Journal of Agricultural and Food Chemistry

Volume 24, Number 2 March/April 1976

Quantitative Structure–Activity Relationships among Selected Pyrimidinones and Hill Reaction Inhibition

Loren K. Gibbons, Edwin F. Koldenhoven, Arthur A. Nethery, Ronald E. Montgomery, and William P. Purcell*

Quantitative structure–activity relationship (QSAR) models were applied to the Hill reaction inhibitory potencies of 17 substituted pyrimidinones. The Free–Wilson model ranked the activity contributions of the rings and substituents and gave reasonable fit to the structure–activity data. Stepwise multiple linear regression analyses on 19 physicochemical parameters using a modified Hansch analysis suggested the equation $-\log [I_{50}(\text{compound})/I_{50}(\text{diuron})] = -0.46(\pm 0.11)\pi_{\text{ring}} + 8.92(\pm 1.85)\sigma_{R3} + 0.045(\pm 0.008)\Sigma MR - 0.15$, with a correlation coefficient of 0.91 at the 99.9% confidence level. This model adequately predicted the activity of an isothiazolo[5,4-*d*]pyrimidinone.

Attempts to correlate the biological activity of a molecule with its physicochemical properties have their origin in medicinal chemistry and date back to the very beginning of the search for drugs (Crum-Brown and Fraser, 1868). Only more recently, however, have these attempted correlations become quantitative (Bruice et al., 1956). From these afforts an area of activity is emerging which has been called quantitative structure–activity relationship (QSAR) studies. Reviews have been published along these lines (Hansch, 1971; Purcell et al., 1973; Redl et al., 1974). Just as the medicinal chemist and pharmacologist wish to maximize potency (desirable activity) and minimize toxicity, the chemists and biologists working in agricultural research wish to maximize pesticidal activity and minimize any deleterious effect on man and the environment.

Inhibitors of photosynthesis are potential herbicides and a number of widely used commercial herbicides are inhibitors of the Hill reaction (Draber et al., 1969). For a discussion regarding the relevancy of the Hill reaction inhibitory potency to herbicidal activity and qualitative structure–activity relationships of substituted amides such as diuron, uracils, *s*-triazines, benzimidazoles, imidazoles, and halogenated benzonitriles, the reader is referred to a review in the area (Moreland, 1969).

A rather detailed description of the mechanism of Hill reaction inhibition and QSAR of NH-acidic π-excessive heteroaromatics has been reported (Buchel and Draber, 1969). It was found that the pI_{50} values correlated nicely with the pK_A values and partition coefficients of the undissociated part of the molecules. In a Hansch analysis of the Hill reaction inhibitory potencies of some 1,2,4-triazinones a good correlation was found in a parabolic dependence of pI_{50} on the partition coefficient (Draber et al., 1969).

This study is an investigation of the QSAR of selected pyrimidinone herbicides which have been evaluated as Hill reaction inhibitors.

EXPERIMENTAL SECTION

Reagents. The compounds used in this report are isoxazolo[5,4-*d*]pyrimidinones (Gibbons, 1972; April 9, 1974; June 11, 1974), isoxazolo[3,4-*d*]pyrimidinones (Gibbons, April 9, 1974; June 11, 1974), isothiazolo[3,4-*d*]pyrimidinones (Gibbons and Ramsey, 1974), and 6-*tert*-butyl-3-isopropylisothiazolo[5,4-*d*]pyrimidin-4(5*H*)-one (FMC 23475). Since FMC 23475 is new, its synthesis and properties are described below.

3-Amino-2-cyano-4-methyl-2-pentenenitrile. A solution of 157 g (1 mol) of 3-chloro-2-cyano-4-methyl-2-

Research Department, Agricultural Chemical Division, FMC Corporation, Middleport, New York 14105 (L.K.G., E.F.K., A.A.N., R.E.M.), and Drug Design Division, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163 (W.P.P.).

pentenenitrile in 300 ml of ethanol was added slowly to a solution of 700 ml of concentrated ammonium hydroxide and 1000 ml of ethanol. The addition took 2 hr and the reaction temperature increased to 40°C. The solution was stirred for 3 hr and then poured over ice. The precipitate was collected by filtration, washed with water, and recrystallized from ethanol to give 122 g (88%) of white solid, mp 186–188°C.

Anal. Calcd for $C_7H_9N_3$: C, 62.20; H, 6.71; N, 31.09. Found: C, 61.73; H, 6.72; N, 31.62.

