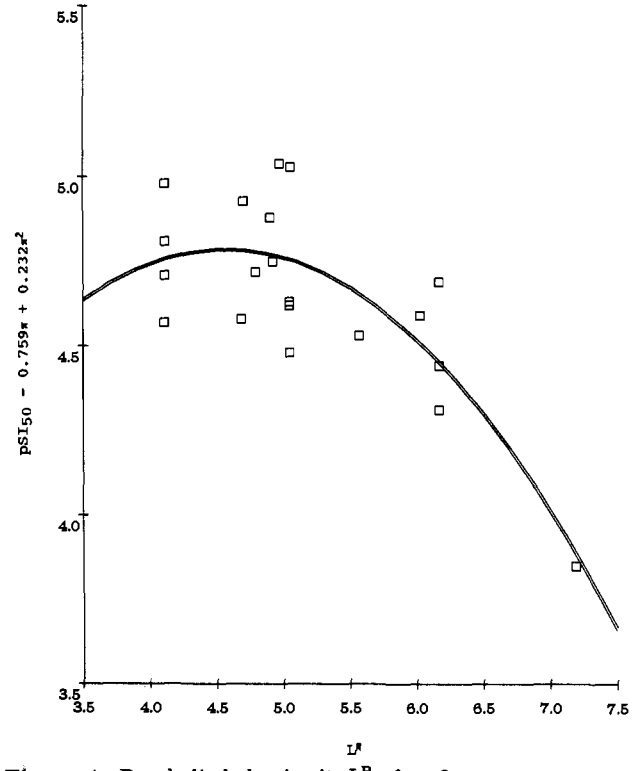


Figure 4. Parabolic behavior in L^R of eq 8.

this generalization appears in *N*-oxide urea **2b** (R = CH_2CH_2OH), which is well predicted by eq 8. However, compound **2x** (R = $CH(CH_2OH)CH_2CH_3$) is poorly predicted as expected. Other negative steric interactions may be involved for more complex R groups.

The basis set for the development of eq 9, examining the effect of pyridine 2-substitution, is very limited but indicates the importance of electronic and receptor binding (MR) properties of the X substituents. Okamoto et al. (1981, 1983) had observed that for *N*-pyridinyl-*N'*-phenylureas (1, R = Ph) electronic and lipophilic properties of X determined cytokinin activity in tobacco callus culture (eq 10).

$$\log (1/C) = 3.81\ (\pm 1.01)\sigma^x_{meta} + 0.52\ (\pm 0.32)\pi^x + 6.50\ (\pm 0.28) \quad (10)$$

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

In contrast, our previous work with arylureas (1 and **2**, R = Ph) using wheat leaf chlorophyll retention data had shown electronic and steric (or receptor binding) properties to dominate (eq 11 and 12), more in line with eq 9 for the analogous alkylureas.

Desoxyureas 1 (R = Ph):

$$pSI_{50} = 3.52\ (\pm 0.50)F^x_{ortho} + 0.275\ (\pm 0.098)B_4^x + 3.96 \quad (11)$$

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

N-Oxide ureas **2** (R = Ph):

$$pSI_{50} = 0.415\ (\pm 0.086)\pi^x + 1.40\ (\pm 0.27)F^x_{ortho} - 0.0519\ (\pm 0.0091)MR^x + 6.29 \quad (12)$$

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

In the development of eq 9 a strong preference for σ_{ortho} over other σ values or F and R was observed. Note also that the coefficient of MR in eq 9 is twice that of eq 12, indicating the differing relative importance of this variable

in the two series. As had been observed for the arylurea *N*-oxides, perfluoroalkyl 2-substitution of the pyridine provides compounds that are much less active than predicted (**2hh** and **2ii**, Table III).