3-Amino-2-cyano-4-methyl-2-pentenethioamide. Excess hydrogen sulfide was bubbled through a solution of 117 g (0.87 mol) of 3-amino-2-cyano-4-methyl-2-pentenenitrile and 87.5 g (0.89 mol) of triethylamine in 117 ml of pyridine at 40–50°C for 1 hr. The reaction was stirred at room temperature for another hour and poured into a beaker of ice. The precipitate was collected, washed with water, and recrystallized from ethanol to give 105 g (72%) of product, mp 114–116°.

Anal. Calcd for $C_7H_{11}N_3S$: C, 49.68; H, 6.55; N, 24.83; S, 18.94. Found: C, 49.58; H, 6.40; N, 25.09; S, 18.93.

5-Amino-4-cyano-3-isopropylisothiazole. Hydrogen peroxide (67.8 g of 30% solution; 0.6 mol) was added dropwise to a solution of 101 g (0.6 mol) of 3-amino-2-cyano-4-methyl-2-pentenethioamide in 700 ml of ethanol at such a rate that the temperature did not exceed 50°C. The solution was stirred at 25° for 15 hr and then concentrated to about half of the original volume. The remaining mixture was heated to reflux, filtered, and allowed to cool to give, after filtration, 96 g (96%) of product, mp 133–136°C. Anal. Calcd for $C_7H_9N_3S$: C, 50.28; H, 5.42; N, 25.13; S, 19.17. Found: C, 50.52; H, 5.52; N, 25.42; S, 18.54.

5-Amino-3-isopropylisothiazole-4-carboxamide. A solution of 25 g (0.15 mol) of 5-amino-4-cyanoisopropylisothiazole in 125 ml of concentrated sulfuric acid was heated for 2 hr at 100°C and poured into a beaker of ice. Ammonium hydroxide was added to adjust the pH to 7 and the precipitate was collected on a filter, washed with water, and recrystallized from ethanol to give 27 g (97%) of product, mp 198–200°C.

Anal. Calcd for $C_7H_{11}N_3OS$: C, 45.39; H, 5.99; N, 22.68. Found: C, 45.40; H, 5.89; N, 22.38.

6-*tert*-Butyl-3-isopropyl-4,5,6,7-tetrahydroisothiazolo[5,4-*d*]pyrimidin-4-one. A solution of 14.3 g (0.077 mol) of 5-amino-3-isopropylisothiazole-4-carboxamide, 13.4 g (0.155 mol) of 2,2-dimethylpropanal, and 1 g of *o*-toluenesulfonic acid in 150 ml of ethanol was heated at reflux under a nitrogen atmosphere for 17 hr. Solvent and unreacted aldehyde were removed at reduced pressure and the residue was recrystallized from ethanol to give 19.4 g (100%) of product, mp 236–243°C.

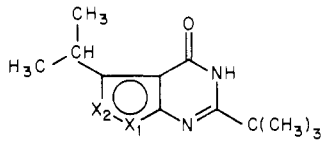
Anal. Calcd for $C_{12}H_{19}N_3OS$: C, 56.89; H, 7.56; N, 16.59. Found: C, 56.60; H, 7.65; N, 15.46.

6-*tert*-Butyl-3-isopropylisothiazolo[5,4-*d*]-pyrimidin-4(5*H*)-one (FMC 23475). A solution of 15.3 g (0.06 mol) of 6-*tert*-butyl-3-isopropyl-4,5,6,7-tetrahydroisothiazolo[5,4-*d*]pyrimidin-4-one and 150 ml of 5% sodium hypochlorite solution in 200 ml of ethanol was stirred for 60 hr at room temperature. Solvents were removed at reduced pressure, water was added to the residue, and the pH was adjusted to 2 by the addition of 10% hydrochloric acid. The precipitate was collected, washed with water, and recrystallized from ethanol to give 5.4 g (35%) of product, mp 172–174°C.

Anal. Calcd for $C_{12}H_{17}N_3OS$: C, 57.34; H, 6.82; N, 16.72. Found: C, 57.05; H, 6.73; N, 16.96.

Partition Coefficients. Partition coefficients (*P*) were determined for compounds FMC 19873, FMC 21844, FMC 23486, and FMC 23475 in cyclohexane–water using Hansch's method (Purcell et al., 1973). Solute concentrations in cyclohexane were measured on a Perkin-Elmer Model 202 UV-Vis spectrophotometer before and after partitioning with water. The partition coefficient of one compound, FMC 23486, was also determined in octanol–water as well as cyclohexane–water using a ^{14}C-labeled sample. The values are given in Table I.

Table I. Log *P* Values for Pyrimidinone Herbicides



FMC	X_1–X_2	Log *P*		
		Cyclohexane–water[a]	Octanol–water	
			b	*c*
21844	O–N	1.22	4.54	3.19
23486	N–O	0.63	3.66	2.34[a]
19873	N–S	1.00	4.21	2.87
23475	S–N	2.45	6.36	4.94

[a] Measured. [b] Calculated from $\log P_c = 0.675 \log P_o - 1.842$ (Leo et al., 1971). [c] Calculated from $\log P_c = 0.7 \log P_o$ derived from the measured P_o and P_c of a ^{14}C-labeled sample of FMC 23486.

Biochemistry. In the screening process used by FMC, the test compounds were studied for their effects on photochemical reduction in chloroplasts by a Hill reaction assay. Chloroplasts from mustard leaves (*Brassica juncea* variety India giant) were prepared by a standard method (Klein and Klein, 1970) and the chloroplast suspension was adjusted to 200 mg of chlorophyll/l. for each test.

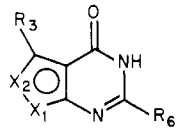
The following reagents were placed in spectrophotometric tubes in this order: 1 ml, chloroplast suspension; 2 ml, 0.01 *M* KH_2PO_4 (pH 6.8); 2 ml, 0.2 *M* KCl; 2 ml, deionized H_2O; 1 ml, inhibitor in aqueous solution; 2 ml, 2.5×10^{-5} *M* 2,6-dichlorophenolindophenol. The liquid in the tubes was mixed thoroughly before reading percent transmittance on a Spectronic 20 spectrophotometer at 605 nm. After the time zero readings were recorded, the tubes were illuminated for 15 min before the second reading was taken. The light intensity from an incandescent source was 20000 lx; the temperature was maintained at 4.0°C.

Several preliminary tests were conducted to determine whether the test compounds were Hill reaction inhibitors and to define the range of activity. For example, each compound was tested at 1.0×10^{-5} *M*, 1.0×10^{-6} *M*, 1.0×10^{-7} *M*, and 1.0×10^{-8} *M*. Diuron [*N*'-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea] was included as a standard treatment in each test and has an I_{50} value of $4.4 \pm 0.2 \times 10^{-7}$ *M*.

Inhibition of the Hill reaction was followed by observing the reduction of dichlorophenolindophenol (DPIP) spectrophotometrically. DPIP acts as a hydrogen acceptor in this in vitro system. As the DPIP was reduced the color of the solution cleared and the percent transmittance increased. The reduction of the DPIP occurred continuously while the tubes were in the light chamber. When the tubes were removed from the light chamber for percent transmittance reading, the process was virtually stopped because the room was nearly dark. The percent transmittance of each treatment was compared to a water blank to determine percent inhibition.

Two concentrations above and two concentrations below the estimated I_{50} value were tested. The percent inhibition

Table II. Free-Wilson Matrix



Compd FMC	X_1-X_2 O-N	N-O	N-S	R_3 C_2H_5	C_3H_7	CH-$(CH_3)_2$	C-$(CH_3)_3$	R_6 CH_3	C_2H_5	CH-$(CH_3)_2$	C-$(CH_3)_3$	RpI_{50} Obsd	Calcd
21868	x			x							x	0.10	0.00
21861	x				x						x	0.59	0.42
21801	x					x				x		−0.30	0.02
21844	x					x					x	0.30	0.30
23491		x		x							x	0.32	0.38
23469		x			x						x	0.68	0.80
23495		x				x			x			0.32	0.33
23494		x				x				x		0.48	0.36
23486		x				x					x	0.77	0.69
23487		x					x	x				−0.27	
23488		x					x		x			0.16	0.04
23493		x					x				x	0.28	0.40
19874			x	x							x	0.39	0.43
25103			x		x						x	0.80	0.85
19850			x			x			x			0.27	0.38
19872			x			x				x		0.57	0.41
19873			x			x					x	0.77	0.73

Table III. Group Contributions from Free-Wilson Analysis[a]

	RpI_{50}
X_1-X_2	
O-N	−0.31
N-O	0.08
N-S	0.13
R_3	
C_2H_5	−0.23
C_3H_7	0.19
$CH(CH_3)_2$	0.07
$C(CH_3)_3$	−0.22
R_6	
C_2H_5	−0.23
$CH(CH_3)_2$	−0.20
$C(CH_3)_3$	0.13

[a] Regression constant, $\mu = 0.41$.

points were plotted on logarithmic graph paper and the I_{50} values determined by dropping an intercept at the 50% inhibition point. A relative pI_{50} value (RpI_{50}) was used for the QSAR analyses and is defined in eq 1.

$$RpI_{50} = -\log [I_{50}(\text{compound})/I_{50}(\text{diuron})] \quad (1)$$

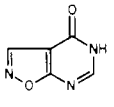
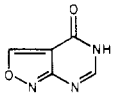
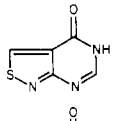
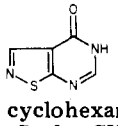
Quantitative Structure–Activity Relationships. The de novo model used has been described elsewhere (Free and Wilson, 1964; Purcell et al., 1973). The linear free-energy related model used was modified from Hansch and Fujita (1964) and has been described previously (Purcell et al., 1973).