Inserting the physicochemical data for R = Ph (**1ii** and **2aa**) into eq 7 and 8 and comparing calculated versus observed pSI_{50} values show that the equations for the alkylureas are not predictive for arylureas, and vice versa. In particular, *N*-oxide **2aa** is very poorly predicted by eq 8, being 2.5 orders of magnitude more active than predicted. Also, in general, the arylureas (**1** and **2**, R = aryl) are much more active than their aliphatic urea *N*-oxide analogues (Henrie et al., 1988) as measured by chlorophyll retention pSI_{50} values, even though the steric length of the phenyl group is supraoptimal (L = 6.28). We interpret these results as indicating that aromatic R groups provide a receptor binding interaction with the π-system that is absent in aliphatic groups.

It is instructive to compare optimum π^R and L^R values for **1** and **2** (see Figures 1–4). π^R(opt) for desoxyureas **1** is 1.09 versus 1.64 for *N*-oxide ureas **2**: The difference (Δ = 0.55) is primarily accounted for by the lower log P (Δ = 0.75) of the *N*-oxide ureas **2**. L^R(opt) for **1** is 5.65 Å versus 4.59 Å for *N*-oxide ureas **2**; the difference (Δ = 1.06 Å) is in good agreement with the average N–O bond length of 1.30 Å derived from 14 X-ray structures of pyridine *N*-oxides found in the Cambridge X-ray Crystal Database, for which the N–O bond lengths ranged from 1.26 to 1.34 Å (Cambridge X-ray Crystal Database molecule codes: BABKIY, CXMPYO, CYPYRO, DCXPYO, DMNPYO, DMNPYO01, DMPOXP, HXTHQO, MNPYDO, NPOAPL, NTPYRO, NTPYRO03, NTPYRO11, NTPYRO12). Clearly, the pyridine *N*-oxide is directly involved in the binding of **2** within the cytokinin receptor. Presumably, the negatively charged *N*-oxide oxygen of **2** replaces the pyridine ring nitrogen of **1** as a polar binding point, thereby shortening the binding region available to the R group at the opposite end of the molecule by the length of the N–O bond.

The preference for $W^R_{max}(B_5)$ as the steric parameter in the QSAR of fused heterocycles **3–5** versus L for **1** and **2** is not easily rationalized. It is interesting to note, however, that the QSAR of other cytokinin-active phenyl- and diphenylureas involve steric terms in length, not width parameters (Iwamura et al., 1980). The absence of a fused heterocycle in **1** and **2** as well as in phenyl- and diphenylureas may allow binding to be somewhat displaced from **3–5**. This seems to be corroborated by the similarity of steric optima for the N′ group, whether described by L or $W_{max}(B_5)$, for **1–5** (4.5–5.7 Å): The binding region to be filled is essentially the same size, but the directionality of the steric component is different (L versus B_5). A final work of caution is appropriate, however, since the majority of the literature QSAR involves cell division biodata [tobacco callus culture or tobacco pith block; cf. Koshimizu and Iwamura (1985)] as opposed to chlorophyll retention biodata used in the present work. The structural requirements for cytokinin receptor binding and the resulting cytokinin activity in various systems may well be quite different.

CONCLUSION

N-4-Pyridinyl-*N*′-alkylureas **1** and their pyridine *N*-oxides **2** are good cytokinin mimics as evidenced by chlorophyll retention in excised wheat leaves. Biological activity is determined by both pyridine substitution and the nature of the *N*′-alkyl group. For *N*-oxide ureas **2**, optimum pyridine substitution appears to be 2-halo or 2,6-dihalo. For both **1** and **2**, biological activity is strongly dependent on R and is parabolic in both π^R and L^R. The optimum pSI_{50} values derived from eq 7 and 8 indicate that the maximum possible biological activities have been achieved in both series: Optimum substitutions are embodied in compounds **1w**, **1x**, and **2r–2u**. For desoxyureas **1**, π^R(opt) = 1.09 and L^R(opt) = 5.65: Optimum R is *n*-propyl or 2-chloroethyl. For *N*-oxide ureas **2**, π^R(opt) = 1.64 and L^R(opt) = 4.59: Optimum R is cyclopropylmethyl, isopropyl, isobutyl, or cyclopentyl. These optimum values reflect differences in binding with the cytokinin receptor in which the *N*-oxide functionality plays a pivotal role. Also, cytokinin receptor binding is apparently different for R = alkyl versus R = aryl, with R = aryl generally providing more active compounds.

ACKNOWLEDGMENT

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Registry No. **1a**, 119108-52-0; **1aa**, 119108-64-4; **1b**, 119108-53-1; **1bb**, 119108-65-5; **1c**, 80194-81-6; **1cc**, 119108-66-6; **1d**, 116652-16-5; **1dd**, 119108-67-7; **1e**, 116652-11-0; **1ee**, 119108-68-8; **1f**, 116652-12-1; **1ff**, 119108-69-9; **1g**, 116652-13-2; **1gg**, 119108-70-2; **1h**, 116681-80-2; **1hh**, 116681-82-4; **1i**, 119108-54-2; **1ii**, 68157-60-8; **1j**, 119108-55-3; **1k**, 119108-56-4; **1l**, 116652-14-3; **1m**, 116681-71-1; **1n**, 116652-15-4; **1o**, 116681-86-8; **1p**, 119108-57-5; **1q**, 119108-58-6; **1r**, 119108-59-7; **1s**, 116714-72-8; **1t**, 116681-70-0; **1u**, 116681-83-5; **1v**, 119108-60-0; **1w**, 116681-72-2; **1x**, 119108-61-1; **1y**, 119108-62-2; **1z**, 119108-63-3; **2a**, 116652-48-3; **2aa**, 97985-35-8; **2b**, 119108-71-3; **2bb**, 116681-78-8; **2c**, 119108-72-4; **2cc**, 116652-53-0; **2d**, 119108-73-5; **2dd**, 119108-80-4; **2e**, 119108-74-6; **2ee**, 116681-79-9; **2f**, 119108-75-7; **2ff**, 116652-51-8; **2g**, 116652-31-4; **2gg**, 116681-77-7; **2h**, 116652-47-2; **2hh**, 119108-81-5; **2i**, 116652-27-8; **2ii**, 119108-82-6; **2j**, 116652-29-0; **2k**, 116652-26-7; **2l**, 119108-76-8; **2m**, 116652-45-0; **2n**, 116667-51-7; **2o**, 116652-41-6; **2p**, 116652-36-9; **2q**, 116652-28-9; **2r**, 116652-44-9; **2s**, 116681-76-6; **2t**, 116652-35-8; **2u**, 116652-46-1; **2v**, 116652-30-3; **2w**, 116652-40-5; **2x**, 119108-77-9; **2y**, 119108-78-0; **2z**, 119108-79-1; **6**, 76947-86-9; **7**, 113567-69-4; DABCO, 280-57-9; mcpba, 64741-01-1; $NH_2CH_2CF_3$, 753-90-2; $NH_2CH(CH_2OH)CH(CH_3)_2$, 473-75-6; $NH_2C(CH_3)(CH_2CH_3)_2$, 3495-46-3; $NH_2CH(CH_2OH)CH_2CH_3$, 96-20-8; $NH_2CH_2CH_2CH_2OH$, 156-87-6; $NH_2CH_2CH_2CH_2OCH_3$, 5332-73-0; $NH_2C(CH_3)_2CH_2OH$, 124-68-5; $NH_2CH_2CH(CH_3)CH_2CH_3$, 96-15-1; $NH_2C(CH_3)_2CH_2CH_2OH$, 42514-50-1; $NH_2CH_2CH_2N(CH_3)_2$, 108-00-9; $NH_2CH_2CF_2CF_3$, 422-03-7; $NH_2CH_2CH_2OH$, 141-43-5; $NH_2CH_2CH_2OMe$, 109-85-3; $NH_2C(CH_3)_2CH_2OH$, 124-68-5; 1-methylbutylamine, 625-30-9; methylamine, 74-89-5; benzylamine, 100-46-9; cyclohexylamine, 108-91-8; 1-ethylpropylamine, 616-24-0; isopentylamine, 107-85-7; *tert*-pentylamine, 594-39-8; neopentylamine, 5813-64-9; 1,2-dimethylpropylamine, 598-74-3; ethylamine, 75-04-7; cyclopropylamine, 765-30-0; cyclopentylamine, 1003-03-8; cyclobutylamine, 2516-34-9; *sec*-butylamine, 13952-84-6; allylamine, 107-11-9; 3-chloropropylamine, 14753-26-5; isobutylamine, 78-81-9; isopropylamine, 75-31-0; butylamine, 109-73-9; cyclopropylmethylamine, 2516-47-4; 2-chloroethylamine, 689-98-5; propylamine, 107-10-8; phenylamine, 62-53-3; 4-amino-2-chloropyridine, 14432-12-3; isopropyl isocyanate, 1795-48-8; butyl isocyanate, 111-36-4; *tert*-butyl isocyanate, 1609-86-5; propyl isocyanate, 110-78-1; *tert*-butylamine, 75-64-9; 4-amino-2-methylpyridine *N*-oxide, 14045-17-1; cycloheptylamine, 5452-35-7.