RESULTS AND DISCUSSION

Table II gives the Free–Wilson matrix along with the observed and calculated RpI_{50} values. The more effective Hill reaction inhibitory compounds correspond to larger positive activity values. FMC 23487 was deleted from the analysis since it represents a compound with a single group (CH_3 at R_6) observation. This Free–Wilson analysis has a correlation coefficient of 0.91, explained variance of 0.67, and is significant at the 97.5% confidence level (Snedecor and Cochran, 1967).

Table III gives the activity contributions of each substituent group which provides a relative ranking of their contributions to the activity. For example, at position R_3 the propyl group is best with isopropyl intermediate and ethyl and *tert*-butyl worst. The "best" compound among the $3 \times 4 \times 3 = 36$ possibilities is FMC 25103 (0.85 calcd and 0.80 obsd).

Table IV. Ring Constants

	π^a (octanol-water)	MR^b
	1.03	50.0
	0.15	50.0
	0.70	56.3
	2.85	56.3

[a] From cyclohexane measurements, $\log P_c = 0.675P_o - 1.842$, $\log P_o$ for $CH(CH_3)_2 = 1.53$, and $\log P_o$ for $C(CH_3)_3 = 1.98$ (Table I). [b] Approximated values.

Table V. Substituent Constants

Group	π^a	$\sigma_p{}^a$	MR^a	MW	$E_s{}^b$
CH_3	0.56	−0.17	5.65	15.0	0
C_2H_5	1.02	−0.15	10.30	29.1	−0.07
C_3H_7	1.55	−0.13	14.96	43.1	−0.36
$CH(CH_3)_2$	1.53	−0.15	14.98	43.1	−0.47
$C(CH_3)_3$	1.98	−0.20	19.62	57.1	−1.54

[a] Hansch et al., 1973. [b] Craig, 1971.

The compounds and activities in Table II were subjected to a Hansch-type QSAR analysis using the stepwise multiple linear regression analysis of eq 2. For the regression analysis π_{ring} values were taken from Table I (first

$$RpI_{50} = a(\Sigma\pi)^2 + b(\Sigma\pi) + c\pi_{ring}{}^2 + d\pi_{R_3}{}^2 + e\pi_{R_6}{}^2 + f\pi_{ring} + g\pi_{R_3} + h\pi_{R_6} + i(\Sigma\sigma)^2 + j\Sigma\sigma + k\sigma_{R_3} + l\sigma_{R_6} + m\Sigma MR + nMR_{R_3} + pMR_{R_6} + qMW + r\Sigma E_s + sE_{s(R_3)} + tE_{s(R_6)} + C \quad (2)$$

Table VI. Correlation Coefficients of RpI_{50} vs.:

$(\Sigma\pi)^2$	0.32	σ_{R_3}	0.56
$\Sigma\pi$	0.33	σ_{R_6}	−0.43
$\pi_{ring}{}^2$	−0.53	ΣMR	0.68
$\pi_{R_3}{}^2$	−0.18	MR_{ring}	0.11
$\pi_{R_6}{}^2$	0.35	MR_{R_3}	−0.20
π_{ring}	0.08	MR_{R_6}	0.58
π_{R_3}	−0.14	MW	0.68
π_{R_6}	0.57	ΣE_s	−0.27
$(\Sigma\sigma)^2$	−0.09	$E_{s(R_3)}$	−0.39
$\Sigma\sigma$	0.08	$E_{s(R_6)}$	−0.53

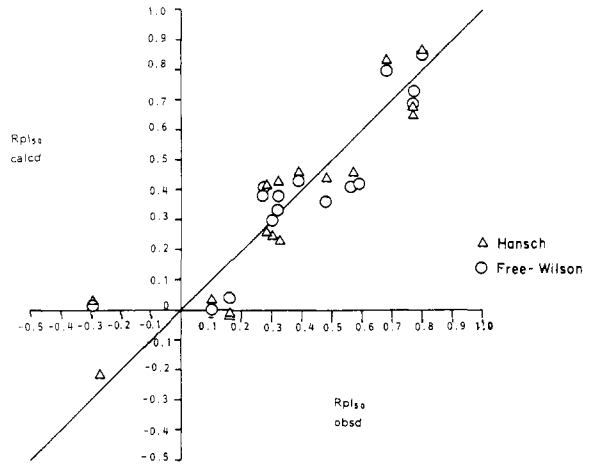


Figure 1. Calculated and observed activities from Free-Wilson and Hansch analyses.