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Herbicidal Diphenyl Ethers: Stereochemical Studies Using Enantiomers of a Novel Diphenyl Ether Phthalide

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Nitrodiphenyl ethers are a highly active class of herbicides that cause light-dependent membrane lipid peroxidation, but the molecular basis of their herbicidal effects is not known. As part of a program to elucidate their primary mode of action, small quantities of the enantiomers of a novel diphenyl ether phthalide have been isolated by high-performance liquid chromatography, using a chiral stationary-phase column, and their relative biological activities have been evaluated. The characteristics of the herbicidal effects of the phthalide diphenyl ether appear to be identical with those of nitrodiphenyl ether herbicides. The *S*-(−) isomer was found to be substantially more active than the *R*-(+) isomer in a test designed to monitor plant membrane breakdown by following ethane production. This selectivity was confirmed in a glasshouse bioassay when the activity of the two isomers on six plant species was determined. The information detailed in this report is the first evidence for the likely involvement of an enzymatic binding process in the mode of action of peroxidizing diphenyl ether herbicides.

Nitrodiphenyl ethers (NDPE's) are highly effective and fast-acting herbicides that are primarily used in the selective control of broadleaf weeds in a variety of crops such as soyabean, barley, wheat, and rice. The effectiveness of this class of herbicides depends on the presence of light (Fadayomi and Warren, 1976; Matsunaka, 1969) and oxygen (Kunert and Böger, 1984; Orr and Hess, 1982; Kenyon and Duke, 1985). Several studies (Vanstone and Stobbe, 1979; Bowyer et al., 1987) have shown that treatment of plants with NDPE's in the light leads to considerable ultrastructural damage after a relatively short time (<12 h after application). The usual symptoms are the initial disruption of the tonoplast membrane and the chloroplast envelope, followed by movement of the ruptured chloroplasts and cytoplasm away from the cell wall, and finally destruction of the thylakoid system. The result of this damage is the evolution of hydrocarbons, predominantly ethane, which are products of decomposition of ω^3-unsaturated fatty acid hydroperoxides. It has been reported that the oxidative damage to plants by NDPE's can be reduced by the presence of antioxidants such as vitamins C and E (Kunert and Boger, 1984; Kenyon and Duke, 1985).

Studies to date have not allowed a distinction to be made between primary and secondary modes of action of NDPE's. A great deal of controversy exists in the literature about the primary mode of action of this class of compounds. From a study (Lambert et al., 1983, 1984) of the mode of action of NDPE's on the green alga *Scenedesmus obliquus* it was stated that photosynthetic electron transport is involved in a manner similar to that in the case of paraquat. This acts as a photosystem I electron acceptor, leading to the production of toxic oxygen species. However, these results are at variance with studies carried out on another green alga *Chlamydomonas eugametos* (Ensminger and Hess, 1985a) and on plants (Fadayomi and Warren, 1976; Matsunaka, 1969, Orr and Hess, 1982; Bowyer et al., 1987; Camilleri et al., 1988) and flower petals

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