column under octanol–water). The π values and other substitutent constants are given in Tables IV and V. *MR* is the molar refraction. Table VI gives the correlation coefficients for the activities and each parameter in eq 2. The stepwise regression gave eq 3 as the best model for

$$RpI_{50} = -0.46(\pm 0.11)\pi_{ring} + 8.92(\pm 1.85)\sigma_{R_3} + 0.045(\pm 0.008)\Sigma MR - 0.15 \quad (3)$$

this correlation; correlation coefficients = 0.91, 99.9% confidence level.

Figure 1 gives the calculated and observed activities from the Free–Wilson and Hansch-type models. One of the objectives of QSAR analyses is to be able to predict the activities of compounds prior to their synthesis and evaluation. Using eq 3 the RpI_{50} of FMC 23475 was predicted to be −0.31. When FMC 23475 was evaluated the RpI_{50} was observed to be −0.55 which is in line with prediction.

The interpretation of these results and eq 3 might be that partitioning properties (through hydrophobic bonding perhaps) of the aromatic ring are important to Hill reaction inhibition, electronic effects at position R_3 are important, and the overall steric effects are manifested in the molar refractions of the molecules. This interpretation is consistent with the mechanisms suggested by others (Büchel and Draber, 1969; Draber et al., 1969; Moreland, 1969).

ACKNOWLEDGMENT

We wish to acknowledge A. A. Ramsey who prepared the isothiazolo[3,4-*d*]pyrimidinones and who contributed to early discussions of the study.

LITERATURE CITED

Bruice, T. C., Kharasch, N., Winzler, R. J., *Arch. Biochem. Biophys.* **62**, 305 (1956).
Büchel, K. H., Draber, W., *Prog. Photosynth. Res., Proc. Int. Congr. 1968* **3**, 1777–1788 (1969).
Craig, P. N., *J. Med. Chem.* **14**, 680 (1971).
Crum-Brown, A., Fraser, T., *Trans. R. Soc. Edinburgh* **25**, 151 (1868).
Draber, W., Büchel, K. H., Dickore, K., Trebst, A., Pistorius, E., *Prog. Photosynth. Res., Proc. Int. Congr. 1968* **3**, 1789–1795 (1969).
Free, S. M., Wilson, J. W., *J. Med. Chem.* **7**, 395 (1964).
Gibbons, L. K. (to FMC Corp.), U.S. Patent 3679682 (July 25, 1972).
Gibbons, L. K. (to FMC Corp.), U.S. Patent 3803148 (April 9, 1974).
Gibbons, L. K. (to FMC Corp.), U.S. Patent 3816090 (June 11, 1974).
Gibbons, L. K., Ramsey, A. A. (to FMC Corp.), U.S. Patent 3816420 (June 11, 1974).
Hansch, C., Fujita, T., *J. Am. Chem. Soc.* **86**, 1616 (1964).
Hansch, C., *Med. Chem., Ser. Monogr.* **11**, 271–342 (1971).
Hansch, C., Leo, A., Unger, S. H., Kim, K. H., Nikaitani, D., Lien, E. J., *J. Med. Chem.* **16**, 1207 (1973).
Klein, R. M., Klein, D. T., "Research Methods in Plant Science", Natural History Press, Garden City, N.Y., 1970, p 588.
Leo, A., Hansch, C., Elkins, D., *Chem. Rev.* **71**, 525 (1971).
Moreland, D. E., *Prog. Photosynth. Res. Proc. Int. Congr. 1968* **3**, 1693–1711 (1969).
Purcell, W. P., Bass, G. E., Clayton, J. M., "Strategy of Drug Design: A Molecular Guide to Biological Activity", Wiley, New York, N.Y., 1973.
Redl, G., Cramer, R. D., Berkoff, C. E., *Chem. Soc. Rev.* **3**, 273 (1974).
Snedecor, G. W., Cochran, W. G., "Statistical Methods", Iowa State University Press, Ames, Iowa, 1967.

Received for review July 16, 1975. Accepted October 20, 1975. One of us, William P. Purcell, would like to thank FMC Corporation for a research contract in support of this research and Cotton Incorporated for their continued sponsored research in the application of QSAR to the search for more effective herbicides and pesticides.